KEY Note Lecture I

SUPRAMOLECULAR INTERFACIAL ARCHITECTURES FOR OPTICAL BIOSENSING

W.Knoll[1,2], F.Yu[1], D.Yao[1], R.Naumann[1], S.Schiller[1] & K.Morigaki[3]

[1]Max-Planck-Institute for Polymer Research, *Mainz, Germany.* [2]*Departments of Chemistry and of Materials Science,* National University of Singapore. [3]National Institute of Advanced Industrial Science and Technology, *Special Division for Human Life Technology, Midorigaoka, Ikeda, Japan.*

INTRODUCTION: Any optical and/or electrical biosensor (transducer) surface requires a functional coating that allows for a highly specific and selective coupling via recognition and binding of the analyte molecule of interest from solution while simultaneously minimizing the response caused by non-specific binding. To this end we design, synthesize, assemble, and characterize (both structurally and functionally) supramolecular interfacial architectures with chemical features that vary for different types of bio-analytes. For the specific case of biosensor applications based on surface plasmon optical detection principles we will discuss in this contribution the different strategies optimized for DNA hybridization and protein binding assays. This will be complemented by examples for the build-up of tethered lipid bilayers for the construction of membrane chips.

METHODS: All optical detection studies were carried out with the recently introduced surface-plasmon fluorescence spectroscopy that combines the optical field-enhancements obtainable at resonant excitation of a surface plasmon mode with fluorescence detection prinicples. This way extreme limits of detection of binding events can be achieved provided the energy transfer quenching mechanisms operating for chromophores located near metal substrates are taken into account. For the tethered lipid bilayer membranes the metal substrate that carries the surface-plasmon mode simultaneously acts as the working electrode in a 3-electrode-electrochemical cell allowing for the simultaneous assessment of membrane properties by electrochemical and impedance spectroscopic techniques.

RESULTS: For surface hybridization studies we use an architecture that is based on a generic binding matrix built from biotinylated mixed self-assembled thiol monolayers and a streptavidin layer. Biotinylated oligonucleotide catcher probes or their uncharged peptide nucleic acid (PNA) analogues are immobilized via this binding matrix at the sensor surface in contact with a flow cell. The injection of target oligonucleotides carrying a chromophore then allows for the on-line observation of the association (hybridization) and dissociation processes, as well as for the evaluation of affinity constants (by titration experiments) as a function of the length of the oligos, matching and mismatching base pairs (for SNPs detection), the temperature, pressure, ionic strength, etc. For protein binding studies we demonstrate that the use of hydrogels or polymer brushes offer a significant sensitivity advantage in terms of a high binding site density but also for the optimization of the distance dependent efficiency for chromophore excitation and fluorescence emission. We demonstrate that detection limits in the attomolar concentration range are possible. Finally, we introduce a novel thiolipid system that allows for the construction of electrically tight bilayers with a capacity in the range of $C = 0.5\mu F/cm^2$ and an electrical resistivity in excess of $R = 10M\Omega cm^2$. The incorporation of the ion-carrier valinomycin results in a reversible increase of the K^+-selective membrane conductivity by more than 4 orders of magnitude. The consequences of this breakthrough will be discussed. Moreover, the use of polymerizable lipids for the preparation of patterned tethered bilayers to be used in membrane chips with multiple corrals will be discussed.

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POLYMER BRUSHES: SURFACE-IMMOBILIZED POLYMERS

W.J.Brittain,* S.G.Boyes, T.Granville, B.Zhao, B.Mirous & M.Baum

Department of Polymer Science, The University of Akron, Akron, OH 44325-3909, USA.

INTRODUCTION: Polymer brushes refer to an assembly of polymer chains that are tethered by one end to a surface or interface.[1] Tethering of the chains in close proximity to each other forces the chains to stretch away from the surface to avoid overlapping. Applications for polymer brushes include colloidal stabilization, tailored surface properties, chemical gates, stimuli-responsive surfaces and cell growth confinement.[2]

METHODS: We have synthesized multiblock polymer brushes using a "grafting-to" technique that first involves the surface immobilization of a polymerization initiator onto a silicate substrate. Most of our brushes have been synthesized using atom transfer radical polymerization (ATRP).[3] We generally use flat substrates for our studies to facilitate surface analytical techniques. We either used freshly cleaned silicon wafers or attenuated total reflectance (ATR) crystals for IR spectroscopy. ATRP is a living radical process that provides the opportunity to perform sequential monomer addition, and thus multiblock copolymer formation. Reactions are conducted under standard ATRP conditions, namely exclusion of oxygen and under inert atmosphere. Our samples have been characterized by X-ray reflectometry and ellipsometry to determine film thickness. Chemical composition at the surface was deduced by IR spectroscopy and X-ray photoelectron spectroscopy. In addition, we routinely used water contact angles to probe surface properties. Because we use free initiator in our polymerizations to maintain control of the polymerization, we generate free polymer in solution. The molecular weight of this free polymer often corresponds to the polymer brush, so we have used gel permeation chromatography to estimate polymer brush molecular weight.

RESULTS: We have synthesized a variety of stimuli-responsive diblock copolymer brushes. Rearrangement (Figure 1) of these diblock co-polymer brushes can be induced by heat, block-selective solvents, pH, or ionic strength. A variety of divergent diblock copolymer systems were studied including a hydrophobic–hy-drophilic diblock, a hydrocarbon–fluorocarbon diblock, and a hydrocar-bon–polyelectrolyte diblock. The extent of re-arrangement of these different diblock systems was primarily deduced using water contact angles. We have found that grafting density and the interaction parameter between the two block segments plays a large role in the rearrangement. If the two blocks are too dissimilar (large interaction parameter), the rearrangement is retarded. We also studied the rearrangement of ABA triblock copolymers. For the Si/SiO_2//polystyrene-*b*-poly(methyl methacrylate), we observed an efficient rearrangement that was accompanied by a unique nanomorphology that we have attributed to pinned micelles.

We have generated metal nanoparticle–brush hybrid systems by making silver and palladium salts of a poly(acrylic acid) block followed by reduction with hydrogen gas. The silver nanoparticle hybrid has the potential to serve as a antimicrobial surface. Further applications may also include patterned reduction to create nanowires.

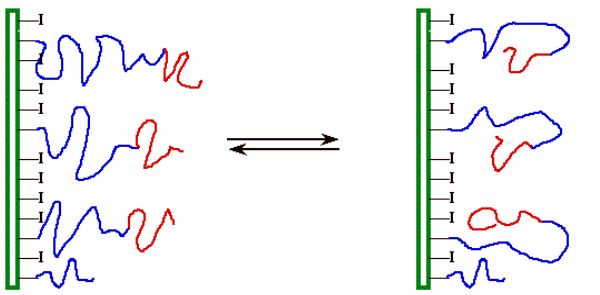

Fig. 1: Proposed rearrangement of diblock co-polymer brushes in response to an external stimulus.

DISCUSSION & CONCLUSIONS: We have synthesized a variety of multiblock polymer brushes that show stimuli-responsive behavior. Key features that dictate the rearrangement include grafting density and the interaction parameter between blocks. We have also devised a synthetic route to metal nanoparticle–polymer brush hybrid systems.

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TEMPLATE DIRECTED SELF-ORGANIZATION OF SOFT MATERIALS

X.Zhang[1,2*], H.P. Xu[1], M.F.Wang[1], F.Shi[1], H.Y.Zhang[1] & Z.Q.Wang[2]

[1]*Key Lab of Supramolecular Structure & Materials, Jilin University, Changchun 130023, P. R. China.*
[2]*Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China.*

INTRODUCTION: Self-organization of soft materials such as amphiphile, dendrimer and polyelectrolytes can be exploited to create diversified structures of technological importance. By deliberately designing the soft materials and choosing the template and conditions, we are able to confine the soft materials in two-dimensional space for development of patterning chemistry and nanostructured materials. Till now, ways of self-organization for pattern formation is greatly restricted in utility, since the size and shape of such a structure is determined by the chemical and physical forces that directs its formation rather than by the requirements of its end application. However, this research is of significance as the method of choice for fabricating various thin film-based devices and sensors using nanoscale patterns in the size range of 1 to 10^2 nm with controlled composition and molecular orientation [1].

METHODS: AFM images of interfacial micelles were captured in situ (tapping mode in fluid) and ex situ (tapping mode in air) on mica substrate and STM images of self-assembled monolayers(SAMs) were taken on gold slide using commercial instruments Nanoscope IIIa AFM Multimode™ (Digital Instrument, CA). Surface-bound dendrons were synthesized through convergent method.

RESULTS & DISCUSSION: To combine the electrostatic layer-by-layer assembly and photo-chemical reaction, we have fabricated photosensitive multilayer thin film by alternating deposition diazo-resins and poly(acrylic acid), and then by taking advantage of the different solubilities of the multilayers prior and after UV irradiation, in a proper solvent, patterned surfaces with designed lateral structure are obtained. This patterned surface can be modified further more and used as a temple for selective adsorption of different functional species [2].

Microporous surface can be also obtained interestingly by post-treatment of the hydrogen-bonding-directed multilayers of poly(4-vinylpyridine) and poly(acrylic acid) in base solution. A two-step mechanism is proposed and confirmed: the first step is the dissolution of poly(acrylic acid) from the film into the basic solution; the second is the gradual reconformation of poly(4-vinylpyridine) remaining on the substrate, producing a microporous film [3].

Going down in scale, we have taken advantage of the interfacial aggregation properties of a series of bolaform amphiphiles bearing mesogenic groups to form cylindrical or wormlike surface micelles that are stable against drying [4]. The enhanced stability can be attributed to the strengthened interaction due to the introduction of mesogenic groups and long spacers in the bolaform amphphiles. The confined geometries of the surface micelles can be fine-tuned by changing factors e.g. chemical structures of the bolaform amphiphiles, substrates, and ionic strength.

To form different structures on the nanometer scale, we have used surface-bound polyether dendrons as nanobuilding blocks for chemisorption onto gold-covered slides [5]. By the precise control of the periphery groups of the surface-bound dendron-thiols, we have obtained surface structures with controlled morphology: heptane chains appear to enhance the interaction between symmetrical backbones, leading to the formation of stripes, while oligo(ethylene oxide) chains appears to weaken the interaction between symmetrical backbones, resulting in a homogeneous structure. Dendrons with both heptane and oligo(ethylene oxide) chains exhibit nanophase separation in a confined state, leading to formation of a honeycomb structure. It has shown clearly that the periphery structure can determine the surface organization.

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CHARACTERIZATION OF TAILORED NON-FOULING POLYMER SURFACES USING TIME OF FLIGHT SECONDARY ION MASS SPECTROMETRY AND MULTIVARIATE ANALYSIS

M.S.Wagner[1], S.Pasche[2], D.G.Castner[3], M.Textor[2]

[1]National Institute of Standards and Technology, Gaithersburg, USA [2]Dept. of Materials, ETHZ, Zürich, Switzerland [3]Depts. Of Bioengineering and Chemical Engineering, University of Washington, Seattle, USA.

INTRODUCTION: Non-fouling polymeric materials are widely used in biomaterials and biosensors. In particular, adlayers of Poly(L-Lysine)-graft-Poly(Ethylene Glycol), PLL-*g*-PEG, on metal oxide substrates resist protein adsorption and cell adhesion [1]. In this study, static Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) was used to study the effect of surface chemistry and structure on the protein resistance of a series of PLL-*g*-PEG adlayers with systematically varied ethylene glycol (EG) surface densities.

METHODS: PLL-*g*-PEG polymers were synthesized with various PEG graft densities (i.e. number of PLL monomers per grafted PEG chain) using 1, 2, or 5 kDa N-hydroxysuccinimidyl esters of methoxy-terminated PEGs and 20 or 300 kDa PLLs and assembled onto Nb_2O_5 substrates as described previously [2]. The EG surface density and protein resistance of the PLL-*g*-PEG adlayers were measured using Optical Waveguide Lightmode Spectroscopy (OWLS). The surface chemistry and structure was monitored using static positive and negative ion ToF-SIMS and Principal Component Analysis (PCA) [3].

RESULTS: As the EG surface density of the PLL-*g*-PEG adlayers increased from 3.9 to 30.9 EG units/nm^2, the amount of protein adsorption decreased from ~300 ng/cm^2 to below the OWLS detection limit (~1 ng/cm^2). Generally, changing the molecular weight of the grafted PEG chain did not affect protein resistance of the adlayers. However, changing the PLL molecular weight from 20 to 300 kDa typically increased the amount of protein adsorption.

PCA of the positive ion spectra showed two distinct trends. The relative intensities of the PEG-related peaks were positively correlated with the thickness of the adlayer and the amount of PEG at the outermost surface of the adlayer. The relative intensities of the peaks related to the methoxy endgroup of the PEG molecules were positively correlated with the amount of methoxy endgroup in the adlayers. The variance in the negative ion spectra was principally due to changes in the thickness of the PLL-*g*-PEG adlayer. The results from PCA were used to generate multivariate peak ratios which corresponded with the thickness, PEG enrichment, and methoxy group enrichment of the adlayer. As the EG surface density and protein resistance increased, the adlayers became thicker and more methoxy endgroups were exposed at the outermost surface. Changing the PLL molecular weight from 20 kDa to 300 kDa increased the amount of protein adsorption of these adlayers, but did not appreciably change the positive or negative ion ToF-SIMS spectra, suggesting that long-range forces (e.g. electrostatic forces) were responsible for protein adsorption for these adlayers.

DISCUSSION & CONCLUSIONS: Protein resistance of the PLL-*g*-PEG adlayers was dependent on the EG surface density and the structure of the PEG chains on the surface. Tightly packed PEG chains that exposed their methoxy endgroups showed enhanced resistance to protein adsorption while less densely packed PEG surfaces revealed substrate (PLL or Nb_2O_5) sites for protein adsorption. Adlayers of PLL-*g*-PEG synthesized using 300 kDa PLL probably looped from the Nb_2O_5 substrates, allowing more protein adsorption than the adlayers of PLL-g-PEG synthesized using 20 kDa PLL.

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HOW BIOLOGY USES POLYMERS TO REGULATE ADHESION IN VIVO

D.Leckband[1,2], C.Johnson[1], C.Perrin-Tricaud[3], I.Fujimoto[3] & U.Rutishauser[3]

[1]Dept. of Chemical and Biomolecular Engineering.
[2]Dept. of Chemistry,University of Illinois, Urbana, IL USA.
[3]Celular Biology and Biophysics, Memorial Sloan Kettering Cancer Center, NY, NY USA.

INTRODUCTION: The neural cell adhesion molecule (NCAM) is one of the most abundant cell adhesion proteins in the central nervous system. This protein mediates both cell adhesion and cell-cell signaling via homophilic interactions with identical proteins on opposing cells (Figure 1).[1] It is also involved in neuronal development and plasticity.

The extracellular regions of NCAM consist of five tandemly arranged immunoglobulin domains (IgSF) followed by two C-terminal fibronectrin type III (Fn III) domains (Figure 1).[1] The functional significance of these different domains is a subject of some controversy.

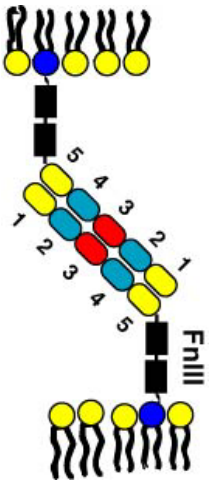

Fig. 1: Proposed mechanism of homophilic NCAM adhesion.

In addition, a unique post-translational modification (PTM) of the protein involving the addition of polysialic acid, (PSA, a long linear, negatively charged carbohydrate polymer) changes the protein function from an adhesion promoter to an adhesion inhibitor.[2,3] This post-translational on domain 5 has significant biological consequences. It increases neuronal plasticity and the measured separation distances between cells.[2,3] However, the physical mechanism by which the attachment of this carbohydrate chain alters the adhesive function of NCAM is still unknown.

This study describes direct force vs. distance measurements of the interactions between NCAM. From these measurements, we determined the mechanism by which NCAM mediates intermembrane adhesion. In addition, we quantified the effect of PSA on the range and magnitude of the intermembrane repulsion, and determine the physical mechanism by which PSA regulates NCAM adhesion.

METHODS: The surface force apparatus[4] was used to measure the normalized force between oriented monolayers of NCAM extracellular segments. The NCAM monolayers were self-assembled onto supported planar lipid bilayers. The bilayers contained lipids with headgroups modified with NTA (nitrilo triacetic acid) moieties (NTA-DLGE). The NCAM was engineered with a C-terminal hexahistidine tag at the position in the sequence where the extracellular domain of the wild type protein begins to thread through the membrane. These engineered proteins bound to the NTA-lipids via their histidine tags. Surface plasmon resonance and X-ray reflectivity measurements were used to characterize the self-assembly of the protein monolayers.

RESULTS: The surface force apparatus[4] was used to quantify the ranges and magnitudes of the attractive and repulsive forces between identical NCAM monolayers with and without the polysialic acid modification. With the unmodified form of NCAM, we demonstrated that the protein forms two bound states at two, different membrane spanning distances. Each of these two states involves different domains of the protein. These data reconcile prior apparently contradictory findings regarding the mechanism of NCAM adhesion and the domains involved in adhesion. Measurements with mutants lacking different protein domains further identified the key modules participating in these adhesive interactions.

Measurements between monolayers of the post-translationally modified form of NCAM show that the addition of the linear carbohydrate chain to domain 5 increases the magnitude of the repulsive force between the membranes. Importantly, the range and magnitude of the increased repulsion is sufficient to abrogate binding via both of the binding alignments measured with the unmodified protein. The enzymatic removal of PSA recovered both binding interactions between the proteins and restored NCAM-mediated intermembrane adhesion. These data suggested that PSA alters cell-cell adhesion by increasing the electrosteric repulsion between cell membranes over a distance that interferes with binding between NCAMs as well as other adhesion proteins on the cell surface.

The molecular origin of the PSA-dependent reduction in adhesion was confirmed by measurements performed at different ionic strengths. Theory predicts that the excluded volume of polyelectrolytes will decrease with the ionic

strength. If the PSA merely impeded binding by nonspecific electrosteric repulsion, then reducing the excluded volume of the polymer should correspondingly increase the intermembrane attraction. Consistent with this, increasing the ionic strength reduced the magnitude of the intersurface repulsion, and the intermembrane attraction increased correspondingly. Conversely, decreasing the ionic strength increased both the magnitude and range of the inter-surface repulsive force. The range and magnitude of the repulsion at physiological ionic strengths suggest that this polymer may influence not only NCAM binding, but also the adhesive interactions of other cell surface adhesion proteins.

DISCUSSION & CONCLUSIONS: These measurements show that NCAM mediates cell adhesion via a complex mechanism involving multiple protein domains. Importantly, we further show that the regulation of NCAM-mediated intermembrane adhesion by the post-translational addition of polysialic acid is due to the corresponding increase in the nonspecific electrosteric repulsion between the membranes. Importantly, our findings directly demonstrate the use of surface attached hydrophilic, waters soluble polymers to regulate of cell-cell interactions *in vivo*.

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SUBSTRATE COMPLIANCE VS LIGAND DENSITY IN CELL ON GEL RESPONSES

A.Engler[1], M.Sheehan[1], H.L.Sweeney[2] & D.E.Discher[1]

[1]*Biophysical Eng'g. Lab, Institute for Medicine and Engineering, University of Pennsylvania, USA.*
[2]*Cell and Molecular Biology, University of Pennsylvania, USA.*

INTRODUCTION: Substrate stiffness is emerging as an important physical factor in the response of many cell types. Like other anchorage dependent cells, smooth muscle cells derived from rat aorta (A7R5 line) are found to spread more and organize their cytoskeleton and focal adhesions much more so on rigid glass and 'stiff' substrates than on 'soft' gels. Collagen density also factors into cell on gel responses, with minimal spreading on very low collagen and a weak maximum in cell spreading on intermediate collagen densities. Bell-shaped curves are readily modeled to highlight the coupling between ligand density and substrate stiffness. Most surprising, however, spreading on soft gels is found to be almost independent of adhesive ligand density: even with high collagen densities, the minimal spreading of cells cannot be over-ridden. On soft gels, however, a fraction of GFP-actin (over)expressing cells do spread. Such cells invariably show an organized cytoskeleton of stress fibers, which suggests that the cytoskeleton is at least one structural node in a signaling network that can override spreading limits typically dictated by soft gels.

METHODS: Gel substrates consisted of polyacrylamide (PA) supported on glass by a method modified from Wang and Pelham [1]. Briefly, coverslips were treated for attaching a 70-100 μm thick gel of PA polymerized from 3-10% acrylamide monomer plus bis-acrylamide (0.03-0.3%). The %ages dictate the final elastic modulus as measured by AFM. Collagen (plus fluorescent collagen) at a desired concentration was chemically crosslinked to the gel surface with a heterobifunctional crosslinker, Sulfo-SANPAH, at 37 ^{o}C overnight.

The A7r5 SMC line is generally known to maintain differentiation markers for α-actinin and myosins. SMC were cultured in polystyrene flasks with DME Medium supplemented by 10% fetal bovine serum. Some cells were pre-transfected in a hypo-serum medium with GFP-paxillin (for focal adhesions) or GFP-actin plasmids using standard Lipofectamine transfection protocol (Invitrogen). Cells were plated on the PA substrates of controlled stiffness and collagen density and observed up to 24 hours.

RESULTS: Varying the collagen density on PA gels and glass was widely found to modulate cell spreading to different degrees (Fig. 1). On soft substrates, however, collagen density could not override the minimal cell spreading; instead it seemed to pin cells down and give a bell-shaped response. The biphasic behavior here is reminiscent of that seen in cell motility versus ligand density [2], which had also been explained by over-attachment at high ligand.

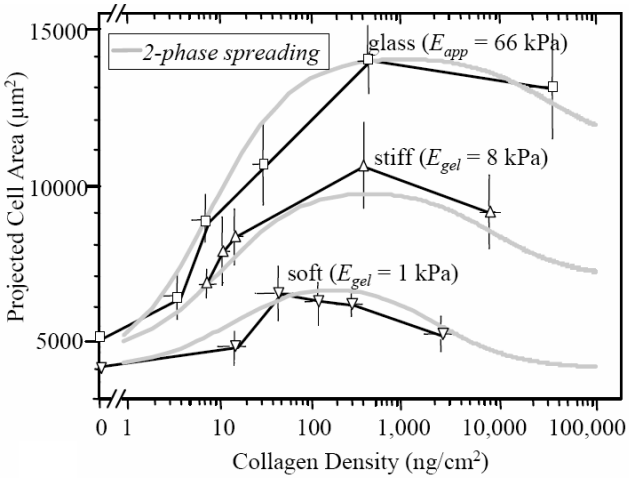

Fig. 1: Cell spreading on substrates of various stiffness and various ligand density.

To make the coupling above explicit, the two phases of cell spreading are modeled as a single function for *Area* dependent on E or E_{eff} (units of kPa) and *coll* = [*collagen*]. The collagen dependence is expressed in the sum of two hyperbolic terms typical of saturable equilibrium associations. The area-promoting association of cell-substrate binding is modeled by the first term of Eq. 1 below (with constant K_1) while the second term models the fractional dissociation in a separate, area-inhibiting reaction (with constant K_2). Importantly, a power law fit of *Area* (μm^2) versus E (kPa) has been used to scale the area-promoting reaction obtained from cell spreading over a 24-hour time course. Cells cultured on glass see $E = E_{\text{eff}}$ based on the power law fit. The baseline area response of a cell in solution appears as a constant, C, and the association constants K_1 and K_2 are taken to be power laws in E.

$$Area = C + 3000E_{\text{eff}}^{0.29}[K_1*coll/(1+K_1*coll)] + 3000[1/(1+K_2\,coll)] \quad \text{(Eq. 1)}$$

with $C = 1000$, $K_1 = 0.07*E_{\text{eff}}^{0.13}$, and $K_2 = 0.0005/E_{\text{eff}}^{0.66}$. A kinetic approach to biphasic cell motility has been described by a ratio of dissociation rate constants, which correspond here to a ratio of K_1 and K_2. The correspondence supports the notion that similar phenomena may underlie the behavior in cell spreading.

Labeling of the actin cytoskeleton with rhodamine phalloidin invariably showed that the most spread cells had a well-organized cytoskeleton of stress

fibers that appeared lacking in the unspread cells (Fig. 2).

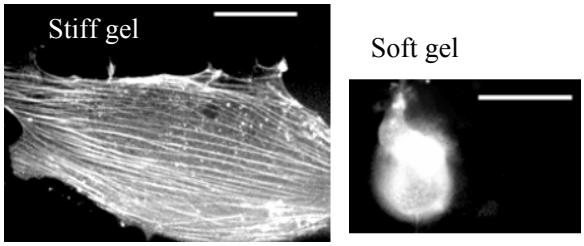

Fig. 2: Range of cytoskeletal organization typically seen on different substrates.

Transfected GFP-actin and GFP–paxillin cells helped to elucidate the coupling of substrate elasticity to cytoskeletal and adhesion expression and structure. On both stiff PA gels and rigid substrates pretreated with collagen, structured actin stress fibers and paxillin focal adhesions were again typically observed as well-spread cells predominate. On the softest PA gel with optimal collagen, the GFP-actin cells were remarkably well-spread as opposed to rounded. Conversely, without collagen, all substrates showed just a small sub-population of highly spread cells with organized actin stress fibers. The results collectively illustrate an emergent rule: spreading has a basis in or at least correlates well with cytoskeletal assembly. GFP-paxillin expression, in contrast, had little ability to over-ride the inadequate signal from a given substrate.

CONCLUSIONS: Overall, cell spreading and cyto-organization are suggested to be a function of a substrate's mechanical as well as chemical properties. Of course, additional physical variables such as substrate topography can be equally important. However, one must remember that cells are highly active, their filaments are incessantly turning over, their stress fibers are constantly pulling on the matrix. Hence, the idea that these stresses on the underlying substrate will strain it and feedback on cell activity is as intuitive as grabbing a small rubber ball in one's hand and successfully squeezing it in approximate relation to its compliance. In a simplistic way, an active cell is as much like your hand - highly structured and responsive to feedback - as a liquid drop that passively wets a surface. It is thus understandable that cells display "durotaxis" [3] as well as haptotaxis and chemotaxis.

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BIOENGINEERED SUBSTRATA TO PROBE SUBSTRATE COMPLIANCE EFFECTS ON VASCULAR SMOOTH MUSCLE CELL BEHAVIOR

J.Y.Wong & X.Brown

Dept. of Biomedical Engineering, 44 Cummington St., Boston University, Boston, MA, USA.

INTRODUCTION: During the development of vascular occlusive disease, stiffening of the vessel is often observed. Recent studies have shown that changes in substrate compliance affect cell adhesion, migration, and differentiation. Vascular smooth muscle cell (VSMC) proliferation is altered during vascular injury and contributes to restenosis. However, the effect of substrate compliance on VSMC behavior is unclear. We hypothesize that the biomechanical properties of the substrate can modulate VSMC behavior. In order to test this hypothesis, we investigated the behavior of VSMCs on polydimethylsiloxane (PDMS) substrata with mechanical properties in the range of human aortas.

METHODS: ***Substrate preparation and characterization.*** Polydimethylsiloxane (PDMS) substrata are prepared using SYLGARD 184 (Dow Corning). Different ratios of silicone elastomer base and curing agent are mixed to obtain PDMS substrata with different elastic moduli. The surfaces of the PDMS substrata are modified with polyelectrolyte layers using the layer-by-layer (LBL) technique. Briefly, layers of polyethyleneimine (PEI, 0.1%) and polystyrenesulfonate (PSS, 0.3%) are alternatively assembled onto the PDMS surface. The substrata are coated with a total of 6 layers and were washed with sterile PBS. The elastic moduli are determined using a standard tensile test.

Cell culture. Bovine vascular smooth muscle cells are maintained in Dulbecco's modified Eagle's medium supplemented with 10% calf serum, L-glutamine and penicillin-streptomycin. Cells are grown to confluence and then serum-starved for 2 days. Cells are re-suspended in either serum-free media or complete media (10% serum) for experiments.

Cell attachment. The number of cells attached after 2 hours is determined by acid phosphatase assay.

Cell proliferation. Serum-stimulated rates of cell proliferation are determined by acid phosphatase assay.

RESULTS: Using three different ratios of silicone elastomer base and curing agent, we were able to generate substrata with elastic modulus ranging from 0.05 to 1.78 MPa. Layer-by-layer treatment of PDMS significantly improved VSMC adhesion (Fig 1).

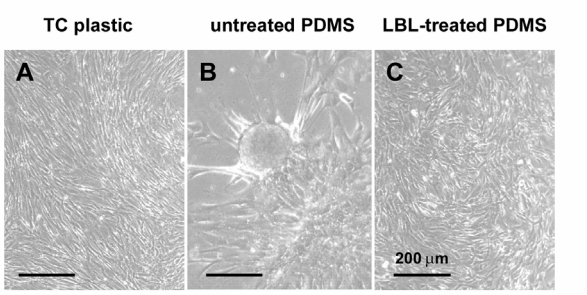

Fig. 1: ***Surface treatment of PDMS is required to maintain VSMC adhesion.*** *Phase contrast images of VSMCs after 5 days culture on (A) tissue culture plastic, (B) untreated PDMS, and (C) layer-by-layer-treated PDMS.*

We found that percent cell attachment increases with substrate elastic modulus (Fig 2). However, this effect is diminished in the presence of serum. Moreover, the amount of attached cells is reduced when 10% serum is added on the substrata with the highest elastic modulus.

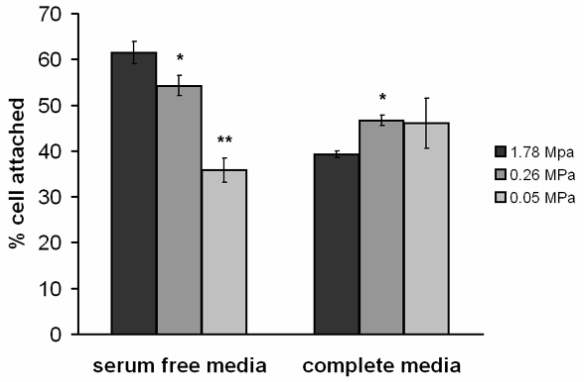

Fig. 2: VSMC attachment to LBL-treated PDMS substrata in the presence or absence of serum. Values are reported as averages and S.E.M.

We found that the rate of serum-stimulated VSMC proliferation increases when the elastic modulus of PDMS decreases (Fig 3).

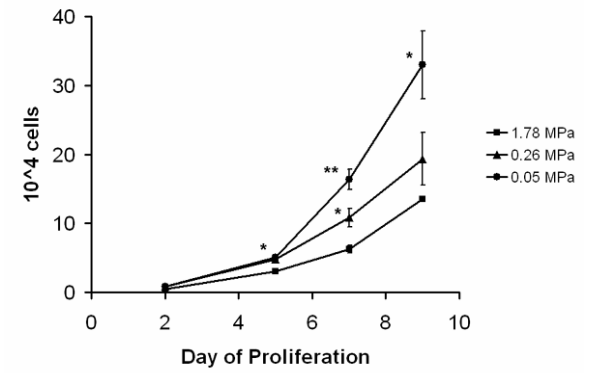

Fig. 3: Serum-stimulated VSMC proliferation on PDMS substrata with different elastic moduli. Values are reported as averages and S.E.M.

DISCUSSION & CONCLUSIONS: The elastic modulus of human aortas has been reported to range from 0.1 to 3 MPa [1]. The elastic moduli of the PDMS substrata in our study fall in this range. Others have used PDMS to study cell-substrate interactions, but a major problem has been to maintain cell adhesion for extended periods of time [2]. Recently, Ai *et al.* [3] showed that layer-by-layer treatment of PDMS improves significantly cell adhesion to PDMS. Applying their method, we find that VSMC adhesion can be maintained for at least 2 weeks (data not shown).

We observe an effect of substrate compliance on cell attachment in the *absence of serum*. It is not surprising that we observe different effects in the presence and absence of serum. Cells are known to respond to soluble growth factors or ECM molecules in serum. While the presence of serum appears to mask the effects of substrate compliance on VSMC attachment, we clearly observe an effect of substrate compliance on serum-stimulated VSMC proliferation.

In conclusion, PDMS is a useful tool for studying the effects of substrate compliance on VSMC behavior. More important, our results show that VSMCs are capable of sensing and responding to changes in substrate compliance in a range of elastic moduli that is physiologically relevant.

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Session 1: Fundamental Aspects of Polymeric Molecules at Interfaces and Biological Responses

MULTILAYERED FILMS MADE OF POLYSACCHARIDES: INFLUENCE ON CELL AND BACTERIAL ADHESION

C.Picart[1], L.Richert[1], E.Payan[2], Ph.Lavalle[1], P.Schaaf[3] & J.-C.Voegel[1]

[1]*INSERM Unité 595, Université Louis Pasteur, 11 rue Humann, 67085 Strasbourg Cedex, France.*
[2]*Laboratoire de Physiopathologie et Pharmacologie Articulaires, UMR 7561 et IFR 111, Faculté de Médecine, 54 505 Vandoeuvre-les-Nancy, France.*
[3]*Institut Charles Sadron (CNRS-ULP), 6 rue Boussingault, 67083 Strasbourg Cedex, France.*

INTRODUCTION: Polyelectrolyte multilayers (PEM) have emerged as a new and versatile way to coat any type of substrate[1]. In the biomaterial field, their potential applications are wide since they can be used to create biomimetic films and can also be functionnalized by insertion of peptides or proteins [2]. Preparation of films made of natural polysaccharide has emerged. The aim of our study was to buildup films of polysaccharides and to investigate their properties with respect to cell and bacterial adhesion.

METHODS: Chitosan (CHI, 120 kDa), poly(L-lysine) (PLL, 30 kDa), and Hyaluronan (HA, 400 kDa) were dissolved in a 0.15 M NaCl solution at pH = 5 or 6.5. Quartz Microbalance (QCM) and Optical Waveguide Lightmode Spectroscopy (OWLS) were used to analyze *in situ* the film growth. Film topography was examined by Atomic Force Microscopy (AFM) and Confocal Laser Scanning Microscopy (CLSM) allowed to visualize the z-structure of the film and to determine its thickness[3]. Primary chondrocytes adhesion was investigated and bacterial adhesion tests are carried out with *E.Coli* –GFP.

RESULTS: OWLS and QCM exhibits an exponential raise for both CHI/HA and PLL/HA films. OWLS signal begins to cycle after around 9 bilayers have been deposited suggesting the diffusion of the polycation, either CHI or PLL within the film[2]. CLSM images confirm that CHI and PLL diffuse within the film while HA remains as a layer (Figure 1). AFM shows that the surface is first covered by small islets that progressively coalesce toward a uniform film (after 9 bilayers). Primary cell adhesion decreases when the number of bilayers in the films is increased, for both CHI/HA and PLL/HA films whatever the outermost layer of the film is (positive or negative) (Figure 2). But, noticeably, $(CHI/HA)_{10}$ are films lead to a ≈80% decrease in bacterial adhesion as compared to bare glass.

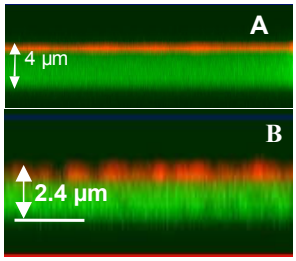

Fig. 1: CLSM images of a $(PLL/HA)_{24}$-PLL-FITC (with HA_{24}-TR) and of a $(CHI/HA)_{36}$ film containing two labeled layers, CHI_{36}-FITC and HA_{36}-TR.

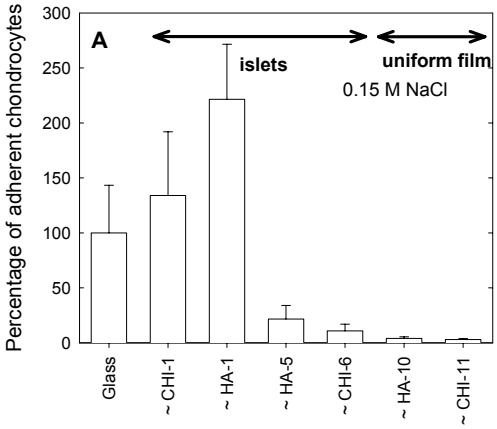

Fig. 2: Primary rat chondrocytes adhesion as measured by ^{3}thimidine after 4 days of contact with $(CHI/HA)_i$ films containing an increasing number of layers (1,5,10) either terminated by ~CHI or ~HA.

DISCUSSION & CONCLUSIONS: Multilayered films made of polysaccharides grow exponentially with the number of deposited layers and can become more than micrometer thick. As these films are highly hydrated, they are cell-resistant but also bacterial resistant. In recent experiments, these films have been crosslinked thereby changing the cell adhesive properties.

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BIOFUNCTIONAL PROCESSING: SCAFFOLD DESIGN, FABRICATION AND SURFACE MODIFICATION

R.Mülhaupt, R.Landers & Y.Thomann

Institut für Makromolekulare Chemie der Universität Freiburg und Materialforschungszentrum, D.

INTRODUCTION: Scaffolds play an important role in tissue engineering (TE). Scaffold architectures have to be designed and fabricated to scaffold has to fit the needs of individual patients. Novel rapid prototyping (RP) technologiesiques meet these requirements by layer-by-layer construction combining computer-assisted design with biomedical diagnostics like computer tomography. In particular the ability of RP techniques to create a well defined shape, makes these techniques so attractive for the fabrication of 3D-scaffolds.[INT6] Among the expanding number of But not all of the commercial RP techniques, very few are suitable for application in TE. are useful for this medical application. They have to meet several criterions, Stringent criteria must be met in biofunctional RP like like the production of scaffolds from non-toxic processing, biocompatible and biodegradable materials, avoidance of elevated temperatures and sterile and mild processing conditions.and the absence of strong heat, which causes the denaturation of sensitive biomolecules like proteins.

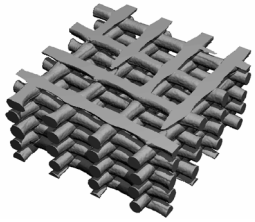

Fig. 1: µcomputertomographic image of a PLGA-3D scaffold. Strand diameter is 200 µm.

METHODS: A new 3D dispensing process (3D Bioplotting) has been introduced at the Freiburger Materialforschungszentrum recently[1]. Key feature of this process is the 3D dispensing of strands of a viscous plotting material into a liquid medium. As a consequence of the buoyancy compensation, architectures can be fabricated mostly without requiring temporary support structures and especially processing of low viscous materials with low viscosities is facilitated[2] profit. The 3D Bioplotter can therefore process a remarkable wide variety of different materials including polymer melts, thermoset resins, polymer solutions, pastes with high filler content and hydrogel precursors[3]. More general is the approach A more viable general approach is based upon dispensing to dispense biomaterials continuously into a liquid medium with a matching density. This commercial RP process technique became known asbecame lknown as 3D Bio The curing after dispensing can be achieved by a physical phase transition or a chemical reaction (i.e. between material and medium). Nevertheless, one main disadvantage of 3D Bioplotter scaffolds is the smooth surface of the strands, which is unfavourable during cell seeding.

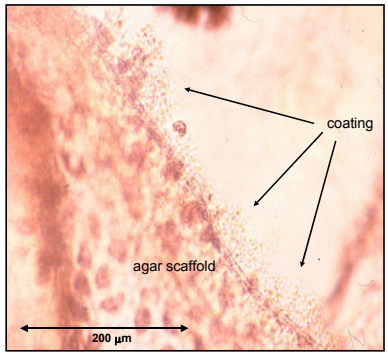

Fig. 2: 25 µm thick calcium alginate/calcium hyaluronate coating on the surface of an agar scaffold.

RESULTS: Different attempts have been made to make the hydrogel (agarose) scaffold strand surface more rough and to change its chemical nature, which has been evaluated by observing the cell attachment. Most sucessful were precipitation reactions performed at the scaffold surface by using a loading technique. The hydrogel scaffolds were placed in an aqueous solution of reactant A, loaded, washed and dipped in an aqueous solution of reactant B. The molecules of A diffusing to the interface, start the reaction with B and the reaction product precipitates in a fur-like coating at the phase border. Suitable reaction types are especially the complex formation of polyelectrolytes with low molecular weight ions and the enzymatic gelation of fibrinogen with thrombin. Cell attachement is significantly enhanced by this type of surface modification.

CONCLUSION: Combination of modern processing technologies for biomaterials and suitable surface modification reactions provides scaffolds highly desirable for TE applications.

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Key Note Lecture II

SURFACE ANALYSIS OF DNA AND PROTEINS IMMOBILIZED IN MICRO-ARRAYING DEVICES

D.W.Grainger[1], P.Gong[1], M.Lochhead[2], S.Metzger[2] & C.Greef[2]

[1]Department of Chemistry, Colorado State University, Fort Collins, CO, USA. [2]Accelr8 Technology Corporation, Denver, CO, USA.

INTRODUCTION: Surface-immobilized microarrays of specific capture reagents are actively studied and marketed for bioassay and drug discovery applications.[1,2] Microarrays of antibodies, nucleic acids, and antigens all encounter problems with prolonged bioactivity and desired capture sensitivity in immobilized formats. Surface chemistry enhances high target capture activity with low non-specific binding. Some microarray surfaces exhibit shelf-life problems from intrinsic hydrolysis of amine-reactive coupling chemistry (active esters, aldehydes) even under protective conditions. Due to the popularity of exploiting amine nucleophiles in biomolecules (e.g., lysines in proteins, derivatized amines in oligonucleotides) for surface coupling, many commercial arraying formats utilize 'amine-reactive' chemistries.[3] Loss of amine-coupling reactivity produces poor reliability, poor signal and sensitivity, high noise and high variability. We have focused on the reactivity of microarray surfaces to both DNA probes and hybridization targets to understand variability and quantitation problems in assay reporting. Due to DNA immobilization variations, we have analyzed the process of regenerating reactive commercial array surfaces targeting amine-reactive nucleic acids or proteins in situ using N-hydroxysuccinimide (NHS) to re-activate amine reactivity, improving both functionalization of the commercial surfaces and immobilization of amine-terminated probes above original capacity. Such methods also offer a possible route to probe binding standardization, and to further understanding quantitative binding issues between immobilized probes and target hybridization.

METHODS: Oligonucleotide selection. Four different synthetic DNA oligonucleotides (MWG Biotech, high purity salt-free) were used: oligonucleotide 5'-CTGAACGGTAGCATCTTGAC (probe) forms a stable duplex with its complementary pair at room temperature with minimal interference due to self-complementarity or secondary structure.[4,5] A 15-T spacer was added to the 3' end of this DNA probe.[6] The complementary 20-base sequence 3'-GACTTGCCATCGTAGAACTG is the target to this probe. Terminal hexylamine-functionalized NH_2-probe-Cy3 and probe-Cy3 molecules were used to study specific surface chemical immobilization vs. non-specific binding (NSB) using fluorescence detection. Terminal hexylamine-functionalized NH_2-probe and target-Cy3 molecules were used for hybridization assays on array surfaces.

DNA probe printing on microarray slides. Commercial polymer-coated amine-reactive slides from Amersham (Codelink™) and Accelr8 Technologies (Optarray™) were purchased and stored per each vendor's recommendations. DNA oligonucleotides were spotted onto microarray slides (pin spotter, spot volumes ~0.7 nL, replicates at 20, 10, 5 and 1 μM DNA in 150mM PBS pH 8.5, with 0.001% Tween20 and 0.001% sarcosine; humidity was 50%), providing dried spots approximately 70-100 μm diameter. Each slide vendor's printing specifications were closely followed for handling, printing, buffers and rinsing. Immobilization was attempted by incubating printed slides overnight, room temperature at 75% humidity.

Microarray surface NHS regeneration. Commercial slides taken directly from vendor storage boxes and printed immediately were denoted "fresh" slides. Other slides were deactivated to amine-reactivity by 10mM NaOH for 0.5 h ("deactivated" slides), or surface 'blocked' using 50mM ethanolamine for 0.5h to quench NHS groups ("blocked slides"). Microarray slides were kept at room temperature in sealed vendor-supplied storage bags for a month and denoted "aged" slides. NHS reactive surface regeneration was then performed on "deactivated" slides, re-named "regenerated" slides post-treatment. Regeneration included full immersion of deactivated slides in 0.5M N-hydroxysuccinimide (NHS) and 0.5M DCC in dry DMF for 3 h at -20°C, then left overnight at +4°C.[7] Slides were rinsed with dry DMF and dried with nitrogen for printing.

Post-print substrate treatment. Residual NHS reactive groups post-printing were blocked using 50mM ethanolamine according to slide manufacturer's specifications. Slides were then rinsed with deionized water, then with 4X saline sodium citrate (SSC), 0.1% sodium dodecyl sulfate (SDS, 50°C) for 30 min., and dried with N_2. Target hybridization was performed with commercial coverslips at room temperature (100% humidity, 4 h) in 5X SSC containing 1μM DNA target. The solution ratio of Cy3-labeled DNA to non-labeled DNA was 1:20. Slides were rinsed with 4xSSC then with 2xSSC/0.1%SDS for 5 min twice, then 0.2xSSC and 0.1xSSC each for 1 min, and finally blown dry with nitrogen.

Microarray scanning, fluorescence detection and array image processing. Microarray slides were scanned using a Packard BioChip Imager (Cy3 channel). Laser power and PMT sensitivity were set

at 70% and 80%, respectively, for probe immobilization scans, and 90% and 100%, respectively, for hybridization scans. Resolution was set to 10 µm. Scanned fluorescence images were processed with ScanAlyze™ software written by Dr. Michael Eisen (University California-Berkeley, http://rana.lbl.gov/EisenSoftware.htm).

RESULTS: Surface regeneration of NHS chemistry on aged microarray slides. Imaging of the efficiency of printed probe immobilization, shown in Figure 1 below, reveals that surface regeneration of 'aged' slides can produce reactivity to amine-modified oligoDNA similar to that from 'fresh' slides. NHS reactivity is lost in storage (data not shown), reducing intrinsic DNA amine reactivity substantially. After surface NHS regeneration, the same slide offered DNA immobilization efficiency equivalent to the original fresh slide. Hybridization yields are also improved by the NHS regeneration treatment as shown in Figure 2.

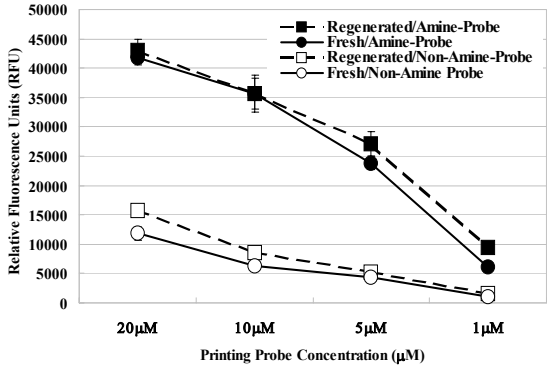

Fig. 1: Fluorescence image quantification for probe printing and immobilization onto commercial reactive polymer-coated microarray substrates under treatments and probe reactivity conditions. RFU data obtained from slide scans show changes in spot pixel average intensities as a function of probe print concentration and slide treatment conditions for regenerated compared to fresh array substrates.

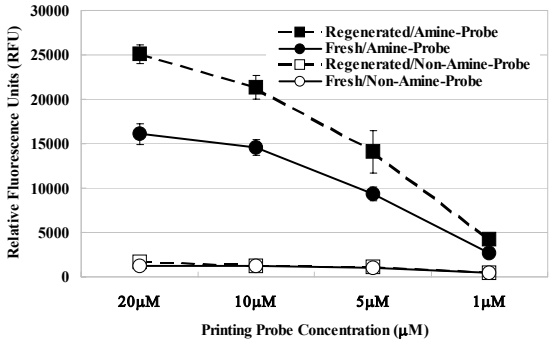

Fig. 2: Fluorescence image quantification for Cy3-labeled complementary target DNA hybridization to oligoDNA probes printed onto commercial reactive polymer-coated microarray substrates under substrate treatments and probe reactivity conditions. RFU data obtained from slide scans show changes in spot pixel average hybridization intensities as a function of probe print concentration and slide treatment conditions for regenerated compared to fresh array substrates.

DISCUSSION & CONCLUSIONS: The NHS regeneration process allows microarray substrates adversely affected by NHS hydrolysis and compromised reactivity to be restored to full or even improved amine-coupling functionality. Regenerated polymer slides subject to hybridization assay perform similarly to fresh NHS-derivatized amine-reactive slides from each vendor, providing enhanced reliability, a standardization 'set-point' for immobilization reactions, and high reactivity for standard microarray printing.

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AMPLIFICATION OF BIOMOLECULAR INTERACTIONS USING LIQUID CRYSTALLINE MATERIALS

N.L.Abbott, Y.-Y.Luk, M.Tingey & B.Clare

Dept. of Chemical and Biological Engineering, University of Wisconsin, Madison WI 53706, USA.

INTRODUCTION: The immobilization of oriented and functional proteins as well as detection of specific binding events to immobilized proteins are two key challenges (amongst many) that must be addressed if surface-based proteomics tools are to be successfully developed. These tools possess the potential to substantially accelerate investigations aimed at understanding issues such as the roles of populations of regulatory proteins in cell signaling processes. This paper reports a strategy for the oriented immobilization of protein receptors on gold films possessing nanometer-scale topographies, and detection of protein binding events to these receptors by using the surface-driven orientational behavior of liquid crystals.[1,2]

METHODS: The approach reported in this paper revolves around the use of self-assembled monolayers (SAMs) formed from nitrilotriacetic acid (NTA)-terminated alkanethiols, **1**, and tri(ethylene glycol)-terminated alkanethiols, **2**. The SAMs are formed on ultrathin gold films that are deposited from a vapor onto silica substrates oriented at an oblique angle of incidence. These polycrystalline gold films possess both in-plane and out-of-plane crystallographic textures as well as an anisotropic topography that can be idealized as a corrugation with an amplitude of 1-2 nm and a wavelength of 10-40 nm. Because the sizes of many proteins are comparable to the spatial scale of the topography of the surface, proteins bound to these SAMs can mask or erase the topography of the surface. The uniform alignment of liquid crystal (e.g., 4-cyano-4'-pentylbiphenyl (5CB) or 4-methoxybenzylidene-4'-butylaniline (MBBA)), which is observed on these surfaces not supporting bound protein, is disrupted by the presence of bound protein. The change in orientation of the liquid crystal is observed by the transmission of polarized light through the liquid crystal.

RESULTS: We have found that single component SAMs formed from **2** on these gold films resist non-specific protein adsorption (using cell lysates) and promote uniform planar anchoring of the nematic liquid crystal, 4-cyano-4'-pentylbiphenyl (5CB). Surprisingly, the azimuthal orientation of nematic 5CB is parallel to the direction of maximum roughness within the gold film when using SAMs formed from **2**, but perpendicular to the direction of maximum roughness when tetra(ethylene glycol)-terminated SAMs are formed on the gold films. Mixed SAMs formed from **1** and **2** bind the hexahistidine-tagged protein MEK via specific complexation of the hexahistidine tags of MEK to the Ni(II)-NTA complexes on the surface.

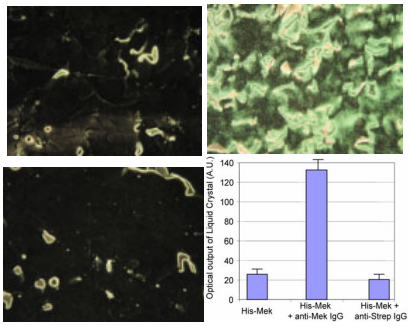

Fig. 1: Optical images (crossed polarizers) of nematic 5CB supported on surfaces that present: ***(A)*** *His-tag Mek,* ***(B)*** *His-tag Mek to which anti-Mek IgG was bound,* ***(C)*** *His-tag Mek exposed to a solution of anti-streptavidin IgG (control experiment). Binding of anti-Mek IgG to the his-tag Mek gives rise to an easily distinguished (and quantifiable) change in light transmission through the LC (error bars indicate standard error of mean luminosity of liquid crystal).*

When gold films are prepared by oblique deposition at an angle of 30° from normal, we measured bound MEK to disrupt the uniform orientation of 5CB, thus leading to an easily visualized change in the optical appearance of the liquid crystal. However, by using gold films deposited at an angle of 40° from normal, we report that bound MEK does not disrupt the alignment of the liquid crystal whereas anti-MEK IgG bound to the MEK does lead to a non-uniform alignment of liquid crystal (Fig 1).

DISCUSSION & CONCLUSIONS: These results, when combined with appropriate control experiments (see Figure 1), suggest that nanostructured surfaces presenting NTA and ethylene glycol-terminated SAMs form a useful interface for liquid-crystal-based reporting of specific binding events between proteins.

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SURFACE ENGINEERING STRATEGIES FOR CONTROL OF PROTEIN AND CELL INTERACTIONS

H.Ma, J.Hyun N.Nath & A.Chilkoti

Department of Biomedical Engineering, Duke University, Durham, NC 27708.

INTRODUCTION: The ability to control the adsorption of proteins and the interaction of cells on a substrate are important for the development of cellular biosensors, biomaterials, and high-throughput drug screening assays. The critical problem in spatially directing cellular interactions at a surface is the rapid adsorption of a complex layer of proteins within minutes of contact with serum in cell culture or upon implantation in vivo.

We describe two different strategies for the synthesis of biologically-nonfouling polymer coatings that are applicable to diverse substrates. The first strategy involves physically coating a surface with an amphiphilic comb polymer that presents short oligoethylene glycol side chains [1]. Reorientation of the oligoethylene glycol side chains at the solid-water interface presents an oligoethylene glycol brush at the interface, thereby rendering the surface protein and cell resistant. A number of different fabrication methods to synthesize these nonfouling coatings will be described including spin-coating, dip-coating and surface-initiated polymerization using an atom transfer radical polymerization (ATRP) initiator immobilized on gold through the formation of an alkanethiol self-assembled monolayer (SAM). The second, "active" strategy involves the grafting of a stimuli-responsive biopolymer, derived from an oligomeric sequence found in mammalian elastin. This polypeptide, which we term an elastin-like polypeptide (ELP), is a biopolymer with the repeat unit Val-Pro-Gly-Xaa-Gly, and exhibits a lower critical solution temperature (LCST) transition in aqueous solution [2]. Surfaces grafted with these polypeptides exhibit a hydrophilic-hydrophobic transition in response to increased temperature or salt concentration. The change in the interfacial properties can be exploited to create "active" non-fouling polymer grafts that enable reversible, triggered binding of proteins onto surfaces in response to an external stimulus [3].

METHODS:

Synthesis of Comb Polymer. The comb polymer was synthesized by free radical polymerization of methyl methacrylate (MMA), poly(ethylene glycol) methacrylate (referred to herein as hydroxy-poly(oxyethylene) methacrylate (HPOEM), M_n ~526 g/mol, corresponding to m ~10) and poly(ethylene glycol) methyl ether methacrylate, (referred to herein as poly(oxyethylene) methacrylate (POEM), M_n ~475 g/mol, corresponding to n ~8.5) [1]. Composition: 61 wt% MMA, 21 wt% HPOEM, 18 wt% POEM. Molecular weight: ~24,000 Da; M_w/M_n ~1.7.

Expression of ELP and ELP Fusion Protein. An ELP with a molecular weight (MW) of 71 kDa and a thioredoxin-ELP (Trx-ELP) fusion protein where the same ELP was fused to the C-terminus of Trx were synthesized by overexpression of a plasmid-borne synthetic gene in *Escherichia Coli*, as reported elsewhere [2].

RESULTS:

Physical Deposition of Nonfouling Comb Polymer Films and Microstructures. The amphiphilic comb polymer was spin-cast onto different substrate from a water/methanol mixture to create a homogeneous coating. Alternatively, we found that microstructures of the comb polymer can be created on most substrate by spin-coating the comb polymer onto a micropatterned polydimethylsiloxane (PDMS) stamp from solution, and then selectively transferring regions of the comb polymer film to the substrate by bringing the stamp into physical contact with the surface. Both homogeneous coatings and microstructures of the comb polymer are stable in water and are protein and cell resistant, as long as the polymer is allowed to hydrate prior to incubation with proteins or cells. These nonfouling microstructures provide an experimentally robust method to pattern proteins and cells, as shown by the fact that cellular patterns are retained in the presence of exogenous serum for up to a month [1]. An important feature of this patterning methodology is that nonfouling topographical structures of the comb polymer can be created, whose height can be varied from ~50 nm up to several microns, and whose lateral dimensions can be controlled from several hundred nanometers to several hundred microns by nano and micro-contact printing. This system allows seamless integration of topographical and biochemical cues to direct cell behavior such that the presentation of both set of cues to cells can be independently controlled. This system has application in fundamental studies of cell-substrate interactions, cell-cell signaling, as well as in biotechnology and pharmacological pharmaceutical screening assays that require isolation of individual cells.

Surface Initiated Polymerization of Comb Polymer. More recently, we have synthesized an alkanethiol functionalized with a terminal ATRP initiator, and have used a SAM of this initiator-functionalized thiol to carry out surface initiated polymerization (SIP) of the amphiphilic comb polymer on gold. A SAM of the ATRP initiator thiol, w-mercaptoundecyl bromoisobutyrate was prepared by immersing a gold-coated silicon wafer

into a 1 mM solution of the thiol overnight. The SAM was rinsed with methanol and dried under a stream of nitrogen. SIP was carried out in an oxygen-free environment in a water/methanol mixture with POEM macromonomer. Results will be presented that demonstrate that oligoethyleneglycol-functionalized polymer brushes of tunable thickness in the 5-50 nm range, a thickness inaccessible to SAMs or polymer grafts, can be easily synthesized by SIP, that these polymer brushes exhibit no detectable adsorption of proteins and are cell-resistant for up to a month under typical cell culture conditions, and that the synthesis method is compatible with a range of patterning techniques from the nano- to the micro-scale, which enables the patterning of cells in a biologically relevant milieu over extended periods of time.

"Active" Control of Protein Adsorption. "Active" approaches to dynamically modulate the binding and release of proteins will also be demonstrated. In one implementation of this concept, which we have named TRAP –Thermodynamically Reversible Addressing of Proteins– we have shown that proteins can be reversibly bound to a surface in a functionally active orientation directly from cell lysate by exploiting a thermodynamically reversible hydrophilic-hydrophobic LCST transition exhibited by a genetically engineered, stimuli responsive ELP [2]. In TRAP, an ELP is covalently micropatterned on a glass surface against an inert BSA background. The ELP patterned surface is incubated with the soluble fraction of E. coli lysate containing an expressed Trx-ELP fusion protein, which is appended with the same ELP as on the surface. The LCST transition of the grafted ELP and the Trx-ELP fusion protein is simultaneously triggered by an external stimulus. The LCST transition results in capture of the ELP fusion protein from solution onto the immobilized ELP by hydrophobic interactions between the grafted ELP and the ELP fusion protein. The captured ELP fusion protein is oriented such that the fusion partner is accessible to binding of its target from solution. We also demonstrate that TRAP is reversible; the bound protein-ligand complex is released from the surface by reversing the LCST transition [3]. The triggered control of interfacial properties provided by an immobilized stimuli-responsive polypeptide at the solid-water interface is an enabling technology that allows reversible and functional presentation of ELP fusion proteins on a surface directly from cell lysate without the necessity of intermediate purification steps and subsequent recovery of the protein-ligand complex for downstream analysis by other analytical techniques. TRAP has application in lab-on-a-chip bioanalytical devices as well as in the fabrication of peptide and protein arrays.

CONCLUSIONS: An amphiphilic comb polymer film can be stably coated onto diverse substrate by a simple spin coating process from an environmentally benign solvent (water/alcohol) such that the comb polymer coating is stable in water and confers nonfouling properties to the substrate. The comb polymer can also be grafted from a gold surface using surface-initiated ATRP, leading to the formation of a polymer brush. These passive nonfouling coatings have diverse applications in the fabrication of biomaterials and biosensors. An active nonfouling coating strategy has also been developed whereby the interaction of a recombinant protein can be reversibly controlled at a surface that is grafted with a genetically engineered stimuli-responsive polypeptide. Such active coatings can be used to selectively and reversibly capture a protein of interest from solution, and are likely to prove useful in microfluidic BioMEMS devices and peptide and protein arrays.

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SURFACE ENGINEERING ON THE NANOMETER SCALE WITH SUPRAMOLECULAR COMPLEX SPECIES

G.J.Vancso[1], H.Schönherr[1], J.Huskens[2] & D.N.Reinhoudt[2]

[1]Dept. of Materials Science & Techn. of Polymers, [2]Dept. of Supramolecular Chem. & Techn., MESA+ Institute for Nanotechnology, University of Twente, The Netherlands

INTRODUCTION: Bio-macromolecules, such as proteins, are synthesized by nature one-by-one with accurate distributions of monomers (amino-acids) in polypeptide chains. Responsible biochemical reactions make use of molecular templates and molecular recognition. The proteins formed are organized on various levels of molecular assembly and fulfill structural and functional roles in living organisms. Relevant aspects of the corresponding biochemical processes and structural organization include non-covalent (supra-molecular) interactions, molecular templates, and recognition on the single-molecule level. Structural organization by "self" assembly eventually results in complex hierarchical structures.

In contrast, most synthetic (industrial) chemical processes are performed in organic solvents, by mixing large bulk amounts of reactants, compared to nature in a crude way. It has been one of the main objectives of science and technology to mimic nature also in man-controlled synthesis, molecular fabrication, and assembly of structures. Recent breakthrough discoveries in supra-molecular chemistry, spatial and temporal confinement of individual molecules, and achievements in applications of various scanning probe techniques have laid the foundations of molecular nanotechnology. This is regarded as the first step to realize bottom-up molecular nanochemistry. The term "nanochemistry" is specifically used here to describe manipulation, structural modification, and spatial and temporal characterization of localized, individual atomic, and/or molecular systems, or their clusters (aggregates) consisting of a small number of elementary units. Interactions with such nanoobjects in nanotechnology must either occur via other nanoobjects directly (single molecule probing), or via mediation by using macro-microobjects (e.g. using scanning force microscopes with contact definition of ultra sharp probes on the nanoscale).

Self-assembled monolayers (SAMs) of thiols, sulfides and disulfides on Au can be used to construct templates for attaching confined, isolated molecules. They can also serve to modify surface properties, or as reactive platforms in surface engineering. Cyclodextrins can act as hosts for the binding of a variety of small, organic guest functionalities in water through hydrophobic interactions [1]. These receptor molecules can be immobilized on Au in SAMs using derivatives with sulfide linkages. These "molecular printboards" [1 (b)] have specific recognition sites, e.g. molecular cavities, to which molecules can be anchored via specific and directional supramolecular interactions.

METHODS: SAMs were assembled on Au using standard procedures. Corresponding monolayers were used as reference (or reactive) templates and to immobilize isolated molecules (e.g. at SAM defect sites). Atomic force microscopy (AFM) was used to image molecular lattices and isolated molecules.

AFM tips were functionalized to tune molecular interactions between tip and templates for force spectroscopy, and for in-situ kinetic studies of surface reactions. Guest molecules were attached to AFM tips in mixed SAMs at different surface concentrations to study guest-host specific binding on the single molecule level.

RESULTS AND DISCUSSION: First the use of AFM will be illustrated to image the head group lattices of SAMs and to locate and image single macromolecules (dendrimers), and their assemblies [1,2]. A discussion on molecular interactions and recognition will follow, based on guest-host chemistry of surface-immobilized β-cyclodextrin receptors and apolar guests in aqueous environments [3]. Single-molecule force spectroscopy was used in the latter case to study individual supramolecular interactions utilizing SFM. Complexes interacting via self-complementary quadruple hydrogen bonds for surface molecular recognition is the next case to be discussed [4]. The complex stability can be controlled in these molecular assemblies by a proper choice of temperature and solvent, which enables us to tackle transitions from non-equilibrium to equilibrium guest-host kinetics in single-molecule force spectroscopy. These complexes also serve as model systems of pyrimidone-based supramolecular polymers at surfaces and interfaces. In the next example, chemistry on individual molecules will be illustrated by a study of surface-confined dendrimers and their attachment (on single molecule level) into self-assembled thiol monolayers on gold [2]. As last example, monitoring surface reactions will be discussed using SAMs with a near-to-molecular resolution [5]. This approach – which we named "inverted chemical force microscopy" – allows us to follow reaction kinetics in real time. As examples ester hydrolysis and aminolysis of SAMs, the latter using "bioreactive" 11,11'-dithio bis(*N*-hydroxy-succinimidyl undecanoate, will be presented.

CONCLUSIONS: By the examples shown in this presentation, including molecular visualization, single molecule force spectroscopy, molecular recognition, and in-situ reaction kinetics studies, we

demonstrate progress in molecular nanochemistry inspired by nature.

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PROTEIN-REPELLING DEPOSITED LAYERS FROM COMPLEX COACERVATE MICELLES

M.A. Cohen Stuart

Laboratory of Physical Chemistry and Colloid Science, Wageningen University, Wageningen, The Netherlands.

In order to suppress fouling of surfaces due to, e.g., protein adsorption and bacterial adhesion, surface are often covered with water-soluble, neutral polymers. Poly ethyleneoxide (PEO, PEG), end-attached to the surface has been used with particular success. The extended hydrophilic chains, often denoted as 'brushes', serve as a barrier for the particles that must be repelled from the surface. For robust and easy application, methods are needed by which one can prepare hydrophilic brushes easily and reliably on a variety of surfaces, preferably with some freedom to tune the brushes to specific needs. Both chemical and physical interactions have been used for this purpose, but many methods have limitations.

We discuss in this contribution a new method, namely anchoring the brush by means of a complex coacervate. This method relies on first preparing micelles by mixing two oppositely charged polymers, one of which carries one or more neutral hydrophilic blocks which are to form the brush. When mixed in the right proportions, micelles appear by electrostatically driven association of the pair of charged polymers. These micelles have a core consisting of the two charged polymers in the form of an insoluble complex, surrounded by a 'corona' of the neutral hydrophilic blocks. We discuss the properties of these micelles, and the forces that drive their formation, and how the solution behaves as a function of polymer composition.

We also discuss the deposition of the micelles on a variety of surfaces: charged, neutral, hydrophobic and hydrophilic, demonstrating that their tendency to adsorb is almost independent of the nature of the surface. Finally, we show that the treated surfaces are protein repellent under a wide variety of conditions, which makes them promising candidates for biomedical applications.

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POLYMER NANOCONTAINERS FOR BIOMEDICAL APPLICATIONS

S.Benito[1], A.Graff[1], R.Stoenescu[1], P.Broz[2], C.Saw[2], H.Heider[3], S.Marsch[2], P.Hunziker[2] & W.Meier[1]

[1]Dept. of Chemistry, University of Basel, Switzerland. [2]Medical Intensive Care Unit, University Hospital Basel. [3]Institute for Biochemistry and Genetics, University of Basel.

INTRODUCTION: During the last decade self-organization of soft materials has shown to be valuable for the creation of a wide variety of nanostructures that could be used for applications in fields ranging from materials science to biology.

In this context amphiphilic block copolymers are of particular interest due to their ability to self-assemble in aqueous media and their broad accessibility to different length and time scales and levels of interaction. Similar to conventional low molar mass surfactants they may form micelles, vesicles or lyotropic mesophases. These aggregates can be significantly more stable than those formed by low molar mass amphiphiles and additionally they can be further stabilized by a subsequent crosslinking polymerization[1]. The long-term stability of these structures makes them well suited for applications and guarantees a constant nonchanging environment for embedded therapeutic or analytic molecules. Moreover, block copolymer chemistry allows introducing easily additional design criteria, like targeting moieties, temperature- or pH sensitivity[1].

RESULTS & DISCUSSION: Here we designed functional block copolymer nanocontainers and tested their potential as generic carriers that can be addressed to specific targets. In particular, we were interested in their receptor- and cell- specific binding and uptake by cells. The nanocontainers were labeled with fluorescent dyes and functionalized on their outer surface with polyguanylic acid (Poly-G) a specific ligand to the scavenger receptor A1 that is found on macrophages, a cell line that plays a major role in infection, autoimmune diseases and cancer.

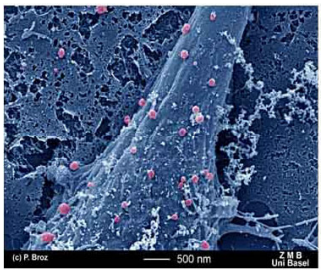

Fig. 1: Scanning Electron Microscopy analysis of endocytosis-inhibited COS-7 cells showing surface bound nanocontainers with diameters of approx. 200 nm.

In the experiments, macrophages derived from the human monocytic THP-1 cell line and the simian tumor cell line COS-7 were used as target cells expressing SRA1. The ligand-bearing nanocontainers showed strong receptor specific binding. Surface-bound nanocontainers were rapidly taken up by the cells through an active process, most probably the endocytotic pathway, without leading to discernible cytotoxicity. In strong contrast, no binding and uptake is observed in cells devoid of the target receptor. Macrophages are unable to bind and to take up those nanocontainers nonspecifically targeted against them, thereby reducing the common problem of conventional targeting approaches, namely, unspecific elimination of carriers by the macrophage system. Thus synthetic polymer nanocontainers appear very promising as novel, target-specific carriers for diagnostic or therapeutic agents.

The walls of the block copolymer containers are formed by membrane-like superstructures that can be regarded as mimetics of biological membranes. Recently, we were able to show that despite their enormous thickness and stability such block copolymer membranes can be used as a matrix for functional reconstitution of membrane proteins[2,3]. This allows an additional control of the exchange of material and provides new interactions between nanocontainers and biological structures.

Generally membrane proteins are vectorial molecules with distinct extracellular and a cytoplasmic parts. In biological membranes these proteins have a well-defined orientation that is also a basic requirement for their function. However, during their isolation from cells and subsequent incorporation into a new artificial membrane system usually the information about their orientation is lost, and the membrane proteins are inserted randomly without any preferred direction. Unfortunately many potential technical applications (e.g., biosensors) of such reconstituted systems depend on a correct and uniform orientation of the membrane proteins.

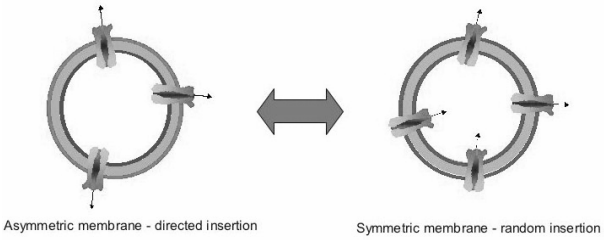

Fig. 2: Directed vs. random protein insertion in ABC and ABA triblock copolymer vesicles.

Amphiphilic block copolymers offer here a particularly interesting approach to overcome this problem by breaking the symmetry of the membranes. For that purpose we recently synthesized a new type of an amphiphilic ABC-triblock copolymer with a water-soluble poly(ethylene glycol) block A, a hydrophobic poly(dimethylsiloxane) block B and again a water-soluble poly(2-methyloxazoline) block C[4]. For certain hydrophilic-to-hydrophobic block length ratio these polymers also form membrane-like superstructures and nanometer-sized vesicles in aqueous media. It is well known that different water-soluble polymers are inherently incompatible and undergo phase separation in aqueous media. Hence, membranes and walls of nanocontainers formed by ABC triblock copolymers are asymmetric: one side is predominantly covered by the blocks A and the other by the blocks C[4].

As a model system to investigate the insertion of proteins into such ABC type membranes we reconstituted Aquaporin 0 labeled with a His-Tag unit on its cytoplasmic side into the walls of ABC block copolymer nanocontainers. Binding studies with fluorescently (Alexa Fluor 555) labeled monoclonal Anti-His antibodies clearly showed that in contrast to 'symmetric' block copolymer and lipid membranes where the insertion occurs randomly (i.e., 50:50 'physiological' to 'non-physiological orientation'), in asymmetric ABC block copolymer membranes 80% of the proteins have a 'physiological' orientation with the His-Tag toward the inside the nanocontainers.

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PEG-MODIFIED NANOPARTICLES FOR NEW MOLECULAR RECOGNITION

Y.Nagasaki[1], T.Ishii[1], K.Uchida[1], H.Otsuka[2] & K.Kataoka[2]

[1]*Department of Materials Science, Tokyo University of Science, Noda 278-8510, Japan.*

[2]*Department of Materials Science,Graduate School of Engineering, The University of Tokyo, Tokyo 113-8656, Japan.*

INTRODUCTION: In the case of microanalysis in a crude sample such as serum, nonspecific adsorption of various proteins and lipids to the surface is an important consideration to achieve specific biosensing with high S/N ratio. In order to avoid the nonspecific adsorption, many types of modification on the sensor surface have been considered. Modification by poly(ethylene glycol) (PEG) tethered chains leads to reduce the nonspecific interaction of biomolecules such as proteins and cells with biomedical devices because PEG is a nontoxic and hydrophilic polymer with low interfacial free energy in water and high-chain mobility inducing excluded volume effects.

In this paper, we are focusing on preparation of complete non-fouling surface by mixed PEG tethered chain, which denotes the introduction of short under-brushed PEG layer to the surface pre-modified with comparatively long PEG chain resulted. By using our original heterotelechelic PEG, which means PEG having a functional group at one end and another functional group at the other chain end quantitatively, ligand-installed non-fouling surface was constructed.

EXPERIMENTS: CHO-PEG-SH and CHO-PEG-b-poly(2-N,N-dimethylaminoethyl methacrylate) (CHP-PEG/PAMA) were synthesized by our original method[1,2]. Protein adsorption measurements were carried out using surface plasmon resonance analyzer (BIAcore 3,000) after the bare gold surface was modified by the prepared PEG samples. Gold and semiconductor nanoparticles were modified in the same manner as the gold sensor surface and used for high sensitive molecular recognition.

RESULTS and DISCUSSION: Figure 1 shows performances of protein adsorption character of SPR sensor chip coated by PEG tethered chains as a function of protein sizes. In the case of dextran gel as a control, non-specific adsorption was avoided to some extent in the case of high molecular weight protein. With decreasing the size of the protein, the non-specific adsorption increased significantly. The conventional PEG tethered chain surface suppressed the non-specific adsorption of the proteins possessing the molecular weight higher than 10kD. However, it is not enough performance for the protein lower than 10kD. In the case of the mixed PEG tethered chain surface, complete non-fouling character was observed. Especially, the mixed PEG tethered chain avoided tetrapeptide (RGDS, MW=450), which is anticipated as ideal biomateials surface.

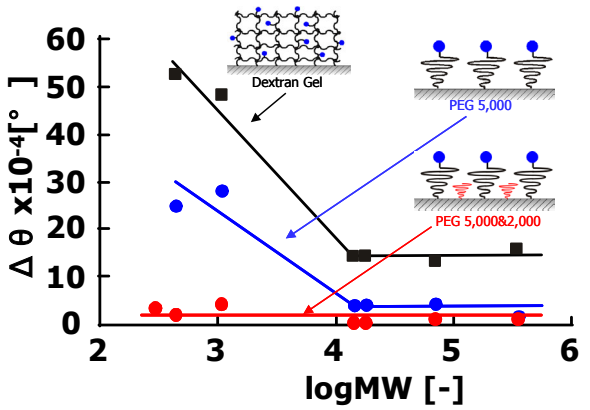

Fig. 1: Protein adsorption on SPR sensor chip modified by heteroPEG as a function of protein size.

By using the same technique, nano-sized gold and semiconductor particles were modified by the heteroPEG in order to improve both non-fouling character and high dispersion stability even in high salt concentration conditions. In this case, CHO-PEG/PAMA was also used as a surface modification agent. For example, stabilized CdS semiconductor nanoparticle was prepared by a simple coprecipitation method of Cd^{2+} with S^{2-} in the presence of CHO-PEG/PAMA block copolymer to form nano-sized crystal. The block copolymer coordinated on the growing CdS crystal to control the size of the particle. At the same time, the block copolymer stabilized the formed CdS particle. The installation of biotin moiety at the PEG chain end made feasible to recognize specific affinity with streptavidin. By the labeling streptavidin with fluorescent probe, TexasRed in this study, an effective FRET was observed, which can be utilized for a high sensitive detection of the protein.

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FUNCTIONAL POLYMERIC COATINGS – FROM POLYMER SYNTHESIS TO HEART VALVE IMPLANTS TO ENDOTRACHEAL TUBES

R.Toomey, H.Murata, B.J.Chang, H.Zhang, R.Konradi, D.Freidank, S.Golze, O.Prucker, H.Klapproth, M.Dahm[1] & J. Rühe

Chemistry and Physics of Interfaces, Institute for Microsystems Technology (IMTEK), University of Freiburg, Germany.
[1]Department of Cardiothoracic and Vascular Surgery, University Hospital Mainz, Germany

INTRODUCTION: The coating of materials with thin layers of polymers, which have been attached to the surfaces of solid substrates through covalent chemical bonds, represents an attractive strategy to improve the surface properties of materials used in biological or medical applications. While layers, which have been deposited through physical processes only, can be removed more or less rapidly through desorption or through the influence of a competing adsorbent present in the environment, layers, which have been coupled to the surface through a chemical bond exhibit strongly improved long term stability. In the paper several new pathways for the synthesis of surface-attached ultrathin polymer layers will be described. Examples are the generation of polymer brushes through growth polymer molecules at the surface of the substrate *in situ*, the photolinking of molecules to surfaces and the generation of thin surface-attached networks (Fig. 1).

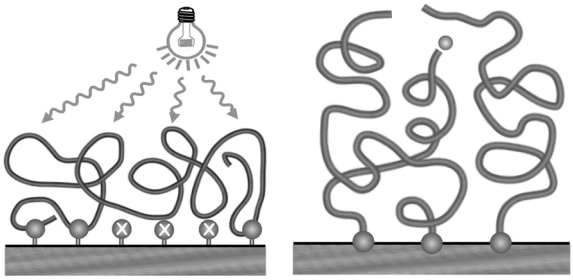

Fig. 1: Methods for the covalent attachement of polymer layers to solid surfaces a) photolinking b) growth of polymer brushes

METHODS: Details on the generation of surface-attached polymer brushes have been described elsewhere [1] as well as the photochemical attachment of polymers using self-assembled monolayers with benzophenone moieties [2]. The fabrication approach for the generation of surface-attached polymer networks is based on the photo-crosslinking of thin copolymer films that contain a photoactive monomer as well as water-soluble comonomers. Irradiation of the film with UV light (λ = 350 nm) activates the benzophenone groups, which extract a hydrogen from almost any kind of neighboring aliphatic C-H group, forming a stable C-C bond cross-link, yielding surface-attached networks of hydrogels. After each deposition, UV illumination simultaneously crosslinks the layer and covalently attaches it to the layer beneath it. Multi-layer structures can then be built up by depositing different copolymer systems on top of one another.

RESULTS: The preparation of highly grafted polymer monolayers in which each chain is tethered to the surface by one end is best done by directly growing the chains on the surface from surface-attached initiator monolayers. In our work we use self-assembled monolayers of azo compounds that are initiators for free radical chain polymerizations. Using this approach one can synthesize polymer brushes in which macromolecules with molecular weights up to 10^7 g/mol are end-attached to the substrate's surface at a density that corresponds to an average anchor distance of less than 3 nm. Furthermore, the free radical process tolerates the use of a large variety of monomers and comonomer mixtures which makes it possible to incorporate chemical and biochemical functionalities and to tailor the properties of the resulting layers in terms of their chemical composition, hydrophilicity, surface free energy, wettability as well as other physical properties. Here we describe one system in which these so-called polymer brushes have been used as the basis for a DNA chip. We show that the attachment of the DNA probes to the polymer chains of a suitably engineered brush instead of direct surface attachment leads to chips that show a significantly improved sensitivity and selectivity.

In a second approach (Fig. 2) towards surface-attached polymer monolayers we use monolayers that carry photoreactive groups such as the benzophenone unit for the attachment of pre-fabricated polymers [2]. Even though the thickness of the resulting layers stays in general lower than that prepared via surface-initiated polymerization this photochemical approach is often advantageous because the particulars of the photochemistry of benzophenone allows for the attachment of almost any polymer. Hence, off-the-shelf polymers can be used to create these layers as well as polymers that contain specific groups to serve a certain porpose. To give one example, we have used this approach to create a wide variety of polymer monolayers that were subsequently tested for ability to promote the growth of endothelial cells on substrates. As a result of this study we identified a number of suitable candidates of polymers that we now use in a slightly different approach for the modification of porcine heartvalve implants. The layers mask the toxic groups generated at the surface of the bioimplants

during a glutar aldehyde treatment that is needed to improve the mechanical stability of these xenografts [3].

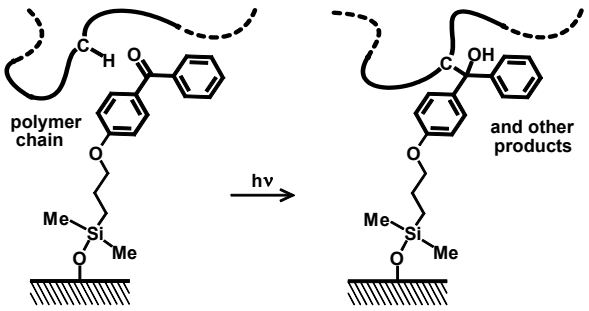

Fig. 2: Schematic illustration of the photochemical process used for the covalent attachment of polymers to solid surfaces.

For the generation of layers of thicknesses adjustable from the nanometer up to the millimeter scale we have chosen another photochemical approach that yields surface-attached polymer networks and that utilizes polymers that carry photoreactive units. If polymeric or biologic substrates are used both the crosslinking and the surface-attachment occur simultaneously. Through the choice of appropriate reaction conditions and (co)polymer composition surfaces with precisely tailored properties can be obtained. Especially systems in which the polymers carry functional groups, which can be used in sensor applications, can be prepared via this method easily.

We show that these surface-attached networks provide a convenient scaffold to host a number of other functionalities. Secondly, they can undergo substantial swelling and contraction in response to a varying environment, making them excellent candidates for "smart" surfaces that respond to specific stimuli. In order to understand and control such stimuli based on swelling, we have studied the swelling behavior of such layers in contact with aqueous solutions with multiple-angle null ellipsometry, the latter being an analytical technique that yields information about the thickness and refractive index profile of the swollen layers.

In order to demonstrate the versatility of the system we will show how such layers can be used as barrier layers on the balloons of endotracheal tubes that keep body liquids from entering into the lungs of ventilated patients. This problem is often encountered in intensive care units at hospitals and in many cases these patients die from pneumonia caused by these liquids. In our system a water-swellable surface-attached polymer network sufficiently seals off all leaks between the tube and the trachea.

DISCUSSION & CONCLUSIONS: The novel methods developed for the coating of organic, inorganic and biological substrates show great perspectives, especially for biological and biomedical applications. The firm covalent bond between the polymer chains and the substrates provide the stability of the coatings that is needed for such systems. Using general and well-established procedures for polymer synthesis it is possible to engineer layers that serve several purposes at the same time such as the swellability of the layers and the chemical nature and number of functional groups. In other words, these chemistries allow a precise tailoring of surface-properties both in a homogeneous as well as in a spatially defined way (surface microstructuring).

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ACKNOWLEDGEMENTS: The authors are indebted to the German Research Council (DFG, SFB 428), Tracoe GmbH and Genescan AG for financial support of this work**.**

CELL SHEET ENGINEETING: FROM TEMPERATURE-RESPONSIVE CULTURE SURFACES TO CLINICS

M.Yamato

Institute of Advanced Biomedical Engineering and Science,
Tokyo Women's Medical University, CREST, JST, Tokyo, JAPAN.

INTRODUCTION: We utilize surface-modified cell culture dishes for tissue engineering and regenerative medicine. Temperature-responsive polymers are covalently grafted onto tissue culture polystyrene dishes by radical polymerization initiated with electron beam irradiation. The grafted surfaces reversibly change the cell adhesiveness depending on culture temperature. Therefore, cultured cells and cell sheets are harvested non-invasively from the dishes without need for proteolytic enzymes such as trypsin. Here, I show the reconstruction of tissue architecture *in vitro* and the clinical applications.

METHODS: A temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PIPAAm), was covalently grafted to surfaces of tissue culture-grade polystyrene dishes by electron beam irradiation of 0.25 MGy with using an area beam electron processing system (Nisshin High Voltage, Japan). For the introduction of bioactive signals on the temperature-responsive surfaces, a newly synthesized monomer, 2-carboxyisopropylacrylamide, was copolymerzied. Cell adhesive peptides including RGDS and YISGR as well as growth factors were immobilized via the carboxyl groups. For the non-invasive cell and cell sheet harvest, culture temperature was decreased to 20°C in a CO_2 incubator.

RESULTS: Temperature-dependent cell adhesion/detachment control was achieved only when the grafted thickness of PIPAAm was around 20 nm. PIPAAm-grafted surfaces were slightly hydrophobic at 37°C, but change to hydrophilic below the lower critical solution temperature (LCST). Various cell types adhered, spread, and proliferated on the surfaces similarly to those on commercial tissue culture dishes. Only by reducing temperature, cells were spontaneously detached from the surfaces. Confluent cells were also recovered as a single contiguous sheet with intact cell-cell junctions and deposited extracellular matrix (ECM). The ECM beneath harvested cell sheets bound the cell sheets onto other surfaces including the other cell sheet surfaces and *in vivo* tissue surfaces.

Corneal epithelium-like tissues were prepared from limbal stem cells obtained from donor eyes on PIPAAm-greafted dishes and harvested by reducing temperature (Fig. 1). Then, the allogenic cell sheets were grafted to corneal stroma of human patients of limbal stem cell deficiencies. Cell sheet engineering technique utilizing temperature-responsive culture surfaces permitted rapid, intact corneal epithelial sheet attachment without any carriers or sutures. The grafted cell sheets containing the stem cells regenerated transparent corneal tissues and the patients recovered their sights.

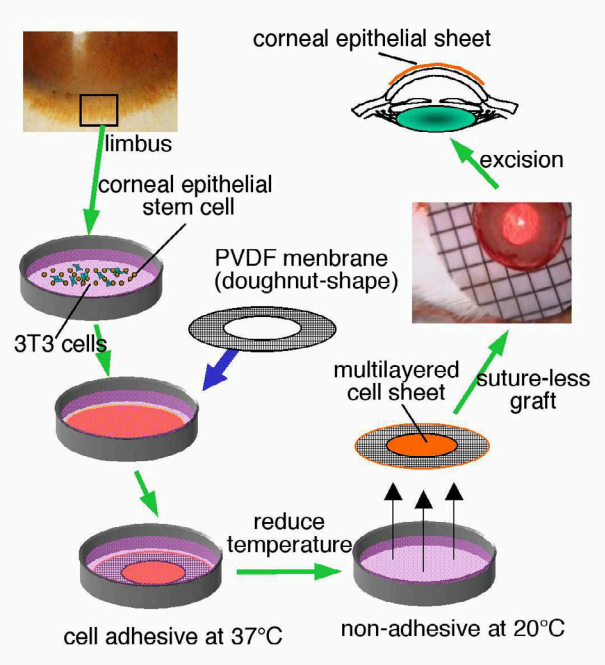
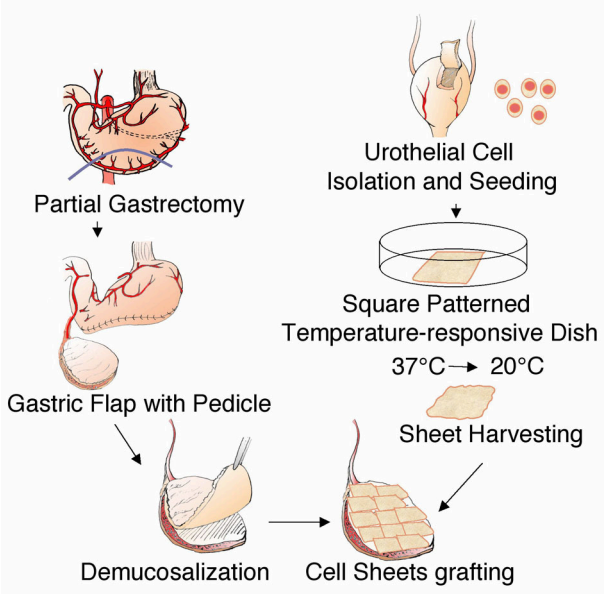


Fig. 1: Scheme of corneal epithelial cell sheet transplantation utilizing temperature-responsive culture dishes.

For the treatment of ischemic hearts, cardiac myocyte sheets were stratified to thick tissues. 3 or 4 sheets gave 100 to 200 μm in thickness (1,2). The reconstructive tissues, cardiac patches, beat spontaneously, and the pulsatile beating was visible even with naked eyes. By grafting these cardiac patches to a rat ischemic heart model, cardiac functions were observed to be significantly improved. For larger animals including pigs, polysurgery with robotic surgery can be useful to prevent necrosis within thicker cardiac patches.

Bladder reconstruction using gastrointestinal flaps often induces severe complications, including lithiasis, urinary tract infection and electrolyte imbalance. The majority of these complications are related to intestinal mucosa. Urothelial cell sheets were autografted onto surgically demucosalized gastric flaps (Fig. 2). Urothelial cell sheets attached to demucosalized tissue surfaces completely without any suture or fixing, and developed into a stratified viable epithelium highly similar to native urothelium. This versatile technology should prove

useful in urinary tract tissue engineering and surgical reconstructions.



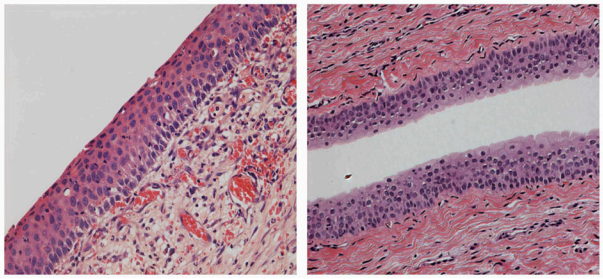


Fig. 2: Bladder reconstruction with cultured urothelial sheets and gastric flaps. Scheme (top) and histology of regenerated urothelium of reconstructed bladder (left) and native ureter (right).

Both cost and safety issues (*e.g.*, prions, bovine spongiform encephalopathy) are compelling reasons to avoid use of animal-derived materials, including serum, in such culture. Synthetic cell adhesive peptides were immobilized onto temperature-responsive polymer-grafted surfaces, and cell adhesion and detachment under serum-free conditions were examined (Fig. 3). Bovine aortic endothelial cells both adhered and spread on these surfaces even under serum-free conditions at 37°C, similar to those in 10% serum-supplemented culture. Spread cells promptly detached from the surfaces upon lowering culture temperatures. These surfaces would be useful for serum-free culture for tissue engineering applications.

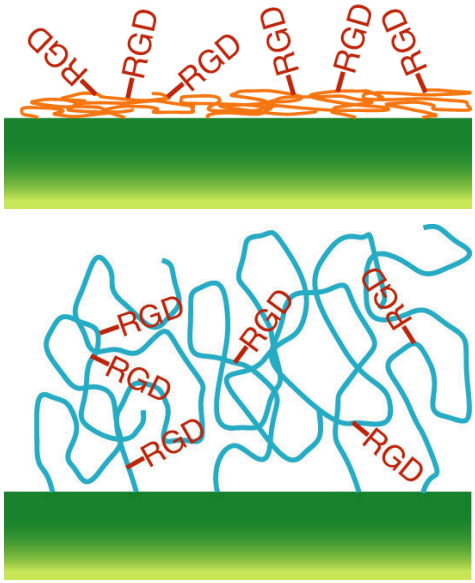


Fig. 3: Cell adhesive peptid-immobilized temperature-responsive culture surfaces. At 37°C, cell adhesive peptides were exposed on the surface (top), but shielded by hydrated PIPAAm chains below the LCST (bottom).

DISCUSSION & CONCLUSIONS: The results shown here suggest promising clinical capabilities of reconstructed tissues by cell sheet engineering for transplantation. Furthermore, the present grafting method without any carriers or suture should be applied to corneal endothelial cells, retina pigmented epithelial cells as well as other epithelial tissues. The cell sheets harvested from temperature-responsive culture dishes can achieve functional and histological integration to host tissues.

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PLASMA POLYMERS AND SOME BIOMEDICAL APPLICATIONS

H.Biederman

Dept of Macromolecular Physics, Charles University, Prague, Czech Republic.

INTRODUCTION: Plasma polymer films that have been extensively studied since the sixties of the last century have been mostly used for surface modification of solids or as functional thin films in microelectronics and optics. In recent ten years a growing interest has been in the applications in biomedical field [1]. In our laboratory an enhanced durable wettability of solids was studied by depositing a hydrocarbon plasma polymer film on a solid surface [2]. This study was inspired by the need of surface modification of hard contact lenses. In the other group of studies amine rich plasma polymer coatings were investigated for modification of CA (cellulose acetate) membranes and quartz crystals. The motivation was the application of a modified membrane for single-layer enzyme electrode and quartz crystal biosenzors [3-5]. Plasma polymerization can be used as a convenient method for preparation of nanocomposite metal/plasma polymer films [6,7]. For example composite Ag/hydrocarbon plasma polymer films have antibacterial properties. The first results of these coatings function in contact with cells and microorganism are considered.

METHODS: The plasma polymerization process is usually carried out by means of the two types of reactors: 1) a tubular (silica or glass) type reactor with an external coil or ring electrodes for the excitation of rf discharge, 2) bell-jar type reactor with the internal parallel plate electrodes. In this case also dc or ac (up to 50 kHz) voltage can be used along the most frequently used rf (100 kHz –30 MHz). For some plasma polymerization processes microwave discharges have been favored usually in the multimode cavity mode.

RESULTS & DISCUSSION: Plasma polymerization of Ar/n-hexane/H_2O mixture and the incorporation of polar groups such as OH and C=O were studied in order to achieve a stable wettability of the coated surface [2]. In the second direction amine rich coatings were deposited on CA membranes in a tubular reactor using EDA (ethylene diamine) and n-BA (n-butylamine) [3]. After the membrane surface activation by glutaraldehyde the covalent binding of the enzyme can be achieved. FTIR-ATR, XPS, contact angle, and enzyme immobilization activity (enzyme immobilization method using radiolabeled glucose oxidase [^{99m}Tc-GOD]) were used to characterize modified AC surfaces. The best modification results were obtained with EDA at a working gas pressure 27 Pa and deposition conditions - power and deposition period: 5W and 30 min and 15 W and 10 min, respectively [3]. In the following studies EDA and DACH (diaminocyclohexane) monomers have been plasma polymerized in a CW (continuous wave) and pulse modes. It has been shown that the variation of the duty cycle does not produce a considerable effect on the retention of amine groups into the film while power and t_{on} play an important role [4,5]. In the next group of studies nanocomposite metal/plasma polymer films have been deposited using a dc unbalanced magnetron with a metallic target (Ag, Ni, and Mo) and operated in argon/n-hexane working gas mixture at 0.4 to 2 Pa. Ag, Ni, and Mo were incorporated into the hydrocarbon plasma polymer in the form of clusters (grains) in size of 1 nm to 100 nm [6,7]. These composite films have been also recently produced by rf co-sputtering from the two magnetrons equipped with polyethylene and e.g. Ag targets, respectively [8]. The behavior of cells (endothelial cells, line CPAE) and microorganism (E. Coli) in contact with this film surface are investigated.

CONCLUSIONS: Plasma polymer films may be used for the modification of surface energy and amine rich coatings for enzyme attachment. Composite metal/plasma polymer films can be considered for antibacterial coatings.

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SURFACE MODIFICATION OF POLYURETHANE SCAFFOLDS WITH NATURAL POLYMERS: THE USE OF SILK FIBROIN

P.Petrini, S.Bozzini, S.Farè & M.C.Tanzi

Dept. of Bioengineering, Politecnico di Milano, Italy.

INTRODUCTION: In this work, silk fibroin, a protein extracted from *Bombyx Mori* silk and sericin-deprived, was used as coating to modify the surface properties of polyurethane (PU) membranes and foams (2D SF/PU and 3D SF/PU).

Silk fibroin (SF) is a highly promising protein: due to its structural properties and its ability to promote cell adhesion, SF has been the object of increasing interest as a potential biomaterial for tissue regeneration and repair [1,2].

The possibility to modify polyurethane membranes and foams could be of interest for the preparation of scaffolds for tissue regeneration.

METHODS: *Bombyx Mori* silk solution was prepared by dissolution in 9.3M LiBr as previously described [3].

2D-substrates were obtained by solvent casting from a THF:Diox (2:1) solution of Bionate 80A (PTG, USA). 3D-substrates were prepared by reacting a polyol mixture (component A, Elastogran, Italy) with polymeric MDI (B141, BASF). Fe-acetyl-acetonate as catalyst and water (5% w/w_A) as expanding agent were used.

Both 2D and 3D PU substrates were coated with SF by dipping in a 3-4% w/w fibroin solution in water. Some samples were then immersed in methanol and allowed to dry at room temperature. The morphology of MeOH treated and not samples was observed by SEM.

SF/PU scaffolds were characterized by ATR FT-IR (FT-IR Magna 560 Nicolet). The SF-coating morphology was investigated by SEM (Stereo Scan S360, Cambridge Instruments).

To test the SF-coating stability, SF/PUs, both treated and not treated with MeOH, were immersed in PBS at 37°C for increasing times (6 hours÷14 days). The PBS extracts were analyzed by UV and the SF-concentration drawn from calibration curves [3].

RESULTS: The presence of an homogeneous coating layer (thickness ~200-600 nm) was observed at SEM. ATR FT-IR of SF/PU 2D scaffold showed the presence of bands which can be attributed to SF, with a small contribution of the polyurethane substrate.

The SF released in physiological-like conditions, was lower than 8% (w/ $w_{coating}$). The methanol treatment induced β-form crystallization, as can be observed by ATR FT-IR. As a consequence, the release of SF was lower, with a decreased rate. The morphology of the coating was not affected by methanol treatment, as shown in SEM images at high magnification (fig 1).

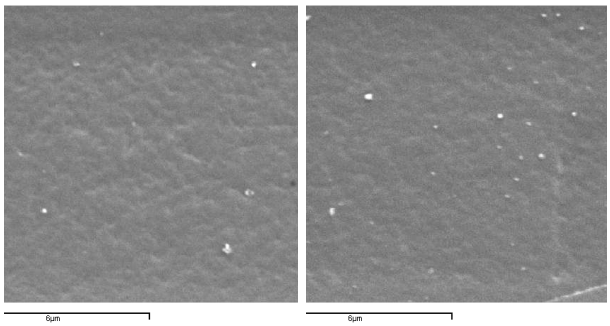

Fig. 1: SEM images(10.000 x) of 2D/SF PU, MeOH-treated (left) and not treated (right).

DISCUSSION & CONCLUSIONS: The dipping technique for the preparation of 2D and 3D SF/PU scaffolds allowed to obtain a thin homogeneous coating with a good stability in physiological-like conditions.

Parallel studies showed that SF-coating enhanced the cellular adherence to the substrate, and promoted higher proliferation rates of human adult fibroblasts [4]. This enhanced cell growth was coupled with a more intense metabolic activity. In addition, HAFs cultured on all types of substrates were never found to secrete any assayable amount of the main pro-inflammatory cytokines [4].

All the obtained results indicate that 2D and 3D SF-coated PU substrates are potentially suitable as scaffolds for Tissue Engineering applications.

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MOLECULAR FORCES AND MORPHOLOGY OF AN ADSORBED POLY(L-LYSINE)-G-POLY(ETYLENE GLYCOL) COPOLYMER

T.Drobek, M.Heuberger & N.D.Spencer

Dept. of Materials, ETHZ, Zürich, Switzerland.

INTRODUCTION: Polyethylene glycol (PEG) surface grafts are used as non-specific protein adsorption resistant surface coatings. In this work, films of PEG grafted to a cationic poly-lysine backbone (PLL-*g*-PEG) were investigated using the surface forces apparatus technique, which provides direct measurements of forces and refractive index using thin-film interferometry. Surface force isotherms were recorded for different molecular architectures and temperatures. The observed interaction force is predominantly repulsive, nearly elastic and can be described by polymer brush scaling theory and electrostatic double layer forces.

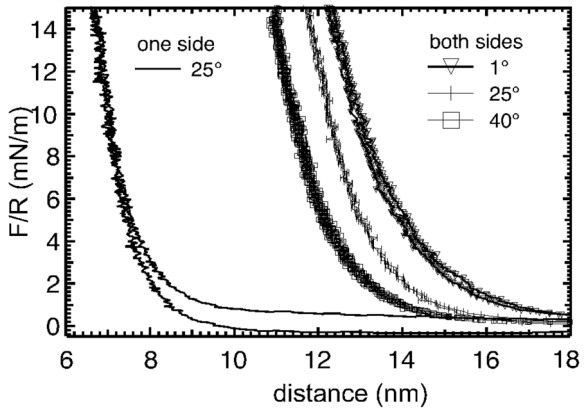

Fig. 1: Compression isotherms of PLL(20)-g-[2,9]-PEG(2) adsorbed layers on one and on both mica surfaces. Loading and unloading tracks are shown.

METHODS: The experimental data were obtained using the extended surface forces apparatus[1,2]. For preparing the mica surfaces, a cutting procedure with scissors was used in order to avoid the formation of nano-particles reported to occur for standard preparation procedure[3-5]. The poly(L-lysine)-*g*-poly(ethylene glycol) films were adsorbed from aqueous solution and HEPES buffer with 0.5 and 1 mg/ml concentration. Compression isotherms were measured in aqueous solution at controlled temperature for one surface (asymmetric) and two surfaces (symmetric) covered with the adsorbed polymer.

RESULTS: A strong exponential repulsive force appeared in the high load regime, caused by the steric repulsion of the PEG chains. In case of the asymmetic setup, a small hysteresis was observed for low loads, as well as transfer of polymers to the second surface at higher loads. The film thickness showed an inverse temperature dependence of -0.5Å/K, which contradicts predictions from brush scaling theory, but is in agreement with the solubility behavior of the PEG molecule. Superimposed to the steric repulsion, discrete film thickness transitions with a variable step size of 0.1 to 0.2 nm could be observed, which reveal the presence of long-lived load-bearing molecular structures of the hydrated PLL-*g*-PEG film.

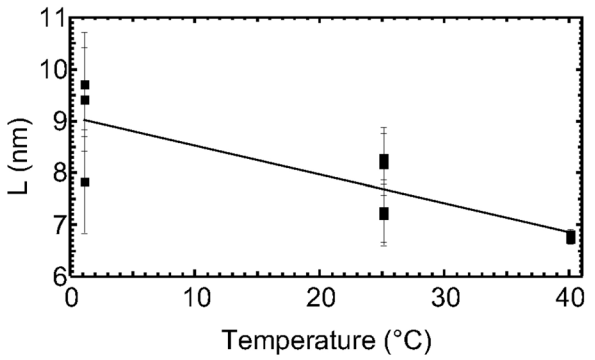

Fig. 2: Polymer brush length L obtained by fitting the compression isotherms of PLL(20)-g-[2.9]-PEG(2) with scaling theory as a function of temperature[6].

DISCUSSION & CONCLUSIONS: Surface force isotherms measured at high resolution reveal ubiquitous sub-nanometer film-thickness transitions suggesting the existence of equilibrium structures superimposed to the well-known steric repulsion.

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FUNCTIONAL POLYMERS FROM POLY(3-HYDROXYALKANOATES): PROTECTION OF SURFACES FROM BIOFOULING

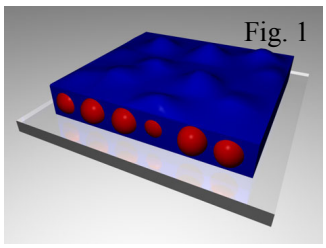
T.Geiger, P.Gasser, R.Hany & M.Zinn

Swiss Federal Laboratories for Materials Testing and Research (EMPA), Dübendorf, Switzerland.

INTRODUCTION: Plants are constantly exposed to harmful attacks by fungi, viruses, bacteria or insects. In order to protect themselves some plants developed various repulsion mechanisms. For example, the eelgrass *Zostera Marina L.*, which has its biotope in saltwater bays and harbors, is attacked by marine bacteria or spores. For self-protection, the eelgrass produces and releases constantly the antifouling compound zosteric acid (ZA), a sodium salt of a sulfated phenolic acid. This compound does not kill the microorganisms but inhibits the adhesion of the biological organism.
The aim of our project was to develop a novel protection strategy, namely the combination of a biodegradable and biocompatible matrix (poly(3-hydroxyalkanoate); PHA) with environmentally friendly antifoulant (ZA) encapsulated in polystyrene (PS) (Fig. 1).

METHODS: Encapsulation of ZA in polystyrene (PS) was performed via an in-liquid-drying process. The particle size of the microcapsules was measured with the Beckman Coulter particle size analyzer LS230. The morphology of the capsule surface was examined by electron microscopy and cross-sections of microcapsules were prepared with a focused ion beam instrument. After dissolving the PS microcapsules in an organic solvent and extraction of the ZA with water the payload was determinated by conductometry. ZA was monitored via ESCA measurements in the inner part of the PS microcapsules. The PHA/PS(ZA) coatings were applied by air brush on glass microscope slides. The release of the ZA out of the coating was monitored again using conductometry.

RESULTS: We demonstrated the possibility to encapsulate the highly water soluble ZA by incorporating the sodium salt in a PS matrix via an in-liquid-drying process. Therefore, a high viscous methylene chloride solution of PS was prepared which represented the oil phase in the OW-emulsion. Subsequently, the ZA powder was dispersed in this polymer solution. Due to the high viscosity of the mixture the ZA was hindered to dissolve in the water phase of the emulsion during dispersion. Shortly after microcapsule formation the methylene chloride evaporated. The solid PS capsules were collected. Capsules were formed with a polydisperse distribution of an average size of 200 µm. The surfaces of the capsules were smooth without failures as proven by SEM. Surprisingly, the inner part of the capsules contained big empty caverns (Fig. 2). ZA crystals were not directly observable in the PS matrix after cross sectioning. Nevertheless, the oxygen originating from the carboxylic acid and the sulfat ester of the ZA was detected by ESCA. The load of ZA in the microcapsules was about 3% (w/w). PHA coatings containing the loaded PS microcapsules showed a constant release of ZA over 5 days in highly purified water. Thereby, a permanent presence of ZA at the coating surface is theoretically maintained in order to avoid biofilm formation.



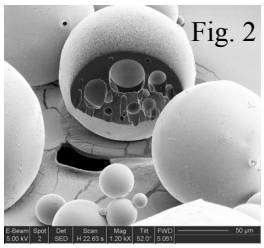


Fig. 1: Scheme of the PHA coating containing microcapsules on a glass substrate; Fig. 2: REM image of a cross section of a microcapsule.

DISCUSSION & CONCLUSIONS: Principally, PS microcapsules allow controlled release of the highly water soluble ZA into aqueous environments over a few days. The presence of the ZA at the surface of PHA coatings should repulse microorganisms and protect the biocompatible material from unwanted settlement. Experiments to proof this antifouling effect are the topic of current experiments.

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CONFORMATIONAL DYNAMICS OF IMMOBILIZED DNA IN EXTERNAL FIELDS

J.Koota, R.Lehner, G.Maret & T.Gisler

Universität Konstanz, Fachbereich Physik, Fach M621, 78457 Konstanz, Germany.

Polyelectrolytes grafted to surfaces are ubiquitous in biology where they are important for cellular recognition. In addition, they are beginning to play an increasingly important role for responsive materials or for biocompatible surfaces.

Despite considerable theoretical and simulation work on surface-grafted polyelectrolytes, experimental data are still scarce, mainly due to the lack of suitable systems with well-defined chain length and homogeneous distribution of charged sites.

These problems plagueing the use of synthetic polyelectrolytes are however largely circumvented when DNA is used. Its large size (λ-DNA has a contour length L=16μm and a radius of gyration R_g=0.8μm) allows visualization by fluorescence microscopy, and the conformational dynamics is sufficiently slow that it may be followed by direct imaging using CCD cameras.

In this contribution we report on the preparation of carpets of end-functionalized λ-DNA and their characterization by confocal fluorescence microscopy (CFM).

We have developed a robust protocol for streptavidin-functionalization of common float glass, mica, as well as indium-tin oxide (ITO) and zinc oxide, to which the biotin-functionalized DNA selectively attaches over a large range of pH values. We find that obtaining a dense and homogeneous silane layer on the primary substrate material is the bottleneck for efficient attachment of DNA to the substrate.

The density of DNA attainable is, however, limited by the excluded volume of the DNA. Simple deposition of end-functionalized DNA results in densities slightly smaller than $1/(\pi R_g^2)$. Higher densities for dilute supernatants were obtained by pushing DNA to the surface by an electric field perpendicular to the surface, using conducting coatings such as ITO or ZnO, and a Pt counterelectrode. ZnO, however, was found to be instable in common DNA buffers containing EDTA due to the complexation of Zn^{2+} ions by the EDTA.

For confocal fluorescence microscopy (CFM) we have labelled the DNA with the intercalation dye YOYO-1 at an average dye concentration of 1 molecule per 5 base pairs.

Applying the electric field perpendicular to the surface and parallel to the optical axis of the microscope then resulted in an extension of the coiled DNA molecules, allowing to distinguish individual DNA molecules (see Fig. 1).

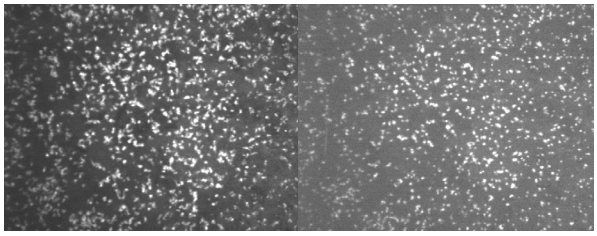

Fig. 1: Confocal fluorescence microscopy images of YOYO-1 labelled λ-DNA grafted to an ITO coated glass slide. Left: relaxed, coiled DNA in the absence of an electric field. Right: the DNA molecules are extended by the electric field. Their cross-sectional area observed by CFM is reduced due to the restricted excursions of the DNA chain transverse to the field direction. Field of view: 70μm x 90μm.

We have also been able to obtain two-sided attachment of λ-DNA which was functionalized with biotin at one end and with a thiol group at the other end. This procedure then allowed to extend the DNA directly using piezo-driven mechanical cantilevers. This method has the advantage over electric field stretching that no currents are required which lead to faster bleaching of the fluorescent marker.

We discuss our results in view of recent theories for the conformation of isolated polyelectrolyte chains extended by electric fields.

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NANO-BUBBLES AT POLYMER SUPPORTED HYBRID BIOMEMBRANES. NEUTRON REFLECTOMETRY AND AFM INVESTIGATIONS.

T.Gutberlet[1,2], R.Steitz[2,3], R.Krastev[2], A.C.Simonsen[4] & B.Kloesgen[4]

[1]Paul Scherrer Institut, LNS, Villigen, Switzerland. [2]Hahn-Meitner-Institut, Berlin, Germany. [3]Technische Universität Berlin, Berlin, Germany. [4]MEMPHIS, Physics Dept., University of Southern Denmark, Odense, Denmark.

INTRODUCTION: The controlled design of supported lipid bilayer probes as model biomembranes is a central challenge in the development of biosensors or as tools for basic biophysical investigations. To guide and control the fabrication of such probes at solid interfaces in-situ surface sensitive spectroscopic and microscopic techniques can be used.

In recent work we have investigated the adsorption of dimyristoyl phosphatidylcholine (DMPC) in water (D_2O) onto a planar hydrophobic polystyrene interface, deposited by spin-coating on a Si-crystal (PS/Si), using neutron reflectivity [1]. Here, formation of a lipid monolayer should be observed at the surface of the soft cushion support and precipitation of further lipid molecules in time. Thus, a free floating fluid membrane should be formed in the vicinity of the solid/liquid interface. The quality of the adsorption of the lipid molecules to the interface is affected by the wetting behavior of the polymer support. Depletion of the boundary layer of water against hydrophobic substrates may occur interfering with the evolution of lipid layers at the solid/liquid interface. By neutron reflectivity and AFM we have studied this behavior at the above PS/Si system [2].

METHODS: PS/Si samples were composed of deuterated or protonated polystyrene layer (PS, M~65kD) deposited onto silicon single-crystal blocks with native oxide layer by spin coating (3500 rpm, toluene solution (5mg/ml)). Thereupon, lipid layers were adsorbed by exposure of the polymer surface to a vesicle suspension prepared by sonication of DMPC in water (0.5 mg/ml). The deposition was monitored by taking consecutive neutron reflectivity patterns at the neutron reflectometer V6 with λ=4.66Å at BENSC, HMI Berlin [1]. The wet samples were investigated by tapping mode AFM, performed with the PicoSPM (Molecular Imaging, Phoenix, AZ) inMACmode (magnetic excitation of the cantilever) and using the medium range scanner (~30 μm maximal scan width) [2].

RESULTS & DISCUSSION: Immediately after injection of the phospholipid vesicle suspension into the sample cell, formation of a lipid monolayer was observed in the reflectivity curve obtained. Further adsorption of an additional lipid bilayer occurred slowly in time. A thickness for each monolayer of about 16 Å was observed in the samples [1] in good agreement with small angle neutron scattering experiments of suspended large unilamellar vesicles. In contrast to measurements of LB deposited phospholipid double bilayers at the bare hydrophilic Si-crystal interface no distinct water gap between the adsorbed lipid monolayer and bilayer was revealed. This may be due to hydrophobic interactions between the polymer interface and the deposited lipid layers. Neutron reflectivity and AFM measurements of the PS/Si interface clearly demonstrated the existence of a nano-meter scale depletion layer before deposition of the phospholipid (Fig. 1) [2]. The origin of this depletion layer has been related to the existence of nanoscopic gas bubbles at the polymer/water interface. They may interfere with the adsorption of lipids to the interface leading to incomplete surface coverage or defects and produce artifacts in the analysis of the reflectivity measurements.

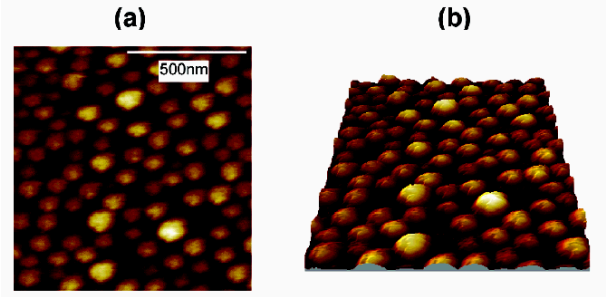

Fig. 1: Tapping mode topology image of nanobubbles on a 1 μm^2 of the surface of a d-PS coated silicon substrate (film) in distilled water (a) and 3D projection (b).

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INTERACTION OF HUMAN OSTEOBLASTS WITH PHOSPHORYLCHOLINE POLYMERS

M.Habib[1,2], R.A.Brooks[1], D.C.Ireland[3], A.L.Lewis[4], N.Rushton[1] & W.Bonfield[2]

[1]*Orthopaedic Research Unit, Box 180, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK.*
[2]*Dept. Materials Science & Metallurgy, New Museum Site, Pembroke St, Cambridge, CB2 3QZ, UK.*
[3]*Bone Research Group, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK.*
[4]*Biocompatible's Ltd, Farnham Business park, Weydon Lane, Farnham, Surrey, GU9 8QL, UK.*

INTRODUCTION: It has been a long-term objective of biomaterials research to develop materials, which can interact with cells and living tissue to replace, repair and enhance biological function. Clearly biomaterials must be thoroughly investigated prior to any clinical use. Although in-vivo studies are essential for a complete picture of healing processes around implants, in-vitro studies are also valuable and can provide answers to questions on cell attachment, proliferation and differentiation on biomaterials. In this study the interaction of primary human osteoblasts (Hobs) with a phosphorylcholine (PC) polymer, containing 20% cationic charge, was investigated. PC polymers are made of hydrophilic PC-based groups and hydrophobic groups such as alkyl methacrylates. They can closely mimic the structure of the naturally occurring cell-surface biomembrane lipids and possess excellent biocompatibility. It has been shown that incorporation of cationic groups into PC polymers increases proetien adsorption and cell attachment.

MATERIALS AND METHODS: 25 ml tissue culture flasks were coated with a 1wt% ethanolic solution of PC (designated PC20) polymer and cured at 72^{o}C for 72 hours. Uncoated tissue culture flasks were used as controls.

Hobs were seeded into flasks at a density of $1.4x10^4/cm^2$ and cultured in McCoy's medium containing 10% foetal calf serum, 1% glutamine and 30µg/ml vitamin C. Cultures were treated with hydrocortisone, in a water soluble cyclodextrin encapsulated form, to stimulate cell differentiation or β-cyclodextrin as a control (HC and DC respectively). Images of cells on PC and control surfaces were captured using an optical microscope and 3-chip colour camera connected to a computer with frame grabber and image analysis software.

Reverse-transcriptase polymerase chain reaction (RT-PCR) was used to measure the cellular expression levels of alkaline phosphatase (ALP) messenger RNA (mRNA). Total RNA was isolated from adherent cells at 12, 24, 36, 48 and 168 hours, following seeding, using phenol/chloroform. PCR was performed using a GeneAMP 5700 Sequence Detection System. mRNA levels for ALP was measured relative to that of the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

RESULTS: Cell adhesion and spreading on the surfaces began about 1 hour after seeding. By 24 hours in culture most cells were fully spread on both PC20 and control surfaces. At the 48 hour time point some mineral deposits were visible on PC20 (Figure.1). The mRNA level for ALP decreased with time in all cultures, however it remained higher in those treated with hydrocortisone compared to those exposed to β-cyclodextrin. There were no significant differences in ALP gene expression between cells on PC20 and control substrates.

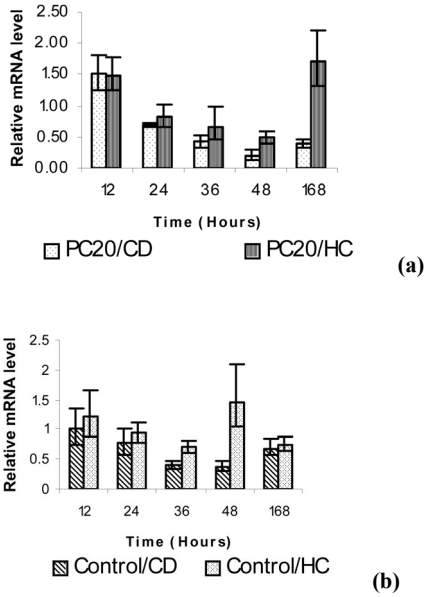

Fig. 1: Graphs showing mRNA levels for ALP on PC20 (a) and control (b) surafces. mRNA levels expressed have been expressed relative to tissue culture plastic exposed to β-cyclodextrin (Control/CD) at 48 hours.

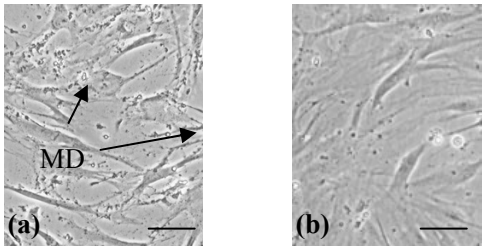

Fig. 2: Optical images of Hobs on PC20 (a) and control (b), Scale bar = 100µm. MD= mineral deposit.

DISCUSSION: The results show that PC polymer is capable of promoting mineral deposition on Hobs. Cationic charge promotes cell adhesion and spreading. The PCR results however indicate that, whilst cell behaviour was altered on PC surfaces, an early change in mRNA levels for markers of osteoblast differentiation was not seen. The higher mRNA level from cells treated with HC indicated that the cells were responding to hydrocortisone.We will measure the message levels for Collagen type I and osteocalcin in future experiments and investigate the mechanism of mineral deposition process.

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CONTROL OF PLATELET AND CELL ADHESION TO TITANIUM WITH HELIUM ION IMPLANTATION

T.Hanawa[1], S.Nakajima[2], A.Yamamoto[1], Y.Suzuki[3] & M.Iwaki[3]

[1]Biomaterials Center, National Institute for Materials Science, Tsukuba, Japan. [2]Faculty of Science, Tokyo University of Science, Tokyo, Japan. [3]RIKEN, Wako, Japan.

INTRODUCTION: Excellent blood compatibility is required on materials used for treatment of cardiovascular disease. In addition, the control of the adhesion of platelet is necessary for the development of metal-base chips. However, blood compatibility of metallic materials is insufficient at present. In this study, helium ion was implanted into collagen-coated and uncoated titanium to control the blood compatibility and endothelial cell adhesion of titanium. The surface layers were characterized using surface analysis techniques.

METHODS: Commercially pure titanium (grade 2) disks were polished and washed. Then, the titanium disk was immersed in 0.3% type I collagen aqueous solution at 4°C for 2 h. Helium ions were implanted into titanium disks with and without collagen coating at an energy of 150 keV. Adhesion blood platelet was measured with 1 x 10^5 ml^{-1} platelet-rich plasma (PRP). Calcium ions were re-added or not added to plasma. After 5-min contact to titanium specimens, the plasma was removed, then the adhesion was evaluated using SEM. To evaluation the adhesion of bovine arteriae endothelial cells (BAEC), droplet of the suspension of BAEC was added to titanium specimens and incubated at 37°C for 24 h. Then, the cells were fixed and the specimens were rinsed, followed by the observation with SEM. The surface of the specimens were characterized using AFM, X-ray photoelectron spectroscopy (XPS), Fourier transformation infrared spectroscopy (FTIR), and laser Raman spectroscopy (LRS) to discuss the results of adhesion test of platelets and cells.

RESULTS & DISCUSSION: *Figure 1* shows SEM images of platelets adhered to collagen-coated titanium. In this case, calcium ion was readded. Adhesion of platelet was inhibited in 10^{13}-ions cm^{-2} specimen. We obtained the same results in collagen-uncoated titanium. Thus, helium ion implantation with an amount of 10^{13} ions cm^{-2} is effective to prevent the platelet adhesion. On the other hand, adhesion of BAEC to collagen-coated titanium is shown in *Figure 2*. Number of adhered BAEC was the largest in 10^{13}-ions cm^{-2} specimen. The same result was obtained in collagen-uncoated titanium. Therefore, 10^{13}-ions cm^{-2} helium ion implantation accelerated the adhesion of BAEC to collagen-coated and uncoated titanium. With helium ion implantation, titanium surface was dehydrated and de-fragmentation of collagen partly occurs while main helix structure is remained.

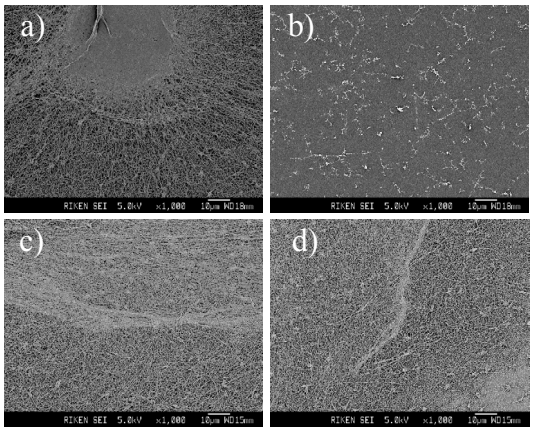

Fig. 1: Adhesion of platelets on collagen-coated titanium in Ca^{2+}-readded plasma; (a) unimplanted, 10^{13}, (b) 10^{14}, and (d) 10^{15} ions cm^{-2}.

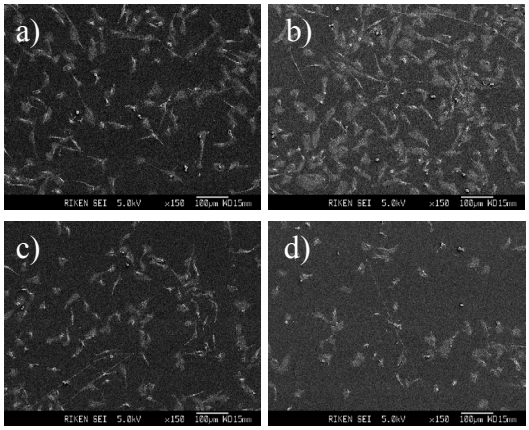

Fig. 2: Adhesion of BAEC on collagen-coated titanium; (a) unimplanted, 10^{13}, (b) 10^{14}, (d) 10^{15} ions cm^{-2}.

CONCLUSIONS: Helium ion implantation with an amount of 10^{13} ions cm^{-2} is the most effective for inhibition of platelet adhesion and acceleration of endothelial cells among conditions tested in this study. Blood compatibility of titanium and other metals could be controlled with collagen coating, followed by helium ion implantation.

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POLYELECTROLYTE MONOLAYERS FOR BIOACTIVE SURFACES

R.Konradi & J.Rühe

Dept. of Chemistry and Physics of Interfaces, IMTEK, Uni Freiburg, 79110 Freiburg, Germany.

INTRODUCTION: Polyelectrolyte brushes are promising candidates for the generation of surfaces with specific biocompatibility requirements, such as to template and coordinate protein or cell adhesion. In these biological systems, a mix of monovalent and multivalent ions is necessary for their proper function. The presence of these ions, however, also modifies the polyelctrolyte brush structure, which in turn modifies its function. The current challenge is to quantify the influence of the complex aqueous environment on the swelling behavior of charged, water-soluble brushes.

METHODS: Poly(methacrylic acid) (PMAA) brushes were synthesized via the "grafting from" technique as described in the literature.[1]

The swelling behavior of these brushes was investigated by multiple angle nulling ellipsometry and the volume fraction profiles were modelled by using a complementary error function.[2]

RESULTS: We report on the swelling behavior of PMAA brushes in contact with aqueous solutions of monovalent, bivalent and trivalent counterions at neutral pH. A fundamentally different behavior was found for the three types of ions. **Figure. 1** shows the evolution of the brush thickness with increasing ionic strength for each of the sodium, calcium and aluminum nitrate solutions.

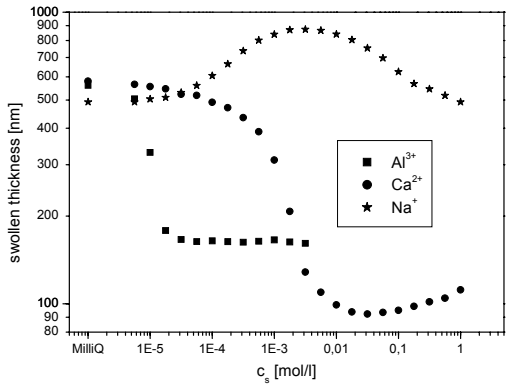

Fig. 1: Swollen thickness of PMAA brushes as a function of the external salt concentration. The respective dry thicknesses were 42 nm (star), 45 nm (circle) and 46 nm (square).

The brush thickness passes through a maximum only for the monovalent sodium ion. With respect to the bivalent alkaline earth metal, calcium, we find that the brush collapses at intermediate concentrations (around 10^{-3} mol/l) and exhibits no maximum in brush thickness. Lastly, PMAA brushes in contact with aluminum solutions show a behavior similar to the calcium case; however, the collapse concentration is found to be lower by roughly 2 orders of magnitude and the collapse already starts at concentrations $<10^{-5}$ mol/l.

DISCUSSION & CONCLUSIONS: Theoretical studies based on ionic interactions alone show that the brush will display a maximum in its thickness with increasing ionic strength. This behavior is predicted for both monovalent and multivalent ions. In contrast to theory[3] we only find such a situation for monovalent ions. The lack of such behavior for the bivalent calcium and trivalent aluminum counterions leads us to believe that ionic interactions alone do not govern the brush structure. Indeed, there have been experimental investigations on the interaction of weak polyacids in different topologies with multivalent cations in the literature that support our findings.[4-6]

We conclude that the swelling behavior of weak polyacid brushes is indeed very sensitive to the presence of bivalent and trivalent cations and that specific interactions must be taken into account. Therefore, as a basis for a predictable engineering of interface properties in biological environments it is crucial to understand the relationship between the structure of weak polyacid brushes and their specific environment.

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DNA-INTERCALATING SURFACE COATING

A.Krieg[1], P.Häfliger[2], S.Laib[1], N.Agorastos[2] & S.Seeger[1]

[1]*Institute of Physical Chemistry, University of Zürich, Zürich, Switzerland.*
[2]*Institute of Inorganic Chemistry, University of Zürich, Zürich, Switzerland.*

INTRODUCTION: A variety of noncovalent coupling techniques are known to bind DNA onto glass.[1] In this study we report a novel efficient method for unspecific binding of double stranded DNA (dsDNA) onto coverslips. This approach is based on the strong affinity between dsDNA and a modified intercalator covalently bound to 3-Aminopropyltrimethoxysilane and 1,4-Phenylenediisothiocyanate coated glass surface (Fig. 1). The intercalation of a Cy5 labelled, doublestranded DNA-fragment (2 kilobase pairs (kbps)) on the surface can be observed by supercritical angle fluorescence (SAF) measurements with a custom-made biosensor [3]. Briefly, after excitation by a HeNe laser a parabolic glass lens collects only the fluorescence emitted into the angular region above the critical angle of refraction, which corresponds to ~61° for a glass/water interface. By detecting only supercritical emission, the detection volume is restricted to a surface distance well below 100 nm, while most of bulk fluorescence is rejected [4].

METHODS: The synthesis of the amine modified pyrene (1) is described in [2]. After fixation of the intercalator on coated coverslips (Genorama SAL, Asper Biotech, Estonia), the surface was treated with CF_3COOH/CH_2Cl_2 (1:1) for 3 h to remove the BOC protecting groups (Fig. 1). Completion of the coupling was checked by absorption spectra.

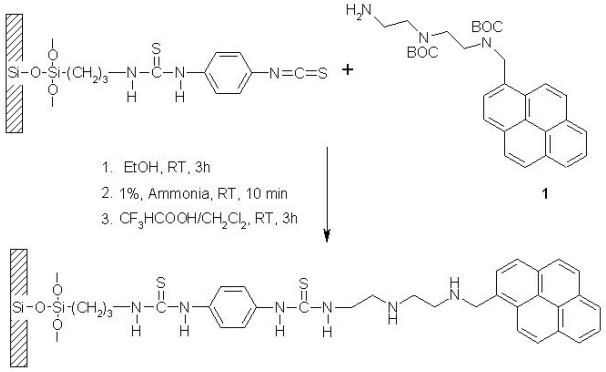

Fig. 1: Reaction scheme for the coupling of the the modified pyrene to 3-Aminopropyltrimethoxysilane +1,4-Phenylenediisothiocyanate coated coverslip.

Subsequently the coverslips were glued onto a measuring cell containing six reaction chambers. For SAF-measurements a solution containing 2 kbps dsDNA-fragments (150µL, 10^{-10} M, TE-buffer, pH 7.1) was pipetted into the chamber. Each fragment was labelled by one Cy5 dye molecule.

RESULTS: As reference for the SAF measurements a SAL slide was prepared without intercalator attached to the surface. The addition of the dye-labelled DNA to this surface caused a rapid increase of the fluorescence to 16±2 kHz, which remained at this level afterwards (Fig. 2). This count rate can be attributed to non-specific interaction of the DNA-fragment with the surface. In contrast the use of DNA-intercalating surface-coating led to a fluorescence increase of more than 80±7 kHz (Fig. 2). Comparisons with FRAP (fluorescence recovery after photobleaching) experiments also revealed this effect and indicated that the noncovalent coupling is caused by intercalation.

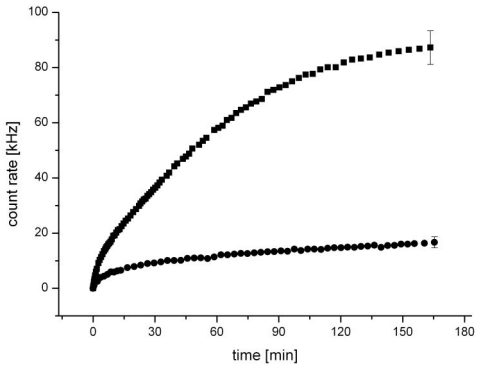

Fig. 2: SAF intensity after addition of Cy5-dsDNA to DNA-intercalating (■) and non intercalating surface (●). The background was subtracted from the data and the start time was set to zero.

DISCUSSION & CONCLUSIONS: Our results stringently demonstrate the binding of the DNA to the surface induced by the immobilized intercalator. Thus, the binding efficiency of dsDNA to surfaces is improved by coupling of an intercalator to the substrate. This method could be an application in different types of DNA analysis.

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A NOVEL METHOD TO STUDY MECHANICAL PROPERTIES OF POLYELECROLYTE MICROCAPSULES

V.V.Lulevich & O.I.Vinogradova

Max-Planck Institute for Polymer Research Ackermannweg 10, 55128 Mainz, Germany.

INTRODUCTION: Polyectrolyte multilayer capsules have been recently introduced as a novel type of nano-engineered microstructures[1]. These capsules are made by layer-by-layer adsorption of oppositely charged polyelectrolytes onto charged colloidal particles with subsequent removal of template core. The relatively easy way of preparation, well-defined shape, and mechanical stability allows their use for such applications as encapsulation of a wide class of molecules, drug delivery or biomimetics.

METHODS: We present a novel approach to study the properties of polyelectrolyte microcapsules which is based on measurement the load-deformation curves of capsule with the Atomic Force Microscope (AFM) related device combined with the laser scanning confocal microscope. Capsule deformed by using piezo manipulator between the flat surface and the big sphere glued on the AFM cantilever. AFM allows us to see the deformation force, and optical or confocal microscope - the shape of capsule (Fig. 1).

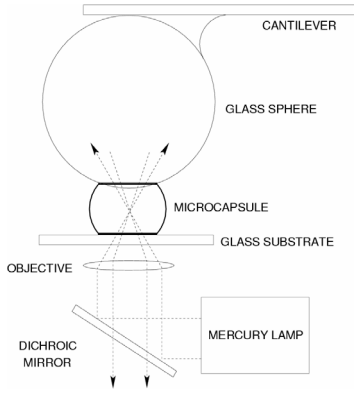

Fig. 1: Schematic picture of the experiment.

RESULTS: Figure 2 shows a typical confocal image of a polyelectrolyte capsule (left) and force-deformation curve (right).

For a small deformation (**ε**) simple model can predict dependence between force **F** and deformation **ε** :

$$F = 2\pi \frac{E}{1-\nu} hR\varepsilon^3$$

where **R** is radius the of capsule, **h** – wall thickness, **E** – elastic modulus of the capsule shell and **ν**- its Poisson ratio. Fitting to the load-deformation profile allows us to determine the Young's modulus of polyelectrolyte multilayer structure (for Poisson ratio of 0.5). It is varies from 5 MPa in the case of melamine formaldehyde (MF) core to 0.3-0.5 for latex core which have got the same order of magnitude with high-elastic polymers[2].

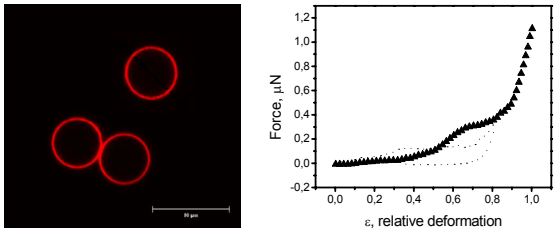

Fig. 2: Hollow polyelectrolyte capsules (left) and their typical load-deformation profile(right).

Hollow capsules were filled with polymer and the same method already presented to study them. It allow us to find the structure of inner polymer network[3] add diffusion properties of polyelectrolyte shell[4].

DISCUSSION & CONCLUSIONS: Our method gives us information about the mechanical and adhesion properties of capsules. Permeability of polyelectrolyte shells and Young modulus of polyelectrolyte multiplayer can be easily obtain. Some extension of this method can be used for characterization micro- and nanoparticles, ultrathin membranes, vesicles and cells.

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A NOVEL METHOD FOR DESIGNING SURFACES TOWARDS BIOSYSTEMS

D.Mädge, O.Prucker & J.Rühe

Institute for Microsystemtechnology (IMTEK), University of Freiburg, Germany.

INTRODUCTION: Functional polymer monolayers are promising architectures for a variety of different applications especially in the life sciences mainly because the surface density of the functional groups can be driven to much higher values as it is possible for self-assembled monolayers of e.g. silanes that carry the same functional group. A technique towards such systems makes use of a monolayer of molecules that carry polymerisable groups. If these materials are added during a free radical polymerisation of other monomers the polymerisable groups at the surface are captured by the growing polymer chains and thin chemically anchored polymer monolayers are formed [1,2]. Despite this synthetic simplicity of this system, however, the general mechanism of the process was investigated in great detail and there is no report on the use of such systems for the construction of biologically relevant devices such as sensor or immuno assay systems.

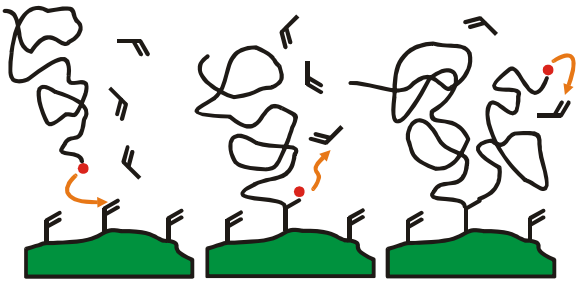

Fig. 1: 2-step mechanism of a radical copolymerisation with immobilised monomers.

METHODS: Basic experimental details can be obtained from the literature [3]. All silanisations were done in dry toluene under nitrogen using dry triethylamine as an acid scavenger and catalyst. Polymerisations were carried out in sealed vessels in a thermostat at 60°C. Prior to polymerisation oxygen traces of all monomer mixtures were carefully removed by at least 4 freeze-thaw cycles. The substrates carrying the polymer films were carefully extracted using a Soxhlet apparatus in order to remove any physically attached chains from the monolayers.

Film thicknesses were measured elipsometrically (planar substrate) and the grafted amount was calculated from elementary analysis (porous substrate).

Activities of immobilised enzymes were derived from UV/vis spectrometre data and biomolecules (e.g. DNA, biotin) detected by fluorescence labelling.

RESULTS & DISCUSSION: A thorough investigation of this process showed that the variation of important polymerisation parameters such as time, temperature and concentration of monomer and initiator allows for the preparation of polymer monolayers with a well-defined thickness and graft density of the polymers. Furthermore, we found that the overall process is largely insensitive to run-to-run variations of hard to control process variables (e.g. residual oxygen content during polymerisation; variation in graft density of immobilised polymerisable groups). This robustness of the technique and the radical polymerisation process used to deposite the monolayers make this method suitable for the tailoring of surface properties and for the incorporation of a wide variety of functional groups. We used (meth)acrylates that carried either N-hydroxysuccinimide ("active ester", NHS) or protected amino functions (phthalimide). The NHS moieties successfully bind enzymes (e.g. glucose oxidase) or DNA with terminal amino group. After deprotection with hydrazine the amino copolymerlayer reacts with isothiocyanate fluorescence labels or NHS-biotin.

Some examples of systems that are interesting for applications in the area of biomaterials are presented: Enzyme immobilisation for biotechnical reactors; reversible linkages via immobilised DNA strands; monolayers with covalently bound peptides or DNA on porous supports for analytical purposes. Furthermore, we show that biotinylisation of the layers are promising candidates for biotin-streptavidin-assays

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PLASMA COPOLYMERIZATION – A RECOMMENDED WAY FOR FORMATION OF DEFINED FUNCTIONALIZED SURFACES

R.Mix, G.Kühn & J.F.Friedrich

Federal Institute for Materials Research and Testing, Berlin, Germany.

INTRODUCTION: The chemical fixation of biological molecules onto functionalized surfaces demands an arbitrary adjustable number of monotype functional groups on the surface to avoid undesirable interactions by redundant functionalities. Common surface treatments as silanization, plasma treatment or plasma polymer deposition of OH, NH_2 or COOH groups-bearing monomers give rise to random functionalized surfaces, which are not perfectly adapted to large molecules. Usual plasma polymer layers, prepared by continuous wave RF plasmas, are often chemically irregular in their structures and chemical compositions. To minimize irregularities low wattages and the pulsed plasma technique was applied to avoid a high degree of fragmentation[1]. Using the pulsed plasma technique we investigated the plasma-initiated chemical copolymerization of allyl alcohol (Aal) with "neutral" molecules ("chain extenders") as butadiene (BD), ethylene (E), acetylene (AC) and styrene (St). The copolymer layers were characterized by XPS, FTIR and contact angle measurements.

METHODS: The copolymerization experiments were performed in a stainless steel reactor (Ilmvac, Ilmenau, Germany) using a RF plasma of 100W/300 W and a duty cycle $t_{on}/(t_{on}+t_{off})$ = 0.1. The X-ray photoelectron spectra (C1s, O1s, F1s) before and after derivatization were recorded with a Sage 150 spectrometer (Specs, Berlin, Germany) using a non-monochromatic Mg K_α radiation for excitation. The energy analyzer was operated at 20 eV pass energy. The analysis area was about 35 mm^2, the pressure in the analysis chamber was hold at $<5\cdot10^{-8}$ mbar. Core-level signals were obtained in the constant analyzer energy (CAE) mode at 90° take-off angle. The X-Ray source was run at a power of 225 W (12,5 kV and 18mA). The contact angles were measured using the Sessile Drop Method with the Contact Angle Measuring System G2 (Krüss, Hamburg, Germany) and interpreted following Owens et al.[2]. The FTIR-spectra were recorded using the Nexus Spectrometer (Nicolet, USA).

RESULTS: In Figures 1-4 the chemical composition of TFAA-derivatized layers of copolymers Aal/BD and the surface energy of the copolymers are shown Dependent on the applied power different regions of plasma deposition of co- or homopolymers were found. Applying 100 W Aal and BD copolymerize, if the concentration of Aal exceeds 30 mole% in the precursor. At lower concentrations of Aal exclusively BD was deposited. The deposition at 300 W restricts the copolymerization region. (cf. Fig. 3). When E instead of BD was used, the copolymerization takes place if the precursor contains 1-30 mole% Aal (100 W). A further increase of the Aal percentage gives rise for deposition of pure Aal layers. The copolymerization regions of Aal with BD, E, AC and St are compared.

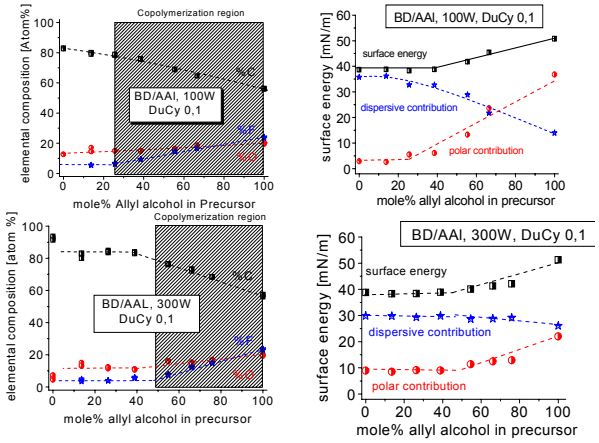

Fig. 1-4: Chemical composition of Aal/BD copolymers (derivatized with TFAA) and surface energy of the (original) copolymers for different Aal l concentrations in the precursor.

DISCUSSION & CONCLUSIONS: The copolymerization of Aal with ´neutral´ monomers like BD, E, AC or St in pulsed plasmas shows an interesting way to produce defined functionalized surfaces such as equipped with hydroxy groups. Depending on the reactivity of the applied comonomers the copolymer formation occurs only in a distinct range of comonomer composition in the precursor. The width of the copolymerization region is mainly influenced by the plasma power.

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MICROSTRUCTURED BIOMIMETIC SURFACES FOR CONTROLLED WETTING

U.Mock, L.Riegger & J.Rühe

Dept. of Chemistry and Physics of Interfaces, Institute for Microsystems Technology, Albert-Ludwigs-Universität Freiburg, Germany.

INTRODUCTION: The water-repellency of biological systems has attracted considerable interest in the last decade due to the thorough investigation of the lotus flower [1]. The superhydrophobic effect of the lotus flower as well as other plants with an apparently smooth surface results from the topography of the leaf, i.e. its combined micro- and nanoroughness. A second category of plants which have hair on their surface also exhibit a strong hydrophobic behavior. For this type of plants a model has been proposed where the elasticity of the plant hair accounts for the water-repellency of the leaf surface. As a representative the lady's mantle surface has been closely investigated [2]. The hair has a medium diameter of 10 µm, a height of 1 mm and an average distance of 500 µm. In the following it is described how to mimic the lady's mantle surface with polymer networks for a systematic study of the influences of elasticity and hair distribution on the hydrophobic effect (see Fig. 1).

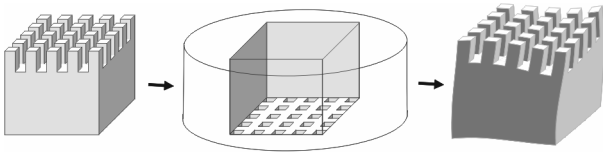

Fig. 1: Concept for the microreplication of a surface. First a plant leaf or a metal master are molded against an elastomer. Second an elastic network can be grown by radical polymerisation in the negative.

METHODS: Materials. 2-Hydroxyethylmethacrylate (HEMA) was purified using an Alox B column, distilled under vacuum from copper(I) chloride and stored under nitrogen at –30°C. All other chemicals were used as received (p.a. grade). **Molding of leaves.** All leaf surfaces were treated with an 0.5 mM solution of 3-(N,N-Dimethylmyristylammonium)sulfonate prior to molding with Sylgard 184 (Dow Corning) and subsequent curing at 70 °C. Then the PDMS master was peeled off the leaf. **Polymerizations.** Polymerizations were per-formed in HEMA/methanol mixtures (1/2 v/v) at 50 °C with 0.01 mol % AIBN as radical starter and 1.0 mol % Ethylene glycol dimethacrylate as crosslinker. All solutions were degassed through at least three freeze-thaw cycles to remove all oxygen traces. After polymerization the swollen network was released from the PDMS master.

RESULTS & DISCUSSION: The hairy lady's mantle surface can be reproduced using polymer networks in combination with two molding steps. First a master is generated by molding a leaf against PDMS. After curing and removal of the leaf the master is filled with degassed monomer solution under vacuum. The polymerization is performed at 50 °C for ~ 2 h until a swollen PHEMA network has formed which is peeled off the master. Figure 2 shows an optical micrograph of an experiment and it can be seen that the polymeric hair has the expected dimensions.

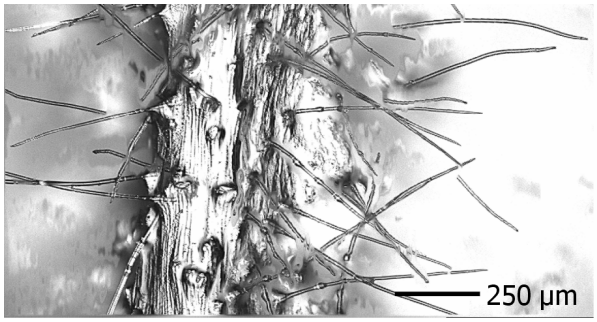

Fig. 2: Extended Focal Microscopy Imaging picture of a PHEMA leaf vein.

CONCLUSIONS: It has been shown that it is possible to mimic biological surfaces using a molding approach which results in the formation of swollen polymer networks even for very high aspect ratios. In order to replicate large surface areas for systematic investigations of the wetting behavior further experiments are necessary.

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ACKNOWLEDGMENTS: Financial support within the DFG priority program "Wetting and structure formation at interfaces" (SPP 1052) is gratefully acknowledged.

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SYNTHESIS AND CHARACTERIZATION OF AMPHIPHILIC POLY(PROPYLENE SULFIDE)-BASED BLOCK COPOLYMERS. MACROAMPHIPHILES FOR SURFACE FUNCTIONALIZATION AND DRUG DELIVERY SYSTEMS.

A.Napoli[1], N. Tirelli[2] & J.Hubbell[1]

[1]*Dept. of Materials, ETHZ, Zürich, Switzerland.* [2]*School of Pharmacy and Centre of Molecular Materials, University of Manchester, United Kingdom.*

INTRODUCTION: In the present communication we report the synthesis and characterization of functional amphiphilic block copolymers able to assemble in water into lamellar and micellar mesophases (also termed *lyotropic*, induced by the solvent).

When designing such macromolecules, a major effort was devoted in having a simple and reliable chemistry and in introducing novel degradation properties. The result has been a macroamphiphile containing as the hydrophobe a linear atactic poly(propylene sulfide) chain (PPS) and as the hydrophile poly(ethylene glycol) (PEG).

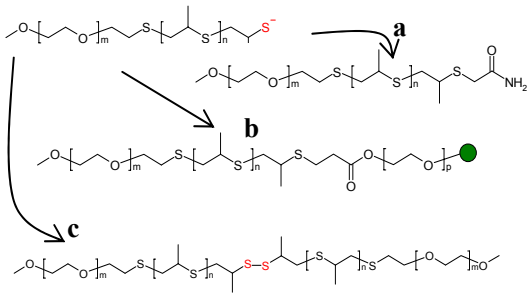
PPS is a "newcomer" in the biomedical field possessing interesting properties like: a low Tg, its conversion into a hydrophile upon exposure to oxidative conditions and the ability to strongly bind to gold (as thiols in SAMs do). Last but not least it is based on thiol chemistry making its functionalization with peptides easy.

METHODS: The synthetic path[1] is based on the anionic ring-opening polymerization of episulfides, initiated by a thiolate group. The initiator is generated in situ from a PEG chain containing a protected thiol (a thio- or dithioester), avoiding the use of free thiols and thus the problems arising from disulfide formation.

The living anionic process produces a polysulfide chain (e.g. poly(propylene sulfide) PPS) with a reactive thiolate end, which was used to couple the polymer with an acrylate-terminated PEG through Michael-type addition or upon exposure to air to form symmetric triblocks bearing a disulfide bridge. The mild character of the end-capping reaction allows the insertion of sensitive biological groups, e.g. peptides and quantitative end-capping with low molecular weight molecules.

The polymerization and the end-capping reaction has been investigated by H^1-NMR and Gel Permeation Chromatography (GPC).

RESULTS & DISCUSSION: We explored the lyotropic behavior and oxidative stability of the lyotropic mesophases in water of PEG-PPS-PEG, using the $EG_{16}PS_{50}EG_{16}$ symmetric triblock macroamphiphile as an example. We have previously described that this liquid polymer forms lamellar phases in water[2].



Scheme 1: End-capping for PEG-PPS living polymerization may lead to: ***a)*** *diblock copolymer using a low molecular weight agent;* ***b)*** *asymmetric triblock using a PEG acrylate;* ***c)*** *by simply exposing the reaction mixture to air, symmetric triblocks are obtained via disulfide bridge formation.*

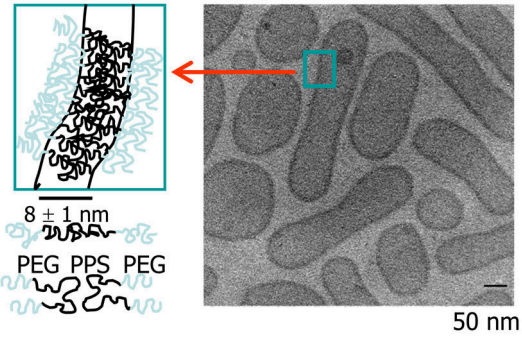


Fig. 1: Vesicles formed by $EG_{16}PS_{50}EG_{16}$ block copolymer imaged by Cryo-TEM (bar represents 50 nm). In the box a scheme of the polymeric membrane with the PPS in black and the PEG chains in cyan.

These copolymers are structurally similar to the well-known poloxamer macroamphiphile (copolymers of PEG and poly(propylene glycol), PPG), but the substitution of S atoms for the O atoms in the PPG block renders the hydrophobe much more hydrophobic and the otherwise relatively unstable poloxamer vesicles into structures that are stable in aqueous environments for months (herein).

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COMPRESSION OF ADSORBED POLY(L-LYSINE)–*G*–POLY(ETHYLENE GLYCOL) MEASURED BY AFM

S.Pasche[1], H.J.Griesser[2], N.S.Spencer[1] & M.Textor[1]

[1]*Laboratory for Surface Science and Technology, Dept. of Materials, ETH-Zürich, Switzerland.*
[2]*Ian Wark Research Institute, University of South Australia, Adelaide, Australia.*

INTRODUCTION: Poly(L-lysine) grafted with poly(ethylene glycol) (PLL-*g*-PEG), a polycationic co-polymer positively charged at neutral pH, has been shown to spontaneously adsorb onto negatively charged surfaces, resulting in grafted PEG layers of controllable architectures, in terms of PEG molecular weight and density.[1] An *in situ* optical waveguide study of polymer and protein adsorption has demonstrated that the amount of protein adsorbed on the PLL-*g*-PEG-coated surfaces is strongly correlated with the EG monomer surface density, in turn determined by the polymer architecture.[2]

PLL-*g*-PEG coatings offer the possibility of varying both the polymer architecture and the degree of biointeraction in a controlled way. This characteristic of tuning the system's properties makes it attractive for a systematic study of the mechanical properties of PEG grafted layers. A colloid-modified AFM technique was used to probe the response of the various PEG layers under compression by a 5µm-SiO_2 sphere, demonstrating the influence of PEG chain length and density at different ionic strengths. Polymer-polymer interactions were investigated after coating both sphere and substrate with the co-polymer.

METHODS: A conventional atomic force microscope (AFM Nanoscope IIIa, DI) was modified by using a 5µ-SiO_2 sphere as a tip, and used to measure the resulting force from the compression between the sphere and PLL-*g*-PEG-modified Nb_2O_5 surfaces *in situ* as a function of the separation distance.[3] PEG molecular weights of 1, 2 and 5 kDa were analyzed, with PEG densities varying from 0 to 0.9 nm^{-2}, in HEPES buffer at pH 7.4, with salt concentrations from 1 to 150 mM.

RESULTS: AFM force measurements show the response to compression of the PEG layer between the underlying metal oxide surface and the microsphere, expressed as an interaction force varying with the separation distance. The measured force is dependent on the co-polymer architecture, PEG length and density. Figure 1 shows different force versus distance curves for adsorbed co-polymers with increasing PEG density.

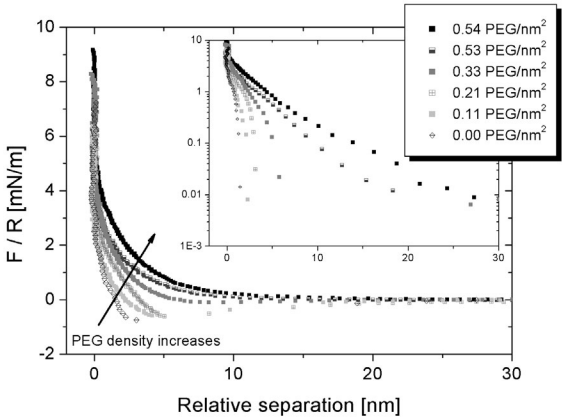

Fig. 1: Forces between a 5µm-SiO_2 probe and PLL-g-PEG-modified Nb_2O_5 substrates with MW 2000 PEG, and varying PEG densities (0 – 0.54 PEG chains/nm^2), in 10 mM HEPES pH 7.4, on a linear and on a log scale.

Performing the AFM measurements at different ionic strengths allowed for the discrimination between the steric repulsive force from the PEG chains and the electrostatic contribution from either the positively charged PLL backbone (attraction) or the negatively charged underlying substrate (repulsion). Polymer-polymer interactions showed additivity of the steric repulsive force.

DISCUSSION & CONCLUSIONS: PLL-*g*-PEG presents a controlled system in terms of interface architecture and protein adsorption. AFM force measurements on these well-defined PEG layers demonstrate the dependence of the surface forces on the PEG interface architecture, and their relationship to protein adsorption. The colloidal AFM is shown to have a high potential to elucidate the mechanisms of protein interaction with PEG layers.

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ACKNOWLEDGEMENTS: G. Gillies, K. Bremmel, F. Assi and S. Lee are thanked for their help with the AFM, J. Vörös and O. Borisov for helpful discussions. This work is financially supported by EPFL and ETHZ (TH-33./01-3).

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NON-SPECIFIC PROTEIN ADSORPTION ON THIN FILM COATINGS

M.Rankl, T.Ruckstuhl & S.Seeger

Institute for physical chemistry, University of Zürich, Zürich, Switzerland.

INTRODUCTION: Chemical composition and topology of a surface determine its physical properties and thus the non-specific interaction with biomolecules. In the present study the surface tension parameters of thin film coatings commonly used in biological assays are determined by contact angle measurements. With this data the relation between chemical composition of the surface films, their energetic properties and the nonspecific adsorption properties is investigated[1]. Therefore coatings with different functional groups and backbone structures are used. Poly-L-lysine (PLL), aminopropyltriethoxysilane (APTES), aminopropyltrimethylsilylethercellulose (ATMSC), cinnamatetrimethylsilylethercellulose (CTMSC) and trimethylsilylethercellulose (TMSC) (see figure 1) provide a suitable variance in chemical composition, as either the backbone or the side chain remains unchanged. To study the biomolecule surface interactions a highly sensitive biosensor was developed to detect surface bound molecules exclusively[2].

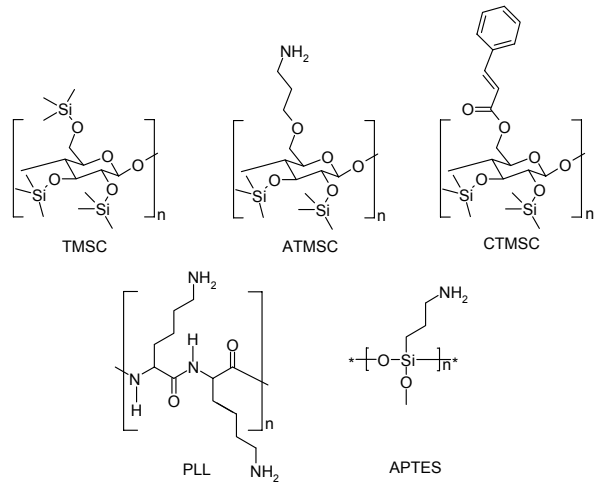

Fig. 1: Chemical structure of the substances used for coating of glass cover slips.

METHODS: The cellulose polymers can easily be coated on common cover slips with the Langmuir-Blodgett-technique yielding very flat and homogeneous films. APTES and PLL are deposited wet chemically on the glass cover slips. The surface tension components are determined by contact angle measurements. Roughness and topology of the surface films are determined by AFM-microscopy. The proteins are labelled with the fluorescent dye Cy5 and adsorption studies are carried out with a supercritical angle fluorescence (SAF) biosensor, which was designed for the selective detection of surface bound molecules. This is achieved by collecting supercritical angle fluorescence emission of the surface bound dipole emitter. Thus the penetration depth of the detection volume into the aqueous medium is reduced to about 100 nm and bulk fluorescence from the solution is rejected successfully.

RESULTS: All films reduce the surface tension of the bare glass cover slip and in particular the biological cellulose LB-films and PLL can be considered to be low tension surfaces. The predominant Lewis-base properties of the cellulose derivatives as well as their electron-acceptor properties coincide with the degree of substitution. While the polarity of the films varies with the covalent attachment of functional groups to the cellulose backbone, there is only a minor effect within the amino-functional films.

AFM-measurements reveal a roughness for all coatings at a nm-scale. The influence of surface roughness on adsorption is found to be rather small.

By SAF-measurements the kinetics and equilibrium density of adsorbed proteins are measured. Celluloses show an extremely weak tendency to adsorb proteins nonspecifically.

DISCUSSION & CONCLUSIONS: Kinetics and yield of the adsorption process are strongly dependent on the surface properties. The adsorption tends to be mainly driven by the backbone properties of the polymer and only to a minor degree by the substituent. Thus cellulose provides an excellent substrate for use in analytics. Different functional groups to bind receptor molecules covalently can be introduced without weakening its low-adsorptive qualities.

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PROTEIN ADSORPTION ON OXIDE PARTICLES: NEW INSIGHTS WITH COLLOID CHEMISTRY METHODS AND APPLICATION

K.Rezwan, L.P.Meier, J.Vörös, M.Textor & L.J.Gauckler

Dept. of Materials, ETHZ, Zürich, Switzerland.

INTRODUCTION: Most metallic biomaterials are covered by a protective, stable oxide film such as titanium oxide on titanium. Hence proteins only interact with the oxide film and not with the underlying metal. Closer investigations of the protein - oxide interface are therefore of great relevance to the biomaterials field.

In the past few years protein adsorption and desorption has been investigated mostly on planar surfaces by in situ techniques such as Ellipsometry, Optical Waveguide Light Mode Spectroscopy (OWLS) and Quartz Crystal Microbalance (QCM).

The main drawback of these methods is the lack of direct information about Surface Charges, which are known to strongly affect protein adhesion and conformation at interfaces. By applying colloid chemistry methods, e.g. Electrokinetic Measurements, supplementary data was obtained about surface charges, protein layer thicknesses and enzyme activity of particle – protein systems.

In addition, colloidal particles provide a very high specific surface area for applications such as Biosensors (see Fig. 1), where enhanced chemical activity is crucial. Furthermore we think that the presented methods and results are essential to the rapidly growing field of Colloidal Patterning.

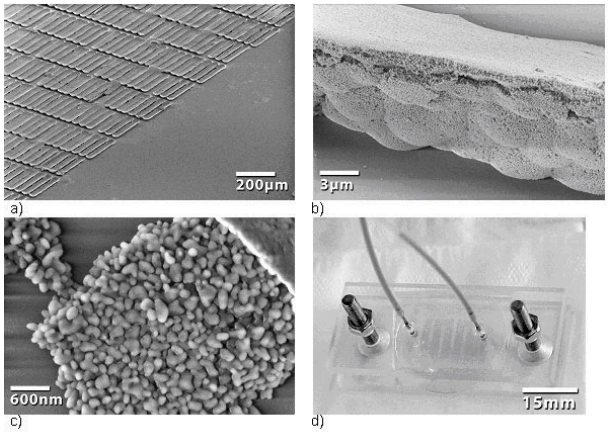

Fig. 1: Miniaturised enzyme reactor based on hierarchically shaped porous ceramic microstruts. [1]

METHODS: For the first time, we used colloid chemistry analysis methods such as colloidal vibration potential (CVP), X - ray disc centrifuge (XDC) measurements and UV – spectroscopy (UVS) to study in detail the adsorption of proteins to well-defined colloidal particles of typically 100 - 300 nm diameter. Combining these methods, the adsorbed amount of proteins and its influence on the zetapotential and the isoelectric point of the particles were determined with great precision and across a wide pH range.

RESULTS: Adsorption of Bovine Serum Albumin was found to alter the Zetapotential of the Oxides and their Isoelectric Points to an extent depending on the mass of the adsorbed protein. Combining UVS and XDC, the volume density and the thickness of the protein layer was determined.

DISCUSSION & CONCLUSIONS: We have shown that simple colloid chemistry methods give a variety of information about particle – protein systems in a very straightforward manner.

Adsorption of Bovine Serum Albumin was found to alter the Zetapotential of the Oxides and their Isoelectric Points to an extent depending on the mass of the adsorbed protein. Combining UVS and XDC, the volume density and the thickness of the protein layer was determined. It was found that adsorption on Al_2O_3 particles occurs only in the "end-on" mode, whereas on SiO_2 particles BSA adsorbs first in the "side-on" mode and then, only at higher amounts (> 200 ng/cm^2), in the "end-on" mode. UVS and XDC measurements showed negligible protein adsorption for TiO_2 particles. In contrast, CVP measurements demonstrated an IEP shift to the protein's IEP of pH 5. We think that the protein was stripped off during centrifugation. This hypothesis is the object of our present research.

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Poster ID: 125

MULTILAYERED FILMS OF POLYSACCHARIDES: INFLUENCE OF IONIC STRENGTH ON THE BUILDUP AND ON BACTERIAL ADHESION

L.Richert[1], Ph.Lavalle[1], P.Schaaf[2,3], J.-C.Voegel[1] & C.Picart[1,3]

[1]INSERM Unité 595, Centre de recherche Odontologie, Université Louis Pasteur, 11 rue Humann, 67085 Strasbourg Cedex, France. [2]Institut Charles Sadron (CNRS-ULP), 6 rue Boussingault, 67083 Strasbourg Cedex, France. [3]Ecole Européenne de Chimie, Polymères et Matériaux de Strasbourg, 25 rue Becquerel, 67087 Strasbourg Cedex 2, France.

INTRODUCTION: Creating biomimetic thin films could allow to give a biological activity to an inert substrate. A new kind of supramolecular assembly based on polyelectrolyte multilayer[1] has been proposed for the nanometer scale modification of material surfaces. Preparation of multilayer entirely made of natural polysaccharides has emerged. Chitosan (Chi) and Hyaluronic Acid (Ha) are two polysaccharides with interesting biological properties. The aim of our study was to investigate the formation of thin film of these polyelectrolytes in different conditions and their properties with respect to bacterial adhesion.

METHODS: Chitosan (Pronova, 120 kDa) and Hyaluronic Acid (Bioiberica 400 kDa) were solubilized in NaCl solution (0.5, 0.15, 10^{-2} and 10^{-4} M) at pH: 5. Quartz Microbalance (QCM-D) and Optical Waveguide Lightmode Spectroscopy (OWLS) were used to analyze *in situ* the film growth. The conformation and topography of the multilayer films were examined with Confocal Laser Scanning Microscopy (CLSM) and Atomic Force Microscopy (AFM). The bacterial adhesion tests are carried out with *E.Coli* transformed with a plasmid bearing the GFPmut3.

RESULTS: The stepwise adsorption of chitosan and hyaluronic Acid was monitored by OWLS and QCM experiments. At 0.15 M NaCl, the growth of the film was exponential and after some layers the raw NTE signal obtained by OWLS became cyclic. This suggests a diffusion of a polyelectrolyte in the film[2]. Observations performed by CLSM using Chitosan-Fitc confirms that Chi diffuses within the film while Hyaluronic Acid-(Texas Red) is deposited as a uniform layers. The QCM experiments indicate a progressive passage from an exponential to a linear regime when the ionic strength decreases from 0.15M to 10^{-4}M (Fig. 1). The thickness (OWLS) of the film decreased of 300 nm to 10 nm for $(Chi/Ha)_{10}$ in 0.15 M and 10^{-4} M NaCl The study of bacterial adhesion show that multilayer buildup in 0.15 M were three times less adhesive that films built in 0.01 M NaCl and five times less that glass. (Fig. 2)

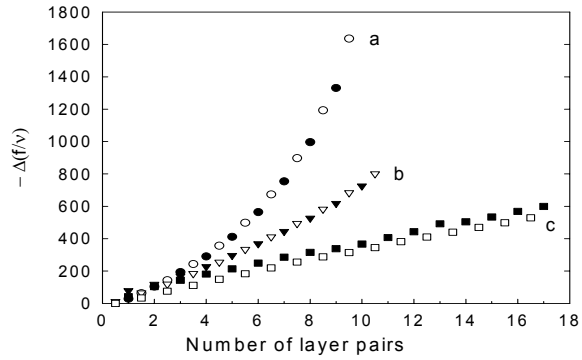

Fig. 1: Frequency shift measured by QCM for the (Chi/Ha) films in the presence of NaCl: 0.15 M (a), 10^{-2} M (b) and 10^{-4} (c). The open and closed symbols represent the Chitosan and Hyaluronic Acid depositions.

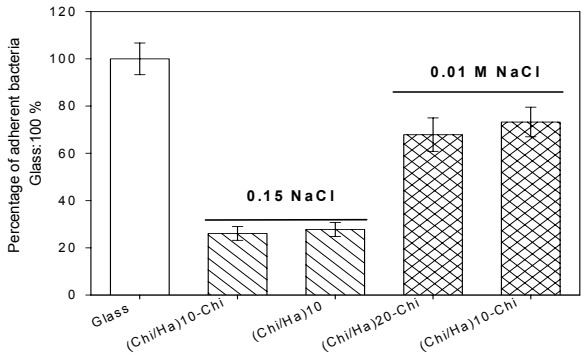

Fig. 2: Bacterial Adhesion (E.Coli) after 30 min on (Chi/Ha) films built in 0.15 M or 0.01 M NaCl versus bare glass.

DISCUSSION & CONCLUSIONS: The growth of thin films made of two polysaccharide –Chitosan and Hyaluronic Acid is strongly influenced by the ionic strength: -exponential growth associated to chitosan diffusion in 0.15 M NaCl, - linear at low salt concentration. The films built in 0.15 M NaCl may be used as anti-bacterial coating.

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MICROPATTERNED NEURAL NETS ON DIFFERENT SOLID SURFACES

M.Jungblut, C.Schwind, W.Knoll, K.Graf & C.Thielemann

Max Planck Institute for Polymer Research, Mainz, Germany.

INTRODUCTION: Precise control over the surface composition of materials allows either the reduction or the promotion of cell growth. Patterning of the surface properties is achieved by microcontact printing (μCP) of adhesion proteins with a PDMS (polydimethylsiloxane) stamp. This enables the structured adhesion of primary cortical neurons by allowing the cells to be placed on well-defined areas of the substrate or device. Our motivation for biopatterning surfaces with glass and gold substrates is the controlled growth of neural nets on a planar microelectrode array (MEA) chip. Coupling between electronic devices and neurons allows for extracellular signal recordings.

For neuropatterning on three-dimensional MEA structures we employed a new, easy-to-handle method based on the local swelling of pre-structured polymer substrates [1]. This method allows us to prepare scalable arrays of regularly shaped microvessels that contain chemically modified gold electrodes at the bottom, representing the adhesive areas for the neural cell bodies.

METHODS: The μCP technique was used to adsorb poly-D-lysine (PDL) in a grid pattern to glass surfaces, thin gold films and to MEAs. We used two types of MEAs. One of which is commercially available from MultiChannel Systems (Reutlingen, Germany); the other of which we designed. Prior to stamping, the gold surfaces were treated with mercaptoundecanoic acid (MUA), an alkanethiol that forms a self-assembled monolayer, allowing the tight binding of a PDL pattern.

The PDMS stamps were soaked in a solution of sterile poly-D-lysine (0,1 mg/ml in PBS) and brought in contact with the surface for 10 minutes at room temperature. We used and optimized grid pattern geometry [2] with a line width of 5 μm an a line distance of 50x100 μm; ideally the cross points would form the adhesion points for the cell bodies, while the lines would guide the outgrowth of the neurites. For the MEAs, the PDL pattern is optically adjusted to the electrode geometry.

The three-dimensional arrays of microvessels with a 50 μm^2-square-range to area contain gold at the bottom of microwells to change their hydrophilicity upon adsorption of thiols containing hydrophilic end-groups. The array was soaked in a solution of laminin (25 μg/ml PBS) which subsequently adsorb to the hydrophilic microvessels.

The cortical cells were obtained from 19-day-old rat fetuses (E19) and plated onto the surface in densities between 100 and 300 cells/mm^2 and cultured at 37 °C an 5% CO_2 in serum free B27/Neurobasal medium with 0,5 mM glutamine.

RESULTS: Cortical cells were found to grow in patterns on glass and the thiol-treated gold surfaces after the PDL pattern transfer by μCP. The surface of the self-fabricated MEAs consisted of silicon dioxide and gold electrodes and so it was possible to form a pattern of neurons on the array. We were also successful in patterning the cortical cells on a commercial microelectrode array. The cells on the arrays of microvessels were growing inside the microwells and form connections to other cells. Furthermore, we proved that patterned neurons formed synapses, which is the condition for interneural communication.

DISCUSSION & CONCLUSIONS: We patterned cortical neurons by μCP on a variety of substrates. The conditions for the formation of a vital, synaptically connected network are an optimal pattern geometry and the right “ink” to form structures that promote cell-growth. The transfer of this neuropatterning technique to the surface of a microelectrode array chip will allow us to record extracellular signals of individual cells in the network. These signals are of fundamental interest for basic neuroscience studies, cell-based biosensor technology and tissue engineering. Neuropatterning on three-dimensional microvessel arrays allows us to substitute the μCP by a self-adjusting cell adhesion process but also offer the cells a compositionally attractive surface. These devices can be modified in order to realize three-dimensional MEA chips. Future work will focus on the recording of the whole network response towards input stimuli patterns.

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GRAFT POLYMERIZATION OF PMMA FROM SILICA SURFACE AND APPLICATION OF THIS METHOD TO THE IMPROVEMENT OF MECHANICAL PROPERTIES OF PMMA BONE CEMENT

O.Shafranska[1], A.Kokott[1], V.Tokarev[2], A.Voronov[3], O.Bednarska[2], S.Voronov[2] & G.Ziegler[1]

[1]Friedrich-Baur Research Institute for Biomaterials, Bayreuth, Germany. [2]Lviv Polytechnic National University, Lviv, Ukraine. [3]University Bayreuth, Bayreuth, Germany.

INTRODUCTION: Synthesis of polymer "brushes" on a solid substrate is an effective way for the control of surface properties which may have important application in medicine and biomaterials production[1].

METHODS: Recently we have synthesized oligomers with a variations in polarity and functionality moieties which could be effectively used for the modification of various inorganic as well as organic (polymeric) surfaces via two-stage process[2].

At the 1st stage, the oligomers are easily adsorbed on the various substrate surfaces due to discrepancy in polarity and reactivity groups present in oligomer macromolecules. This allows to immobilize the functional groups on the modified surface which are able to initiate the "grafting from" polymerization. At the 2nd stage, the immobilized macromolecules of oligomer, containing the peroxide groups, could serve as an initiator of radical polymerization at the elevated temperatures. Using N,N-dimethyl-p-toluidine as a catalyst of peroxide initiation permits grafting of the polymer layer at the reduced and even room temperatures.

Two peroxide oligomers were investigated in order to synthesize polymer "brush" on the surface of silica. PMI-1 oligomer was derived from peroxide monomer and maleic anhydride while the second oligomer PMI-2 was synthesized from the first one via its interaction with (diethylamino)ethanol. Both oligomers have been firstly adsorbed on the silica surface and afterwards used for grafting of poly(methyl methacrylate) (PMMA) chains.

Samples of ultrafine silica modified with PMI were used as a filler for PMMA bone cement. Filled bone cements were examined in mechanical tests.

RESULTS: The thickness of the adsorbed peroxide oligomers as well as the grafted polymer "brushes" was distinquished by spectroscopic ellipsometry. An essential influence of the solvent nature on the adsorption of peroxide oligomer was found[3]. The amount of the oligomer adsorbed from the polar solvents was usually lower in comparison with an adsorption from the solvents with a lower polarity. At low adsorption density an island-like type of covering was formed. With an increase of the amount of adsorbed macromolecules the morphology turns into the like-wise type. Presumably, it occurs in the range from 0.8 mg/m^2 to 1.4 mg/m^2. Interesting, that higher grafting density of PMMA "brushes" was nevertheless in both cases obtained for PMI-2 modified surface. The morphology of the grafted polymer layers was investigated by atomic force microscopy and the expected mechanism of PMMA "brushes" grafting has been proposed.

CONCLUSIONS: Using of two various peroxide oligomers immobilized on the SiO_2 surface as an initiators of polymerization allows us to synthesize PMMA "brushes" with a various thickness and density. Using of SiO_2 as a filler leads to an increase of the compressive strength and Young's Modulus, but sufficiently reduces the bending strength of the PMMA bone cements. Modification of the silica filler with PMI oligomers increases the bending strength of the bone cements up to 15% in comparison to unmodified one.

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ACKNOWLEDGEMENTS: AV thanks Alexander von Humboldt Foundation for the financial support. Authors thanks Prof. Georg Krausch for the fruitful discussion.

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SELF-ASSEMBLED PEPTIDE FUNCTIONALIZED HYDROGELS

S.Tugulu[1], H.-A.Klok[1], A.Bernd[2], M.Möller[3], J.Groll[3] & J.P.Spatz[4]

[1]Institut des Matériaux, EPFL, Lausanne, Switzerland. [2]Zentrum der Dermatologie und Venerologie, Klinikum der Johann Wolfgang von Goethe-Universität, Frankfurt, Germany. [3]Deutsches Wollforschungsinstitut an der RWTH Aachen, Aachen, Germany. [4]Physikalisch-Chemisches Institut, Biophysikalische Chemie, Universität Heidelberg, Heidelberg, Germany.

INTRODUCTION: Integrins play a major role in the mechanosensory system enabling cells to sense the topology and mechanical properties of their environment as well as to detect mechanical stimuli. The main events in the function of this mechanosensory system are the binding of integrins to extracellular ligands, the clustering of integrins in the plane of the cell membrane and the aggregation of signalling molecules, linker proteins and other transmembrane receptors for extracellular signalling molecules. The resulting large protein complexes, known as focal adhesion complexes transduce the mechanical stimulus into a chemical signal, which results in an alteration of the expression of specific gene products[1]. We present the development of a well defined model system enabling the systematic investigation of the (combined) effects of (bio)chemical, topological and mechanical stimuli on integrin dependent cellular behaviour. The model system consists of an intrinsically bioinert hydrogel layer of cross-linked PEO-PPO starpolymers deposited on a flexible PDMS support. The functionalization of the hydrogel with short peptide ligands acting as integrin specific recognition motifs promotes adhesion of human keratinocytes and fibroblasts to its surface. The resulting substrate allows mechanical deformation of the cells in elongation experiments. Furthermore topological aspects are taken into account by elaborating several complementary approaches to control the concentration, spatial distribution and clustering of peptide ligands on the surface of the substrate on a length scale ranging from several nanometers up to a few micrometers.

METHODS: PDMS and silicon substrates are coated with the hydrogel layer by cross-linking isocyanate terminated PEO-PPO starpolymers[2]. Peptide ligands are prepared by standard Fmoc-SPPS and grafted on the substrate by using maleimide succinimide cross-linking chemistry. Peptide functionalized starpolymers are synthesized by organic chemistry procedures and characterised by standard analytical methods (RHPLC, NMR, Maldi, GPC, etc.). Surface characterisation is carried out by XPS and radiolabeling. Cell adhesion experiments are performed using human keratinocytes (HaCaT 41), which are stained with Hoechst reagent and analysed by fluorescence microscopy.

RESULTS: First results were obtained using a system consisting of a 25 nm thick hydrogel layer on a silicon substrate. Peptide ligands bearing a cysteine residue were covalently bound to residual amino groups on the surface of the hydrogel via a maleimide succinimide cross-linker. A direct proof for the successful functionalization was obtained from XPS experiments by the appearance of a sulfur signal on the peptide functionalized surfaces resulting from the sulfhydryl group of the cysteine moiety. An indirect evidence was given by the clearly enhanced adhesion of HaCaT 41-cells after the peptide functionalization.

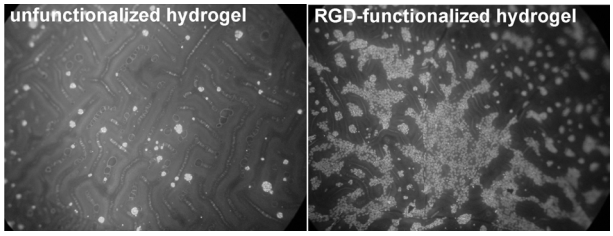

Fig. 1: Fluorescence microscopic images of HaCaT 41 cells after Hoechst staining adhering to an unfunctionalized hydrogel (left) and to a hydrogel functionalized with the peptide sequence GRGDSC (right).

DISCUSSION & CONCLUSIONS: We were able to show, that integrin specific cell adhesion can be accomplished on an otherwise bioinert hydrogel by the functionalization with peptide ligands. Future work will include extending the functionalization strategy to elastic substrates and the introduction of topological variations in the presentation of the peptide ligands on the surface.

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POLYMERIC SPACERS FOR ANTIMICROBIAL AGENTS

C.Waschinski & J.C.Tiller

Freiburger Materialforschungszentrum (FMF), Albert-Ludwigs-Universität Freiburg, Germany.

INTRODUCTION: There is a great interest in modifying surfaces of common objects to render them antiseptic. In the most common applications, surfaces are impregnated with antimicrobial agents, which are released into the surrounding environment. Another approach is based on grafting macromolecules that repel microorganisms (e.g., poly(ethylene glycol)), onto a surface. Grafting antimicrobial polymers [1], such as *N*-alkylated poly(4-vinylpyridine) results in surfaces that are capable of killing microbes on contact [2]. Although the latter concept is the most effective one, it is not fully understood. One hypothesis is that the antimicrobial functions of the grafted polymer are carried through the thick cell wall of, e.g., bacteria using the polymer backbone as long spacer. In order to verify this proposed mechanism, we decided to immobilize biocidal groups via polymeric spacers of well defined and uniform length. It is expected that a minimal effective length of the polymer chain is required to remain the antimicrobial activity of the immobilized function.

Here, we report on the synthesis of narrowly distributed polymeric spacers based on 2-ethyl-1,3-oxazoline carrying functional groups at both ends with one of these functions being able to attach covalently to a surface and the other group possessing antimicrobial properties.

METHODS: Initiators were synthesized coming from 1-aminohexanol and 1-aminopentanol. The amino-functions were protected with BOC (*tert*-butoxycarbonyl) groups by conversion with BOC carbonate. Subsequently, the hydroxy-groups were transformed into the polymerization initiating sulfonic acid esters by reaction with trifluoromethanesulfonic anhydride and 4-toluenesulfonyl chloride, respectively. The polymerizations of 2-ethyl-1,3-oxazoline using the prepared initiators were carried out in chloroform at 70°C. After 48 h, the reaction was terminated with different primary amines. The introduced secondary amino end groups were converted into potentially antimicrobial functions by permethylation with methyl iodide.

The bacterial susceptibility test of the synthesized spacers was carried out in bacterial suspensions of *Staphylococcus aureus* (gram-positive bacterium) and *Escherichia coli* (gram-negative bacterium).

The determined minimal inhibitory concentration (MIC) is the concentration of the tested polymer at which 99% of the microorganisms are prevented from growing.

RESULTS & DISCUSSION: By using the new BOC-aminoalkyl sulfonic acid esters as initiators 2-ethyl-1,3-oxazoline was polymerized to give polymers with degrees of polymerization in the range from 20 to 100 exhibiting a narrow molecular weight distribution of 1.11-1.17. Upon termination and subsequent permethylation followed by deprotection of the BOC-protected NH_2 groups of the starting group poly(oxazoline)s with an NH_2 anchor group and a terminal antimicrobial function could be generated. The MICs (*Table 1*) showed antimicrobial activity comparable to biocides of low molecular weight if the concentrations were related to the actual amount of antimicrobial groups bonded to the one end of the polymer chain. So, polymeric spacers were synthesized with functional groups at one end of the polymer able to attach covalently to a surface and an antimicrobial function at the other end. These polymers can readily be used for surface modifications in order to investigate the mechanism of surface grafted antimicrobial polymers.

Table 1. MICs of a polymer terminated with dodecane bromide followed by quarternaryzation with methane iodide (DP=44, PD=1.11) and the low-molecular biocide dodecyltrimethylammonium iodide.

	S.aureus [µmol/ml]	*E.coli* [µmol/ml]
Polymer	15	30
Biocide	3-6	3-6

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SURFACE MODIFICATION BY PLASMA POLYMERIZATION AND APPLICATION OF PLASMA POLYMERS AS BIOMAERIALS

Z.Zhang, R.Foerch & W.Knoll

Max Planck-Institute for Polymer Research, Germany.

INTRODUCTION: Polymer surface modification is an elegant method for generating functional poylmer surfaces combined with the desirable attributes of bulk polymers[1]. In present work, di(ethylene glycol) mono vinyl ether (EO2)[2] and allylamine (AA) [3]were employed as plasma monomers. Further more, these plasma polymers were investigated as biomaterials, which included protein adsorption, cell attachment, and DNA immobilization/hybridization.

METHODS: The plasma polymers were deposited in the plasma reactor at contiueous wave (cw) and pulsed plasma conditions. The plasma equivalent power is definated as

$$P_{eq} = P_{peak}(t_{on}/t_{off}+t_{on})$$

The chemical properties of the plasma polymers were investigated by Fourier Transform Infrared Spectroscopy (FTIR). Protein adsorption and DNA immobilization or hybridization were detected by Surface Plasmon Resonance Spectroscopy (SPR) and Surface Plasmon Resonance Fluorescence spectroscopy (SPFS)

RESULTS: Chemical properties: For plasma polymerized EO2, according to the defination of the equivalent power, $P_{eq} = P_{peak}(t_{on}/t_{off}+t_{on})$, rising the input power or decreasing t_{off} can make P_{eq} increase, and the ratio of C-O to C-C groups decreases. For plasma polymerized allylamine, the density of amino group increases with decreasing the input power and DC.

Protein adsorption on Plasma polymerized EO2: There is a clear dependence of the protein adsorption affinity on the plasma polymerization condition employed during the film formation, for cw and high DC generated films the extent of adsorption is also somewhat dependent on the plasma polymer thickness. The use of low DCs during the polymer synthesis results in a strongly reduced affinity for protein adsorption. However, the data indicate maximal non-fouling surface structures can be achieved at DCs which are less than the lowest values available for film deposition.

Cell attachment*:* Compared with the nake glass, it is difficult for cells to attach onto the low DC plasma polyEO2.

DNA immobilization and hybridization on PPA films: DNA adsorption was found to be dependent on the DC, the probe concentration, and the polymer film thickness. The probe DNA thickness increases (d_{probe}) with the DC decreaing. Further more, d_{probe} increases with the low DC and inputer power plasma polymer thinkess increasing within 50 nm thickness of the polymer. At the same time, d_{probe} increases with the probe concentration increasing under 100 nM of probe. It is very easy to distinguish the mismatch two, mismatch one, and mismatch 0 hybridization behaviors for this plasma ploymer matrix.

CONCLUSIONS: The plasma polymer posses the potential properties, whick could be applied as biomaterials. In present work, protein adorption, cell attachment, and DNA immobilization or hybridization were investigated successfully.

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FUNCTIONAL POLYSILOXANE-G-POLY(ETHYLENE GLYCOL) LAYERS ON GOLD SURFACE: SURFACE CHARACTERIZATION AND RESISTANCE TO PROTEIN ADSORPTION

C.Zhou[1,3], V.K.Khlestkin[2], W.Laureyn[1], G.Reekmans[1], A.Campitelli[1], G.Borghs[1], W.Dehaen[2] & Y.Engelborghs[3]

[1]Interuniversity Microelectronics Center (IMEC), Belgium. [2]Dept. of Chemistry, KULeuven, Belgium. [3]Lab. of Biomolecular Dynamic, Dept. of Chemistry, KULeuven, Belgium.

INTRODUCTION: Protein resistant surface is a prerequisite for bioaffinity sensing, where the selectivity and sensitivity of the antigen/antibody assay is directly related to how well the nonspecific adsorption can be suppressed. From the point of view of stability, polymeric monolayers are better candidates for surface modification compared to SAMs [1]. In addition, it is well known that poly(ethylene glycol) (PEG)-containing surfaces show outstanding protein resistance properties [2]. In this report, the synthesis, deposition and performance of a series of PEG grafted polysiloxanes is described. These PEG functional polymers can chemisorb onto Au surface by means of the grafted alkyl disulfide side chain. These polymeric monolayers show enhanced protein resistance properties.

METHODS: The synthesis was achieved by simultaneously grafting alkylenyl Disulfide and allyl-PEG onto poly(hydrogen methylsiloxane) (figure 1). Polymeric surfaces were prepared by immersing Au substrate into 1mM polymer solutions in toluene or water for a designed time. Fibrinogen (FB) (0.46mg/ml) was selected for protein resistance tests. For Surface Plasmon Resonance (SPR), the degree of protein adsorption is reported in refractive units (RU). For the QCM-D™, the degree of protein adsorption is reported in frequency shift (ΔF (Hz)).

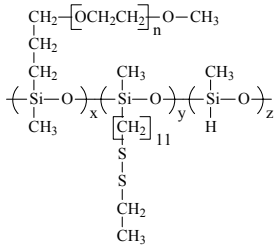

Fig. 1: the structure of the grafted polymer, nomenclature is as follows: S_{13}-$PHMS_R$-PEG_W, R= x/y, which is the graft ratio of PEG to disulfide. W is the MW for grafted PEG.

RESULTS: The Cyclic Voltammetry (CV) was performed (in $Fe(CN)_6^{3-/4-}$) for polymer surfaces prepared by 5hrs immersion. The typical oxidation/reduction peaks for clean bare Au were greatly suppressed after polymer deposition. The integrated area of CV curve for clean bare Au was 1.45mC, while integrated areas of CV curves for polymeric surfaces were lower than 0.14mC.

Water contact angles were measured for polymer surfaces prepared using different immersion times. (Table 1)

Table 1. Advancing Water Contact Angles of polymers on Gold.

Polymers	Immersion time		
	2hr	4hr	6hr
S_{13}-$PHMS_5$-PEG_{600} (toluene)	56	66	65
S_{13}-$PHMS_8$-PEG_{600} (toluene)	51	54	56
S_{13}-$PHMS_8$-PEG_{1100} (toluene)	44	43	44
S_{13}-$PHMS_8$-PEG_{1100} (water)	28	29	29

The protein resistance was test by SPR and QCM. (Table 2)

Table 2. The FB protein resistance property.

	SPR (RU)	QCM (Hz)
Hydrophobic surface		405
Clean bare Au	5000	
S_{13}-$PHMS_8$-PEG_{1100} (water)	45	15

DISCUSSION & CONCLUSIONS: CV analysis indicated that these polymers chemisorb to Au and the surface coverage is high. The polymer surfaces demonstrate hydrophilic property, which depends on the PEG ratio, MW of PEG and solvent. The polymer surface shows outstanding protein resistance property.

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AFM, NEUTRON SCATTERING AND NEUTRON REFLECTOMETRY INVESTIGATION OF POLY(L-LYSINE)-*GRAFT*-POLY(ETHYLENE GLYCOL) CO-POLYMER CONFORMATIONS IN SOLUTION AND ON THE ADSORBED STATE

F.Assi, L.Feuz, S.Pasche, N.D.Spencer & M.Textor

Dept. of Materials, ETHZ, Zürich, Switzerland.

The development of protein-resistant surfaces is of central interest in the context of biosensor chip development and for the design of medical implants in contact with blood. Self-organized films of PEG based co-polymers on different oxide surfaces are one system that has been investigated by a variety of surface characterization techniques in order to establish systematic correlations between the polymer composition/structure, interface architecture and interaction with protein-based biological media.

A class of co-polymeric molecules of special interest is based on a poly(L-lysine) backbone, charged positively due to the presence of protonated amine groups at a neutral pH, and grafted with poly(ethylene glycol) side chains (short: PLL-*g*-PEG). Although the protein-resistant properties of these films have already been demonstrated, little is known about the effect of the co-polymer interfacial architecture on the resulting protein resistance. One of the most important factors turns out to be the polymer conformation in the adsorbed state, which depends not only on the polymer architecture, but also on the environment the polymer is exposed to.

We report results that elucidate the surface conformation of PLL-*g*-PEG of different molecular architecture at various humidity levels in air as well as in contact with aqueous solutions, studied by means of atomic force microscopy (AFM). The experimental results for different polymers are finally compared with neutron scattering and reflectometry results.

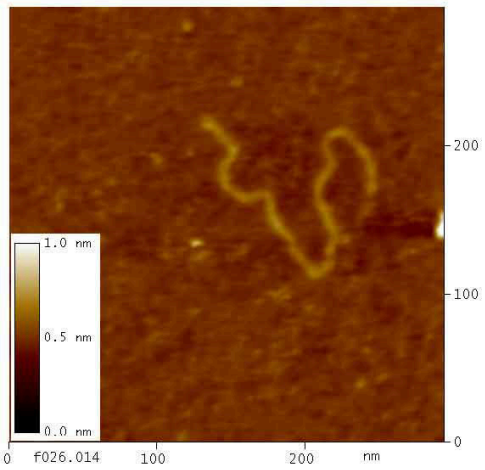

Fig. 1: Single PLL-g-PEG molecule adsorbed on Mica observed with tapping mode AFM in air.

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NEW PHOSPHATES/PHOSPHONATES; A MODULAR APPROACH TO FUNCTIONAL SAMS

F.Durmaz, S.Zürcher & N.D.Spencer

Dept. of Materials, ETHZ, Zürich, Switzerland.

INTRODUCTION: An alternative and complementary surface treatment to the PLL-PEG strategy is the use of functionalized self assembled monolayers (SAMs). Alkylthiols on gold and alkylphosphates and phosphonates on metaloxid surfaces have been shown to form stable well ordered monolayers. The functionalisation of these molecules, to use them as docking site is possible, but not straightforward. The application of simple alkane phosphate or phosphonate SAMs to produce biointerfaces has furthermore proven to have a number of limitations, in particular stability in aqueous media. Therefore it is proposed to use a modular approach for the synthesis of new functional self-assembling substances. In the same strategy, we include a reactive group, which can be used to crosslink, respectively polymerize the molecules once they have self-assembled on the surface. This is to increase the final stability of the films. A sketch of this strategy is depicted in Figure 1.

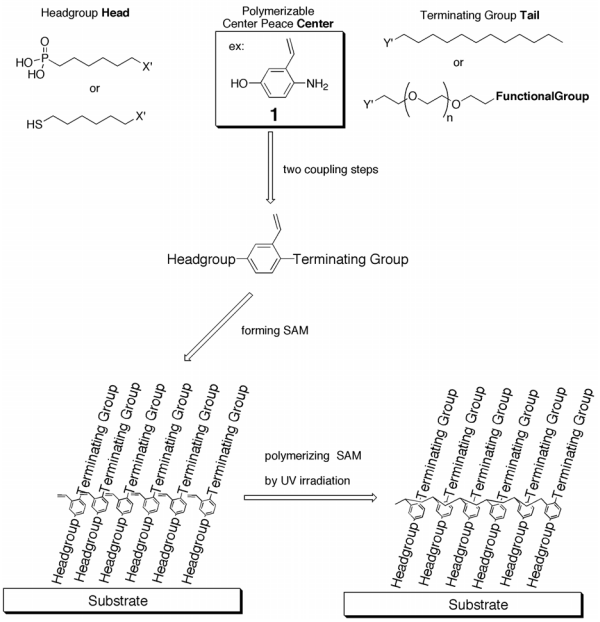

Fig. 1: Sketch of the modular synthesis of self-assembling functional molecules. Possible heads and tails are depicted.

METHODS: This approach is expected to eliminate the main disadvantages of the simple alkane phosphate SAMs, i.e. the inferior stability in aqueous solutions and the diffulty to introduce reactive docking sites for the attachment of biomolecules.

A candidate for the center molecule is 4-amino-3-vinyl-phenol **1**. The phenol group can be used to attach the headgroup while the aminogroup can be coupled to a fraction of the end-functionalized terminating groups. The vinyl group will be the polymerizable group.

RESULTS: A new synthesis for **1** has been worked out and already proven to function (Figure 2). Using an amino group for the attaching site for the functional end groups, makes it theoretically possible to attach up to three equal or different chains in the same molecule. This could be advantageous to increase the density as well as the order of the SAM.

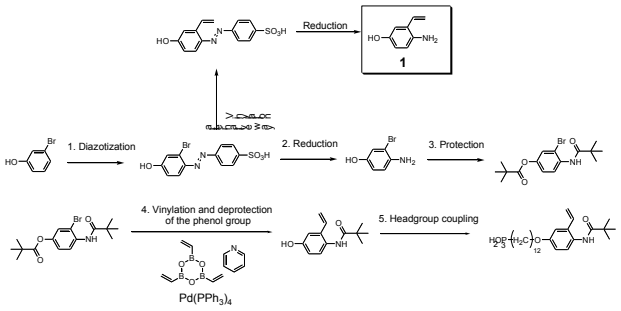

Fig. 2: New synthesis route.

DISCUSSION & CONCLUSIONS: This is an alternative and complementary surface treatment to PLL-PEG strategy. Introduction of specific docking sites have relevance for applications in areas such as: biomaterials, biosensors and adhesion. Alternative synthesis routes and polymerization will be carried out.

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CREATION OF SURFACE MACROMOLECULAR DOCKING SITES FOR THE REVERSIBLE IMMOBILIZATION OF PROTEINS IN ACTIVE CONFORMATION AND CONTROLLED ORIENTATION

G.Zhen[1], E.Zobeley[2], V.Eggli[2], S.Zürcher[1], J.Vörös[1], R.Glockshuber[2], M.Textor[1] & N.D.Spencer[1]

[1]Laboratory for Surface Science and Technology, ETHZ, Zürich, Switzerland.
[2]Institute of Molecular Biology and Biophysics, ETHZ, Hönggerberg, Switzerland.

INTRODUCTION: Interfaces are key elements in the design and fabrication of bioaffinity sensor chips with directed biological response. At the same time, the knowledge of the conformation, specific activity and orientation of proteins bound to surfaces constitutes a crucial prerequisite for understanding the functional properties of proteins bound to surface and for the development and optimization of highly specific and sensitive biosensors.

METHODS: Two docking site platforms based on biotin-(Strep)avidin and NTA-Ni^{2+}-histag linkage techniques, in combination with polycationic, PEG-grafted, biotinylated or NTA(nitrilotriacetic acid) functionalized copolymers were synthesized. One of the most efficient enzyme known β-lactamase served as the model protein. Five different variants of β-lactamase with single cysteine site-directed mutagenesis on the surface, one 6xHis-tagged β-lactamase and Green Fluorescent Protein (GFP) were engineered. The five β-lactamase variants were biotinylated at free thiol-group with a cleavable biotinylation reagent allowing for release of the surface-bound enzyme after adding DTT. The immobilization was achieved on niobium oxide surface coated with biotinylated Poly(L-lysine)-g-poly(ethylene glycol) monolayer. The biotinylated β-lactamase was subsequently bound to the surface via NeutrAvidin. The amount of immobilized β-lactamase on the chips was measured by two different techniques: OWLS and specific enzymatic activity via photospectroscopic detection of the turnover of the chromogenic substrate nitrocefin. Furthermore two protocols for the control of the amount of β-lactamase on chip are compared and investigated. Specific immobilization could be discriminated from non-specific adsorption. The catalytic activity of enzyme was firstly quantitatively compared when they are in solution and on chip. 6xHis-tagged GFP bound to NTA functionalized polymer modified surface were measured by OWLS and CLSM.

RESULTS: Michaelis kinetics of β-lactamase variants with and without biotinylation in solution are the same. No significant effects on the activity of biotinylated β-lactamase variants for NeutrAvidin binding were found. Enzymatic study shows surface-bound β-lactamase lost 75% to 95% relative activity per molecule. OWLS studies of 6xHis-tagged GFP bound to NTA functionalized polymer modified surface showed that the binding was required the presence of Ni(II) on the surface and could be desorbed by treatment with 500 mM imidazole.

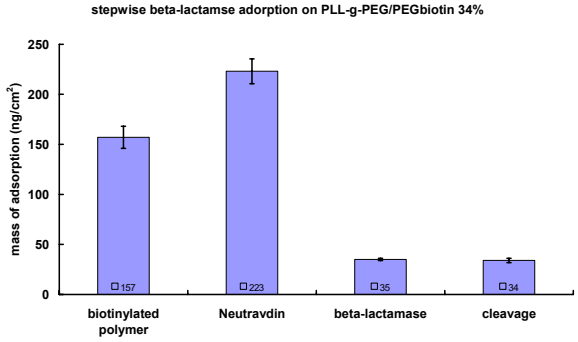

Fig. 1: Adsorption of PLL-g-PEG/PEGbiotin polymer, neutral avidin and biotinylated β-lactamase on chip (Nb_2O_5) measured with Optical Waveguide Lightmode Spectroscopy (OWLS).

DISCUSSION & CONCLUSIONS: OWLS shows that the different surface platforms are successfully build up and the mass adsorption of molecules were quantified. Enzymatic study shows surface-bound β-lactamase is less active than enzyme in solution because the reaction is extremely fast and diffusion becomes a limiting factor upon immobilization of the enzyme. NTA functionalized polymer shows the potential to be a new surface docking site interface for protein immobilization.

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Poster ID: 136

REDUCTIVE DESORPTION OF SELF-ASSEMBLED MONOLAYERS OF OCTADECYL PHOSPHATE (ODP) FROM TI/TIO2 SURFACES

S.Lee[1], T.Sannomiya[2], C.Tang[1,3], J.Vörös[1], S.Tosatti[1], M.Textor[1] & N.D.Spencer[1]

[1]Dept. of Materials, ETHZ, Zürich, Switzerlan., [2]Dept. Of Metallurgy and Ceramics Science, Tokyo Institute of Techonology, Japan. [3]Swiss Federal Laboratories for Materials Testing and Research (EMPA), Dübendorf, Switzerland.

INTRODUCTION: Self-assembly of alkylphosphate is a powerful approach to modify various surface properties of titanium (Ti) and titanium oxide (TiOx), which enables extended applications of Ti-based materials in biosensors and corrosion-resistant systems etc.[1,2] Electrochemical reductive desorption has been established as a useful way to examine the electrochemical properties as well as surface coverage of self-assembled monolayers (SAM), mostly thiol-based SAM on gold.[3,4] By applying this methodology to *n*-octadecylphosphoric acid (ODP) SAM formed on Ti(metal) surface, we attempt to broaden our understanding of the surface coverage and bind characteristics of ODP films on Ti surfaces.

METHODS: (1) *Sample preparation:* Ti (metal) substrates were prepared by physical vapor deposition (PVD) onto silicon wafers as previously reported.[2] The self-assembled monolayer of *n*-octadecyl phosphoric acid (ODP) was generated by immersing a Ti(metal) substrate in a organic solvent (mixture of *n*-heptane and propan-2-ol, 100:4 v/v ratio) containing ODP with a concentration of 500 μM. (2) *Monolayer characterization*: Formation of ODP self-assembled monolayer was confirmed by water contact angle measurement (~105° static contact angles), atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS). (3) *Cyclic voltammetry*: Cylic voltammetry was performed in a three electrode cell (working electrode: ODP SAM-coated Ti metal, reference electrode: Ag/AgCl(sat'd), and counter electrode: Pt) using a potentiostat (AMEL Instruments, Model 2053). The scan rate was 20 mV/sec and all the measurements were performed under pH 12 condition (adjusted by NaOH).

RESULTS: A representative cyclic voltagmmogram of reductive desorption ODP SAM from Ti(metal) surface is presented in Figure 1. In this figure, the first and the second forward potential scans (zero to negative potential) of ODP SAM, together with that of a bare Ti substrate are presented. The cathodic currents starting from ~0.5V at bare Ti substrate, which is attributed to H_2 evolution, is considerably reduced by ODP SAM and the commencement of the H_2 evolution is delayed to ~1.0V. The broad shoulder peak at ~ 1.5V is attributed to the desorption of ODP films from Ti (metal) surface.

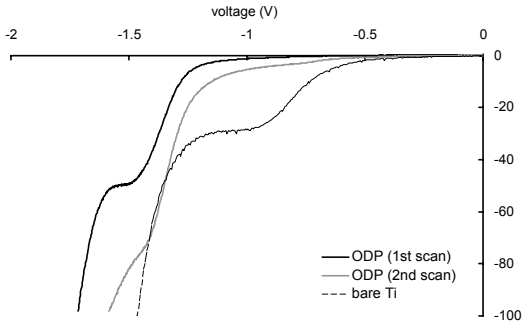

Fig. 1: A representative cyclic voltammogram of reductive desorption of ODP SAM from Ti(metal) surface.

DISCUSSION & CONCLUSIONS: The total (or observed) currents during potential scan are composed of (a) double layer charging (b) H_2 evolution (c) desorption of ODP

$$Q_{tot} = Q_{dl} + Q_{H2} + Q_{des}.$$

Through a quantitative analysis method suggested by T. Kawaguchi et al,[4] we extracted the currents due to desorption process of ODP film, $Q_{des.}$, and they were further used to estimate the ODP film coverage on Ti (metal) surface. In contrast to alkanethiols, which bind exclusively as monodentate on gold surface, a mixture of monodentate and bidentate (or even tridentate) coordination of alkylphosphate on Ti (metal) surface leads to a complicated interpretation of surface coverage based upon this method. The surface coverage of ODP on Ti (metal) surface will be discussed in comparison with *n*-allkanethiols on gold surfaces.

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Poster ID: 137

GLASS SURFACE FUNCTIONALIZATION WITH ZnS:Mn QUANTUM DOTS USING POLYELECTROLYTES

S.Oberti[1], Y.Axmann[2], I.Beliaev[1], H.Hofmann[2] & A.Stemmer[1]

[1]Nanotechnology Group, ETHZ, CH-8092 Zürich, Switzerland. [2]The Powder Technology Laboratory, EPFL, CH-1015 Lausanne, Switzerland.

INTRODUCTION: Semiconductor nanoparticles or Quantum Dots (QD) are novel materials with unique physical characteristics caused by the so-called quantum confinement effect [1]. QD have higher photo stability and luminescence intensities than conventional organic fluorophores. Changing the size and composition of QD allows precise control of their absorption and emission spectra. QD applications include biology (biocompatible coatings, biosensors, multicolor labeling), electronics (tunable lasers, flat panel displays, LED) and some other areas. Doping of QD, a recently developed technique, results in increased photoluminescence quantum efficiency and decreased lifetime in comparison with other QD. Here we report on studies to functionalize glass surfaces with Mn-doped ZnS (ZnS:Mn) QD using polyelectrolytes.

METHODS: We used the layer-by-layer technique (LbL) [2] to adsorb nanoparticles from dispersion onto a surface of oppositely charged polyelectrolytes. To this end, clean glass cover slips (7 mm diameter, 0.17 mm thickness) were immersed in an aqueous polyelectrolyte solution of poly(diallyldimethylammonium chloride) (PDDA) or polyethylenimine (PEI) and subsequently in a dispersion of QD. Solutions of polyelectrolyte with concentrations of 0.5% to 5% and QD-dispersions with concentration of 0.07 to 7 mg/ml in 0.01 M NaOH were used. ZnS:Mn QD exhibited a strong optical absorption for wavelengths shorter than 310 nm and an emission maximum at 600 nm. AFM and light microscopy were used for the characterization of QD layers.

RESULTS: Experiments showed that microscopic aggregates of QD (ranging from several micrometers to several hundred micrometers in size) were formed on the glass surface. The size and the density of these aggregates significantly decreased for lower QD concentrations. We found that layers with dispersed QD were formed between aggregates. Figure 1 shows the typical AFM topography image of such QD layer. These layers were not perfectly uniform and contained QD clusters with diameters ranging from several ten to several hundred nanometers. The thickness of the composite polyelectrolyte-QD layer was measured by AFM and found to be several tens of nm.

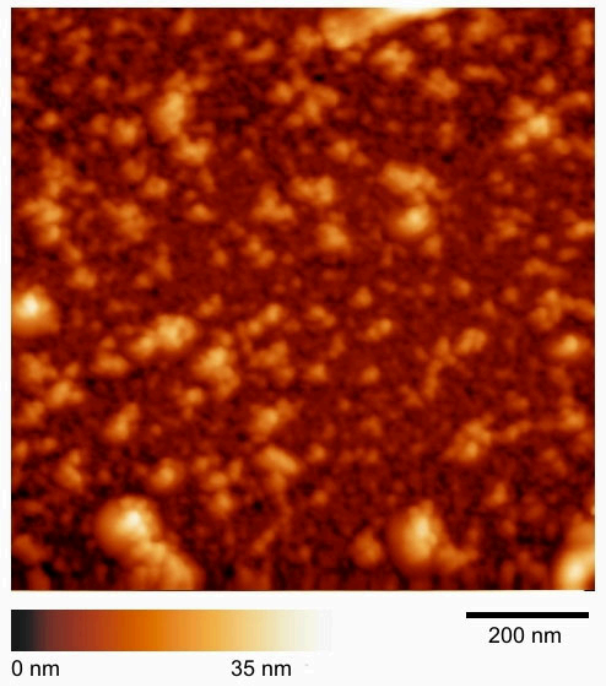

Fig. 1: AFM image of glass surface functionalized with ZnS:Mn QD (0.7 g/ml) on PDDA (0.5 %).

DISCUSSION & CONCLUSIONS: Even though monolayers of single QD (QD size is several nm) were not achieved in these experiments, the topography of the ZnS:Mn QD layers was somewhat intermediate between layers of CdSe QD on PDDA and PEI reported in [3]. Uniformity of layers strongly depends on the combination of QD and polyelectrolyte [4] and on the respective concentrations, which should be fine-tuned later. Choosing different substrates (for example Si wafer [3,4]) may also be considered.

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INTERFACIAL BINDING OF BETA-LACTAMASE TO DIFFERENT MATRICES MONITORED BY SURFACE-PLASMON SPECTROSCOPY

F.Xu[1], G.Zhen[2], E.Zobeley[3], V.Eggli[3] & R.Glockshuber[3], M.Textor[2] & W.Knoll[1]

[1]*Max-Planck-Institut fuer Polymerforschung, Ackermannweg 10, 55128 Mainz, Germany.*
[2]*Laboratory for Surface Science and Technology, ETHZ, Zürich, Switzerland.*
[3]*Institute of Molecular Biology and Biophysics, ETHZ, Hönggerberg, Switzerland.*

INTRODUCTION: Surface-plasmon resonance spectroscopy (SPR) is a well-accepted analytical tool for the characterization of interfaces and thin films. One of the major advantages of surface-plasmon spectroscopy is the fact that it allows for the label-free detection of binding events. In this study, we used SPR to investigate the binding of biotin labelled β-lactamase.

METHODS: Three kinds of matrices were compared for the protein binding. Biotin-terminated Self-Assembly Monolayers (SAMs) were used for the binding of Neutravidin and further attachment of lactamase. On the other hand, we compared the binding of Biotin-PLL-PEG to carboxyl-terminated SAMs via electrostatic interaction and by covalent interactions (NHS ester activation), separately. In these two matrices, biotin-PLL-PEG was used for the binding of a pre-incubated Neutravidin and lactamase mixture. For all the systems, the non-specific binding and regeneration possibilities were tested. In order to calculate the amount of bound lactamase, DTT was used to release lactamase from the surface by opening the disulfide bond between lactamase and Neutravidin.

RESULTS: 1) Biotin-terminated SAMs. We found a layer thickness (n=1.45) for Neutravidin of 4.15nm and for Biotin labelled β-lactamase (n=1.41) of 2.78nm. For Neutravidin, this corresponds to the coverage of 60%. No non-specific binding of β-lactamase was found. These results are summarized in Table 1. **2) Carboxyl-terminated SAMs.** 3.63nm Biotin-PLL-PEG and 3.5nm Neutravidin/Biotin labelled β-lactamase bound on SAMs. HCl can be used to eliminate the elctrostatic interation between SAMs and Biotin-PLL-PEG in order to regenerate the matrix. **3) Carboxyl-terminated (NHS ester) SAMs.** 3.2nm Biotin-PLL-PEG and 3.5nm Neutravidin/ Biotin labelled β-lactamase bound on SAMs.

DISCUSSION & CONCLUSIONS: Our results show that all three matrices are well suited for the binding of the protein, although quantitative differences are found and will be discussed. Furthermore, it is found that the data obtained by SPR are quite comparable to those derived from optical waveguide measurements.

Table 1. Comparision of data obtained by SPR and derived from optical waveguide measurements, respectively, based on Biotin-terminated SAMs

	Results from SPR		Results from Optical Waveguide measurements
	Thickness	Mass	Mass
Biotin thiol mixture	2.92 nm		
Neutravidin	4.15 nm	294 ng/cm2	270 ng/cm2
Biotin labelled β-lactamase	2.78 nm	134 ng/cm2	130 ng/cm2
Cutting amount by DTT	2.78 nm	134 ng/cm2	130 ng/cm2

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Poster ID: 139

X-RAY PHOTOELECTRON SPECTROSCOPY AS A TOOL FOR BIO-SURFACE ANALYSIS

C.J.Blomfield[1], A.J.Roberts[1], S.J.Hutton[1], H.Ma[2], J.Hyun[2] & A.Chilkoti[2]

[1]Kratos Analytical Ltd, Manchester UK. [2]Dept of Bio-Medical Engineering, Duke University, USA.

INTRODUCTION: X-ray photoelectron spectroscopy has found many applications in polymer surface modification in particular.

Recent advances in instrument performance in the areas of charge neutralisation, spectroscopic sensitivity and the spatial resolution of XPS imaging have broadened the areas of application to include modifed bio-surfaces. Results will be presented showing how XPS can help with the characterisation of self-asssmebled monoloayers in terms of composition, structure, orientation and spatial patterning.

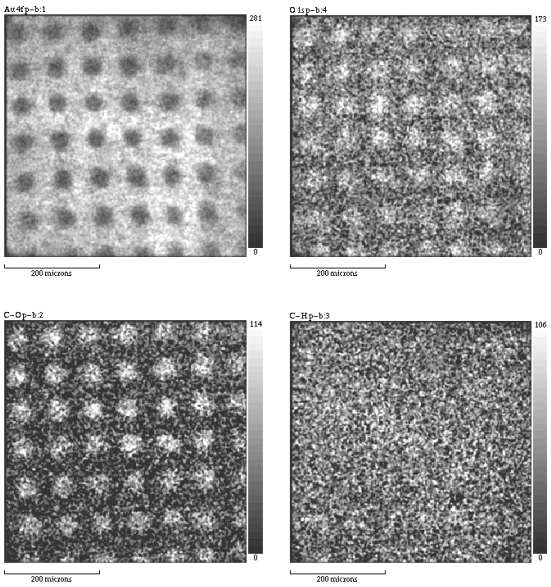

Fig. 1: XPS images of polymer film grown from 40µm patterned initiator SAM on Au surface.

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MODELING THE MULTILAYER STRUCTURE OF PLL-g-PEG ON Nb_2O_5 SURFACES

A.Rossi[1,2], L.Feuz[1], M.Textor[1], & N.D.Spencer[1]

[1]*Dept. of Materials, ETHZ, Zürich, Switzerland.* [2]*Dept. of Inorganic and Analytical Chemistry, Università degli Studi di Cagliari, Cagliari, Italy.*

INTRODUCTION: The functionalization of metal oxide surfaces with adsorbed molecules allows the production of new tailor-made nanosized-bio-films. X-ray photoelectron spectroscopy allows the assessment of biocompatibility and the composition of the overlayer. So far the thickness of the layer can be determined using ellipsometry and angular-resolved XPS measurements, but both techniques have their limitations. In this study a novel approach to calculate the thickness of PLL and PEG layers was developed based on the XPS intensities of the spectra collected at only one emission angle. The first results obtained studying the influence of the molecular weight of the PEG chains are presented.

METHODS: A Physical Electronics 5700 X-ray photoelectron spectrometer was used to characterize the surface of niobium pentoxide surfaces before and after immersion in PLL(20)-[3.5]-PEG(x)[2]. Measurement conditions are the same as those described in a previous paper[2].

RESULTS: C1s, O1s, N1s and Nb3d photoelectron spectra were resolved into surface and bulk components using parameters determined on the pure substances[2]. All the spectra were multicomponent: the O1s peak at 531.5 ± 0.2 eV assigned to the oxygen of the PLL group NH-CH-**C**O contains also the contribution of niobium hydroxide and this contribution was taken into account for the quantitative analysis. The intensities of each component were used to calculate thickness and composition of the PLL and PEG layer[3,4] simultaneously.

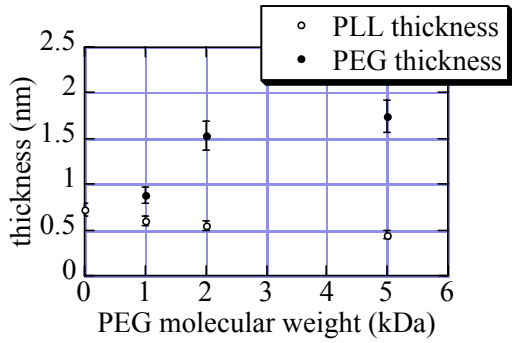

Fig. 1: PLL and PEG layer-thickness determined with the new model

DISCUSSION & CONCLUSIONS: XPS analysis of the films in this and previous work[2] has shown that binding-energy values for the different functional groups remain unchanged varying the PEG molecular weight. The intensity ratio of C1s to oxygen O1s in the (NH)C=O group is always lower than unity. This finding substantiates the assumption that the oxide substrate is hydroxylated. In agreement with the modified model (figure 2), a computer algorithm was written based on earlier work [2,3,4].

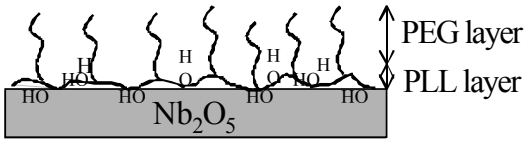

Fig. 2: Assembly of the PLL-g-PEG film on niobium pentoxide.

The results calculated with this model are in excellent agreement with the total thickness measurements obtained with ellipsometry. The three-layer model (fig. 1) provides clear evidence at high molecular weight of a deviation from a linear dependence of the PEG chain thickness.

In conclusion, the model applied in this study allows one to determine the thickness of both PLL and PEG layers, their sum being in agreement with ellipsometry. The model yields very good results for molecular weights of PEG up to 2kDa; the deviation at higher MW values requires further investigation.

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STRUCTURED PEG-DEXTRAN SURFACES FOR BIO-APPLICATIONS

N.Blondiaux[1], S.Jeney[2], M.Liley[1], R.Pugin[1], H.Sigrist[1], H.Heinzelmann[1] & N.D.Spencer[3]

[1]*Centre Suisse d'Electronique et de Microtechnique (CSEM), Neuchâtel, Switzerland.*
[2]*Institute of Physics* of Complex Matter, EPFL, Lausanne, *Switzerland.*
[3]*Dept. Of Materials, ETHZ, Zürich, Switzerland*

INTRODUCTION: Polyethylene-Glycol (PEG) / Dextran aqueous biphasic systems have been widely studied in solution for cell and macromolecule separation [1]. The demixing properties of PEG and Dextran were here exploited to create structured polymer thin-films by polymer demixing. Polymer demixing exploits the ability of a solution of two different homopolymers to phase separate and form micro- to nano- structured films when spread on a substrate. Use of the technique to create structured polymer films was previously reported [2] but has been here extended to water-soluble polymers.

METHODS:

The two polymers were first diluted in water (the common solvent). Polymer blend solutions were then spin-coated on silicon wafers, leading to a polymer film composed of dextran-rich and PEG-rich domains. The size of the structures could be tuned adjusting parameters such as the polymer ratio (PEG:Dextran from 50:50 w/w to 90:10 w/w) , the initial polymer blend concentration (from 0.5 %wt to 5 %wt), spin-speed (from 2000 rpm to 5000 rpm) and polymer molecular weight (from 5 kDa to 100 kDa for both polymers). A selective solvent was also used to remove one of the two polymers, thus coarsening the structures. The structured films were then characterized using Atomic Force Microscopy (AFM) or fluorescence microscopy.

RESULTS & DISCUSSION:

The phase-decomposition of assymetric PEG/Dextran polymer blends follows a nucleation & growth process. Dots of Dextran embedded in a matrix of PEG can thus be seen on the surface (figure 1). The size of the structures was found to increase with the solution concentration and decrease with the spin velocity. The molecular weight also influences the demixing process since it affects the viscosity of the solution.

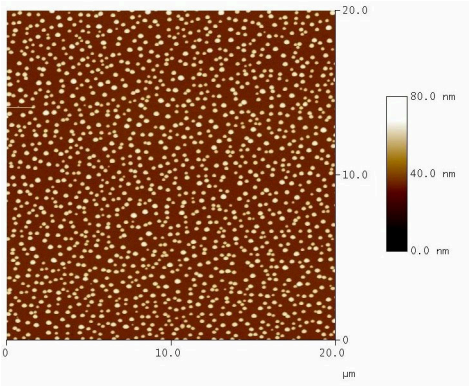

Fig. 1: Typical AFM image of PEG-Dextran polymer blend demixing: PEG 77k / Dextran 70k, 80/20, 2%wt diluted in H_2O spin coated at 5000 rpm on hydrophilic Substrate.

POTENTIAL APPLICATIONS:

The water-solubility and the biocompatibility of the polymer used make biological applications possible. First experiments were tried adding proteins to the polymer blend. When phase separation occurs, the protein goes preferentially in the dextran phase. This can be an interesting way to pattern proteins on surfaces, combining both structuring (with polymer demixing) and functionalisation (with the protein). Potential application may be found for instance in activation or passivation of surfaces (cell or bacteria adhesion) [3].

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ENZYMES IN AMPHIPHILIC CONETWORKS

N.Bruns & J.C.Tiller

Freiburger Materialforschungszentrum (FMF), Albert-Ludwigs-Universität Freiburg, Germany.

INTRODUCTION: The technological and synthetical utility of enzymes can be enhanced greatly by using them in organic solvent rather than their natural aqueous reaction media [1]. However, drawbacks of enzymes in organic media are their insolubility resulting in lower catalytic activity and their reduced stability. We have developed a method to significantly reduce these problems: incorporating an enzyme into the hydrophilic phase of an amphiphilic conetwork.

METHODS: Amphiphilic conetworks on the basis of polyhydroxyethylacrylate (PHEA) and polydimethylsiloxan (PDMS) were synthesized by radical copolymerisation of α,ω-methacryloyl-terminated PDMS-macromonomers and trimethylsilyl-protected HEA and subsequent cleavage of the protecting group [2]. The networks were produced as thin films covalently attached to a glass substrate. These films were incubated in an enzyme containing aqueous solution and thus loaded with the biocatalyst. After drying, the enzyme loaded networks were placed in organic media, allowing the enzyme to catalyze a reaction of organosoluble substrates.

Enzymatic activity of horseradish peroxidase (HRP) was studied through the oxidative coupling of phenol and N,N-dimethylphenyldiamine with *tert*-butylhydroperoxide in n-heptane. The reaction was carried out directly in a cuvette and was monitored by the increase of absorbance at $\lambda = 536$ nm.

RESULTS: Amphiphilic conetworks were synthesized as thin films to minimize diffusion problems in enzymatic reactions and to allow their use as catalytic coatings. The films show nanophase separation on the surface and in their bulk (Fig.1) and can be swollen in water as well as in organic solvents.

By loading the film with HRP, this biocatalyst was entrapped into the 10-100 nanometer sized domains of the network's hydrophilic phase. This phase acts as a nanoreactor by providing enzyme compatbile surroundigs and simultaneously allowing good accessibility for organosoluble substrates through a swollen hydrophobic phase.

HRP was used as model enzyme to catalyse a reaction in heptane. It showed an up to 100-fold increased catalytic activity as well as increased stability when entrapped into the amphiphilic film, compared to native HRP directly suspended into the reaction mixture (Fig. 2).

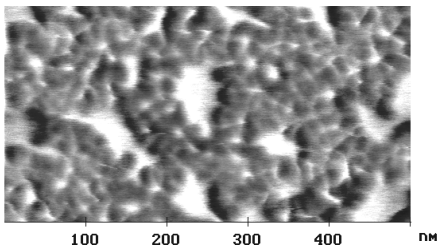

Fig. 1: Nanophase separation on the surface of a thin amphiphilic conetwork film (PHEA-l-PDMS, 50 % PHEA) revealed by phase contrast AFM. PDMS shows dark, PHEA light.

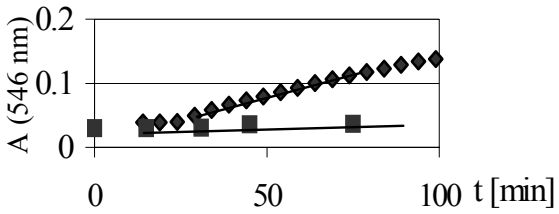

Fig. 2: Increased catalytic activity of HRP entrapped in an amphiphilic conetwork (♦) compared to native HRP (■): HRP-catalysed oxidative coupling of phenol and N,N-dimethyl-phenyldiamine with tert-butylhydroperoxide in n-heptane monitored by the increase of absorbance at $\lambda = 536$ nm.

DISCUSSION & CONCLUSIONS: For the first time it could be shown that nanophase separated amphiphilic conetworks posses the ability to stabilize and enhance the catalytic activity of enzymes in organic solvents dramatically. This could lead to new biocatalytic systems (e.g. catalytic coatings for reactors) as well as extend the application of biocatalysts in organic synthesis.

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SURFACE PROPERTIES OF METAL OXIDES – FROM MACRO- TO NANO-SCALE

G.Busch, X.Cai, E.Jähne & H.-J.P.Adler

Institute for Macromolecular Chemistry and Textile Chemistry, Dresden University of Technology Mommsenstrasse 4, D-01069 Dresden, Germany.

INTRODUCTION: Metal oxide surfaces are the most common supports in technical applications. Occasionally, they can be coated with several substances having abilities to self-assemble[1], to modify or to improve their capabilities. Especially, corrosion inhibition, adhesion promotion or protein resistance are of huge interest for further coatings. Special adhesion promoters are necessary to apply known systems of appropriate polymers on important metal oxide surfaces like aluminum, tantalum or titanium oxide. Therefore, different bifunctional alkyl phosphonic acid derivatives have been designed and synthesized.

METHODS: Freshly prepared metal oxide surfaces are immediately covered by organic residues from the atmosphere. For that reason they must be cleaned carefully, e.g., by ultrasonification before adsorption. Several chemically and physically induced surface pretreatment methods have been used, including oxygen plasma treatment, wet chemical staining and etching. Detailed information of the chemical and physical properties of the used metal oxides are necessary to improve the properties of the substances as well as the procedures by applying adhesion promoters.

We use several surface sensitive methods of different resolution capabilities to obtain this information. Contact angle measurements and microdroplet analysis were used to obtain macro-scale information (mm to µm) about the treated surfaces. Microdroplet analysis bases on characteristics of condensation of vapors on cooled surfaces[2]. Atomic Force Microscopy (AFM) and Scanning Electron Microscopy (SEM) with EDX are the methods to get structural and chemical information about surfaces in the nano-scale (µm to nm). X-ray Photoelectron Spectroscopy (XPS) and angle-resolved XPS give information about the atomic composition of the metal oxides and adsorbed layers as well as the orientation of the adsorbed molecules.

Surface plasmon resonance spectroscopy (SPR)[3] is used to follow kinetics during the adsorption processes of different surface active molecules on alumina substrates. For SPR special substrates[4] are necessary e. g., consisting of BK7 glass, 45nm Au and 2.5nm Al_2O_3.

RESULTS: Contact angle and SPR- measurements revealed that phosphonic acid derivatives adsorb readily and spontaneously onto alumina. The intermolecular organization and ordering processes of these self-assembled layers require more time (see figure 1, 60-1200 min). Angle-resolved XPS of bifunctional phosphonic acids revealed ordered layers where the phosphorus is attached to the substrate surface and the functional moiety faces away from it.

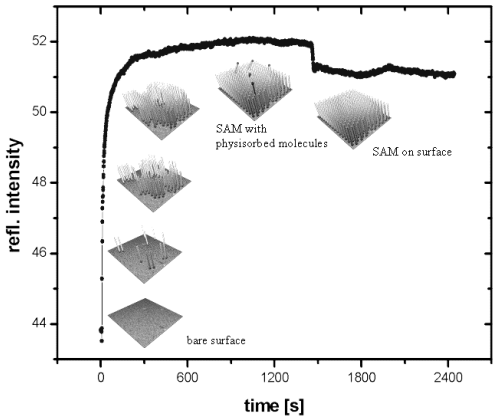

Fig. 1: Typical SPR measurement in kinetic mode.

DISCUSSION & CONCLUSIONS: Several surface-sensitive methods were used to characterize different metal oxide surfaces. SPR is able to resolve kinetic processes on alumina surfaces. Contact angle measurements give first indication about successful treatment as well as adsorption. Microdroplet analysis reveals disordered areas on the surface in the range of some µm. Surface coverage and molecular order normal to the surface can be concluded from XPS results. AFM and SEM show characteristics of surface morphologies. Kind of material and pretreatment have a great influence on the self-assembly monolayers resulting from adsorption.

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MICRO- AND NANO-STRUCTURES FOR OPTICAL DETECTION OF BIOMOLECULES

A.Perentes, A.Bachmann, M.Leutenegger, G.Benvenuti, I.Utke, T.Lasser & P.Hoffmann

Institute for Biomedical Imaging, Optics, and Engineering, EPFL, 1015 Lausanne, Switzerland.

High accuracy, very low surface roughness of deposits, and extended versatility leads focused electron beam induced deposition (FEBID) to be perfectly suited to nano-optic device production e.g. photonic band gap (PBG) structures [1]. Here we present the fabrication of a transparent two-dimensional PBG structure.

Our FEBID equipment is based on a Cambridge S100 scanning electron microscope (SEM) with thermionic tungsten filament. The electron beam is controlled with a Nabity Systems patterning software (NPGS). A modified internal syringe supplies the volatile precursor in the SEM chamber to the substrate.

The classical SiO_2 CVD precursor Tetraethyl orthosilicate (TEOS) was chosen as it also decomposes into a transparent dielectric material under electron irradiation. Refractive index and absorption of deposits prepared in our SEM were subject of investigation. High resolution ($5x5\mu m^2$) spectroscopic micro-ellipsometry measurements were carried out with a Nanofilm EP3 (Nanofilm Technologie GmbH, Göttingen, GE) on deposited rectangles of 70nm thickness. We obtained the complex index of refraction ñ between 350nm and 900nm wavelength. At λ = 632nm ñ equals 1.85 + i $3.9 \cdot 10^{-4}$. These values were used as input parameters for the photonic structure design (Fig. 1) with maximum PBG at this wavelength.

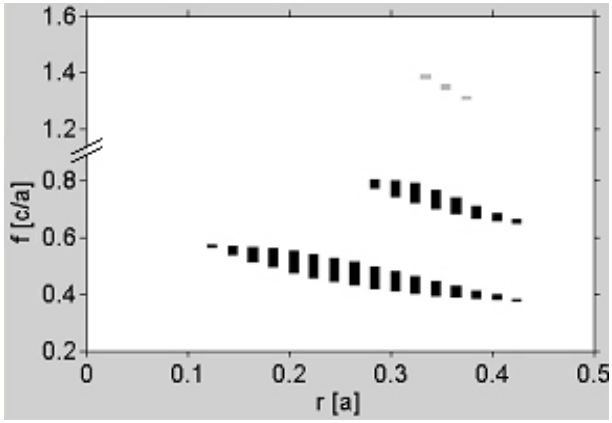

Fig. 1: The photon frequency f normalized to the lattice constant (a) as function of the pillar bottom radius r shows the forbidden photonic states for the two polarizations (black =TM, grey = TE) of a two-dimensional cylindrical pillar hexagonal arrangement.

Furthermore, growth rates and geometrical dimensions of the deposits for different irradiation conditions were determined.

The proposed PBG structure is based on a 2-D hexagonal lattice with missing central pillar in order to create a micro-cavity. Parameters are: lattice constant (a) 470nm, pillar diameters 320nm, pillar heights 500nm.

Deposition parameters were based on the dynamic and geometrical properties of FEBID tips with TEOS: beam current 100pA, dwell time for each pillar 120s, deposition divided in 30 overlays, 30 automatic realignments in total.

Observation (Fig. 2) shows that the deposited pillars have dimension errors below 3% of the targeted values. Deposition on a 2mm thick microscope slide covered by a 10nm thick antimony doped tin oxide layer suppressed structure's distortions due to charging effects. Implementation of multiple beam re-alignments on alignment marks compensated electron beam drift.

Optical analysis of the PBG structure is currently under investigation.

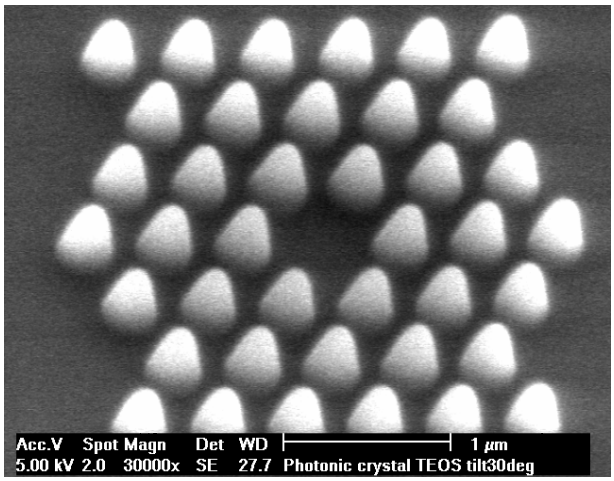

Fig. 2: SEM image of the PBG structure, 30° tilt view. Hexagonal lattice and micro-cavity are well observable.

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COMBINING POLYMER DEMIXING AND MICROPHASE SEPARATION OF BLOCK COPOLYMERS TO OBTAIN STRUCTURING ON DIFFERENT SCALES

K.Sivashankar[1], R.Pugin[1], J.Brugger[2], J.Vancso[3], H.Heinzelmann[1] & Ch.Hinderling[1]

[1]Centre Suisse d'Electronique et de Microtechnique (CSEM), Neuchâtel, Switzerland. [2]Institute of Microtechnology and Microsystems, EPFL, Lausanne, Switzerland. [3]MESA+ Research Institute, Department of Materials Science and Technology of Polymers, University of Twente, Netherlands.

INTRODUCTION: Patterning of surfaces has been of particular interest in trying to achieve chosen functionalization at desired areas of the substrate[1,2,3]. Block copolymers have been a favorite choice due to their ability for nanoscale self - organization. This work demonstrates the use of phase separation of homopolymer/block copolymer blends to form larger structures (~100nm) and incorporate the smaller microphase phase separated structures (~30nm) of block co-polymer domains within them. The importance of the work lies in the tunability of structures on two different length scales

METHODS: A polymer blend consisting of 1% solutions of the block co-polymer Polystyrene block Polyferrocenyldimethylsilane (PS-b-PFS, 60 kg/molPS, 17.7kg/mol PFS) and the homopolymer PMMA were prepared in toluene. The solution was spin coated at 3000 rpm on Si substrate to obtain thin films (<100nm). The samples were annealed in vacuum at 150°C for 17h and reactive ion etched with O_2 plasma (50 mT, 30 W, 3min). This leaves the substrate with iron containing oxide nanoparticles in the areas initially occupied by the block copolymer [3]. The ratio PMMA: PS-b-PFS in the blend was varied from 6:1 to 24:1. The structures were characterized using Atomic Force Microscopy (AFM).

RESULTS & DISCUSSION: The phase separation of the PS-b-PFS block co-polymer/PMMA homopolymer blend forms PS-b-PFS domains in a continuous phase of PMMA. The size of these domains and their spacing are tunable by varying the composition of the initial blend and decreases monotonously with decrease in PS-b-PFS proportion in the blend. The additional microphase separation of the PS and PFS blocks in the block copolymer forms PFS dots in a PS matrix within these domains. Annealing of the samples increase the ordering of PFS domains.

APPLICATIONS: The iron containing oxide nanoparticles formed after O_2 RIE have been shown to act as etch masks to transfer the structures into Si using RIE in earlier publications[4,5]. Iron containing oxide nanoparticles on the patterned substrates are currently explored for use as catalyst in carbon nanotube growth.

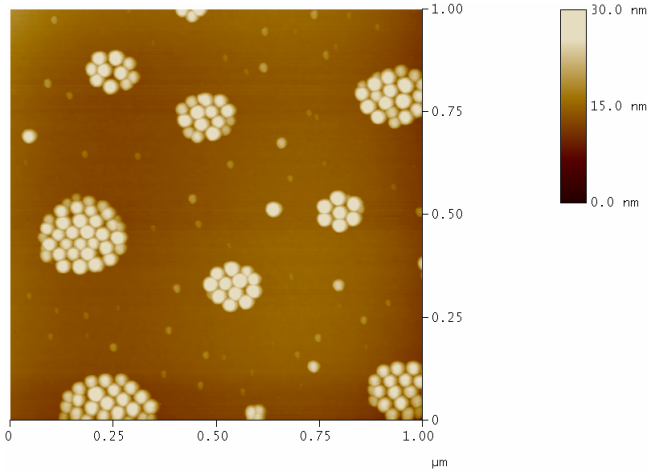

Fig. 1: AFM image of oxide nanoparticles within microstructures obtained by polymer demixing of a 6:1 PMMA: PS-b-PFS blend after O_2 RIE

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SURFACE STRUCTURING METHOD FOR DEFINED GROWTH OF NEURAL NETWORKS

D.Kurzbuch, S.Seeger

Institute for Physical Chemistry, University of Zürich, Zürich, Switzerland.

INTRODUCTION: Culture studies in vitro provide better control over the extracellular milieu, thereby allowing one to change systematically the environmental conditions around cells. Surface patterns in high resolution have been produced down to the micrometer scale, e.g. by using standard photolithography [1], combination of laser ablation techniques with lithographic masks [2] and microcontact printing [3]. Most of these methods are based on precise templates, which are used as aperture stops, punching tools or they are engraved onto the cell substrate itself. A method is presented to fabricate cell patterns. By designing patterns with a computer controlled laser ablation process the desired structures can be realized within few minutes without the need of a precasted template.

METHODS: Glass slides were silanized with OTS (octadecyltrichlorosilane) and a silicone chamber was fixed on it. Then the hydrophobic surface within the chamber was coated with laminin which is a protein of the extracellular matrix. A pattern was burned in the laminin coated layer by a computer controlled UV-laser ablation. The pheochromocytoma cells (PC12) were positioned on nodes of the pattern by the use of optical tweezers. Nerve growth factor (NGF) was added to induce neurite growth and to inhibit repeated cell divisions.

RESULTS: We formed an array of three times three octagons on the laminin-molecules coated glass slides with a node size of about 10 to 30 µm and a line thickness of 2 to 10 micrometers, whereby the interior of the octagons was ablated with the help of an UV-laser.

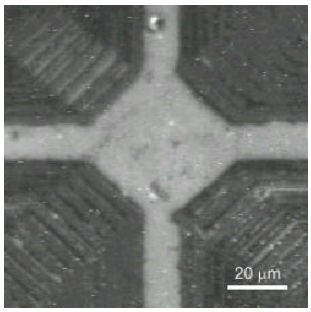

Fig. 1: Fluorescence image of a laminin coated, node between ablated areas which were incubated with Cy3.5-labeled anti-laminin at RT.

The fluorescence image (Fig 1.) showed, that the ablation process was effective and that the laminin molecules were removed or destroyed. After 3-4 days the PC12 cells formed a small network according the burned pattern, which was only affected by some cell divisions (Fig 2.). The cells and their neurites don't grow on the ablated areas.

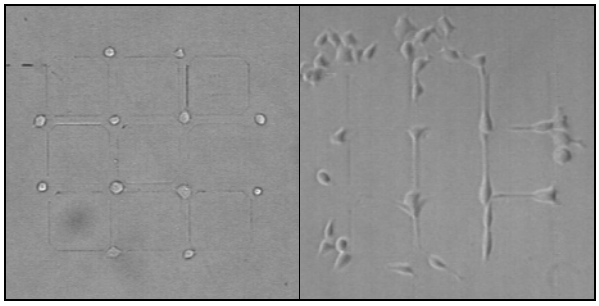

Fig. 2: PC12 cells on laminin coated glass slides

DISCUSSION & CONCLUSIONS: This method is very versatile and can be performed in absolute sterility. No substantial preparatory work like the execution of complex surface chemistry or mask fabrication is necessary. After the unspecific absorption of laminin molecules on the OTS-coated cover glasses, a computer-controlled ablation process is started which follows a programmed pattern and is in our case an arrangement of octagons with laminin-coated lines and nodes between them. It is possible to keep the time between desired design and realization of the pattern short especially in comparison to other methods. This is an enormous advantage if one wants to test cell growth on many patterns which by comparison takes a much longer time with other methods and is considerably extensive.

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AMPHIPHILIC DENDRIMER/DNA SELF-ASSEMBLIES FOR CELL TRANSFECTION. A SCANNING PROBE MICROSCOPY STUDY.

M.Losson[1], D.Joester[2], E.Walter[3], M.Guillot[2], J.L.Gallani[4], H.P.Merkle[3], R.Pugin[1], H.Heinzelmann[1] & F.Diederich[2]

[1]*Centre Suisse d'Electronique et de Microtechnique, Neuchâtel, Switzerland.* [2]*Laboratory of Organic Chemistry, ETH Zürich, Switzerland.* [3]*Institute of Pharmaceutical Sciences, ETH Zürich, Switzerland.* [4]*Institut de Physique et Chimie des Materiaux de Strasbourg, France.*

INTRODUCTION: Amphiphilic dendrimers were designed to combine a high charge density and spontaneous self-assembly properties to condense DNA [1-2]. After promising biological transfection results, Scanning Probe Microscopy gives insight into structure-activity relationships.

METHODS: The amphiphilic vectors **1-4** *(Fig. 1)* have been synthesized. The two parts, lipophilic and hydrophilic dendrons, are connected to a rigid scaffold. After extended biological investigations, the study on surfaces has been focused by using AFM in tapping mode on freshly cleaved mica surfaces with drop cast of dendrimer, plasmid and a mixture of dendrimer/plasmid depending of the charge excess (CE).

To a better understanding of the amphiphilic compounds behavior, measurements were done at the air-water interface by recording the isotherms and exploiting Brewster Angle Microscopy, X-Ray reflectivity and tapping mode AFM on Langmuir Blodgett films.

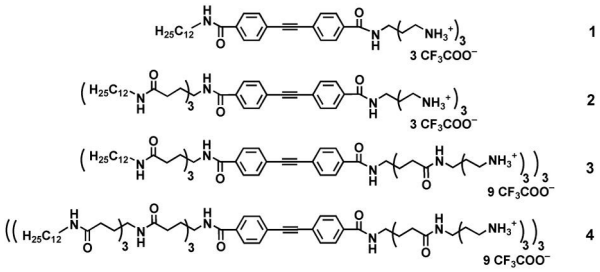

Fig. 1: A novel class of amphiphilic dendrimers.

RESULTS & DISCUSSION: Compounds **2** and **3** were found to be highly efficient transfection agents *(Fig. 2)*. The study purchased in AFM showed *(Fig. 3)* complexation properties of the dendrimer **2** with the same plasmid used for the cell transfection.

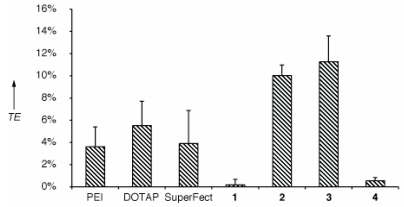

Fig. 2: Gene transfection efficiency study

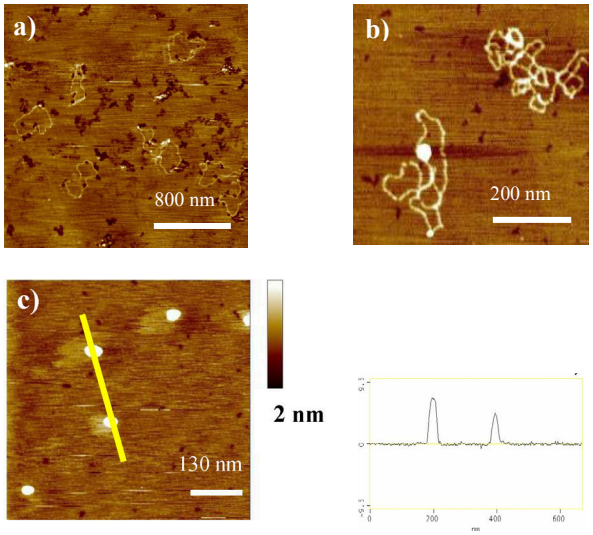

Fig. 3: Tapping mode AFM images on freshly cleaved mica: a) Plasmid deposition. b) Plasmid deposition followed by dendrimer **2** drop-cast showed 'prior' coiling state. c) Plasmids and **2** were mixed prior to deposition, showing polyplexes formation.

CONCLUSIONS: Our approach allows to tune the complexation properties of amphiphilic compounds and we will use AFM as a predictive tool for their transfection properties [3].

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SURFACES WITH A HYDROPHOBICITY GRADIENT: POSSIBLE APPLICATIONS IN BIOLOGICAL TESTING

S.Morgenthaler[1], S.Lee[1], P.Schär-Zammaretti[2], J.Ubbink[3] & N.D.Spencer[1]

[1]Laboratory for Surface Science and Technology, Dept. of Materials, ETH- Zürich, Switzerland. [2]Institute for Biomedical Engineering, Dept. of Materials, ETH-Zürich, Switzerland. [3]Nestlé Research Center, Vers-chez-les-Blanc, Lausanne, Switzerland.

INTRODUCTION: Surfaces with well-designed surface properties are important for gaining more information about biological interactions, such as microbial or cell adhesion. Such surfaces also show a potential for applications in various fields, such as diagnostics, nanotribology, biomolecular interactions or cell-motility studies.

In this study a novel method for the preparation of gradient surfaces with varying functionalities based on the formation of self-assembled monolayers has been developed. Gradients are prepared by simple control of the kinetics of thiol adsorption on gold [1].

METHODS: Samples, cut from silicon wafers, were coated with a 6-nm-thick Cr adhesion layer and 80 nm Au. Prior to immersion in thiol solution the substrates were rinsed with ethanol and plasma-cleaned for 30 sec in nitrogen plasma. Dodecanethiol ($CH_3(CH_2)_{11}SH$) and 11-mercapto-1-undecanol ($HO(CH_2)_{11}SH$) have been used to prepare wettability gradients.

Wettability gradients were prepared by using a linear-motion drive to control the immersion. In a first step single-component gradients of methyl-terminated thiols were generated. The single-component gradient is then completed by full immersion in a solution of hydroxyl-terminated thiols. The adsorption kinetics can be controlled by the concentration of the solution, the solvent and the immersion time.

Gradients were characterized by dynamic water-contact-angle measurements and x-ray photoelectron spectroscopy.

RESULTS: Fig. 1 depicts a wettability gradient of a length of 4 cm that covers a range of 20° to 85° in water-contact angle. The gradient was generated from a gradual immersion in 0.005mM methyl-terminated solution by a speed of 50 µm/sec and subsequent saturation in 0.005mM hydroxyl-terminated solution. By varying the concentration of the solutions, the sequence of the immersion and the immersion speed of the substrate, wettability gradients of different slopes can be generated.

A gradual change in the composition along the gradient was also monitored by XPS.

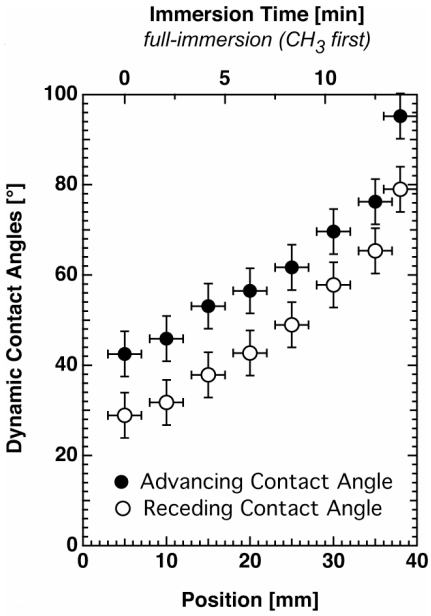

Fig. 1: Results of dynamic water-contact angle measurements and a photo taken of water droplets placed along a wettability gradient.

DISCUSSION & CONCLUSIONS: The preparation of wettability gradients by controlled immersion is easy and reproducible. Gradients with a variable slope are easily generated by changing the immersion speed. Thiols with many different functionalities are commercially available or can be generated, so that the surface properties can be tailored for different purposes.

Preliminary experiments on microbial adhesion to gradient surfaces were carried out and lead to interesting results. Microbes with a variation in surface properties adhered to different wettability regions.

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TWO-DIMENSIONAL CONTROL OVER PROTEIN AND CELL ATTACHMENT ON MODIFIED POLY(ETHYLENE) SURFACES BY LASER ABLATION

B.Muir[1], H.Thissen[1], G.Johnson[1] & J.Hayes[2]

[1]*CSIRO, Molecular Science, Melbourne, Australia.* [2]*Industrial Research Institute Swinburne, Melbourne, Australia.*

INTRODUCTION: In the field of biomaterials and related research, there is currently an increasing amount of interest in obtaining two-dimensional control over the location of cells and bioactive compounds on a surface by manipulation of the surface chemistry and topography. Having control over location of proteins or cells on a surface with high resolution is of great importance for applications such as diagnostic tests, cell based sensors and tissue-engineered medical devices[1,2].

METHODS: Spatially controlled surface chemistries were created as follows; a poly(ethylene) co-polymer [PE-co-GMA] (Atofina Chemicals) with a high degree of glycidyl methacrylate functionalities was used to provide a surface suitable for cell attachment. Coupling of thiol functionalised poly(ethylene oxide) [PEO] (Sigma-Aldrich) at a pH of 9.0 under cloud point conditions was used to create a surface resistant to protein adsorption.

Patterning of the surface chemistry of injection moulded samples was achieved by laser ablation using a 248 nm excimer laser. X-ray photoelectron spectroscopy (XPS) and atomic force microscopy were used to characterize the surface topography and surface chemistries. Protein adsorption tests were performed on the surfaces using collagen [Coll] (Vitrogen®) and the biological response to two-dimensional patterned substrates was analysed in cell culture experiments using bovine corneal epithelial cells [BCEp]. After fixation of the cells with formol-saline and staining cell attachment patterns were imaged using a confocal scanning laser microscope.

RESULTS: XPS experiments were perfomed to monitor protein adsorption on the PE-co-GMA substrate material and PEO functionalised surface. The presence of adsorbed proteins is clearly evident from analysis of the C1s high resolution spectra of the PE-co-GMA–Coll sample as seen in Figure 1. A broad shoulder representing amine/hydroxyl and amide components (286.5 eV and 288.5 eV respectively) of the protein are clearly visible. However, no protein is detected after collagen adsorption to PE-co-GMA-PEO surfaces indicating low fouling characteristics.

Figures 2A and 2B show light microscopy images of a tissue culture poly(styrene) surface after collagen adsorption and BCEp cell culture and an ablated PE-co-GMA-PEO sample respectively. The ablated rectangular area is clearly visible and well resolved. Cell attachment clearly follows the pattern provided by excimer laser ablation, while the non-ablated area only shows very few cells with little or no spreading.

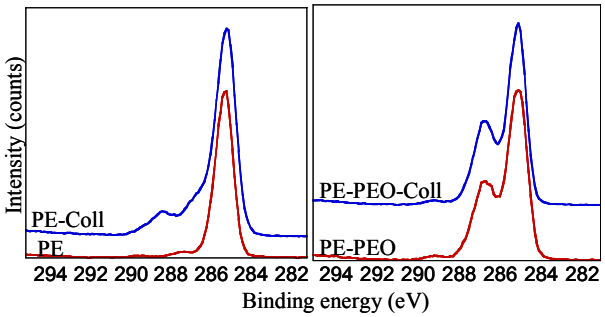

Fig. 1: (A) PE-co-GMA before and after adsorption of collagen; (B) PE-co-GMA – PEO before and after adsorption of collagen

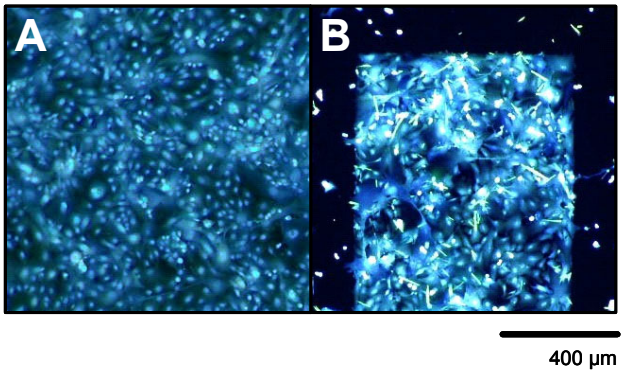

Fig. 2: Microscope images of (A) TCPS control surface and (B) PE-co-GMA-PEO after laser ablation and BCEp cell attachment.

DISCUSSION & CONCLUSIONS: Cell culture experiments using bovine corneal epithelial cells confirmed that cell attachment is controlled by the surface chemistry pattern. The method is an effective tool for use in a number of *in vitro* and *in vivo* applications.

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PATTERNING OF SURFACES WITH X-RAY INTERFERENCE LITHOGRAPHY AT MACROMOLECULAR LENGTH SCALES

H.H.Solak, C.Padeste & J.Gobrecht

Laboratory for Micro- and Nanotechnology, Paul Scherrer Institut, 5232 Villigen PSI , Switzerland.

INTRODUCTION: Interaction of biological systems with artificially patterned surfaces attracts interest due to diverse potential applications. For example influencing growth of cells on patterned surfaces may have great impact in tissue engineering and implant applications, or controlling the molecular self assembly processes may lead to new ways of synthesis and analysis of biological materials. Until recently most experiments in the field have used surfaces patterned with micrometer sized or slightly smaller features. However when the feature sizes approach the length scales of the macromolecules involved in biological processes we can expect to have more and potentially stronger ways of influencing the interaction between the artificially patterned surface and the biological system the surface is in contact with. Here we introduce a new way of patterning surfaces that can be used for this purpose.

METHOD: X-ray Interference lithography (XIL) offers a way to achieve high resolution patterning that is beyond the capabilities of today's production techniques. In IL two or more coherent light beams are brought together to form an interference pattern which is then recorded in a photosensitive material. In general the patterns achieved with IL are periodic; e.g. one-dimensional line/space patterns or two dimensional dot arrays are readily obtained. Lasers are commonly used as light sources in IL due to their coherent properties. We have extended the technique to the x-ray region where the extremely small wavelength has allowed us to achieve patterns with periods as small as 40nm[1]. The theoretical limit for pattern period is equal to the half of the wavelength, which is about 6nm in our system. The technique is based on the coherent light available from modern synchrotron sources. We have recently completed the construction of a unique X-ray interference lithography facility at the Swiss Light Source in Switzerland. The main goals of the project are to produce patterns with sub 50nm periods, over areas as large as several square millimeters with high throughput. The technique has advantages over alternative techniques; e.g. it has higher throughput and higher resolution for dense patterns than electron beam lithography.

In this project we target applications that require high resolution periodic patterns which are not easily available from alternative methods. This includes production of patterned templates that can later be used for guided self-assembly of macromolecular systems such as block copolymers[2] or crystallization of proteins. Periodic patterns with nanometer scale resolution can be used to influence the growth of cells and tissues. Even though cells are often as big as tens or hundreds of micrometers, the processes and intracellular structures have much smaller length scales. There is growing evidence that through these processes high resolution surface patterns influence cell growth behavior[3].

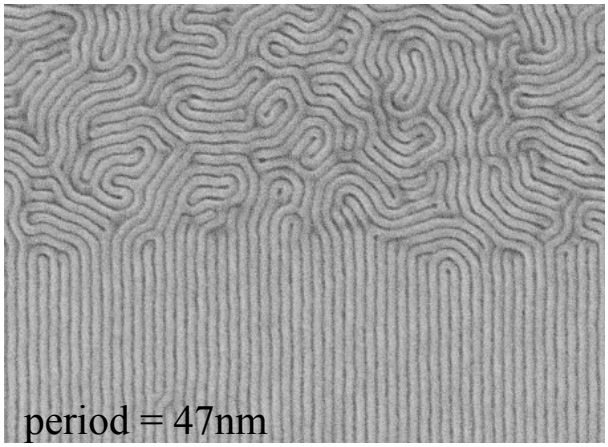

Fig. 1: Epitaxial self-assembly of a block copolymer film on a surface patterned with X-ray interference lithography. The upper part of the SEM image shows the random orientation of the block copolymer domains in the un-patterned area of the sample whereas the domains in the lower part have aligned themselves to the lithographically defined surface template.

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SELF-ASSEMBLED MICROARRAYS OF ATTOLITER MOLECULAR VESSELS

D.Stamou[1], C.Duschl[2], E.Delamarche[3] & H.Vogel[1]

[1]ISB–VO, EPFL, Lausanne, Switzerland. [2]Fraunhofer Institute, AMBT, Berlin, Germany.
[3]IBM Research, Rüschlikon, Switzerland.

INTRODUCTION: We believe that combining self-assembly (SA) principles with the use of biologically important building blocks, is key to the development of functional nano-sized architectures of far greater complexity than the one attainable by "conventional" microstructuring. In this context we describe a method that allows the massively parallel isolation of attoliter experimental volumes and their self-assembled positioning with 100-nm precision in ordered arrays on surfaces.[1]

RESULTS: The strategy employed to realize arrays of surface-immobilized single vesicles (SVs) is illustrated in Figure 1. Using high resolution micro contact printing[2] (μCP), we defined regions on the substrate that specifically bind vesicles and are surrounded by areas that prevent nonspecific attachment. In this manner, the positioning of vesicles and their content becomes a diffusion-limited SA process guided by the patterned surface functionalisation.

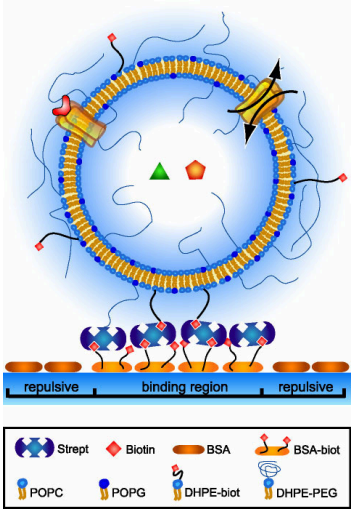

Fig. 1: Biotinylated bovine serum albumin (BSA-biot) is patterned on the surface by means of μCP. The nonprinted regions are passivated by adsorbing BSA from solution. Streptavidin is then bound to the printed BSA-biot. Biotinylated lipids mediate the specific immobilization of vesicles. The vesicles carry charged and PEG-derivatised lipids to prevent their nonspecific interactions with the surface.

The μCP technique allowed us to create patterns with feature sizes from a few microns down to 100 nm. These patterns were used to immobilize groups of vesicles and most importantly to isolate single individual vesicles and their content, Figure 2 A and B.

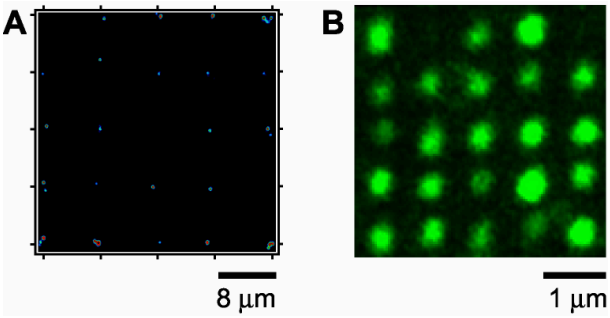

Fig. 2: Confocal fluorescence microscopy characterization of vesicles arrayed on a glass surface. Binding sites in A) and B) were 2 μm in diameter and 100 × 400 nm² respectively. Vesicles were labeled with 1% rhodamine-lipid in the bilayer and carboxyfluorescein in their interior.

DISCUSSION & CONCLUSIONS: In this work we combined hierarchical SA and receptor-ligand interaction first to define attoliter volumes, and then to order them on surfaces. The lipid-bilayer vesicles we used as molecular vessels are arrayed at high densities, ~10^6 per mm². Nevertheless, each one maintains its cargo dissolved in a protective environment of defined chemical composition (pH, ionic strength etc.). Such ultra-small volume libraries allow simultaneous screening of (bio)chemical properties, molecular function or confined chemical reactions over millions of samples, while consuming total reagent volumes of a few picoliters.

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FAST AFM-IMAGING OF BIOLOGICAL SPECIMEN

G.Schitter, R.W.Stark[1] & A.Stemmer

Nanotechnology Group, Swiss Federal Institute of Technology, ETH Zentrum CLA, CH-8092 Zurich, Switzerland.

INTRODUCTION: Imaging and characterization of biological material requires imaging methods that provide a spatial resolution beyond the diffraction limit of light microscopy. The atomic force microscope (AFM) is a well-established tool for imaging as well as for tribological characterization of surfaces with nanometer resolution[1].

In order to investigate friction on the nanometer scale at technical relevant velocities as well as to monitor biological processes in real-time the operation speed of the AFM has to be improved significantly. We present a method to increase the imaging speed of an AFM by one order of magnitude by utilizing modern model-based control methods.

METHODS: The imaging speed of the AFM is limited by the dynamic behavior of the scanning system[2] and the bandwidth of the proportional integral (PI) controller in the vertical direction[3]. To suppress the lateral dynamics of the scanning system an open-loop controller based on the model of the X- and Y-directions is designed[2]. To improve the system performance in the vertical direction a model-based two-degrees-of-freedom controller is implemented[3].

As test specimen an aluminum structure on a glass surface was used for friction measurement. The sample was made hydrophobic by exposure to the vapor of octadecyltrichlorosilane (OTS).

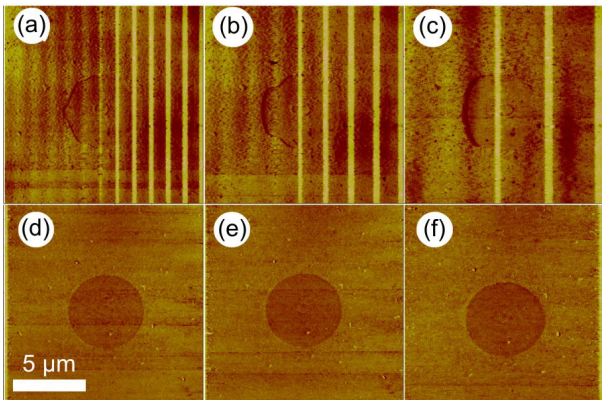

Fig. 1: Lateral force images on aluminum test pattern on glass. (a-c): without compensation, (d-f) with compensation. Scan velocity: (a,d) 1.1 mm/s, (b,e) 1.7 mm/s, (c,f) 3.3 mm/s.

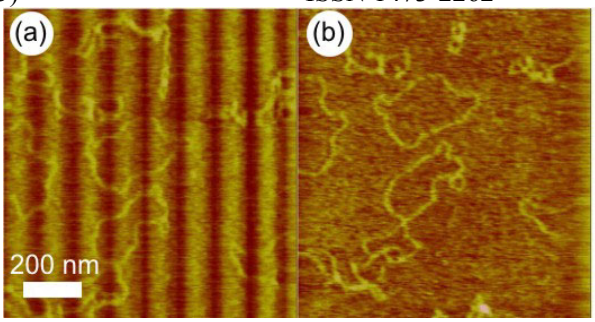

Fig. 2: Topography of pUC-18 plasmid DNA imaged at 61Hz line- scan rate. (a) Standard PI controlled system and (b) model-based controlled AFM. Images are recorded from right to left. Height scale: 3 nm.

Topographic measurements were carried out on pUC-18 plasmid DNA immobilized on muscovite mica.

RESULTS: To demonstrate the performance of the AFM with the new controller as compared to the uncompensated system a test specimen was imaged in lateral force mode (Fig. 1). The images in the upper row are dominated by vertical stripes that are induced by the lateral vibrations of the scanner. The images in the lower row show the performance of the compensated AFM system[2]. There is a clear image contrast and the scanner artifacts are absent even at technically relevant velocities. Scanning with the standard AFM system similar imaging artifacts can be observed in the topographic images of plasmid DNA (Fig. 2). The shape of the specimen is distorted and the sample surface shows an apparent corrugation (Fig. 2a). In case of the model-based controlled AFM the imaging artifacts vanish (Fig. 2b).

Concluding, the new controller allows us to perform fast AFM imaging together with reliable friction force measurements at mm/s speed.

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[1] present address: Section Crystallography, University of Munich, Theresienstr. 41, 80333 Munich, Germany.

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PATTERNING OF LIPID MEMBRANES BY SELF-ORGANIZATION OR MICROCONTACT PRINTING OF LIPOPOLYMERS

H.Müller, P.Theato* & R.Zentel

Institute of Organic Chemistry, University of Mainz, Duesbergweg 10-14,55099 Mainz, Germany.

INTRODUCTION: Recently, the patterning of lipid membranes gained interest, as the mimic of different, separate addressable cell membranes on a single substrate would lead to novel integrated bio-devices [1-3].

Different approaches have been presented to form patterns on a solid substrate, which all suffer from the fact of their complex preparation. Within this study we compare two simple methods, patterning on a LB-trough and microcontact printing (μCP). Investigations of the phase separation of a lipid mixture on a LB-trough showed a pattern formation [4]. Furthermore a regular pattern could be prepared using microcontact printing on solid substrates [5]. However, to stabilize such a pattern in a lipid membrane, further efforts like the polymerization of lipids on a trough, or the stabilization of microcontact printed areas on a substrate have to be made.

METHODS: On a LB-trough a mixture of stearic acid and amphiphilic monomer **1**, together with a dye is spread on a water subphase. The phase separation is visualized using a fluorescence microscope. Polymerization of the monomer **1** is carried out by illumination with a UV-lamp for 10 minutes. μCP of the lipopolymer **P2** [6] has been performed using a water/THF solution. The carefully loaded stamp has been transferred onto a gold substrate. Dewetting has been performed by dip-coating the substrate into a polystyrene solution. Drying of the substrate resulted in a patterned substrate.

RESULTS: The fluorescence image in figure 2, left, shows the phase separation in stearic acid enriched crystal analogous domains (dark) and a monomer **1** enriched area (bright). UV-polymerization of monomer **1** results in a highly decreased fluidity of the monolayer, which results in a stabilization of the pattern formed. Thereby, a patterned lipid monolayer with long term stability is created.

Microcontact printing of **P2** resulted in a pattern on a solid substrate. This pattern could be stabilized by controlled dewetting of polystyrene, as it is shown in figure 2 on the right side. Afterwards, the dewetted areas can be filled with a lipid bilayer by a vesicle fusion process.

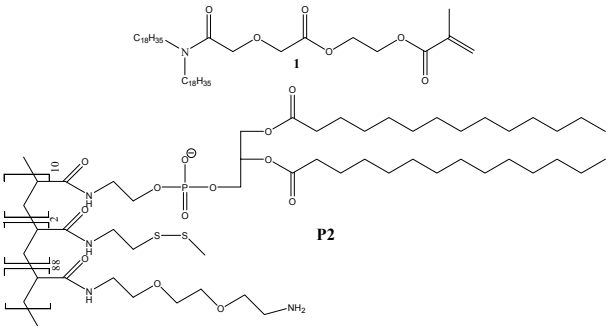

Fig. 1: Structures of used compounds.

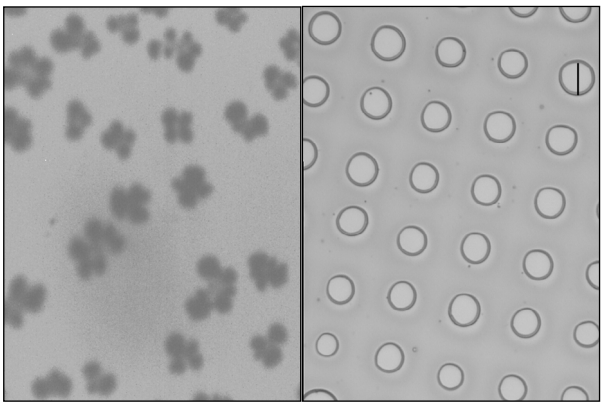

*Fig. 2: Left: Fluorescence image of a mixture of monomer **1** and stearic acid (3:2) at π=15mN/m. Right: PS dewetted pattern of lipopolymer **P2**.*

CONCLUSIONS: We could present two different ways to pattern lipid membranes. In each case the fluid lipid layer was surrounded by a polymer. Thereby, a regular array of lipid membranes was assembled.

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MICRO-PATTERNING OF FLUOROPOLYMER SURFACES FOR ELECTRONIC AND BIOMATERIALS APPLICATIONS

W.Prissanaroon, N.Brack, P.J.Pigram & J.Liesegang

Centre for Materials and Surface Science and Department of Physics,
La Trobe University, Melbourne, Australia.

INTRODUCTION: Micro-contact printing (μCP) is a soft lithography process that offers an alternative route for the formation of conducting polymer structures on a variety of substrates for application in polymer-based sensor and electronic devices. The present study is focused on creating polypyrrole-based sensor structures on a flexible, bio-compatible substrate (poly(tetrafluoroethylene), PTFE). The key requirements are to optimize polypyrrole (PPy) growth on an active metal (copper)[1], to form an adherent multi-layer film with appropriate interlayer grafting[2] that incorporates PPy and copper layers[3], and to pattern this structure using μCP to create sensor arrays.

METHODS: PPy films doped with a surfactant (dodecylbenzene sulfonic acid), PPy(DBSA), were deposited on mechanically polished and electropolished copper substrates in an aqueous solution of 0.05 M pyrrole and 0.05 M DBSA at a constant potential in the range 1.0 – 3.5 V.

Multi-layer films were deposited on poly(tetra-fluoroethylene) (PTFE) film. The film was cleaned ultrasonically in acetone (5 min.), Ar plasma-treated for 0 to 300 s (Harrick Scientific PDC-32G, Ar pressure 5 X 10^{-2} mbar; power 18 W), air exposed for 10 min., then immersed N-[3(trimethoxysilyl) propyl] diethylene triamine (TMS) (1 wt% solution in 1,4 dioxane). The film was washed in 1,4 dioxane to remove excess TMS and dried in nitrogen. The film was activated in an aqueous solution of 0.1 wt% $PdCl_2$ and 1.0 wt% HCl for 10 min. and rinsed in deionised water. Electroless copper was deposited in a $CuSO_4$-based plating bath for 20 min., followed by rinsing in deionised water. PPy(DBSA) was electro-deposited at a potential of 1.5V for 10 min. in an aqueous solution of 0.05 M pyrrole and 0.05 M DBSA. The completed multilayer was rinsed using deionised water and dried for 24 hours at 60°C.

RESULTS: PPy(DBSA) films were successfully deposited on electropolished copper at potentials of 1.5 V and higher due to partial surface passivation by Cu_2O. XPS showed doping levels were highest, (ratio of charged N species to total N species = 0.55) at 1.5V deposition potential. In contrast, deposition on mechanically polished copper failed with substrate dissolution dominating in the presence of a much thinner surface oxide.

XPS and TOF-SIMS showed the formation of oxygen-containing functional groups during plasma treatment and subsequent reaction of these with TMS. $PdCl_2$ formed a complex with nitrogen groups in the TMS and activated the surface for electroless deposition of copper. The copper surface oxide was similar to that observed for electropolished copper, with a significant concentration of Cu_2O. PPy(DBSA) was successfully deposited on this surface due to the passivation action of the oxide, completing the desired multi-layer film (Table 1).

Table 1. Multi-layer film forming basis of μCP-fabricated conducting polymer sensor devices.

Layer	Material/Treatment
substrate	poly(tetrafluoroethylene)
plasma treatment	Ar^+ plus air exposure
silane coupling	TMS
activator	palladium chloride
metallization	electroless copper
conducting polymer	PPy(DBSA)

μCP, via a polydimethylsiloxane stamp, was used to apply a TMS silane pattern on a plasma-treated PTFE surface, allowing the formation of copper patterns and providing a pathway to PPy(DBSA) sensor arrays.

DISCUSSION & CONCLUSIONS: The formation of patterned structures on PTFE has been demonstrated. Migration of silicon-containing species remains a potential limitation to reducing features sizes from the current 200 μm to 10μm and below. Current work is directed at identifying enhanced μCP pathways.

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Poster ID: 215

MICROFABRICATION OF A BIODEGRADABLE POLYMER BY ION BEAM IRRADIATION FOR A NEW CO-CULTURE SYSTEM OF CELLS

A.Yamamoto[1], T.Yotoriyama[2], Y.Kohyama[1], Y.Suzuki[3], M.Iwaki[3] & T.Hanawa[1]

[1]Biomaterials Center, National Institute for Materials Science, Tsukuba, Japan.
[2]Graduate School, Tokyo University of Science, Tokyo, Japan. [3]The Institute of Physical and Chemical Research (RIKEN), Wako, Japan.

INTRODUCTION: For the visualization of the advanced medical services such as an order-maid medical treatment and a point-of-care system, improvement and generalization of diagnosis materials and technologies were requested. From the ecology, biodegradable materials are expected to enlarge their application to the fields of diagnosis and biosensing. Poly-(L-lactide) (PLLA) is one of the biodegradable polymers which are already used as implanting materials, and it remains in a human body for years after implantation. However, ion beam irradiation into PLLA film accelerates its degradation, and the irradiated layer detaches to be a thin sheet rom the remaining film. When this film is used for cell culture, the irradiated layer can detach with cells cultured on it. This phenomenon can be utilized to produce patterned culture of cells, which are useful for tissue engineering, regenerative medicine, and diagnosis techniques. In this study, we attempt the microfabrication of biodegradable polymers by ion beam irradiation for the patterning culture of human and mammalian cells to establish a new technique of patterning culture.

METHODS: PLLA films (LACTY, SHIMADZU, Japan) were irradiated by He^+ ion beam at the acceleration energy of 50 keV, 100 keV, 150 keV and fluency of 1×10^{13} ~ 1×10^{15} ions/cm^2 at the beam currency of 0.1μA/cm^2 over a stainless steel mask with several kinds of patterns. Then, laminin (extracted from EHS tumors of mouse, Harbor Bio-Products, USA) or laminin pentapeptide (AnaSpec Inc. USA) was coated at the concentration of 1-10μg/mL for 2h at 37˚C. Then, human hepatocellular carcinoma cells, Hep G2, were cultured on the PLLA film for 2h in a CO_2-incubator. The irradiated layer was detached from the PLLA film with cells over them, and human embryonic fibroblasts HEL299 were inoculated and cultured for 1h in the CO_2-incubator.

RESULTS & DISCUSSION: Figure 1 shows the depth of the grooves prepared by detaching the irradiated layer. By changing the acceleration energy of He^+ ion beam, the depth can be controlled between 1.4-2.7 μm.

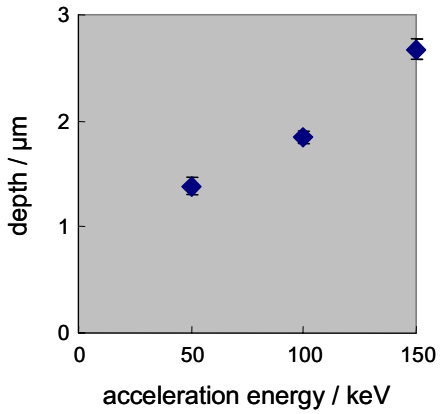

Fig. 1: Depth of the grooves fabricated on biodegradable polymer by ion beam irradiation.

An example of the images of cells after the detachment of the irradiated layer was shown in Figure 2 (a). Hep G2 was cultured in a pattern because the cells on the irradiated layer were removed. Then, HEL299 was inoculated over Hep G2 and cultured for 1d. An example of the images of co-cultured cells was shown in Figure 2 (b). HEL299 was mainly adhered to the detached area whereas Hep G2 still remained on unirradiated area.

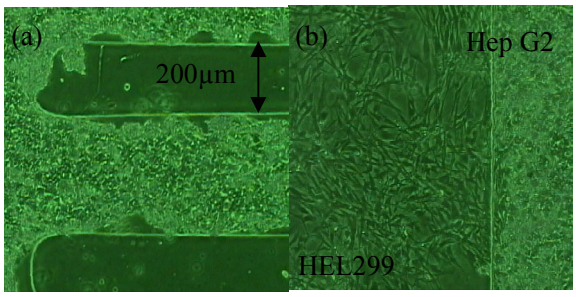

Fig. 2: Hep G2 cells cultured in a pattern(a) and patterned co-culture of Hep G2 and HEL299 cells (b).

CONCLUSIONS: As a conclusion, modification of PLLA surface with ion beam irradiation is effective to produce patterned co-culture system after the detachment of the irradiated area as a thin sheet.

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A NEW APPROACH TO MICRO AND NANO PATTERNING FOR CELL STUDIES AND PROTEOMICS APPLICATIONS

D.Falconnet, D.Pasqui, F.Assi, A.Koenig & M. Textor

Laboratory for Surface Science and Technology, Dept of Materials, ETH Zürich, Switzerland.

INTRODUCTION: Patterning of surfaces into bioadhesive and non-adhesive areas has attracted increasing interest over the past 10 years. The motivation for the production of such patterned surfaces ranges from basic investigations of cell adhesion, cell function and cell-cell interaction to cell based sensor development for drug testing. Designed scaffolds for tissue engineering also benefit from the development of these biologically designed surfaces. The common goal is to produce geometrically defined patterns that promote cell attachment in a background that is resistant to protein adsorption and cell interaction. A more recent topic of interest is the production of submicrometer patches containing proteins or bioactive ligands to study ligand-receptor activation and focal contact formation. Furthermore, in the area of microarray chips for sensing DNA/RNA (genomics) or proteins (proteomics), chemical patterns may be useful to better control spatial arrangement of recognition units and improve spot quality as well as signal to noise ratio.

METHODS: A simple photoresist lift-off process is exploited in conjunction with the spontaneous assembly of cationic poly(L-lysine)-*grafted*-poly(ethylene glycol) (PLL-*g*-PEG) onto negatively charged metal oxide surfaces. The process is termed molecular assembly patterning by lift-off (MAPL). A positive photoresist on a metal-oxide-coated substrate (e.g. niobium oxide) is developed resulting in a pattern of resist and bare metal oxide areas. Bio-functionalized (biotin or cell-adhesive peptide) PLL-*g*-PEG is adsorbed onto the pattern and immobilized at the bare metal oxide areas. The photoresist is lifted-off in an organic solvent without affecting the integrity of the adsorbed functionalized PLL-*g*-PEG monolayer. Subsequently the background is backfilled with protein- and cell-resistant PLL-*g*-PEG. Various functionalizations of the PEG chains are possible, in this study part of the PEG chains were modified either with biotin to specifically bind to streptavidin or with Arg-Gly-Asp for integrin mediated cell attachment. Pattern quality was investigated with AFM, XPS, ToF-SIMS and fluorescence microscopy.

Standard photolithography was used for structures ≥ 1 μm and nano-hot embossing for producing sub-micron features.

RESULTS: Patterns of constant quality can be achieved over several centimeters with excellent contrast as shown in Fig. 1.

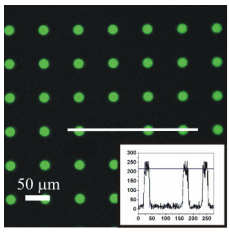

Fig. 1: PLL-g-PEG/PEG-biotin immobilized in the spots and PLL-g-PEG in the background. Fluorescent signal: alexa-488 labeled streptavidin. One spot has been bleached for comparison with the background fluorescence intensity.

The ligand surface density can be decreased by diluting with non-functionalized PLL-*g*-PEG. It was quantified through the measurement of the adsorbed polymer mass with the optical waveguide lightmode spectroscopy (OWLS) technique.

Exposing cells to patterns with different peptide densities is believed to be interesting for cell surface interactions studies. Pattern shape and area are also relevant parameters for a better understanding of cell mechanism.

MAPL was also successfully applied to produce nano-patterns of bioactive molecules (100 nm lines) in a non-fouling background.

DISCUSSION & CONCLUSIONS: MAPL is a simple technique for patterning interactive micro and nano structures in an inert background. When peptides are patterned cells attach to these ligands and not to the PLL-*g*-PEG background. A further potential application of MAPL are protein chips with high spot definition and homogeneity.

ACKNOWLEDGEMENTS: H. Schift, S. Park, C. Padeste, M. Horisberger for providing the nano-embossed sample and metal oxide coatings. S. Pasche and F. Durmaz for the different PLL-*g*-PEG.

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POLYANILINE/GOLD NANOPARTICLE FILMS: ASSEMBLY AND ELECTROCHEMICAL PROPERTIES

S. Tian, J. Liu, T. Zhu & W. Knoll

Max-Planck-Institute for Polymer Research, Ackermannweg 10, D-55128, Mainz, Germany.

INTRODUCTION: Recently, much research work concentrated on how to shift the redox activity of polyaniline (PANI) from acidic conditions to a neutral pH environment in order to apply it for bio-purpose. One approach is to introduce acidic groups into the PANI chain and to form a so-called "self-doped" PANI[1]. Another method is to dope PANI with negatively charged polyelectrolytes either by electrocopolymerization [2] or by the layer-by-layer(LBL) method [3]. All the obtained PANI films showed good redox activity at neutral pH and some of them have been successfully utilized to immobilize enzymes[4].

Here, we report another novel method to shift the redox activity of PANI to neutral conditions, i.e., to dope PANI with mercaptosuccinic-acid-capped gold nanoparticles(MSAG) instead of the normally used polyelectrolytes, by using the LBL self-assembly method. The obtained PANI/MSAG multilayer films showed excellent redox activity in neutral solution and good electrocatalytic efficiency toward the oxidation of NADH, as well as potential applications for biosensing.

METHODS: The preparation of water dispersible MSAG followed Kimura's method[5].The average particle size (TEM)was around 2 ± 0.5 nm. PANI (Aldrich, MW ~65000) can be made water soluble according to the procedure used by Rubner[6]. The LBL self-assembly process was carried out by first modifying the freshly prepared Au subtrate with 3-mercapto-1-propanesulfonic acid (MPS). Next, the modified Au substrate was alternately exposed to the PANI solution (1mM, pH2.6) and the MSAG solution (30µg/ml, in MilliQ water), each for 15min, with rinsing steps in between, until the desired number of layers was achieved.

RESULTS: The LBL self-assembly process was monitored in situ by both SPS and cyclic voltammetry(CV)(Fig.1). Both the gradual SPS minimum angle shifts and the almost linear peak current increase with the increase of the number of bilayers indicate a progressive deposition of PANI and MSAG layers in each cycle. Also from the cyclic voltammograms, it is clear that the PANI/MSAG multilayer films show very good redox activity in pH 7.1 PBS buffer. The films are very stable upon repeated potential scans. CV measurements at different scan rates showed that the redox process is surface-controlled.

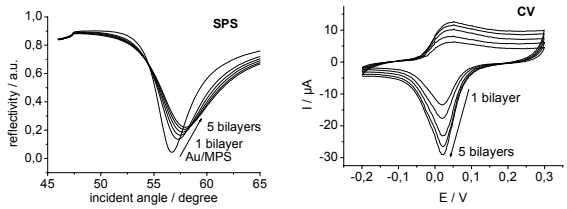

Fig. 1: SPS spectra and CVs of different bilayers of PANI/MSAG recorded in situ in 0.1M PBS buffer (pH 7.1). Scan rate was 50mV/s.

It has been shown that PANI doped by negatively charged polyelectrolytes can electrocatalyze the oxidation of NADH[2-4]. The electrocatalytic capability of PANI/MSAG films toward the oxidation of NADH was also clearly observed (Fig.2).The anodic catalytic peak current increases with the increase of NADH concentration. Further experiments show that the catalytic peak current increases almost linearly with the increase of the film thickness up to at least 12 bilayers.

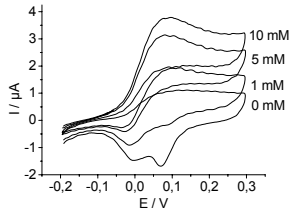

Fig. 2: CVs of 5 bilayers of PANI/MSAG measured in 0.1M PBS buffer,pH7.1 in the presence of different amounts of NADH, scan rate 5mV/s.

DISCUSSION & CONCLUSIONS: The stable and good redox activity of the prepared PANI/MSAG multilayer films in neutral pH solution suggests their use in bioengineering and biosensor development.

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METALLIZATION OF THE OUTER AND INNER SURFACES OF A PLANT VIRUS

A.M.Bittner[1], M.Knez[1], S.Balcı[1], A.Kadri[2], C.Wege[2], H.Jeske[2] & K.Kern[1]

[1]*Dept. of Nanoscale Science, MPI für Festkörperforschung, Stuttgart, Germany.*
[2]*Abt. Molekularbiologie und Virologie der Pflanzen, Universität Stuttgart, Germany.*

INTRODUCTION: Electroless deposition from metal ion solutions yields metal structures on and inside the Tobacco Mosaic Virus (TMV). Coatings, clusters and 3 nm wide nickel and cobalt wires were obtained. Adsorption, covalent binding and MicroContact Printing (μCP) of pure TMV were investigated with atomic force microscopy (AFM) for a range of crystalline oxide and metal surfaces.

METHODS: Electroless deposition is a process by which metal ions, e.g. Ni(II), in aqueous solution are reduced by molecules such as hypophosphite or borane. This process is catalyzed ("activated") by noble metal nanoclusters, e.g. Pd, and thus starts at nanocluster nuclei. This cluster is formed on an organic surface from a solution of the respective ion (e.g. Pd(II)); various interactions such as electrostatic binding and complex formation come into play.

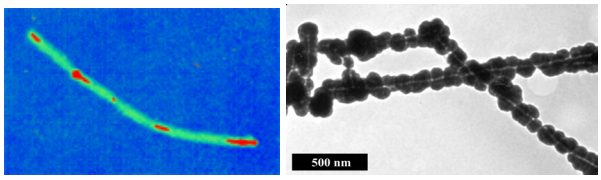

Fig. 1: Transmission electron micrographs of two tubular viruses (each 300 nm long) with five 3 nm Pd/Ni clusters in the inner channels. Right: Metallization of the exterior surface by Pt/Ni.

This "bottom-up" process of nanofabrication is ideally complemented by "top-down" μCP with a (sub)micrometer-patterned stamp: The stamp is inked with a virus suspension, and TMV is then transferred to a surface in the desired pattern. The adsorption properties of TMV were tested for a range of pH values and surfaces. Monitoring by AFM was conducted in noncontact mode.

RESULTS: The electroless deposition occurs selectively on the nanoclusters. In this way metal/metal core/shell clusters such as Pd/Ni were positioned on the tube-shaped virus particles (Fig. 1). The presence of hydrophilic groups, but also complex formation with Pd(II) and Pt(II) allowed producing clusters and wires even *inside* a nanotube, i.e. in the 4 nm wide inner viral channel (Fig. 1). With strong reductands, Ni and Co wires of only 3 nm diameter, but several 100 nm length formed (Fig. 2).

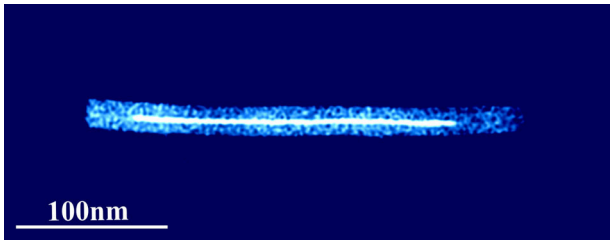

Fig. 2: Transmission electron micrograph of a 300 nm long virus with a 150 nm long and 3 nm wide Co wire (white) in the inner channel.

Adsorption and μCP (Fig. 3) showed that TMV can be immobilized by hydrogen bridges, or by covalent binding on reactive monolayers. On graphite it adsorbed only weakly by van der Waals interactions.

DISCUSSION & CONCLUSIONS: Bio-metallization of a viral nanotube is an elegant way to build nanostructures like aligned clusters and wires whose optical and magnetic properties are of great interest. A combination with spatially selective transfer by soft lithography should allow placing virus/cluster composites on a surface with nanometer precision.

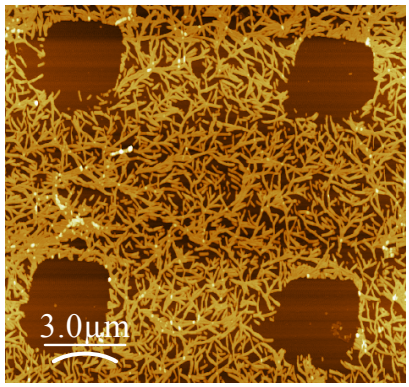

Fig. 3: Atomic force microscopy of a virus pattern (yellow) printed on a silicon wafer.

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SURFACE CHARACTERIZATION OF HYDROGEL-BASED MICROSPHERES

D.Sainz Vidal[1], M.Lekka[2], A.J.Kulik[3] & C.Wandrey[1]

[1]ISP-FBS-EPFL, Lausanne, Switzerland. [2]Institute of Nuclear Physics, Cracow, Poland.
[3]IGA-FBS-EPFL, Lausanne, Switzerland.

INTRODUCTION: Microspheres with alginate as the main component are widely used in pharmacy, biotechnology, and medicine. Surface characteristics including roughness, charge, and mechanical properties may have a significant impact for applications where biocompatibility is a crucial requirement. Indeed surface imaging of hydrogels, in particular if they are spherical, is complicated due to the high water content and the curvature. AFM provides the possibility to obtain three-dimensional images of surfaces within an aqueous environment, at high resolution. In addition to imaging, the elastic properties can be evaluated by analyzing force-distance curves (1). Recent study aims at comparing surface topography and elastic properties of alginate-based microbeads and multi-component microcapsules prepared from various components under different conditions.

METHODS: Microbeads were manufactured by atomizing 1.5 wt% sodium alginate (SA) Keltone HV (Kelco, San Diego, USA), in PBS, with a coaxial-air-flow encapsulator. The droplets were gelled during 3 min in a bath containing 1.5 wt% $BaCl_2$ and 0.9 wt% NaCl (20°C). After gelling, Ba-alginate beads were washed three times in 0.9 wt% NaCl. The beads were stored in their washing solutions at 4°C. Microcapsules were prepared from beads of 1.5 wt% SA/sodium cellulose sulphate (SCS), 1:1, gelled by reacting with 1.5 wt% $CaCl_2$ in 0.9 wt% NaCl (20°C) and subsequently transferred into 0.1 wt% poly(L-lysine) (PLL) or 1.2 wt% poly(methylene-co-guanidine) hydrochloride (PMCG) in 0.9 wt% NaCl (pH 7.4) to produce the capsule membrane (2). Capsules were rinsed three times with 0.9 wt% NaCl solution. Additional coating was performed using 0.15 wt% SCS (Acros Organics, Geel, Belgium) in 0.9 wt% NaCl during 10 min under gentle mixing.

A home built AFM (Institute of Nuclear Physics, Cracow) working in contact mode was employed. Measurements were performed with a standard "liquid cell" (NaCl 0.9%, at room temperature) and unsharpened Si_3N_4 cantilevers (Park Scientific Instruments, Switzerland). Their spring constant was 0.03 N/m for a tip radius of 50 nm. Roughness was calculated according to (3). Young's modulus was determined assuming a conical or parabolic shape for the AFM tip (4).

RESULTS: Surface scanning revealed remarkable differences of both surface roughness and Young's modulus. *Figure* 1 shows selected AFM images of two different types of microcapsules studied. (Note the difference in the gray scale.) The values of the average roughness and Young's modulus calculated for both microspheres and Ba-alginate microbeads are compared in *Table* 1.

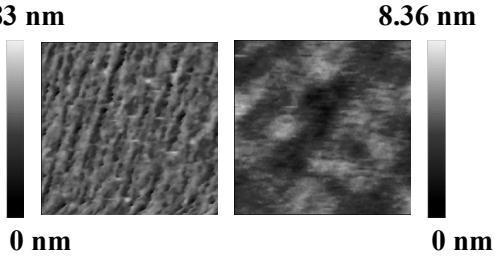

Fig. 1: AFM images of SA-based mocrosphere: SA/SCS//Ca//PMCG//SCS (left) and SA//Ca//PLL (right).

Table 1. Average roughness (AR) and Young's modulus (YM) of selected SA-based microspheres.

	AR (nm)	YM (kPa)
SA//Ca//PLL	1.18	1.04
SA//Ba	3.03	6.95
SA/SCS//Ca//PMCG//SCS	26.14	369

DISCUSSION & CONCLUSIONS: The study identified and quantified the influence of the components and preparation conditions on the morphology and elasticity of alginate-based microspheres and, therefore, the results may serve to adjust these parameters for specific applications.

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SELF-ASSEMBLING OF NANO-SIZED SiO_2-PARTICLES ON PATTERNED SUBSTRATES

Ch.Huwiler, F.Assi & M.Textor

Dept. of Materials, Laboratory for Surface Science and Technology, ETHZ, Zürich, Switzerland.

INTRODUCTION: The structuring of surfaces on a nanoscale level - both chemically and topographically - has become an increasingly relevant field of research in nanotechnology with widespread application potential in various fields of science (e.g. biotechnology, optics, electronics).[1] Among patterned surfaces, organized particle layers and single particles attached to a surface have attracted special attention due to their interesting properties and application potential.

A major aim of nanotechnology is to produce nanoscopic functional entities and to incorporate these features into devices with a structural hierarchy ranging from the molecular to the macroscopic level [3]. One way to achieve such hierarchic structures is the production of a pre-pattern with conventional methods in the micrometer range on which nanoscale objects (in this case SiO_2 colloids) are self-assembled by choosing appropriate adsorption conditions.

METHODS: In this work, two different patterning techniques are used to generate a chemical / wettability contrast pattern that directs particle adsorption. With these two patterning methods – called SMAP (Selective Molecular Assembly Patterning) [4] and MAPL (Molecular Assembly Patterning by Lift-Off) – any kind of 2D structures in the micrometer range can be produced and the colloid assemblies formed subsequently on them can be controlled by adjusting the adsorption parameters (i.e. ionic strength, pH, concentration). These two patterning methods allow the production of pure metal oxide contrasts as well as the production of chemical contrasts by adsorbing different chemical species (Self-assembled monolayers, polyelectrolytes, polymers). With these variable patterning methods, influence of different chemical backgrounds (alkane phosphate SAMs, polymers, polyelectrolytes) and inter-actions between colloids and surfaces can be investigated. Furthermore, possible modifications of these pattering methods (with nano-imprinting techniques) will allow the production of structures in the 100nm range.

RESULTS: Different sub-monolayer to multilayer colloid patterns with 35nm silica colloids have been achieved with the patterning methods described above. It was found, that particle-substrate interactions are generally weaker in the studied systems compared to the capillary forces occurring during the drying process. Thus, the most important effect that leads to the colloid pattern formation is the wettability contrast between the background and the pattern. Such a wettability contrast causes de-wetting of hydrophobic regions and the colloid suspension retreats to the hydrophilic regions draging colloids along due to capillary forces.

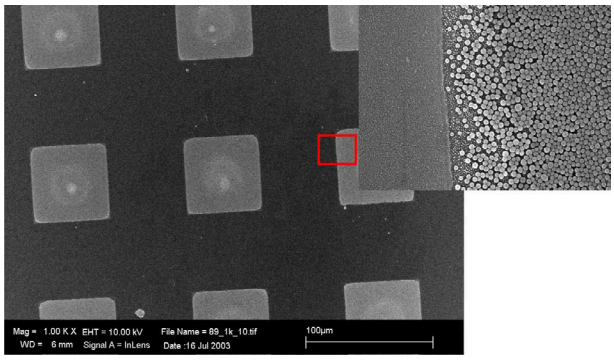

Fig. 1: SEM image of 60µm squares (SiO_2) covered with self-assembled SiO_2 particles (35nm). The TiO_2 background is modified with a hydrophobic alkane phosphate SAM and is particle free due to de-wetting during drying.

DISCUSSION & CONCLUSIONS: With the presented patterning systems it is possible to study drying processes and self-assembly properties of nano-colloids onto chemically customizable substrates and the influence of adsorption parameters on this process. Due to the ability to tailor the chemistry of the substrate and to vary the adsorption parameters, the nature of the self-assembled colloid structures can be controlled.

REFERENCES: [1]Masuda, Y., Itoh, M. et al. (2002). Langmuir **18**(10): 4155-4159. and references therein. [2]A. Blaaderen, R. Ruel, P. Wiltzius, Nature 385 (1997) 321. [3]Jonas, U. and C. Krüger (2003). Journal of Supramolecular Chemistry, in press. [4]Michel, R., J. W. Lussi, et al. (2002). Langmuir **18**(8): 3281-3287.

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Poster ID: 304

IMMOBILIZATION AND DETECTION OF FUNCTIONALIZED NANOCONTAINERS ON (PATTERNED) SURFACES.

F.F.Rossetti[1], I.Reviakine[2], G.Csucs[1], F.Assi[1], J.Vörös[1] & M.Textor[1]

[1]*Dept. of Materials, ETHZ, Zürich, Switzerland.*
[2]*Dept. of Chemical Engineering, University of Houston, TX, USA.*

INTRODUCTION: The focus of the project is to develop chips carrying immobilized native or artificial lipidic or block copolymer vesicles (termed "nanocontainers") for the rapid detection, identification, and manipulation of biological compounds. In a first stage, a general, one-step method for modifying surfaces of different types of vesicles was investigated. The method was based on coating negatively charged vesicles with functionalized Poly(L-Lysine)-*g*-poly(ethylene glycol) (PLL-*g*-PEG), a polyelectrolyte with a positively charged backbone which forms protein-resistant ("stealth" [1]) layers. Applications of this strategy to immobilized and free vesicles are summarized in this Poster.

METHODS:

- Quartz Crystal Microbalance with dissipation measurement (QCM-D, QE301/QAFC301 from Q-Sense AB, Göteborg, Sweden).
- Confocal Laser Scanning Microscope (CLSM 510 Carl Zeiss, Oberkochen, Germany).
- Atomic Force Microscopy (Nanoscope IIIa, DI, San Diego, CA). Measurements were performed in liquid, in contact mode.

RESULTS: Our approach for immobilization focussed mainly on the so-called "docking sites" technique [2], combined with the coating of vesicles with PLL-g-PEG.

The docking sites build-up starts with the coating of a metal oxide surface with biotinylated PLL-g-PEG and the subsequent binding of streptavidin to the biotinylated surface (Figure 1). Biotinylated polymeric and/or lipidic vesicles are then immobilized on the streptavidin layer.

Figure 1 illustrates the immobilization of negatively charged, biotinylated lipidic vesicles on docking sites and subsequent modification with PLL-g-PEG. The process was followed by Quartz Crystal Microbalance with Dissipation (QCM-D), which permits the layer-by-layer build up of the surface to be followed *in situ*. The docking sites technique is very versatile and can easily be combined with patterning methods such as Selective Molecular Assembly Patterning (SMAP) [3], for example for sensor applications.

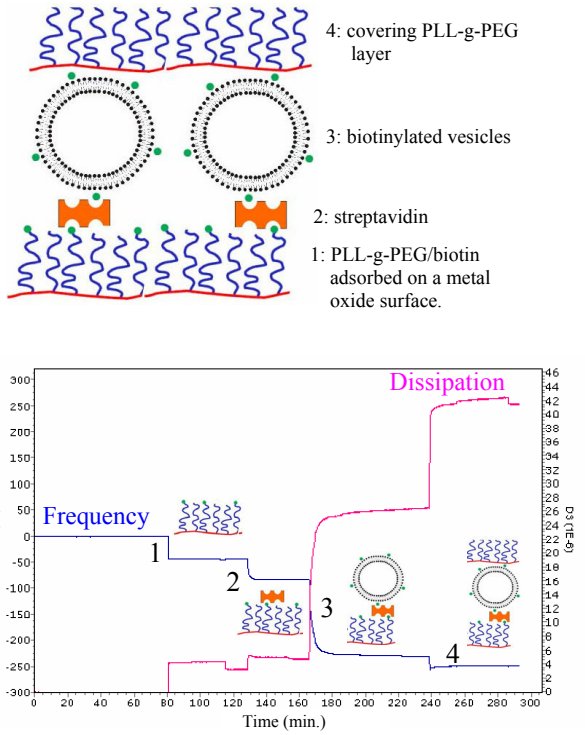

Fig. 1: Schematic diagram of biotinylated vesicles immobilized on docking sites and covered by a protecting PLL-g-PEG layer (top). The experiment was followed by Quartz Crystal Microbalance with Dissipation (bottom).

REFERENCES: [1]MC Woodle, DD Lasic, *Biochim. Biophis. Acta*, **1113**: 171-199 (1992). [2]N-P Huang, J Vörös, SM De Paul, M Textor, ND Spencer, *Langmuir* **18**(1): 220-230 (2002). [3]R Michel, JW Lussi, G Csucs, I Reviakine, G Danuser, B Ketterer, JA Hubbell, M Textor, ND Spencer, *Langmuir*, **18**(8), 3281-3287 (2002)

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FLUORESCENCE LIFETIME IMAGING ON A SURFACE IN UV RANGE: MODEL EXPERIMENTS

G.Ananchenko, Q.Li & S.Seeger

Physikalisch-Chemisches Institut, Universität Zürich, Zürich, Switzerland.

INTRODUCTION: Developments in fluorescence detection have made it possible to detect single molecules at room temperature in a solution or at interfaces with good signal-to-background ratios.[1] So far, dyes have been used for ultrasensitive detection by one-photon excitation (OPE) in a wavelength region from green to near-IR. Since a large number of biological species have intrinsic fluorescence when excited by the UV light, UV laser excitation is an attractive alternative that allows to avoid labeling of these compounds.

RESULTS: The setup for the single molecule detection in the UV region created in our group employs time-correlated single-photon counting microscopy using a pulsed mode-locked diode-pumped UV laser. In order to estimate sensitivity limits of the setup, several model objects with various fluorescence intensities at 266 nm excitation were chosen (Figure 1): *p*-terphenylacetic acid (**1**); β-galactosidase – a protein with high content of tryptophan; and commercial poly(2-vinylnaphthalene) (M_w~175000). In addition, samples (**2**) of poly(2-vinylnaphthalene) with various degrees of polymerization and bearing COOH end group were synthesized using atom transfer radical polymerization (ATRP)[2] aiming to control polydispersity and fluorescence properties of the polymer.

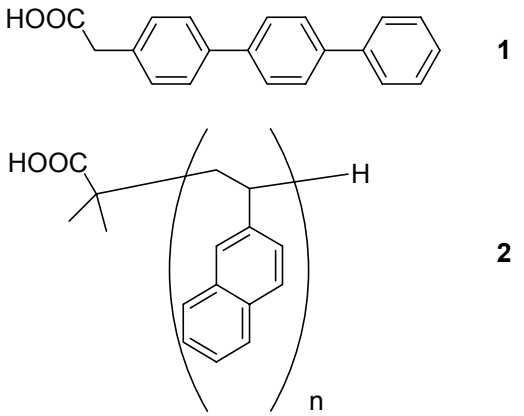

Fig. 1: Model compounds for FLIM at 266 nm excitation.

Two methods were used to deposit these compounds onto quartz glass slides: spin coating for all species and chemical deposition onto surface treated with aminopropyltriethoxysilane for compounds with COOH moiety.

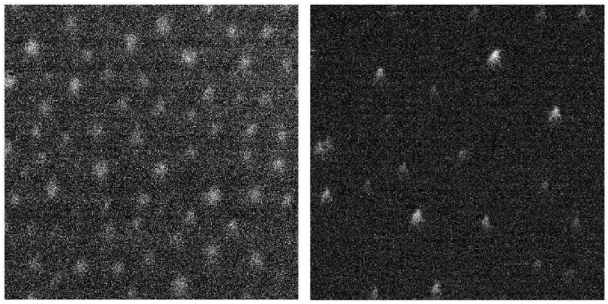

*Fig. 2: Fluorescence intensity images of p-terphenylacetic acid (**1**, 1/9 with isovaleric acid) after chemical deposition onto NH_2-functionalized quartz glass surface (left) and poly(2-vinylnaphthalene) (**2**, DP~50) after spin-coating deposition (right). Size of each image is 80×80 μm, optical resolution – 1 μm.*

A mixture of **1** and isovaleric acid was used to achieve spatial resolution of molecules of **1** (Figure 2, left). The spot size becomes larger at the fraction of **1** in the mixture higher than 10%. The maximum of photon counts exhibits similar values, the amount of bright spots decreases, and the photostability dramatically drops down at the fraction of **1** below 10%. We attribute the bright spots in the image to the fluorescence from single molecules of the [surface]amide derivative of **1**.

Poly(2-vinylnaphthalene) proved to be more stable against photobleaching than **1** and utilization of different samples of **2** with various chain lengths allowed to estimate the detection limit of the setup as >100 vinylnaphathalene units in the chain. Taking the similarity of fluorescence properties of tryptophan and ethylnaphathalene into account, one can conclude that similar sensitivity can take place upon detection of autofluorescence of tryptophan in proteins.

REFERENCES: [1]*New Trends in Fluorescence Spectroscopy: Applications to Chemical and Life Science* (2001) (eds. B. Valeur and J.-C. Brochon). Springer. [2]K. Matyjaszewski and J. Xia (2001) *Chem Rev* **101**:2921-90.

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THE USE OF X-RAY PHOTOELECTRON SPECTROSCOPY (XPS) IN THE CHARACTERIZATION OF THE BIOTRANSDUCER NANOSTRUCTURED INTERFACE.

A.Azioune[1], P.Louette[1], B.Lindner[2], A.Neubauer[2], S.Muyldermans[3] & J-J.Pireaux[1]

[1]Laboratoire Interdisciplinaire de Spectroscopie Electronique (LISE) – FuNDP 61, rue de Bruxelles B5000 Namur (Belgium). [2]TSP Nanoengineering, Hettenkofergasse 13/46 A-1160 Wien (Austria). [3]Department of Immunology, Parasitology and Ultrastructure, VUB, Brussels (Belgium).

INTRODUCTION: In this work, we show the use and the importance of X-ray photoelectron spectroscopy (XPS) in the characterization of a nanostructured biotransducer interface. Indeed, the gold substrate has been decorated by the highly nanostructured S-layer protein layer before the immobilization of the Camel antibody (cAb) which is the responsible of the prostate specific antigen (PSA) recognition. The homogeneity of the crystallized S-layer onto gold substrate has been checked before the immobilization of cAbs. In order to take a finger print of the immobilized cAbs, we have labelled it with iodine, one of the most sensitive element detectable by XPS.

METHODS: S-layer protein was crystallized onto IMEC gold substrates.

The cAb-PSA-N7 antibody was prepared at the institute of biotechnology at VUB (Brussels). A volume of this solution was labelled with cold iodine, according to the following protocol: 100 µl of the antibody was incubated with 4 µl of KI under stirring with a rate of 800 t/min, at RT during 1 h. The resulting yellow colour product was dialyzed three times in PBS during 90 minutes until it became uncoloured.

The S-layer/Au substrates were subsequently incubated at pH 4.75, during 1 h at RT with water soluble carbodiimide (EDC). Finally, different concentrations of cAb-PSA-N7 were incubated overnight with S-layer in PBS 10 mM, pH 9 and RT. The unbound proteins were removed by washing the samples three times with 2% of Tween 20 in PBS (150 mM), pH 7.4; three times with PBS 10 mM and three times with pure water. The plates were blown dried with a stream of pure argon, before analysis. The fresh samples were analyzed by XPS using an SSX100 spectrometer.The monochromatic Alkα X-ray source (1486.6 eV) was used. The X-ray spot size was 1000 and 300 µm for the acquisition of the survey and narrow scan regions, respectively.

RESULTS: Table 1 reports the measured composition of S-layer, expressed in atomic percent.

Table 1. Surface atomic composition of S-layer – coated IMEC gold substrate

Au	C	N	O	C/N	O/N
5.3	60.9	13.5	20.4	4.5	1.5

The C/N and O/N ratios are in good agreement with those calculated for the pure S-layer .These ratios may also be taken as a fingerprint of the complete S-layer to measure its lateral homogeneity and stability with time on the solid substrates. For this purpose, we have analyzed different positions of the samples using the facility of the XPS small-spot focalization. The thickness of the layer is about 3 nm as determined by ARXPS.

The immobilized cAb onto S-layer was studied through its iodine marker. The detection of the iodine was possible from 1 µg/ml initial concentration. The immobilization of cAb was also followed from the XPS survey spectra. Indeed, the shape of the background of gold spectrum (between the Au signal at 84 eV and the carbon one at 285 eV) changed when a "thick" layer is deposited. This is more clear at high cAb immobilized amount.

DISCUSSION & CONCLUSIONS: S-layer is very complete and homogeneous on the gold substrates; it is 3 nm thick; the detection of immobilized cAb through its iodine marker is possible at high initial concentration.

The mechanism of the interaction between S-layer and cAb may be obtained from the XPS survey spectra. cAb may be attached covalently via its amino groups with the carboxyl groups of S-layer which are exposed at the extreme surface; then, it may intercalate between the pores of the S-layer (since carboxyl groups exist also in the pores).

ACKNOWLEDGEMENTS: The work is performed with the financial support of the EU project "PAMELA"(IST-1999-13478).2000-2003. http://www.imec.be/PAMELA/

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SURFACE MODIFICATION OF PVC ENDOTRACHEAL TUBE SURFACES TO REDUCE PSEUDOMONAS AERUGINOSA ADHESION: PLASMA PROCESSING AND CHEMICAL METHODS

D.J.Balazs[1], K.Triandafillu[2], P.Wood[3], E.Sardella[4], H.Harms[2], C.van Delden[3], P.Favia[4], R.d'Agostino[4], C.Hollenstein[5] & H.J.Mathieu[1]

[1]Materials Science Institute, EPFL, Lausanne, Switzerland. [2]Institute of Environmental Science and Technology, EPFL, Lausanne, Switzerland. [3]Division of Infectious Diseases, HUG, Geneva, Switzerland. [4]Department of Chemistry, University of Bari, Bari, Italy. [5]Plasma Physics Research Center, EPFL, Lausanne, Switzerland.

INTRODUCTION: *Pseudomonas aeruginosa* is one of the most prevalent bacterial strains in a clinical environment, responsible for 30% of pneumonia cases occurring in intubated and mechanically ventilated patients [1]. Colonization of the intubation device leads to death for over 40% of these cases, despite aggressive antibiotic therapy. This paper presents the development of a strategy to reduce bacterial adhesion based on the surface modification of the polymer commonly used in endotracheal devices, medical grade poly(vinyl chloride) (PVC). The surface properties of biomaterials, such as chemistry, wettability, and morphology play an important role in their interaction with the surrounding environment and the success of their application. Several different surface modification strategies are at a scientist's disposition to alter the physicochemical properties of substrates and make them less attractive to proteins and bacteria. Various methods are evaluated in the scope of this paper. First, a oxygen (O_2) plasma treatment was used to alter the hydrophilicity of native PVC and form an anti-adhesive surface. Secondly, non-fouling surfaces were created through the physisorption of poly(ethylene oxide)-containing (PEO) co-polymers, Pluronics®, to a fluoropolymer film deposited on native PVC. Thirdly, silver-containing/PEO-like coatings were plasma-deposited in the effort to combine the non-fouling properties of PEO [2] and the well-known bacterial-toxic effects of silver [3]. Fourth, O_2 plasma treatments, followed by a wet chemical treatment in sodium hydroxide and silver nitrate solutions were used to incorporate monovalent silver as a germicidal agent.

METHODS: XPS analysis was used to investigate the chemical nature of the modifications. Surface wettability was determined by contact angle measurements of water sessile drops. Protein adhesion to the various surfaces was studied by incubating the samples in albumin and fibrinogen. Initial bacterial adhesion studies were completed using four different strains of *P. aeruginosa.*

DISCUSSION & CONCLUSIONS: O_2-plasma treatments provided a 70% reduction in bacterial adhesion as compared to native PVC [4]. The O_2-plasma-treated surfaces were prone to ageing effects and are not ideal for an anti-bacterial modification without further steps. The creation of a non-fouling surface by deposition of PEO-like coatings and the physisorption of PEO-containing copolymers, Pluronics®, was not sufficient to produce an effect on *P. aeruginosa* adhesion. Although the Pluronic® F127 and PEO-like surfaces were demonstrated to be non-fouling, they did not prevent bacterial adhesion. The PE-CVD deposition of Ag/PEO-like coatings and $NaOH/AgNO_3$ wet treatments were the only methods tested that completely prevented bacterial adhesion, due to the bacterial toxic effects of silver. Furthermore, the efficacy of the $NaOH/AgNO_3$ treatment to reduce biofilm formation over a 72h period was demonstrated as a 7-logarithmic drop in biofilm population at 24h and an 8-logarithmic reduction at 72h, compared to native PVC. It can be concluded that surface modifications that incorporate silver are the most promising of the methods explored in this paper, and do indeed create an anti-*P. aeruginosa* surface. Although a modification may create a non-fouling surface, this property is not sufficient to prevent bacterial adhesion, and a germicidal agent is necessary prevent colonization.

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ACKNOWLEDGEMENTS: The authors would like to thank Nicolas Xanthopoulos for his assistance. We greatly acknowledge the financial support of the Common Program on Biomedical Engineering and Research, Universities of Lausanne and Geneva and EPFL, (1999-2002).

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SURFACE MODIFICATION OF PORCINE HEART VALVE IMPLANTS FOR REENDOTHELIALISATION

B.Berchtold[1], B.J.Chang[1], P.Böhringer[1], O.Prucker[1], A.Wallrath[2], M.Dahm[2] & J.Rühe[1]

[1]*University of Freiburg, Institute for Microsystems Technology (IMTEK), Freiburg, Germany.*
[2]*Department of Cardiothoracic and Vascular Surgery, University Hospital Mainz, Germany.*

INTRODUCTION: Porcine heart valves are interesting as replacements for aortic valves because they exhibit good blood compatibility, i.e. low thrombogenicity. However, due to the need for mechanical strengthening of the collagen surface of these bioimplants by Glutaraldehyde crosslinking the surfaces become toxic and are now prone to calcification. This process finally leads to implant failure and reduces the life time of the valve to 7 - 10 years in an adult. It has been shown previously that the seeding of endothelial cells on the implant surfaces significantly reduces the calcium uptake [1]. But cell growth on the surfaces is rather difficult due to their toxicity. Our approach to make the collagen surface of porcine heart valves again attractive for endothelial cell growth is to "mask" the toxic groups by coverage of the surface with a thin polymer coating. These polymers contain benzophenone moieties as photo crosslinkable group and to covalently attach the layer to the tissue-surface.

RESULTS & DISCUSSION: In this paper we present a robust and versatile technique for the photochemical generation of surface-attached polymer networks on the surfaces of tissue material. The approach is based on a copolymer that contains one comonomer that carries a Benzophenone moiety. This group can be activated to form a biradical triplet state if illuminated with UV light (λ = 350nm). This triplet can react with almost any aliphatic C-H group to form a crosslink. The process is schematically depicted in Figure 1. If a polymeric or biological substrate is used the same reaction takes place between the BP-Groups and surface-bound C-H groups and, thus, the polymer network is also anchored to the surface. The process is schematically depicted in Figure 2. We will present data from investigations on the overall photochemical process along with results that aim at understanding of how such layers interact with model liquids that contain biological material that is also found in the blood stream. Furthermore we present preliminary results of cell seeding experiments on these surfaces.

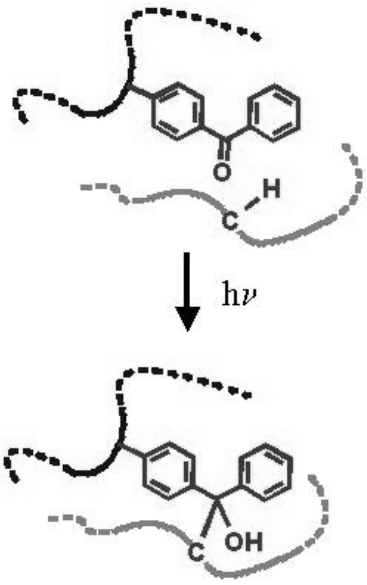

Fig. 1: Illustration of the photoinduced crosslinking of benzophenone containing polymers.

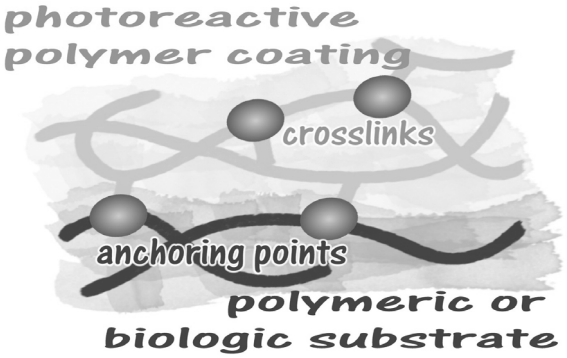

Fig. 2: Schematic illustration of the photochemical process used to generate surface attached polymer networks.

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MULTIPARAMETRIC OPTICAL ANALYSIS DEVELOPMENT FOR CONTROL OF BIOLOGICAL MICRO- AND NANO- PARTICLES

A.Bezrukova

Faculty of Medical Physics and Bioengineering, St.Petersburg State Polytechnic University, St.Petersburg, 195 251, Russia.

INTRODUCTION: Multiparametric analysis of optical data for biological and other three-dimensional (3D) disperse systems with nano- and micro- particles can provide further progress for characterization and "on-line" or "in-situ" control of such dispersions. Taking into account optical theory [1,2] and results of investigations [3,4] can help to elaborate sensing elements for the control of specific particles.

METHODS: 3D disperse system multiparametric optical characterization includes: a) the simultaneous measurements of dispersion by different compatible non-destructive optical methods such as refractometry, absorbency, fluorescence, light scattering (integral and differential, static and dynamic, unpolarized and polarized); and b) solution of inverse optical problem by different methods including modern technologies of data interpretation by information-statistical theory. For this purpose it is necessary to have information about optical properties of different 3D disperse systems.

RESULTS: Our research has investigated different 3D water dispersions for the Bank of Optical Data for Disperse Systems (BODDS) with nano- and micro-particles: proteins, nucleoproteins, lipoproteids, viruses, lipid emulsions, blood substitutes, latexes, liquid crystals, cells with various form and size (especially E.coli cells), metal powders, clays, kimberlite, zeolites, oils; and mixtures - proteins with nucleic acids, liposomes and viruses, liquid crystals with surfactants, mixtures of clay with cells, samples of natural waters, etc. [3,4]. Fig. 1 demonstrates the nanostructures of rotavirus (which look like influenza virus, coronavirus or virosome artificial structure). Rotavirus and E.coli cells can be considered as test-objects for natural water virus and bacteria contamination.

DISCUSSION & CONCLUSIONS: By optical methods it is possible to estimate mean diameter and number of particles, disperse phase mass and number of particles size distributions, form and inner structure of particles [1,2]. Due to the comparison of data from integral and differential light scattering it is possible to explain light scattering intensity changes at aggregation of such particles as lyposomes, latexes, viruses, etc. Mixtures can be considered as polycomponent and polymodal disperse systems (such as natural waters). Optical data indicate complex interactions between particles of different nature in mixtures. Electrokinetic data are evident in favor of an assumption that there is heteroaggregation in mixed dispersions. Due to the fusion of various optical data it is possible to solve the inverse physical problem by methods of information-statistical theory on the presence of impurities in mixtures (biological cells, viruses, oil, metallic particles, etc.) [4,5]. At this case the polymodality of particle size distributions is not an obstacle.

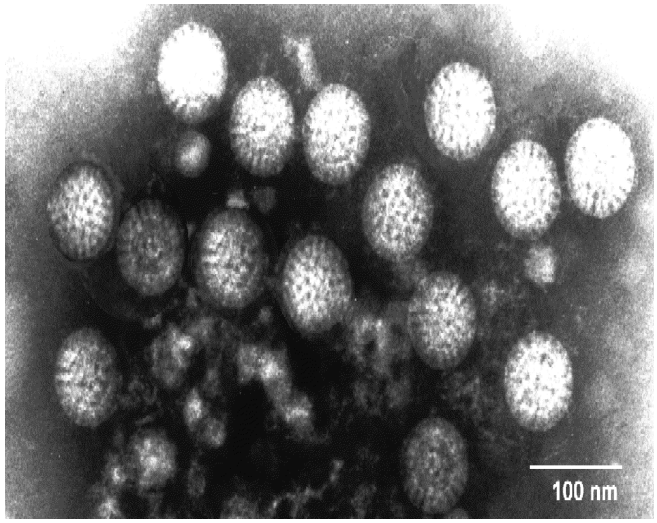

Fig. 1: Electron microscope photograph of Rotavirus.

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PROBING CELL MIGRATORY AND PROLIFERATIVE BEHAVIOR ON MICROPATTERNED FIBRONECTIN LANES

G.A.Cooksey[1], W.L.Little[1], A.T.Chang[1], P.D.Mourad[2,3] & V.Vogel[1]

[1]Dept. of Bioengineering, University of Washington, Seattle, USA. [2]Dept. of Neurological Surgery, University of Washington, Seattle, USA. [3]Applied Physics Lab, Seattle, USA.

INTRODUCTION: Cell migration plays fundamental roles in development, wound healing, immune response, and tumor invasion[1]. Migrating cells in particular must dynamically sense their extracellular environment for signals that could guide them to a target in the body, induce them to divide, or cause them to secrete and/or modify extracellular matrix or growth factors[2]. Migration is enabled by the attainment of a polarized cell shape, which results from coordinated binding to and release from adhesion molecules, a dynamic cytoskeleton, and constructive force production[1]. Our lab is using protein micropatterning to address mechanistic questions about how cell shape affects migratory behavior and how these behaviors are altered in transformed cell types.

METHODS: NIH 3T3 fibroblasts are maintained in CO_2-independent media supplemented with 10% fetal bovine serum and 1-3% Bovine serum albumin (BSA). Fibronectin (FN) protein adsorbed on an elastomeric stamp is transferred onto glass or into 6-well plates made of tissue culture polystyrene to create FN micropatterned lanes. BSA is adsorbed between the lanes to block nonspecific cell adhesion. Lanes with 14, 23, 35, and 65 um widths as well as flat (control) stamps have been fabricated. Timelapse videos are captured on an inverted-fluorescence microscope and analyzed using Metamorph and Matlab software.

RESULTS: NIH 3T3 cells preferentially adhered, elongated, and migrated on micropatterned lanes of fibronectin for greater than 24 hours (*Figure 1*). Cell aspect ratio, defined as the cell length divided by the cell width at the nucleus, significantly increased when lane widths decreased from 65 to 14 um. The average cell speeds on different lanes was not found to be significantly different. Inspection of single cell behavior reveals that speed may be maximum for cells with intermediate aspect ratios. Cell speeds have also been found to be dependent on FN density, with speeds increasing with decreasing absorbed FN densities for both patterned and unpatterned surfaces (from 100ug/ml to 0.1ug/ml). Furthermore, we find that upon division, cell migration speeds are increased for periods of several hours on all lane widths.

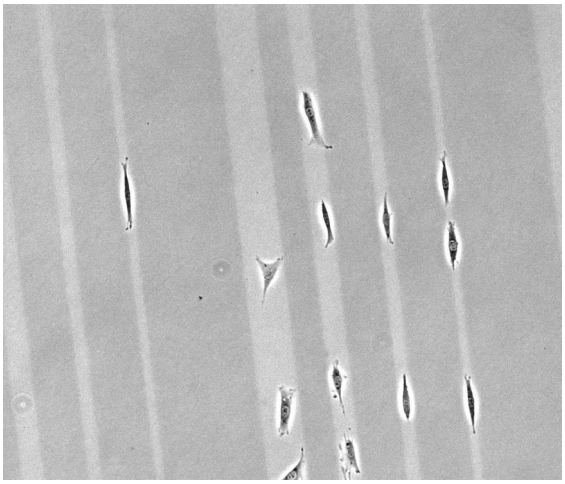

Fig. 1: NIH3T3 cells adhered for 8 hours to a gradient pattern of 65, 35, 23, and 14μm-wide fluorescently labeled FN lanes.

DISCUSSION & CONCLUSIONS: We have demonstrated that cell shape can be defined on micropatterned lanes of FN. Single-cell analysis techniques provide important insight into the complex relationship between cell shape and cell speed—it is likely that maximal speed is attained at an intermediate aspect ratio. Increased cell speeds observed following cell division are in agreement with increased cell speeds at lower FN densities. One study showed that cells selected to express fewer integrins behaved as normal cells but with speeds shifted to higher relative FN densities[3]. Presumably, when a cell divides, each daughter cell receives half the number of adhesion receptors, which makes the surface relatively less adhesive, thus allowing the cells to move faster on a surface that was previously "too sticky". In conclusion, our data show that migration speed is a complex function of cell shape that can be strongly influenced by cell division.

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SURFACE BIOENGINEERING FOR MICROANALYTICS AND BIOMATERIALS

H.Gao, S.Angeloni, F.Crevoisier, S.Guinchard & H.Sigrist

CSEM Centre Suisse d'Electronique et de Microtechnique SA, Neuchatel, Switzerland.

INTRODUCTION: Bioarrays – made of oligonucleotides, proteins or complex carbohydrates, viruses, cells or cell extracts - require the immobilization, preferably covalent, of differing molecular species, differing molecular structures or mixtures of different molecules on material- or transducer surfaces. Research and development in this interdisciplinary field lead to new products and create new markets.

A NOVEL TECHNOLOGY*: arrayon*® technology relies on the unique properties of the photolinker polymer OptoDex® to immobilize probe (bio-) molecules on material surfaces [1,2]. Light activation of OptoDex® leads to generation of highly reactive intermediates (carbenes) which form covalent bonds with biomolecules on any type of material. To attain this, probe (bio-) molecules are mixed with the photolinker polymer and applied to the surface. Subsequent activation with light leads to covalent irreversible binding of the probe molecules. This feature makes OptoDex® and its analogues versatile products for i) specific surface functionalization and ii) surface passivation. The technology sates fundamental requirements for the investigation of biological systems. It is applicable in genomics, proteomics, functional proteomics, metabolomics and cellomics.

APPLICATIONS: In one type of application, photolinker polymers are thin-film coated on blank platforms such as plain glass. Specific products are *arrayon*® PhotoChips: generic platforms for biochip manufacturing. PhotoChips are ready for microprinting of biomolecules. Immobilized biomolecules to date include proteins (allergens, antigens, enzymes, peptides), carbohydrates and nucleic acids. Photobonded enzymes remain catalytically active, allergens and antigens can be immunocomplexed, oligosaccharides respond to carbohydrate-specific lectins, and photobonded oligonucleotides hybridize with their complementary strands.

When used in a more specific way, photolinker polymers with specific chemical functional groups are immobilized on biochip platform resulting in *arrayon*® functional platform (Fig. 1).

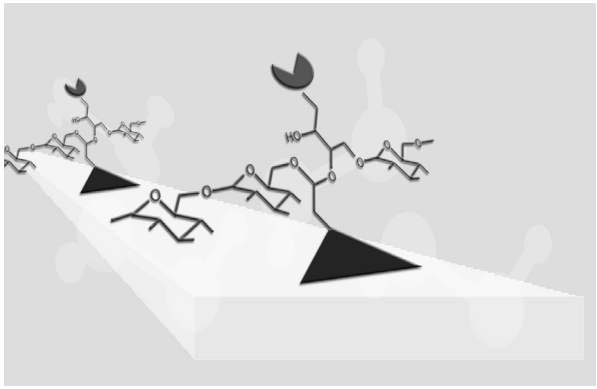

Fig. 1: Schematic representation of OptoDex® based functional platform. After photo-immobilization photolinker polymer provides secondary functional groups (amino-, carboxyl-, maleimido-, activated thiol- or biotin functions, lactose or fluorophores) that modulate the physical properties of the surface, and/or enable binding (covalent or affinity) of probe molecules.

OptoDex® is most beneficial for surface passivation [3] of any type of material. Passivation is a recommended or even mandatory treatment for surfaces that are prone to uncontrolled, heterogeneous or undesired physisorption. OptoDex® treatment unifies the surface properties of devices that are composed of different materials and renders them chemically uniform. Materials, bioengineered with *arrayon*® procedures withstand severe washing conditions and show low non-specific binding to ambient system components. This applies to *arrayon*® PhotoChips, *arrayon*® functional platforms, OptoDex® treated textiles and medical devices.

DISCUSSION & CONCLUSIONS: Exclusivity of the technology is challenged by a unique chemistry and its versatility. Wide-reaching applications open promising new markets, promoted by beneficial overall cost projections.

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NEURON GUIDANCE ON BIO-ELECTRONIC CHIPS

F.Greve[1], A.Hierlemann[1], H.Baltes[1] & H.Hall[2]

[1]*Physical Electronics Laboratory*, [2]*Institute for Biomedical Engineering*, *ETH-Zurich, Switzerland.*

INTRODUCTION: Two-dimensional electrode arrays fabricated using microelectronic circuit processing are an ideal tool for studying the electrical activities of neuronal networks [1]. Prior to measurements, neurons are placed onto the electrodes and cultured until a neuronal network is formed. Once the network is established, electrophysiological tests can be performed where specific neurons get electrically stimulated and extracellular potentials are subsequently recorded using the array of electrodes.

As a pre-requisite, neuronal cell growth on different surface layers of the microelectronic electrode array (silicon nitride and platinum) has to be characterized. Also, the influence of adhesion proteins such as laminin-1 and poly-L-lysine is important to analyze.

In this study, Dorsal Root Ganglia (DRGs) from chicken embryos have been used. It turned out that the neurite extension is essentially the same on silicon nitride and on platinum. However, differences occur using the two adhesion proteins.

METHODS: Figure 1 shows a schematic of the chip layout, which was used for electrical characterization. Platinum was used as measurement- (20 x 20 μm^2) and as reference electrodes, while silicon nitride (1000 nm) was deposited as insulation layer to cover the conducting lines between electrodes and bondpads [2].

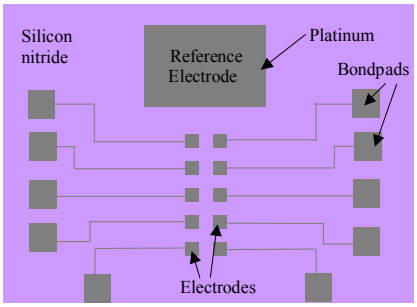

Fig. 1: Layout of the chip.

Biocompatibility of the chip surfaces was analyzed using either laminin-1 (20 µg/ml) or poly-L-lysine (PLL) (0.05%). The surface has been pre-treated with 30% H_2O_2 followed by sterilization under UV-light. DRGs were harvested from 10-day-old chicken embryos and cultured for up to 7 days in EAGLE's MEM 10% FCS, 5% chick serum and 100 ng/ml NGF.

RESULTS: Figure 2 shows a DRG placed at the border between a platinum reference electrode and the nitride passivation. The surface was coated with laminin-1 and after 4 days in culture, neurite growth was very similar on platinum and nitride surfaces.

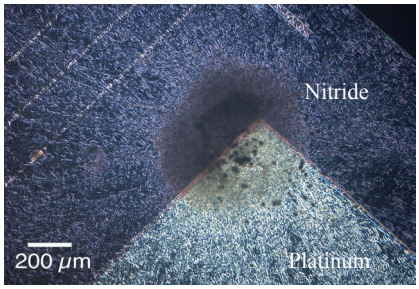

Fig. 2: DRGs on laminin-1 coated microchip surface (4 days culture).

Different proteins, however, seem to affect the neurite growth substantially (Fig. 3). Here, silicon nitride was coated with laminin-1 (left panel) and PLL (right panel). The DRG placed on laminin-1 spread well on the surface, while the DRG on PLL remained smaller in diameter and very densely packed. Neurite outgrowth was different as well, since neurites extended longer and more frequent on laminin-1, whereas they remain few and short on PLL. These results correspond well with results observed for DRGs cultured on laminin-1 and PLL absorbed to tissue culture polystyrene (not shown).

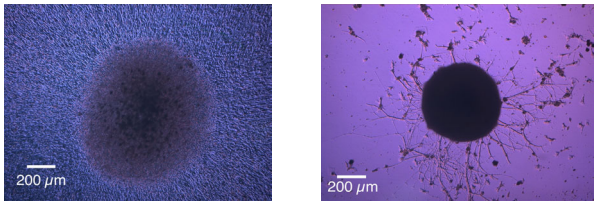

Fig. 3: DRG placed on silicon nitride, absorbed with laminin-1 (left) and PLL (right).

DISCUSSION & CONCLUSIONS: Laminin-1 provides a good adhesive substrate, however forms, because of its large molecular mass (800kDa), a thick protein layer. Therefore, electrical measurements could be impaired as the signals from the neurons are minute. Future aims are to find an adhesion layer as good as laminin-1, but having low molecular mass.

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SCANNING PROBE MICROSCOPY FOR BIOLOGICAL APPLICATIONS

D.Haft[1], O.Sqalli[1], T.Lindenberg[1], C.Bödefeld[1], K.Höfling[1], M.Vogel[2], C.Schulhauser[2], A.Högele[2] & K.Karrai[2]

[1]*attocube systems AG, Viktualienmarkt 3, 80331 München, Germany.*
[2]*Ludwig-Maximilians-Universität, Sektion Physik, Lehrstuhl für experimentelle Halbleiterphysik, Geschwister-Scholl-Platz 1, 80539 München, Germany.*

INTRODUCTION: Confocal microscopy and scanning probe microscopy have drawn considerable research interest in recent years since they allow the measurement of both the topography and the optical contrast of a sample with sub-wavelength resolution.

SCANNING PROBE MICROSCOPES: The instruments work by scanning a sub-wavelength sized probe in the near-field of a sample surface. The near-field probe acts simultaneously as a topographic sensor, which allows controlling the tip-sample distance, and as a nanometric optical aperture that records an optical signal. Scanning near-field optical microscopy requires a performant sensor to measure the tip-to-sample distance. In this letter, we report on a novel fiber based AFM[1] and a novel shear-force detection scheme[2] for scanning near-field optical microscopy applications. They are based on an all fiber lowcoherence interferometer. This setup makes it possible to measure a tip oscillation amplitude of less than 50 pm both in air and aqueous environment with a precision of 160 fm/Hz$^{1/2}$, thus demonstrating the ability to perform topographic measurements both in air and in liquids with a tipsample distance resolution better than 1 nm. Stable feedback in air and fluids is obtained with tipsample interaction forces below 1 pN.

CONFOCAL MICROSCOPES: Confocal microscopes work by scanning a tiny light spot on a sample and by measuring the scattered light in the illuminated area. Confocal imaging systems achieve out-of-focus rejection by two strategies: a) by illuminating a single point of the specimen at any one time with a focused beam, so that the illumination intensity drops off rapidly above and below the focus plane and b) by the use of blocking with a pinhole aperture in a conjugate focal plane to the specimen so that light emitted away from the point in the specimen being illuminated is blocked from reaching the detector. By scanning many thin sections through a sample, one can build up a very clean three-dimensional image of the sample. Confocal imaging can offer another advantage in favorable situations (small pinhole size, bright specimen): the resolution obtained can be better in comparison with the microscope operated conventionally (see fig. 1). Here we present a very compact and easy to use confocal microscope that is compatible with low temperatures, high magnetic fields and high vacuums. The stability of the microscope is higher than three months: indeed three months long spectroscopy measurements have been achieved on a single semiconductor quantum dot of about 10nm size[3].

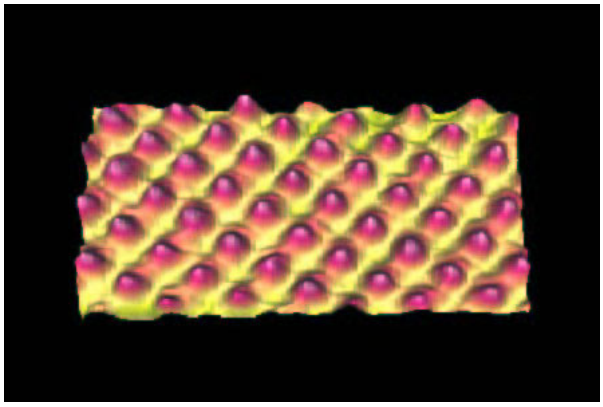

Fig. 1: Confocal image of a chess board with 1 micron of period.

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FIBRIN MATRICES FOR CELL TYPE SPECIFIC DIFFERENTATION

H.Hall & J.A.Hubbell

Institute for Biomedical Engineering and Department of Material Sciences, ETH and University of Zurich, Zurich, Switzerland.

INTRODUCTION: Fibrin clots get assembled at the surface of injured blood vessels and provide a provisional matrix for invading cells that induce the wound healing process. Finally they get proteolytically degraded and disappear. Our approach is to use modified fibrin matrices as 3D-scaffolds that induce cell type specific differentiation such as angiogenesis and nerve regeneration. Fibrin matrices become covalently modified by L1Ig6, a specific receptor for αvβ3-integrins that is expressed on human umbilical vein endothelial cells (HUVECs) and on chicken DRG- neurons. Both cell types are able to interact specifically with L1Ig6 (Hall et al., 2001; Yip and Siu, 2001) and form processes and myelinated axons, respectively. Modified fibrin matrices provide an interesting way to design a material that can be adsorbed to any surface shape, polymerize and provide a provisional guidance cue for specific tissue regeneration.

METHODS: HUVECs were purchased from PromoCell, Heidelberg, Germany and maintained under low serum conditions (2%) in the absence of additional growth factors. DRG neurons were dissected from E10 chicken embryos and cultured in Dulbecco's modified Eagle's medium containing glutamax-1, 10% fetal calf serum, 5% chick serum and 100 ng/ml 2.5S NGF (Sigma). DRG-neurons were induced to form myelin by 50µg/ml ascorbic acid. Both cell types were cultured in covalently L1Ig6-modified fibrin matrices (Hall et al., 2001), matrices filled with laminin-1 (Sigma) or native fibrin matrices. Process extension was determined after 8 days for HUVECs, and neurite length and myelinization of DRG-neurons after 14 days. Living cells were fluorescently labeled with fluorescein diacetate and the morphology of the cells was analyzed by confocal or phase contrast microscopy.

RESULTS: Cell-matrix interactions are responsible for cell-type specific morphology and/or differentiation. Both cell types extend cell type specific processes in L1Ig6-modified fibrin hydrogels. HUVECs form multi-cellular extensions that become interconnected. These processes have been described as first indications of angiogenic differentiation *in vitro* (Pepper et al., 1996).

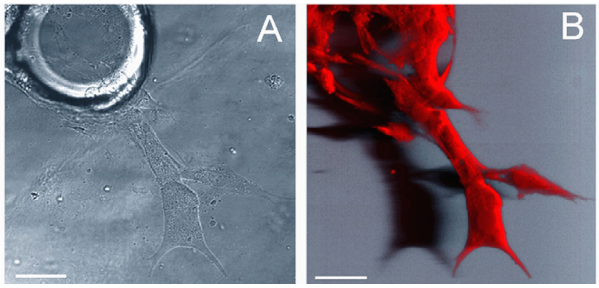

Fig. 1: HUVECs cultured on microcarrier beads in L1Ig6-modified fibrin hydrogels migrate and extend processes into the matrix. Processes were analyzed by phase contrast (A) or confocal microscopy (B). The scale bar represents 50µm.

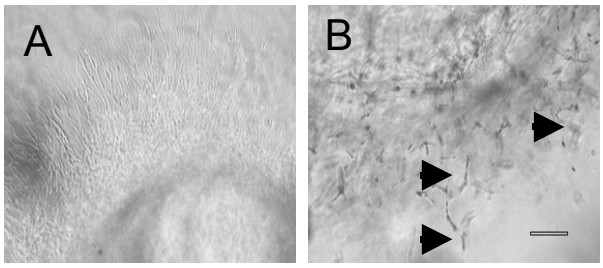

Fig. 2: Chick-DRG neurons in L1Ig6-modified fibrin matrices (A) and matrices containing soluble laminin-1 (B) extend neurites into the matrix and get myelinated (arrow heads). The scale bar represents 200µm.

DRG-neurons extend neurites and form networks in L1Ig6-modified or laminin-1 containing fibrin matrices that eventually get myelinated *in vitro*.

DISCUSSION & CONCLUSIONS: The fibrin matrix designed to interact specifically with αvβ3-integrins on angiogenic HUVEC and on neurite extending DRG-neurons is able to induce communication between the cell and the extracellular matrix that results in cell type specific differentiation. Therefore we think, that by using artificially designed matrices we are able to place a specific stimulus at the site of injury to induce specific tissue regeneration. Furthermore, it will be possible to use these matrices as guidance cues to direct cells towards their target organs.

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EFFECT OF DIFFERENT HYDROPHILIC POLYURETHANES ON CELL AND BACTERIAL ADHESION

L.G.Harris, K.Gorna, S.Gogolewski & R.G.Richards

AO Research Institute, Davos, Switzerland.

INTRODUCTION: Studying morphology and adhesion of cells can give an indication to the cytocompatibility of a surface and its suitability for possible further application for orthopaedic implants. In most anatomical areas, soft tissue adherence to implanted material is regarded as one sign of material compatibility and is also important for the prevention of infection. Cells adhere to substrates using special adhesive sites known as focal adhesions. *In vitro* cell adhesion has been used to predict 'implant surface-soft tissue' compatibility[1]. The morphology and adhesion of hTERT human fibroblast cells, and the adhesion of *Staphylococcus aureus* (SA) and *S. epidermidis* (SE) were studied on experimental polyurethanes (PUs) with different hydrophobic:hydrophilic (pho:phi) content ratios to determine their cytocompatibility.

METHODS: The surfaces studied were 3 different PUs with different pho:phi content ratios (100%, 70:30 and 30:70), PVC, both sides of Thermanox (controls) and poly(L-DL-lactide) 70/30% (control; results not shown). hTERT fibroblasts were cultured in DMEM with 10% FCS at 37ºC. Approximately 20,000 cells were seeded onto each surface for 48h before fixation or immunogold labelling. SA and SE were cultured on the surfaces in brain heart infusion broth (BHI) for 1h at 37°C prior to fixation or fluorescent labelling. For scanning electron microscopy (SEM) study, hTERT, SA and SE were fixed with 2.5% buffered glutaraldehyde for 5 min, post-stained with 1% buffered osmium tetroxide for 1h, dehydrated, critical point dried, coated with Au/Pd, and visualised with an SEM using a backscattered electron detector[2]. Immunogold labelling of vinculin was carried out as previously described, Richards *et al*.[3]. To quantify the amount of SA and SE adherence on the different surfaces, cultured bacteria were stained with fluorescent redox dye, 5-cyano,2-ditolyl tetrazolium chloride (CTC)[4] for 1h, and visualised with a Zeiss Axioplan 2 Epifluorescence microscope. The density of live bacteria adhering to the surface observed in each image were counted using KS400 software. All results were statistically analysed to determine whether the adherence of cells and bacteria on the different surfaces varied significantly.

RESULTS: hTERT cells showed the greatest degree of cell spreading and total cell area on the 70:30 and 30:70 pho:phi surfaces, and on both sides of the control Thermanox (Fig. 1).

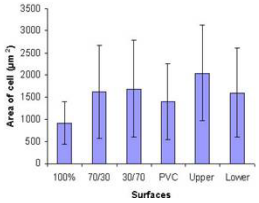

Fig. 1: Graph showing the results of average cell area on each surface.

Less cell spreading was observed on the 100% hydrophobic surface (Fig. 1), most cells were observed in a round state in comparison to flat cells on the 70:30 pho:phi surface (Fig. 2).

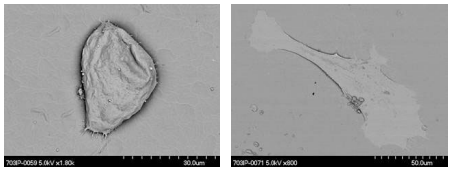

Fig. 2: SEM images of a cell on 100 % hydrophobic surface and on 70:30 hydrophobic/hydrophilic surface.

The adhesion of SA and SE varied depending on the surface, with results from the fluorescence labelling suggesting less SE adhesion than SA, whilst such differences were not observed with the SEM (Fig. 3).

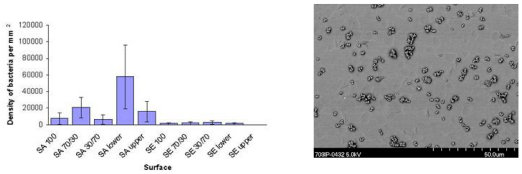

Fig. 3: Graph showing the average density of bacteria on each surface (SA – S. aureus; SE - S. epidermidis) and an SEM image of SA on the 100% hydrophobic surface.

DISCUSSION & CONCLUSIONS: Initial results have shown that hTERT cells adhere less to the 100% hydrophobic surface in comparison to the 70:30 and 30:70 pho:phi surfaces (Fig. 2). Results so far suggest, neither SA nor SE have a preference to any of the surfaces tested (Fig. 3).

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CMOS NEUROCHIP FOR STIMULATION AND RECORDING OF ELECTROGENIC CELLS

F.Heer[1], S.Hafizovic[1], W.Franks[1], A.Blau[2], C.Ziegler[2], S.Taschini[1], A.Hierlemann[1] & H.Baltes[1]

[1]Physical Electronics Laboratory, ETH Zurich, 8093 Zurich, Switzerland.
[2]Dep. of Physics, Kaiserslautern University of Technology, 67663 Kaiserslautern, Germany.

ABSTRACT: An array of platinum electrodes [Fig. 1] has been integrated in standard CMOS technology that allows each electrode to be simultaneously used for stimulation and recording. The microelectrode array exhibits a shifted-electrode design for improved electrode stability. The system architecture includes A/D converters for each row and a digital control unit that scans the array and provides a digital interface with the outside world.

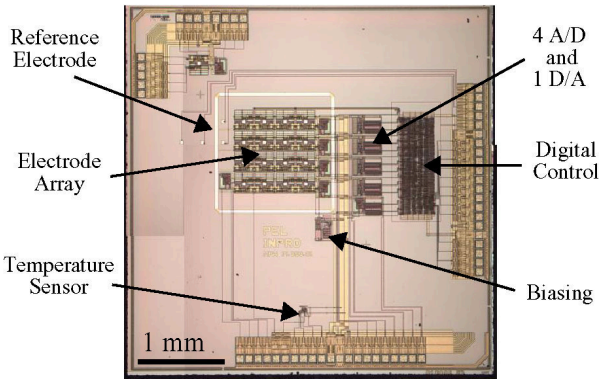

Fig. 1: Micrograph of CMOS Chip with main components.

SYSTEM ARCHITECTURE: The 4.4 x 4.4 mm^2 CMOS chip presented here consists of a 4-by-4 electrode array with an integrated reference electrode [Fig. 1]. Fabrication was performed using an industrial 0.6 µm CMOS process at Austriamicrosystems AG, Austria. The electrodes are 30 x 30 μm^2 with a 250-µm pitch.

Any electrode subset can be used for stimulation at any time, recording from all electrodes is possible during the measurement period. Stimulation and readout operations are controlled by the on-chip digital circuitry.

Each electrode is equipped with a band-pass filter for readout. The corner frequencies of the filter are specified at 100 Hz and 50 kHz, since neural signals have a frequency of about 1 kHz [1-3]. Extracellular neural signals are weak, up to hundreds of microvolts [1-3], therefore an overall gain of the filter of 1000 was used. Buffering of the signals is mandatory in going to larger arrays with several hundred electrodes because it allows for fast multiplexing, resulting in a high sampling frequency at a small number of A/D converters.

TRANSDUCER DESIGN: A simple 2-mask post-processing procedure is used to cover the metal of the CMOS process (aluminum) with biocompatible platinum. The transducer has been electrically characterized and the experimental results have been fitted to an equivalent circuit model presented in [1].

RESULTS: Operation of the stimulation circuitry is demonstrated by generating a square-wave stimulus on chip. The resulting signal on a selected electrode was measured using a probe tip [Fig. 2A]. The band-pass filter has been electrically characterized, using a gain-phase analyzer (HP-4194A), with results shown in [Fig. 2B]. The equivalent input noise of the filter is below 9 μV_{RMS} (100 Hz - 50 kHz) and was measured with a spectrum analyzer (HP-4195A). First tests were performed using heart cells from chicken embryos, the signals of which are shown in [Fig. 3].

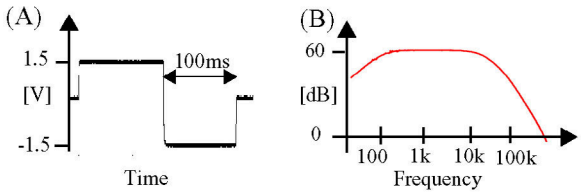

Fig. 2: Tests with on-chip generated stimulation signal (A). Transfer function of the band pass filter (B)

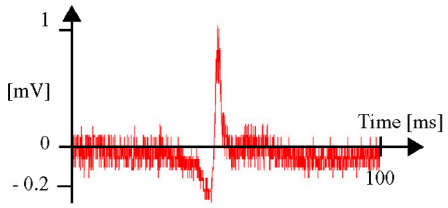

Fig. 3: Typical Spike from a chicken heart cell. Sampling frequency was set to 20 kHz.

CONCLUSION: A microelectrode array capable of simultaneous recording and stimulation of electrogenic cells has been presented. The functionality of the circuitry components has been verified with regard to the initial specifications. Mammalian neural signals are much smaller than the signals from chicken heart cells, therefore the performance of the measurement setup needs further improvement.

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URETHRAL TISSUE AUGMENTATION USING PRECIPITABLE POLYMERIC SOLUTIONS: AN EX VIVO STUDY

O.Jordan[1], A.Caviezel[2], E.Doelker[1] & C.Iselin[2]

[1]*School of Pharmacy, University of Geneva, Quai Ernest-Ansermet 30, 1211 Geneva, Switzerland.*
[2]*Urology Clinic, University Hospital, Rue Micheli-du-Crest 24, 1211 Geneva 14, Switzerland.*

INTRODUCTION: Stress urinary incontinence (SUI) is a highly prevalent disorder in women and also concern men after prostatectomy. The endoscopic injection of a bulking agent under the bladder mucosa is an attractive therapy for SUI. Although collagen has proven efficient, its effects are limited in time, spurring the search for long-lasting bulking agents. We propose to use solutions of preformed polymers in organic vehicles. Once injected in the tissue, an implant is formed following solvent exchange with water and polymer precipitation. Such implants are known for treatment of vascular malformations and drug delivery. They avoid *in situ* polymerization reactions and related toxic side effects, although the presence of an organic solvent may rise some concerns.
The goal of this study is (i) to show the feasibility of such implants for incontinence (ii) to investigate the interactions between implant and tissue *ex vivo*.

METHODS: The solvents used are pharmaceutical excipients: dimethyl sulfoxide (DMSO), n-methyl pyrrolidone (NMP), glycofurol 75, dimethyl isosorbide (DMI). We used acrylic, vinylic or cellulosic polymers. In a first phase, polymer solutions were assessed with respect to their ability to form a high bulk, comparable to a collagen control. The solutions were injected in fresh porcine urethra using an endoscopic needle. In addition, we required a stable bulk after 4 hours and a low backward flow following needle withdrawal. Implants elasticity was measured in compression and their microstructure examined by scanning electron microscopy.
In a second phase, selected implants and solvents were injected in urethras (n=3) and left 4 hours in a low potassium dextran solution designed for organ conservation. Hematoxylin-eosin histological sections of the urethras were then examined.

RESULTS: We selected 12 formulations producing an efficient bulking (bulk higher than 5 mm and stable over 4 hours) with no backward flowing. They were based on EMA-co-MMA, PMMA, cellulose acetate butyrate or ethylene vinyl alcohol copolymer. The implants have a microporous structure (Fig 1, left) and elasticity ranging from 7 to 42 kPa, comparable to the urethral muscle (21 kPa). The histological examination revealed implants well integrated into the urethra, and some swelling of subepithelial tissues with DMSO-based agents. However, using NMP, DMI or Glycofurol 75, preserved subepithelial tissue were seen (Fig. 1, right). The mucosal layer did not rupture in any case. Compared to saline injection (Fig. 2, left), slightly thinner mucosae were observed with DMI and DMSO (Fig. 2, right).

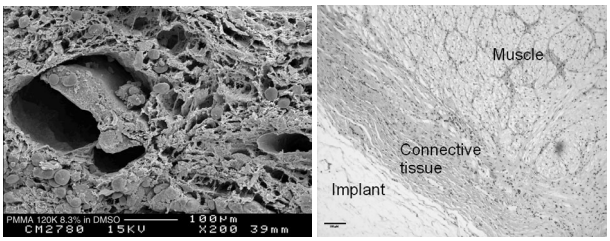

Fig 1: Porous microstructure of the implant shown by SEM (left) and integration into the urethral tissue of EMA-co-MMA in NMP (right).

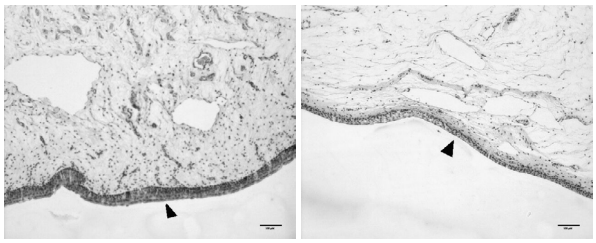

Fig 2: Mucosal layer histology (arrows). Left, saline control and right, implant EMA-co-MMA in DMSO. Scale bar 100 μm.

DISCUSSION & CONCLUSIONS: Bulking agents based on polymer precipitation may lead to long-lasting implants for an efficient therapy of SUI. The microporosity and elasticity are of interest for implant integration into the urethra. The organic solvents such as DMSO may be used to form tissue implants although some tissue swelling was observed; alternative pharmaceutical excipients may circumvent this problem. Ongoing *in vivo* experiments are performed to demonstrate these implants for the treatment of urinary incontinence.

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TIME AND REGIOSELECTIVE DRUG RELEASE BY TWO-PHOTON ABSORPTION FOR APPLICATION IN EYE DISEASE THERAPY

S.Kreiling[1], H.-C.Kim[1], N.Hampp[1,2] & A.Greiner[1,2]

[1]Department of Chemistry, University Marburg, D-35032 Marburg, Germany.
[2]Material Science Center, D-35032 Marburg, Germany.

INTRODUCTION: Cataract is world-wide one of the most widespread eye diseases. Its treatment is carried out by operative removal of the opaque human lens followed by implantation of a polymeric intraocular lens implant. In Germany approximately 400,000 intraocular lenses are implanted every year. Post capsule opacification (secondary cataract) is the major complication leading to reopacification due to the proliferation of lens epithelial cells at the implants surface. It is not desirable to add cell toxic drugs at the time of the implantation because wound healing is negatively affected. The goal of our research is the development of reservoirs for the postoperative, noninvasive medical treatment of post capsule opacification based on drug-loaded methacrylate-type polymers [1,3].

Controlled drug release is accomplished by photo-triggered cleavage of the covalently bound drug from the polymer. In this context we have investigated [2π+2π] cycloreversion reactions of cyclobutane-type photodimers which act as a photolabile linker between drug and polymer [2]. The photochemically induced cleavage is performed by two-photon absorption processes with help of 532 nm pulses of a conventional frequency-doubled Nd:YAG laser system.

Two-photon organic photocleavage reactions offer several advantages over their customary single-photon induced analogues. Using pairs of VIS photons rather than single UV photons confines the release to areas which cannot be directly optically adressed due to cover layers, i.e. the cornea, which have a high absorption at the single-photon absorption wavelength (Fig. 1). Moreover two-photon absorption processes are dependent on a spatial and temporal overlap of the incident photons. Therefore precise three-dimensional spatial as well as temporal control of the drug release can be achieved noninvasively.

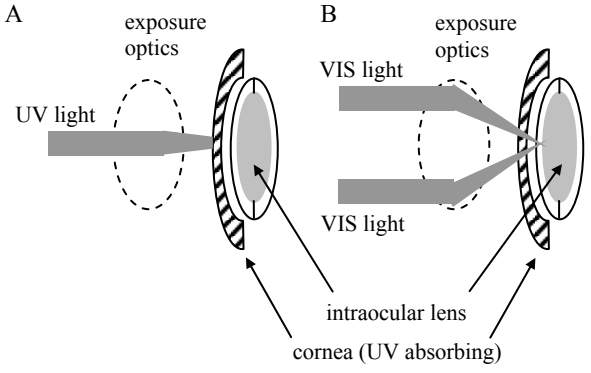

Fig. 1: Photochemically triggered drug release from a polymeric intraocular lens. (A) UV light is strongly absorbed by the cornea. (B) Two-photon absorption (TPA) of photons of the visible allows to induce drug release because in the visible the cornea has very low absorption.

RESULTS & CONCLUSIONS: The synthesis of the polymeric lens material was accomplished in only two steps. First, a methacrylate based copolymer modified with drug linking sites was prepared. In a second step drug molecules were photochemically loaded onto the copolymer.

Drug release from the copolymer was performed photochemically via single-photon excitation with UV light and via two-photon excitation with laser light of the visible. In both cases the photochemical deliberation of the drug from the copolymer was investigated by UV/VIS spectroscopy and HPLC analysis. Drug diffusion out of copolymer films was monitored after photochemical release.

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DNA-SIZING BY SURFACE SCANNING MICROSCOPY

S.Laib, M.Rankl, T.Ruckstuhl & S.Seeger

Institute for Physical Chemistry, University of Zürich, Zürich, Switzerland.

INTRODUCTION: The determination of DNA fragment sizes is of major importance in the investigation of genetic material. The presented work describes an approach to determine DNA-fragments on poly-l-lysine (PLL) coated glass coverslips [1]. Double stranded DNA-fragments were labeled with the intercalation dye TOTO-1-iodide stoichiometrically such that the amount of dye intercalated is proportional to the length of that fragment [2]. The fluorescence intensity of surface-bound DNA-TOTO-1 complex was measured by confocal scanning microscopy, revealing the brigthness of every individual fragment.

METHODS: At a DNA concentration of 10 ng/µl fragments of different lengths were stained separately with TOTO-1-iodide in MES buffer (50 mM 2-Morpholino-ethansulfonic acid monohydrate, 50 mM NaCl, pH 6) yielding an average bp:dye ratio of 1:1. 250 µl DNA solution diluted to a final concentration of 10^{-13} M was applied to PLL coated coverslips [3]. A surface area of 50×50 µm^2 was scanned by a confocal microscope with resolution of 640×640 pixels, which took less than 8 minutes.

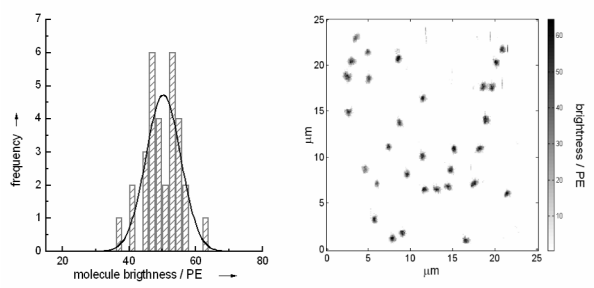

Fig. 1: Histogram and section of the scanning image of adsorbed pBR322 DNA fragments.

RESULTS: For calibration and examination of the proportionality of fragment length and brightness on single molecule level, DNA samples of defined sizes were measured.

Table 1: Calibration of the method with different DNA-fragment sizes

DNA-Fragment	Fragment size / bp	Centroid / PE	σ / PE	CV / %
PCR product	1 985	18.8±0.5	2.6	13.8
pUC 19	2 686	26.7±0.8	3.3	12.4
pBR 322	4 361	49.9±0.8	3.7	7.4
M13mp18 RF1	7 250	80.5±1.2	6.9	8.6
pHyR15AoriKncagGF	14 200	151.5±1.7	10.3	6.8

The obtained intensity distribution of the fragments was fitted with a Gaussian curve, resulting in average fragment intensity in photon electrons (PE) for each DNA-fragment (fig. 1). The results of the Gaussian fits are summarized in table 1. The coefficient of variation (CV) is defined to be the standard deviation of a distribution divided by its means. For different sizes the CV varied from 7% to 13%.

Subsequently, the obtained calibration was used for DNA sizing of different fragments between 2 000 and 14 000 base pairs (bp). An intensity histogram of five different fragment sizes is shown in figure 2.

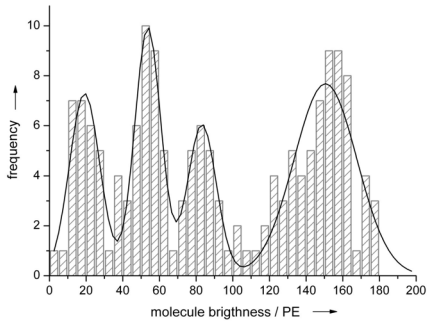

Fig. 2: Histogram of the molecule brightness from a mixture of different DNA-fragment sizes.

Fragments of 4.3, 7.2 and 14.2 kbp are clearly resolved with a CV of approximate 12%. Due to the CV's of table 1 we cannot expect to identify the fragments of 1.9 and 2.6 kbp in two separated peaks, although the broader peak (CV 25%) indicates the occurrence of more than one fragment.

DISCUSSION & CONCLUSIONS: The fluorescence based DNA-sizing technique is capable of determining fragments within an accuracy of typically 7-13%, which is comparable to data obtained by established methods and already sufficient for most applications. In comparison with gel electrophoresis, the new sizing method has high sensitivity concentration requiring several orders of magnitude lower amount of DNA. To ascertain the sizes of particular fragment, a quantity of 10^{-17} mol was sufficient. Additionally, the time exposure for the measurement is about ten times lower than for gel electrophoresis.

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LOCALIZED IMMOBILIZATION OF BIO-AFFINITY MOLECULES ON STATIC AND RESONANT CANTILEVER SENSOR ARRAYS

J.Lichtenberg & H.Baltes

Physical Electronics Laboratory, ETHZ, Zürich, Switzerland.

INTRODUCTION: The detection of biochemical analytes by mechanical interactions such as mass change or surface stress is highly sensitive. A typical sensor element is a micromachined silicon cantilever, which is coated with a bioaffinity probe and contains sensors to detect the mechanical response.

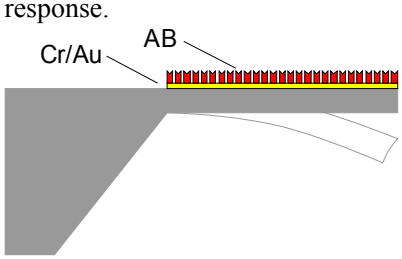

Fig. 1: A silicon cantilever with a single-side antibody coating for static measurements. When analyte molecules attach to the antibody layer, the resulting surface stress bends the cantilever.

We present a silicon-based microchip combining up to four of these cantilevers with dedicated, microelectronic circuits for signal read-out and device operation. Thiol chemistry is studied as possible candidate for surface immobilization. The focus of our research is directed towards applying concepts of microfluidics to achieve local confinement of the immobilization reagents. Thus, each cantilever can be coated with a different bioaffinity probe.

METHODS: Cantilever chips with integrated piezo-resistors for deflection measurement are fabricated in a commercial CMOS (complementary metal-oxide-semiconductor) process [1]. This is followed by back-end processing in-house to release the cantilevers (between 100 × 100 and 300 × 300 µm large and 7 µm thick, see Fig. 2). Finally, a thin chromium/gold (5/40 nm) layer is sputtered locally onto the cantilevers.

The microfluidic functionalisation tool is based on a precision-molded elastomeric holder made from poly(dimethylsiloxane) (PDMS). The holder aligns directly with the chip via special alignment guides. Antibody reagents are brought in contact with the cantilever surface via PEEK capillaries embedded in the PDMS to avoid protein adsorption to the siloxane. The antibody solution is confined between the capillary outlet and the cantilever surface by meniscus forces (Fig. 3).

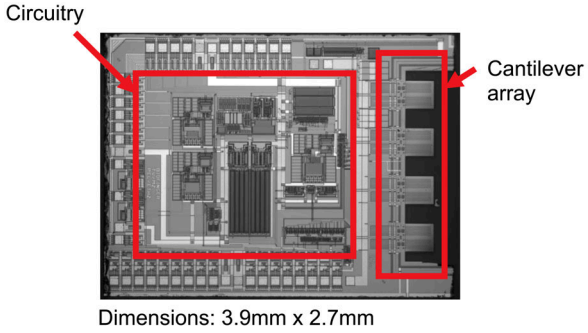

Fig.2: A CMOS-compatible cantilever array with four cantilever probes on the right. To the left, the signal processing circuit can be seen.

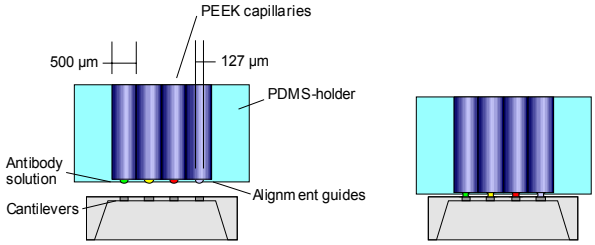

Fig.3: Four PEEK capillaries embedded in a PDMS holder are used to deposit the solutions.

RESULTS & CONCLUSION: The meniscus approach was studied by depositing fluorescently labeled bovine serum albumin (BSA) on flat, gold-coated chips. Deposited spots were clearly confined locally although the spot diameter was twice that of the capillary used (Fig. 4). However, this widening will not occur on cantilevers thanks to surface tension around their edges. The meniscus-confined deposition technique allowed the local functionalisation of a gold surface with very little reagent consumption.

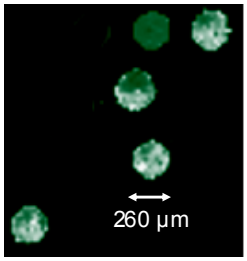

Fig. 4: Single spots of fluorescein-labeled BSA (100 µg/mL) in PBS, incubated for 10 min. Subsequent wash in PBS.

REFERENCES: [1]Y. Li et al. (2003) *IEEE Sensors Conference*, Vancouver, Canada.

ACKNOWLEDGEMENTS: The Biofinger project is funded by the Swiss Office of Education and Science.

European Cells and Materials Vol. 6. Suppl. 1, 2003 (page 100) ISSN 1473-2262
Poster ID: 418

FUNCTIONAL INVESTIGATION OF IMMOBILIZED G PROTEIN-COUPLED RECEPTORS

K.L.Martinez[1], D.Ott[2], B.H.Meyer[1], R.Hovius[1], A.Plückthun[2] & H.Vogel[1]

[1]Laboratory of Physical Chemistry of Polymers and Membranes, Swiss Federal Institute of Technology Lausanne (EPFL), CH-1015 Ecublens, Switzerland. [2]Institute of Biochemistry, Univerity of Zurich, Zurich, Switzerland.

External signals (light, hormones, odorants, drugs) interact with G protein-coupled receptors (GPCRs) and provoke the activation of intracellular heterotrimeric G-proteins. It triggers their dissociation and results in the production of second messengers, due to the interactions of both functional units with the respective enzymes or ion channels. GPCRs represent therefore a major target for therapeutic compounds, but also for molecules involved in smell and taste perception.

Here we present generic procedures for immobilization of GPCRs on glass surfaces in order to perform pharmacological and functional studies. We used an optical evanescent wave technique: Total Internal Reflection Fluorescence spectroscopy which offers a selective excitation of the fluorophores at surfaces and therefore on-line detection of molecular interactions occurring at the surface. Native membrane fragments containing the neurokinin-1 receptor protein, grafted with a biotin tag were immobilized to functionalized surfaces with streptavidin, whereas detergent solubilized Kappa-opioid receptor carrying affinity tags was immobilized to surfaces functionalized with antibodies. In both cases, the immobilized GPCRs did not show any alteration of their pharmacology features due to their immobilization.

Our approach delivered functionaly active GPCRs immobilized in a uniform orientation by using minute amounts of non purified membrane proteins in native or artificial environments. This will be of general interest for investigating the function of membrane proteins, but also for screening of potential therapeutic compounds.

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NANOSCALE STRUCTURE OF POLY(ETHYLENE GLYCOL) HYBRID BLOCK COPOLYMERS CONTAINING AMPHIPHILIC β-STRAND PEPTIDE SEQUENCES

A.Rösler[1], H.-A.Klok[2], H.Nuhn[2], I.W.Hamley[3], V.Castelletto[3] & O.O.Mykhaylyk[3]

[1]Max Planck Institute for Polymer Research, Mainz, Germany. [2]Institut des Matériaux, Laboratoire des Polymères, EPFL, Lausanne. [3]Department of Chemistry, University of Leeds.

INTRODUCTION: Peptides and proteins are receiving increasing attention as building blocks for the design of self-assembled nanostructured materials.[1] The interest in peptides and proteins is, at least to a large extent, driven by their ability to hierarchically self-organize into precisely defined nanostructures with an unpretended complexity and functionality. Although a lot of work has focused on pure peptides and proteins, only limited attention has been devoted to the selfassembly properties of conjugates of peptides or proteins and synthetic polymers. Attachment of well-defined peptide sequences to synthetic macromolecules may be a versatile strategy to generate novel polymeric materials with unprecedented nanoscale order.

METHODS: Polyethylene glycol (PEG)-peptide-PEG and peptide-PEG tri- and diblock copolymers were prepared by standard Fmoc solid-phase peptide synthesis (Fmoc SPPS) on a Tentagel PAP-resin.[2] The primary structure of the peptide was as follows: GELXELXQQKLKLKLKG.[3] In case of two of the three block copolymers the X, normally Gly, is replaced by Asp. Acylation of the N-terminal R-amino acid residue of the resin-bound peptide with a carboxylic acid end-functionalized monomethoxy-PEG (mPEG) derivative affords two triblock co-polymers. After cleavage from the resin, the crude block copolymers were purified by extensive dialysis. The final products were characterized by 1H NMR spectroscopy, MALDI-TOF mass spectrometry, and reversed-phase HPLC. The block copolymers were isolated in 7-20% yields with purities > 95% according to HPLC.

The nanoscale structure of the block copolymers was investigated by ATR-FTIR and small- and wide-angle X-ray scattering (SAXS/WAXS).

RESULTS & DISCUSSION: The investigation by ATR-FTIR show a strong amide I band at ~ 1620 cm^{-1} and a weaker band at ~ 1690 cm^{-1}. This suggests an antiparallel β-sheet conformation of the peptide chains.[4] The β-strand character is supported by the presence of the amide V band at ~ 700 cm^{-1} and an amide I band at ~ 1660 cm^{-1}, which could be considered as reverse turns.[5,6] The results for the three block copolymers differ a little, i.e. the molecule, where X is Glu, shows apparent a mixture of different structures.

The temperatur dependent X-ray scattering experiments suggest that at low temperatur the PEG crystallized in a monoclinic unit cell.[7] Above 45 °C, which is the melting point for PEG, X-ray scattering experiments suggested a lammelar phase separated structure composed of alternating peptide and PEG domains. Within the peptide domains, the scattering experiments reveal the presence of an anti-parallel β-strand structure.

CONCLUSIONS: In conclusion, the FTIR and X-ray scattering experiments indicate that the ability of peptide sequences to form hierarchically organized structures is retained upon conjugation to PEG. The amphiphilic β-strand sequences investigated in this contribution mediate self-assembly of the PEG-based di- and triblock copolymers in lamellar superstructures consisting of alternating PEG layers and peptide domains with a hierarchically organized β-strand internal structure. The structures formed by the three block copolymers are difficult to obtain with conventional block copolymers and illustrate the potential of combining biological structural motifs with synthetic polymers to prepare novel complex, self-assembled materials, with the ability to interface with biology.

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ACKNOWLEDGEMENTS: This work was financially supported the Bundesministerium für Bildung und Forschung (MaTech-Neue Materialien, 03N8641), and the Engineering and Physical Sciences Research Council U.K. (EPSRC, Grant GR/N22052).

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FUNCTIONALIZED PEG-BRUSH LAYER FOR CONTROLLING PROTEIN AND CELL INTERACTIONS

H.Otsuka[1], T.Satomi[1], J.H.Itadani[1], Y.Nagasaki[2], T.Okano[3], Y.Horiike[1] & K.Kataoka[4]

[1]Biomaterials Center, National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan. [2]Department of Materials Science, Tokyo University of Science, Chiba 278-8510, Japan. [3]Institute of Advanced Biomedical Engineering and Science (IABES), Tokyo Women's Medical University, Tokyo 162-8666, Japan. [4]Department of Materials Science and Engineering, Graduate School of Engineering, The University of Tokyo, Tokyo 113-8656, Japan.

INTRODUCTION: A poly(ethylene glycol) (PEG) coating has most widely been used to minimize non-specific fouling of the device surface with biocomponents, including plasma proteins. Although the PEG coating can be performed by a variety of methods, most of the PEG-coated surfaces so far reported possess no reactive group on the PEG chain end. To provide the further functionality on the PEG-coated surface, we designed a block copolymer having end-functionalized PEG as a hydrophilic segments. An AB-type block copolymer composed of α-acetal-PEG as the hydrophilic segment and polylactide (PLA) as the hydrophobic segment was synthesized, and utilized to construct the functionalized PEG layer on the device surface by simple coating [1,2]. In this paper, a PEG-brushed layer with a terminal aldehyde group was readily prepared which may have both non-fouling and ligand-binding properties, and thus the surface will show to support the growth of anchor dependent cells with ligand-specific manner.

On the other hand, because cellular adhesion and spreading is regulated by protein adsorption, patterning of proteins responsible for cellular adhesion leads to spatially directed cellular adhesion. Micropatterned PEGylated substrates with two-dimensional arrays of plasma-etched circular domains (ϕ100 μm) were prepared on silanized glass slide dishes. Here, a microarray of ten thousand (100x100) hepatocyte hetero-spheroids, underlaid with endothelial cells, was successfully constructed with a 100 μm spacing in an active area of 20x20 mm on micro-fabricated glass substrates coated with PEG-brushes. The spheroid array constructed here is highly useful as a platform to detect a wide variety of pharmacologically and toxicologically active compounds through a cellular physiological response.

METHODS: The glass substrates, which were cleaned by a Piranha etch, were placed in 2 % (v/v) ethanol solution of 3-(trimethoxysilyl)propyl methacrylate. The PEG-brushed layer was constructed on this silanized glass surface by the spin coating of toluene solution of PLA (4 % (w/v)), followed by the α-acetal-PEG-PLA (2 % (w/v)). Micropatterned PEGylated substrates with two-dimensional arrays of plasma-etched circular domains (ϕ100 μm) were prepared by plasma-etching through a metal mask pattern with circular holes. Bovine aortic endothelial cells (BAECs) were seeded onto the patterned surfaces with ϕ100 μm glass-circular domains that were edge-to-edge spaced in l100 μm intervals. Obviously, BAECs adhered only onto the circular domains exposing a glass substrate. Rat primary hepatocytes, suspended in a culture medium, were then applied to the patterned dishes with cultured endothelial cells selectively located in the circular domains.

RESULTS & DISCUSSION: As demonstrated in Introduction, inhibition of protein (bovine serum albumin) adsorption was achieved on PLA surfaces modified with α-acetal-PEG-PLA copolymers, depending on the PEG molecular weight. Furthermore, ligands including proteins, peptides and sugars can be immobilized to the distal end of these PEG chains utilizing aldehyde functionality, which converted from α-acetal groups, constructing substrates that recognize a specific molecule with a least non-specific adsorption of other components. We further showed that α-acetal-PEG-PLA modified surfaces, which are not adhesive for hepatocytes, can be converted into the selective substratum for hepatocytes by covalent linkage of a carbohydrate ligand (lactose) specific for the hepatocyte asialoglycoprotein receptor to the distal end of PEG chains.

On micropatterned PEGylated substrates, rat primary hepatocytes formed spheroids within 24 h only on the circular regions of existing endothelial cells, generating a 2D-arrayed structure of the hepatocyte spheroids (Fig. 1). Hepatocyte spheroids were characterized by an immuno-histochemical double staining method: these hepatocyte hetero-spheroids exhibited stereotypical polygonal morphology with distinct nuclei, and well-demarcated cell-cell borders for at least 3 weeks, expressing liver-specific proteins such as albumin.

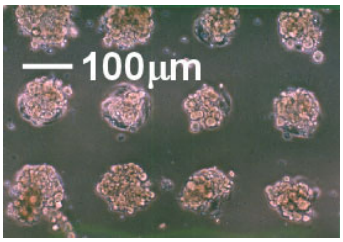

Fig. 1: Micro-array of ten thousand (100 x 100) hepatocyte hetero-spheroids prepared on ϕ100 μm circular glass domains with l100 μm spacing on 20 x 20 mm glass substrate coated with α-lactosyl-PEG/PLA.

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FOETAL RAT FIBROBLAST ADHESION ON BIOSTRUCTURED PVDF-SURFACES

J.Salber[1], Z.Ademovic[1], D.Klee[1], B.Hafemann[2], N.Pallua[2] & H.Höcker[1]

[1]Dept. of Textile and Macromolecular Chemistry, RWTH, Aachen, Germany. [2]Dept. of Plastic, Burn and Hand Surgery, University Hospital, RWTH, Aachen, Germany.

INTRODUCTION: Failure of the precise control of the physiological processes at the biomaterial-tissue interface leads to unfavorable host responses such as thrombus formation and undesirable inflammation. These responses promote the formation of fibrous capsules, which repel the surrounding functional tissue from the implant [1]. *Reparatio* instead of *restitutio ad integrum* is not only a problem of the surface biocompatibility of implants. Biomaterial research exhibit a growing need in new materials which combine necessary mechanical and physicochemical properties with improved biocompatibility in terms of structure mimicry and surface biocompatibility. The synthetic and non-resorbable homopolymer poly(vinylidene fluoride) PVDF has excellent mechanical and physicochemical properties for further technological processing. The development of the PVDF based *Aachen Keratoprothesis* and of the next generation of surgical meshes show impressively the importance of structure mimicry of the used polymer [2, 3]. Enhancement of the biomimetic character of the PVDF surfaces is achieved by their modification with different compounds of the basement membrane of the extracellular matrix [4, 5].

METHODS: PVDF SOLEF™ is used for manufacturing sheets, films, filaments and different textile structures [6]. Because of its chemical stability surface modifications of PVDF were performed by plasma induced graft copolymerisation of acrylic acid. The provided carboxy-functionalised PVDF-g-PAAc surface was used to immobilise type IV collagen (Biomol) covalently by the EDC/NHS strategy. All modification steps were verified by means of XPS, IR-ATR and confocal Raman microspectroscopy. Surface topography was studied by atomic force microscopy measurements (AFM). Foetal rat fibroblasts (FRF) were prepared from 18-day-old foetal Sprague-Dawley rats, cultured, and passaged serially. All cultures were maintained in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) foetal calf serum, 100 units/ml penicillin, and 100 µg/ml streptomycin.

RESULTS: Surface biocompatibility of implants or scaffolds is generally achieved by adsorptive or covalent immobilisation of ECM compounds as mentioned in literature. Little is published about the consideration or verification of the in vivo-typical tertiary or quaternary structure and thus the influence of their 3D-structure-based highest biological activity. Although this activity controls the quality of the interaction between the integrin receptors of the adhesive cells and the modified implant surface. Our approach is the reconstitutive refolding of type IV collagen on technical manufactured PVDF surfaces.

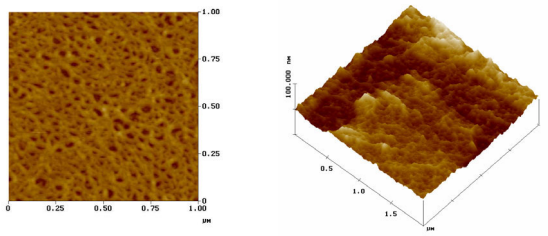

Fig. 1: AFM-images of type IV collagen: absorbed on a Si model surface (left), covalently immobilised on PVDF-g-PAAc (right).

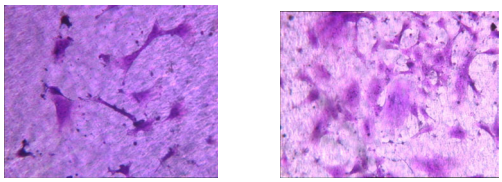

Fig. 2: FRFs response to PVDF-g-PAAc-Coll-IV$_c$:24 h (right) and 120 h (left).

DISCUSSION & CONCLUSIONS: Preliminary results suggest, that, concerning the viability and morphology of the FRFs, PVDF-g-PAAc-Coll-IV$_c$ is better suited for a tissue like cell adhesion compared to PVDF-g-PAAc-Coll-IV$_{ad}$.

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CHARACTERIZATION AND AGEING OF BRAIDED CARBON FIBER/PEEK BONE PLATES

T.Schambron[1,2] & A.Lowe[2]

[1]*Dept. of Materials, ETHZ, Zürich, Switzerlan.* [2]*Dept. of Engineering, ANU, Canberra, Australia.*

INTRODUCTION: Fractures in human limbs are often treated by internal fixation with stainless steel bone plates. Although these bone plates are strong and highly biocompatible, they have a very high resistance to bending (bending stiffness), because the elastic modulus of steel is 10 times that of bone. This higher bending stiffness leads to stress shielding, resulting in bone resorption, and potentially causing bone refracture upon plate removal. A bone plate with a lower bending stiffness, but a similar strength and biocompatibility to steel could potentially reduce stress shielding problems. One group of materials that match these criteria are polymer matrix composites, such as carbon fiber reinforced poly-ether-ether-ketone (CF/PEEK). However, the effects of body fluids on the mechanical properties of CF/PEEK are still relatively unknown and are thus investigated in this study.

METHODS: The CF/PEEK bone plates used for these experiments had the same dimensions as standard AO steel bone plates for the human shinbone, except for the thickness, which was 15 percent lower[1]. 9 specimens were aged in a 0.9% saline solution at 40°C (simulated body environment) for several days to a few months. The specimens were then mechanically tested in 3 and 4 point bending tests to measure bending stiffness and strength. Changes in weight, bending strength and bending stiffness were monitored, using both destructive (static and fatigue) and non-destructive bending tests. Scanning electron microscopy (SEM) was used to investigate failure mechanisms in destructive tests.

RESULTS: The specimen weight increased by around 0.25 weight percent during 12 weeks in saline solution at 40°C. Bending stiffness and strength in the aged specimens remained unchanged at around the pre-aged values of 0.85 Nm/deg and 10.2 Nm, respectively (Fig. 1). Fatigue testing showed that the fatiguing behavior remained unaffected by the ageing process. In addition, SEM images showed no different failure modes for different ageing conditions, indicating that no chemical changes occurred during the experiments.

DISCUSSION & CONCLUSIONS: Braided CF/PEEK shows a high chemical resistance to saline solution at body temperature. Both bending stiffness and strength remained unaffected during the ageing experiments, thus fulfilling one of the essential requirements for any implant material.

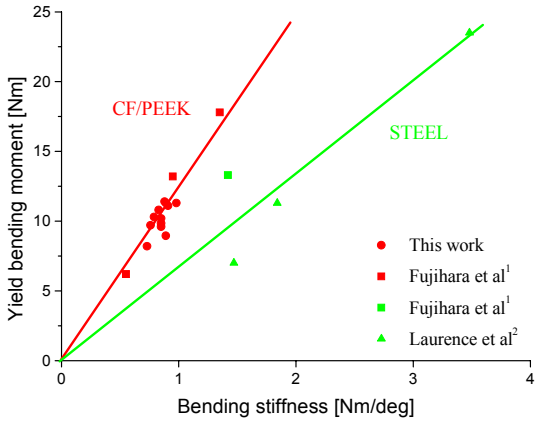

Fig. 1: Comparison of yield bending moment and bending stiffness of steel and CF/PEEK bone plates, showing that for any given bending strength, the stiffness of CF/PEEK bone plates is only half that of steel.

Results from this study show that the thinner braided CF/PEEK bone plates have a similar strength to steel bone plates but have half the bending stiffness. The lower stiffness should reduce stress shielding, while a thinner bone plate could make the implantation process easier. Thus braided CF/PEEK is a suitable material for constructing bone plates and should be further investigated for this application.

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IMPEDANCE OF ELECTRODES FOR STIMULATION AND RECORDING OF NATURAL NEURAL NETWORKS

I.Schenker[1], W.Franks[1], P.Schmutz[2], A.Hierlemann[1] & H.Baltes[1]

[1]*Physical Electronics Laboratory, ETHZ, Zurich, Switzerland.*
[2]*Institute of Materials Chemistry and Corrosion, ETHZ, Zurich, Switzerland.*

ABSTRACT: Electrochemical impedance spectroscopy was used to study the impedance of an electrode-electrolyte system. For the stimulation and recording of natural neural networks a low impedance is required; hence the need for a full impedance characterization. The electrode materials investigated were bright Pt, Pt-black and TiN; the electrolyte was 0.9% aqueous NaCl. An equivalent circuit model has been established. The effect of surface roughening has been investigated. Cyclic voltammetry was used to obtain the exchange current density of bright Pt.

MEASUREMENTS & RESULTS: The measurements were performed using a three electrode apparatus: calomel reference electrode and Pt counter electrode with a commercially available potentiostat (Autolab PGSTAT 30) and frequency response analyzer (ECO Chemie B.V., NL). Both Pt-black and TiN have dendritic surface structures, and are used here to explore the effect of surface roughness.

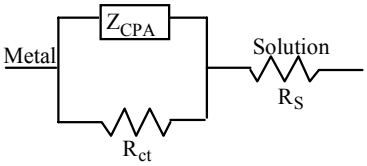

Fig. 1: Equivalent circuit model.

The equivalent circuit model, shown in Fig. 1, consists solution resistance R_s, a constant phase element Z_{CPS}, representing the interface capacitance, and the charge transfer resistance R_{ct} [1]. Fig. 2 and 3 show the modulus and the phase of the measured impedance of bright Pt, Pt-black and TiN electrodes and the modeled values.

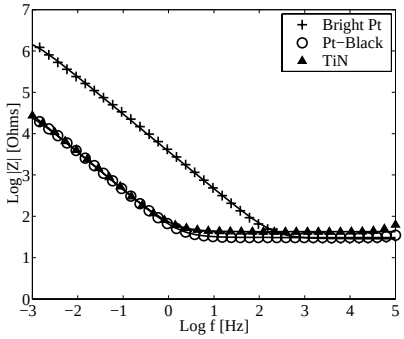

Fig. 2: Impedance modulus experimental results for 1 cm² bright Pt, Pt-black and TiN electrodes. Modeled results given by solid lines.

The exchange current density, J_0, of Pt was measured using cyclic voltammetry and a value of 42.5 μA/cm² was obtained.

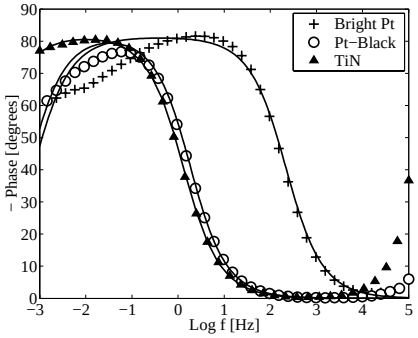

Fig. 3: Phase experimental results for the 1 cm² bright Pt, Pt-black and TiN electrodes. Modeled results given by solid lines.

DISCUSSION & CONCLUSION: The close fit of the modeled and experimental results indicates that a model has been experimentally verified, and can therefore be used to optimize future electrode designs. For frequencies below 2 Hz the impedance modulus for Pt-black is 65 ± 6 times lower than for bright Pt; similar results were obtained for TiN. Thus, as expected, roughening the electrode surface results in a lower impedance modulus. The measured exchange current density of 42.5 μA/cm² is closer to that of the O_2 reaction (J_o = 4.5 μA/cm²) than to that of the H_2 reaction (J_0 = 794 μA/cm²) [2]. In fact, the H_2 reaction does not take place given the open circuit potential of 0.35 V [3]. This finding leads to the omission of a Warburg impedance element in the model over the measurement frequency range used here.

REFERENCES: [1]W. Franks, et al (2003) *Transducers'03*: 963 – 966. [2]D.A. Stenger, et al (1994) *Enabling Technologies for Cultured Neural Networks*, Academic Press. [3]M. Pourbaix (1974) *Atlas of Electrochemical Equilibria in Aqueous Solutions*, Nat. Assoc. of Corrosion Engineers.

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INCREASED CELL MEMBRANE SENSITIVITY TO SHEAR FORCE RESULTING FROM ADHERENCE ON AN ANISOTROPIC POLYMER SURFACE

J.Lau[1], P.Cooke[2] & R.Tchao[1]

[1]University of the Sciences in Philadelphia, Philadelphia, PA 19104 USA. [2]The United States Department of Agriculture, ARS, ERRC, Wyndmoor, PA USA 19038.

INTRODUCTION: We have previously reported that squamous epithelial cells, such as NBT II derived from a rat bladder tumor; show sensitivity to irregularities of plastic tissue culture flasks when grown in serum-free (SF) media.[1] On areas of the flask radiating from the neck in a V-shape, cells attach and grow. However, when these cells are subjected to a fluid shear force generated by the flow of warm (37°C) SF media, some cells grown in this region immediately rupture. Coating the surface of the flask with serum or extracting the surface with 100% ethanol did not prevent cell death. Furthermore, a similar phenomenon was seen with NBT II cells grown on collagen. Conversely, cells do not rupture under fluid shear on amorphous substratum, such as glass. We hypothesize that the organization of the polymer molecules may affect the cohesiveness of the cell membrane by either interacting directly with the lipid cell membrane or by altering the cell adhesion process and the cell cytoskeleton.

METHODS: Our experimental model developed to further study this phenomenon is as follows: We cast thin plastic discs onto clean glass slides by slowly drying a solution of 20% pure, atactic polystyrene in chloroform overnight, sealing with clear silicone glue, and sterilizing with 90% ethanol. After NBT II cells adhere to the plastic surface, cells are sheared with warm media and observed under phase microscopy and scanning electron microscopy (SEM). Interestingly, cell rupture occurs mostly at the peripheral ~1mm zone of the plastic disc and rarely in the center.

RESULTS: When NBT II cells were observed under phase microscopy and SEM prior to applied shear force, there was no morphological difference between cells grown on the edge and the center of the plastic. However, after attached cells were sheared with warm media, most cells at the edge of the plastic disc ruptured while cells in the center of the disc remained alive as shown in the following scanning electron micrographs. (Figure 1) Dead cells were also easily stained with propidium iodide. Using polarized light, we demonstrated that optical birefringence occurred only at the edge of the plastic disc. This suggests a more orderly arrangement of polymer molecules.

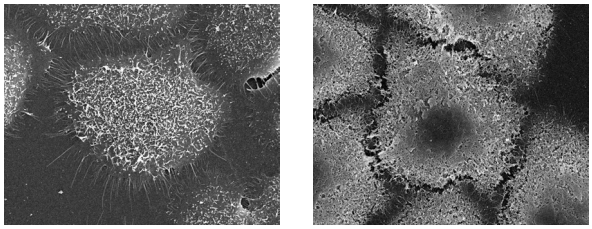

Fig. 1: Live (left) and dead (right) cells on the center and edge of the plastic disc, respectively, after a warm fluid shear force.

DISCUSSION & CONCLUSIONS: Our studies show the cellular membrane of NBT II cells grown in SF media have an increased sensitivity to rupture at the edge of a plastic disc. Cells grown in serum media were protected from rupture. However, a coat of serum on the plastic surface did not prevent this effect indicating that serum in media may only act as a surfactant on the cell membrane. Since this effect is absent on a glass surface, we speculate there may be differences in the molecular organization of polymers on certain areas of the plastic. Optical birefringence seen around the edge of the plastic disc, where cell death occurs, indicates a more orderly molecular arrangement. The anisotropic state of the polymer may cause differential interaction with the cell membrane or cell adhesion proteins. This may lead to altered cytoskeletal structure and membrane integrity. Currently, we are examining the f-actin arrangement of cells on the edge vs. center of the plastic disc using confocal microscopy.

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ACKNOWLEDGEMENTS: Special thanks to the United States Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center for use of the SEM and confocal microscope.

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HIGHLY ELECTRICALLY INSULATING TETHERED LIPID BILAYERS FOR PROBING THE FUNCTION OF ION CHANNEL PROTEINS

S.Terrettaz, M.Mayer & H.Vogel

Institute of Biomolecular Sciences, Swiss Federal Institute of Technology Lausanne, CH-1015 Lausanne, Switzerland.

INTRODUCTION: A method is presented to form gold electrode-tethered lipid bilayers with exceptionally high electrical resistances. Electrical impedance spectroscopy is used to monitor the bilayer incorporation of a ligand-gated ion channel protein and the modulation of its channel activity by the selective binding of an antibody. Due to the low defect density of the tethered membrane, the effect of a few channels can be resolved thus opening the way to single-channel experiments on this highly stable and versatile platform. In turn a minute quantity of analyte, here antibodies, can be measured which is of great interest for bioanalytics.

RESULTS: We synthesized a new thiolipid consisting of a single phytanoic acid coupled to a hydrophilic spacer which is terminated by a thiol group.[1] This thiolipid has a smaller cross-section than phospholipids and therefore is expected to form a first monolayer with a higher lipid density on a solid support. Values above 2 x 10^8 Ω have been reached for the newly reported bilayer membranes, which translate into an unprecedented membrane resistance of 7 MΩcm^2.[1] For comparison, the electrical resistances of BLM are around 10 MΩcm^2.

A synthetic ligand-gated ion channel SLIC comprising four channel-forming amphipathic membrane-spanning α-helices each connected to a $(NANP)_3$ sequence has been shown to bind the monoclonal antibody Sp3E9 with high affinity and selectivity in a former study.[2] SLIC inserted into a preformed highly insulating tethered lipid bilayer at low concentration from the aqueous phase, in analogy to protocols used in classical BLM experiments. The incorporation of SLIC in a tethered lipid bilayer is reflected as a drop in membrane resistance measured on the real part of the impedance (Fig.1). The decrease of the membrane resistance after incorporation of SLIC in tethered lipid membranes can be explained by the presence of only about 200 open SLIC channels of 90 pS conductance. Selective antibody binding to SLIC in the lipid bilayer increased the membrane resistance as a function of the concentration of the antibody Sp3E9 in the aqueous phase. The observed response corresponds to a total closure of 95 SLIC channels of 90 pS conductance. With the present signal-to-noise ratio the closure of a few individual channels could be detected.

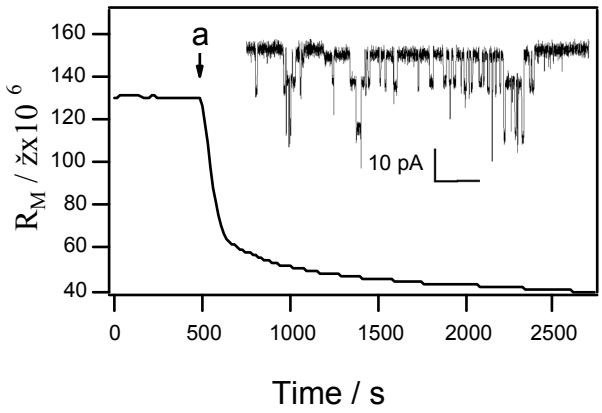

Fig.1: Electrical resistance of a tethered lipid bilayer after the addition of 7 x 10^{-7} M (a) of SLIC to the aqueous phase. Single channel recordings on BLM were done at -30 mV in 1M NaCl, 10mM Tris-HCl, pH 7.4. The single channel events shown correspond to a conductance level of up to 90 pS at buffer conditions used for impedance spectroscopy.

DISCUSSION & CONCLUSIONS: Tethered lipid bilayers with an extremely high resistance can be formed on a gold electrode. With a further optimization of membrane composition and electrode miniaturization gigaOhmic resistances should be obtained. The resolution in our present impedance measurements is so high that the blocking of only a few large pore molecules can already be detected. Measuring channel modulation of a few SLIC's offer in turn the possibility to detect very small quantities of analytes such as antibodies. The ability to modify the detector element in SLIC could thus deliver a generic tool for ultra sensitive biosensing.

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ACKNOWLEDGEMENTS: This work was financially supported by the TopNano 21 program. The authors are grateful to Andreas Heusler for synthesizing the thiolipid.

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THE CELL WALL OF LACTIC ACID BACTERIA: SURFACE CONSTITUENTS AND MACROMOLECULAR CONFORMATIONS

J.Ubbink[1,*] & P.Schär-Zammaretti[1,2]

[1]Nestlé Research Center, Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland.
[2]Current affiliation: ETH and University of Zürich, Institute of Biomedical Engineering, CH-8044 Zürich, Switzerland.

Bacterial surfaces display an impressive variation in physico-chemical properties. Apart from the chemical nature of the surface constituents and the organization of these constituents within the cell wall, these properties are determined by the conformational degrees of freedom of the polymeric surface constituents. Detailed knowledge of the bacterial surface properties is of major practical importance, as they determine the propensity of a bacterium to adhere to surfaces, to bind polymeric constituents of the growth medium and they are implied in bacterial (auto) aggregation and clustering. Of a number of lactic acid bacteria, the physico-chemical surface properties are studied in relation to the conformational properties of the surface polymers. Techniques giving coarse-grained information on the whole bacterial cell wall, like electrophoretic mobility, Dynamic Light Scattering and adhesion to liquid interfaces, are combined with Atomic Force Microscopy from which information on the morphology, elasticity and interactions of the bacterial surface on nanometer scales is extracted. The S-layer, which is an important constituent of the cell wall of many *lactobacillus* strains and which consists of a para- crystalline layer of globular proteins, contributes strongly to the overall surface properties if present on the outer layers of the cell wall, like in *L. crispatus*, but not if polymers protude through this layer, like in *L. helveticus*. Polymers at the bacterial surface can vary widely in their chemical nature, physico-chemical properties and macromolecular conformations, as witnessed for instance by *L. johnsonii* DSM20533, which has a rough, heterogeneous surface consisting of neutral polysaccharides, *L. johnsonii* ATCC33200 which is covered by a homogeneous layer of single polymers and *L. johnsonii* ATCC332 which is covered by lipoteichoic acids.

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REVERSIBLE IMMOBILIZATION OF PEPTIDES: SURFACE MODIFICATION AND IN SITU DETECTION BY ATR-FTIR SPECTROSCOPY

P.Rigler, W.-P.Ulrich & H.Vogel

Laboratoire de Chimie Physique des Polymères et Membranes, EPFL, Lausanne, Switzerland.

INTRODUCTION: A generic method is described to reversibly immobilize polyhistidine-bearing polypeptides and proteins on attenuated total reflecting (ATR) sensor surfaces for detecting biomolecular interactions by FTIR spectroscopy.[1] Nitrilotriacetic acid (NTA) groups are covalently attached to self-assembled monolayers (SAMs) of either thioalkanes on gold films or mercaptosilanes on silicondioxide films deposited on germanium internal reflection elements (IREs). Complex formation between Ni^{2+} ions and NTA groups activates the ATR sensor surface for the selective binding of polyhistidine sequences. This approach not only allows a stable and reversible immobilization of histidine-tagged peptides (His-peptides), it also simultaneously offers the direct in situ quantification of surface adsorbed molecules via specific FTIR spectral bands.

METHODS: Ge IREs were coated with a 2 and 10 nm thin film of Au or AuPd on one face by thermal evaporation. SiO_2 layers of 20 nm thickness were deposited by plasma enhanced chemical vapor deposition. Au and AuPd surfaces were covered by a mercapto-NTA via self-assembly from D_2O solution.[2] SiO_2 surfaces were coated in the gas phase with 3-(mercaptopropyl)trimethoxysilane and then reacted with NTA-maleimid.[3] A 15 amino acid polypeptide His_6-Gly-Pro-Gly-His_6 was dissolved in deuterated buffer and equilibrated therein for several days to assure complete H-D exchange. Infrared measurements were recorded using a FTIR spectrometer equipped with an MCT detector. A sketch of the home-built flow-cell is shown in Fig. 1.

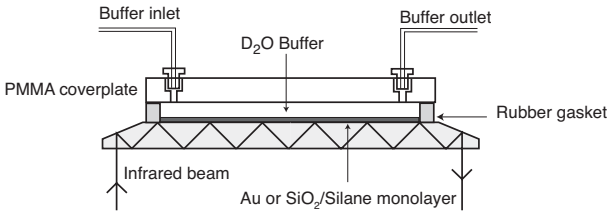

Fig. 1: Flow cell featuring a transparent polymethylmethacrylate coverplate which is pressed against a thin perfluorinated elastomer gasket to form a watertight seal. A computer-controlled piston pump enables rapid and efficient exchange of the buffer.

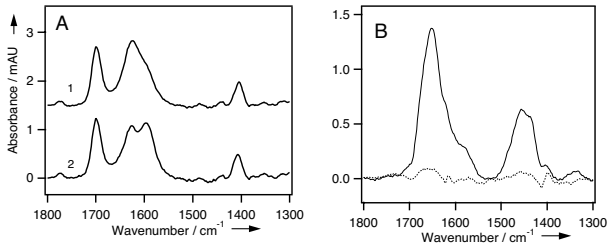

Fig. 2: Binding of His-peptide to silanized Ge IRE observed by ATR-FTIR in D_2O. (A) Surface tethered NTA after buffer wash (1), and after complexation with Ni^{2+} (2). (B) His-peptide bound to NTA-covered surface (solid line) and after desorption adding an excess of imidazole (dotted line).

RESULTS & DISCUSSION: Fig. 2 shows FTIR spectra following the stepwise build-up of the sensing surface and the reversible binding of His-peptide for the case of SiO_2 surfaces. The experimentally determined surface concentrations of both NTA on silanized surfaces was calculated to be 1.1 molecules/nm^2. Although the surface density of NTA on gold was almost threes times higher,[2] a coverage of 0.4 molecules/nm^2 for the His-peptide was found in both cases showing that the surface is densely covered with the peptide. The kinetics of His-peptide binding was investigated by recording consecutive FTIR spectra. A plot of the amplitudes of the amide I' band at 1645 cm^{-1} versus the concentration of the His-peptide in the bulk solution shows saturation with half saturation reached at 2-3 µM His-peptide.[1] A comparison of experimental FTIR spectra with simulated spectra reveals in the case of gold surfaces a surface enhancement effect of one order of magnitude. The presented sensor surfaces open up new possibilities to investigate in situ and with high sensitivity and reproducibility protein-ligand, protein-protein, protein-DNA interactions and DNA hybridization by ATR-FTIR spectroscopy.

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ACKNOWLEDGEMENTS: The authors thank Patrik Hoffmann, EPFL and Michael Mayer, Harvard University for helpful discussions and advice concerning silylation.

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BIO-INSPIRED APPROACHES TO AQUEOUS LUBRICATION BY MEANS OF A SURFACE BOUND BRUSH-LIKE POLYELECTROLYTE

M.Müller[1], X.Yan[2], S.Lee[1], S.S.Perry[2] & N.D.Spencer[1]

[1]*Dept. of Materials, ETHZ, Zürich, Switzerland.* [2]*University of Houston, Houston, USA.*

INTRODUCTION: The design of water-based biocompatible tribosystems is of increasing interest not only for implanted tribosystems (e.g. hip or knee joints) but also for many non-medical applications, where traditional, oil-based lubricants are inappropriate (e.g. food and textile industries). However, the low pressure-viscosity coefficient of water impairs the formation of a satisfactory elastohydrodynamic separating film between sliding surfaces, limiting practical applications of aqueous lubricants in engineering systems [1]. On the other hand, nature solves this problem by a mechanism involving the squeezing out of a protein-containing lubricating fluid from the porous cartilage surface and by coating the cartilage with highly water-soluble biomolecules. In the present study, we describe the use of the polyelectrolyte graft copolymer, poly(L-lysine)-g-poly(ethylene glycol), as a surface coating. The choice of PLL-*g*-PEG as a model additive for aqueous lubrication is strongly related to its unique adsorption behavior: the positively charged PLL backbones are electrostatically attracted to negatively charged oxide surfaces, while forcing the PEG side chains in a dense brush like structure coordinating large amounts of water.

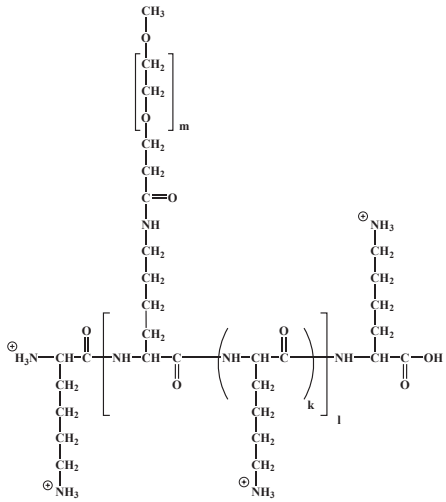

Fig. 1: Structure of PLL-g-PEG

METHODS: PLL(x)-*g*[y]-PEG(z)—a grafted co-polymer with a PLL backbone of molecular weight x kDa, a grafting ratio of (lysine-mer)/(PEG side chains) of y, and a PEG molecular weight of z kDa—was synthesized according to a previously described method [2].The lubrication properties of PLL-*g*-PEG and its influencing parameters will be investigated on the macroscopic and microscopic length scale using: pin-on-disk, mini-traction machine (MTM), ultra-thin film interferometry, and atomic force microscopy (AFM).

RESULTS: As shown in Fig 2, PLL-*g*-PEG forms a boundary lubricating film at very slow sliding speeds and promotes the entrainment of a fluid separating film up to 20 nm thick at intermediate to high speeds. The increase of film thickness with increasing speed is accompanied by a drop of the coefficient to a value of 0.0003 at a speed of 2.5 m/s.

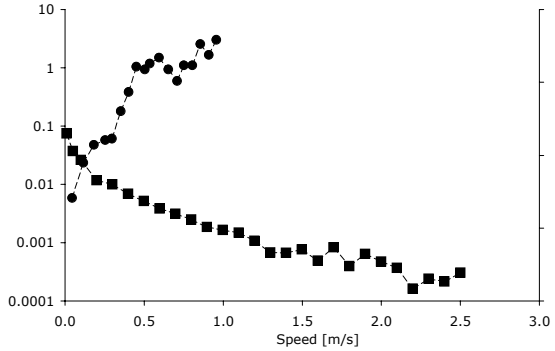

Fig. 2: A direct comparison of the coefficient of friction (y-axis on the left side) and the lubricant film thickness (y-axis on the right side).

The lubrication properties of PLL-*g*-PEG are strongly influenced by the polymer architecture. Both increasing the molecular weight of the PEG side chains and reducing the grafting ratio were found to result in an improvement in the lubricating properties of aqueous PLL-*g*-PEG solutions.

DISCUSSION & CONCLUSIONS: The lubrication properties of PLL-*g*-PEG polymers were found to be strongly dependent on the architectural parameters. The observations of this study suggest that a technological application of such bioinspired, aqueous boundary lubricant systems might be possible, in macro-, micro-, or nano-systems.

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3D-DNA CHIPS
SURFACE ATTACHED FUNCTIONAL HYDROGELS FOR OLIGONUCLEOTIDE MICROARRAYS

D.Freidank[1], S.Zirker[2], S.Mohry[2], P.O.Prucker[1], Zeltz[2] & J.Rühe[1]

[1]IMTEK, Universität Freiburg, 79110 Freiburg. [2]GeneScan Europe AG, 79108 Freiburg.

INTRODUCTION: One of the most prominent applications of the "lab-on-a-chip" concept is the so-called DNA-chip for the simultaneous detection of different DNAs from a biological sample. Labeled analyte DNA hybridises with probe oligonucleotides immobilized in a well-defined micro-array on a chip surface. Prominent applications of such DNA microarrays are the detection of genomically modified organisms, the detection of genetically inherited diseases, gene expression analysis and applications in the area of food quality control. A key issue for the generation of well-defined microarrays is the immobilization of the probe molecules on the surfaces of the chip substrates. This is especially of importance as a high reproducibility of the surface reactions is required and as the surface-attached films have to withstand quite stringent conditions (in some cases boiling in aqueous buffer for extended periods of time). We developed a fast and relatively simple procedure to prepare functional surface attached polymer networks. The suitability of the substrates has been shown by using them as oligonucleotide microarrays.

RESULTS AND DISCUSSION: In order to enhance the signal intensity, improve the sensitivity of chip systems and to control the surface properties of the substrates we have developed a system, where the DNA probes are not directly attached to the chip surface but rather to surface attached polymer monolayers or networks.

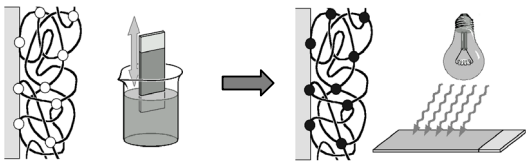

Fig. 1: Polymers, containing photocrosslinkable groups (○) are coated on a surface. Upon UV-Irradation a suface attached polymer network is formed (● crosslink).

Properties such as the mesh size of the network or its swelling behaviour can be easily tailored by the polymerisation process. Additionally functional groups such as amino groups, acrylic acid groups or reactive moieties like active esters (NHS) or epoxy groups can be incorporated in the network.

Here we used a copolymer made from N,N-dimethylacrylamide and a benzophenon derivative to yield a highly swellable, soft and hydrophilic interface that is able to covalently bind DNA-probes via a photochemical pathway (Fig. 1). The biofunctionality with respect to the amount of DNA-binding sites incorporated into the polymer layers can be tuned by adjusting the polymerisation parameters. Finally, following standard printing or spotting protocols, an array of different probe molecules can be formed.

In order to demonstrate the applicability of our DNA-probe immobilization strategy, we have chosen concentration series or a model oligonucleotide as an example. First results (see Fig. 3) show that the graft density of the probe molecules on such a "3D-polymer" chip is significantly enhanced and, consequently, the sensitivity of these chips towards the target DNA is much higher. It is further evident, that the probe molecules are very firmly attached and the microarray survives the complete hybridization protocol as well as a melting of the obtained hybrids.

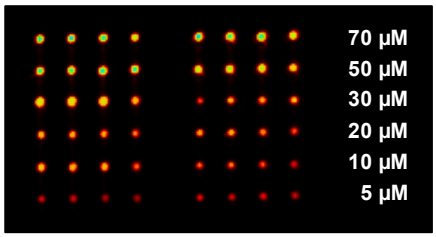

Fig. 2: Fluorescent image of an fluorescent labeld analyte DNA, hybridised with surface attached DNA-probes of different grafting density.

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IN SITU HYBRIDIZATION KINETICS OF PNA/DNA BY ELECTROCHEMICAL IMPEDANCE SPECTROSCOPY

J.Liu[1], D.Yao[1], S.Tian[1], W.Knoll[1] & P.E.Nielsen[2]

[1]*Max-Planck-Institute for Polymer Research, Ackermannweg 10, Mainz, Germany.*
[2]*Panum Institute, Blegdamsvej 3c, DK-2200, Copenhagen, Denmark.*

INTRODUCTION: The detection of DNA hybridization is of significant scientific and technological importance, as manifested in, for example, the growing interest in chip–based characterization of gene expression pattern and detection of pathogens. From a fundamental point of view, the details of hybridization reactions for a surface confined DNA transducer, are important for the *in situ* control of binding events and for selecting the optimal reaction conditions. A number of groups have described successful attempts to monitor surface hybridization reactions. Optical detection of DNA was mostly accomplished by scanning the fluorescence from correspondingly labeled oligonucleotides in an array format [1], by the use of surface plasmon resonance spectroscopy [2], or by surface plasmon fluorescence spectroscopy [3]. Electrochemical concepts have also proven to be very useful for sequence-specific biosensing of DNA, because they provide simple, rapid, lable-free and low-cost detection of nucleic acid sequences [4,5]. However, there is no report yet about the reaction kinetics of DNA hybridization by electrochemical methods. Here, we present the *in situ* control of the hybridization process of label free DNA on a mixed monolayer of peptide nucleic acid (PNA) and 6-mercapto-1-hexanol(MCH) on Au electrodes using electrochemical impedance spectroscopy (EIS). From the titration curves binding constants were calculated.

METHODS: The freshly deposited gold films were mounted to an electrochemical flow cell, and PNA probe solution was injected for overnight incubation followed by a 1mM MCH solution for 1h in order to detach physically adsorbed segments. During detection the solution was constantly pumped through the flow cell. EIS data were analyzed by applying a nonlinear least squares fit to the theoretical model represented by a Randles equivalent electrical circuit. $Fe(CN)_6^{3-/4-}$ was employed as the electroactive substrate.

RESULTS: The PNA probe density strongly affects the hybridization kinetics and efficiency. By changing the concentration of PNA, assembling time and the exchange time of MCH, the conditions were optimized for EIS detection. Figures 1 shows the EIS original data (A) and the fitted charge transfer resistance, Rct (B) obtained at different hybridization times. It is important to note that a single base mismatch (MM1) can be discriminated against a fully complementary target sequence (MM0) from the kinetics curves. By changing the concentration of the DNA target from 1nM to 500nM, a Langmuir isotherm could be obtained from the titration experiments. The binding constants for MM0 and MM1 were found to be $K_A=2.8\times10^7$ M^{-1} and 8×10^6 M^{-1}, respectively. If the PNA/DNA and DNA/DNA hybridization kinetics were compared it was found that the DNA/DNA hybridization is slower because of the electrostatic repulsion between two strands.

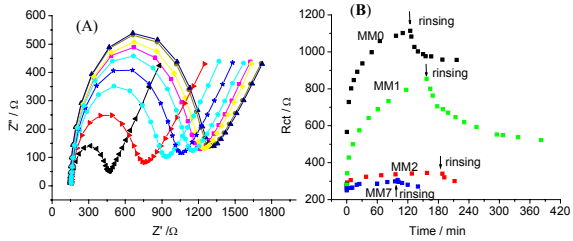

Fig. 1: (A) EIS of PNA/DNA hybridization in 20 mM PBS containing 500nM complementary target 1mM/1mM $Fe(CN)_6^{3-/4-}$ with increasing hybridization time(from bottom to up); (B) PNA/DNA association and dissociation kinetics (black: MM0, green: MM1, red:MM2, and blue curve: MM7). Rct were got from fitting EIS curve.

DISCUSSION & CONCLUSIONS: Using EIS, the association and dissociation kinetics, as well as, the affinity constants of PNA/DNA- and DNA/DNA-hybridization was investigated. Without any modification to the target, the complementary and mismatch sequence can be differentiated easily.

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MICROFABRICATED BIOCHIPS WITH A SELECTIVE SURFACE CHEMISTRY TO CONTROL 3-DIMENSIONAL CELL SHAPE

M.Dusseiller, A.Koenig & M.Textor

Deptartment of Materials, ETHZ, Zürich, Switzerland.

INTRODUCTION: At the interface between an artificial material and a biological environment a number of processes take place at different time and length scales; adsorption of water, biomolecules, activation and binding of cellular receptors, attachment and regulation of cells and finally the organization and response of the whole tissue. Therefore, there is a general interest to study interfaces at different levels; the findings have a relevant impact in the fields of implant materials, tissue engineering, cell culture systems and cell-based sensing[1].

Biochip technology has emerged from the fusion of biotechnology and micro/nanofabrication technology. Biochips enable us to realize revolutionary new bioanalysis systems that can directly manipulate and analyze the micro/nano-scale world of biomolecules, organelles and cells.

METHODS: Silicon wafers showing arrays of circular holes with different diameters and depths were produced by standard photolithography and inductive coupled plasma etching (ICP). Whole wafers were replicated into silicone rubber (PDMS, Sylgard 184), which could then be used to hot-emboss polystyrene (PS) films.

Wet-etched glass wafers were used to study different methods to produce a selective surface chemistry, showing a protein resistant background on the plateau and a specific biochemistry inside the wells. One relies on the SMAP technique[2], developed in our lab, modified for use on 3D microstructures. The other method relies on a special wetting and adsorbtion phenomena of small glass microstructures.

RESULTS: A high-throughput method to produce large quantities of structured PS films could be established and the limits of the method were found to be at high aspect ratio structures (more than 2) and small structures (below 10 µm) due to the mechanical properties of the soft intermediate PDMS master. Thus other materials have also been tested to serve as replicated masters.

The surface chemistry of the PS films has been analysed with XPS and showed a very similar composition as the commonly used tissue culture polysterene (TCPS).

First results were obtained to create a combination of topographical and chemical patterns.

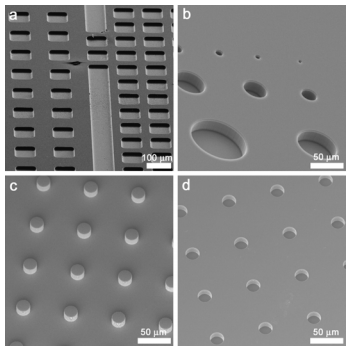

Fig. 1: SEM micrographs of a) wet-etched structures in glass; b) ICP-etched Si-Master; c) replicated PDMS structures; d) hot-embossed structures in PS. (scale: a: 100 µm, b,c,d: 50 µm)

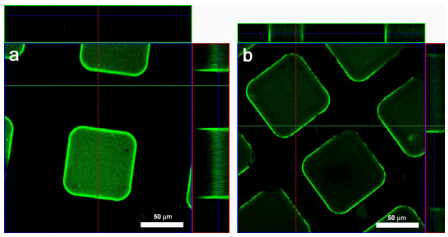

Fig. 2: CLSM images of glass structures with adsorbed fibrinogen (Alexa488 labelled). a) chemical contrast by selective capillary wetting of plateau with PLL-g-PEG solution. B) TopoSMAP by lift-off of a non-adhering TiO_2 layer on the plateau surface. (Scalebar = 50 µm)

DISCUSSION & CONCLUSIONS: The use of replication techniques and biocompatible polymers might lead to low-cost biochips in order to study cell function and drug development. Furthermore, controlled etching depths and diameters will allow for the analysis of single cells or clusters of cells

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ACKNOWLEDGEMENTS: The authors would like to thank Dr. Stefan Blunier, IMES - ETHZ, for help in Si-microfabrication; Dr. Arash Dodge, IMT - Université de Neuchatel, for wet-etching of glass; M. Gössi, Polymer Technolgy - ETHZ, for help with the polymer processing.

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Last MinutePoster

3D PLOTTING OF SCAFFOLDS DERIVED FROM BIO DEGRADABLE POLYMER MATERIALS AND TAILORED FOR APPLICATIONS IN TISSUE ENGINEERING

Rüdiger Landers, Yi Thomann, Rolf Mülhaupt [1]

[1] *Freiburger Material Research Center, University Freiburg, 79104 Freiburg, Germany*

Scaffolds are of great importance for tissue engineering because they enable the production of functional living implants out of cells obtained from cell culture. A new rapid prototyping (RP) technology was developed at the Freiburg Materials Research Center to meet the demands for desktop fabrication of scaffolds, in which liquids and pastes could be plotted and dispensed in liquid media in three-dimensional (3D). This technique can apply a much larger variety of synthetic as well as natural materials, either as aqueous solutions or pastes, to fabricate 3D scaffolds for application in tissue engineering. Soft tissue scaffolds such as out of hydrogel material and polyelectrolyte complexes was, for the first time, manufactured for cell engineering. Surface coating in the pore formation were achieved to get better biofunctionality and cell compatibility, as well as for a better cell adhesion and cell growth.

The versatile applications of this new type of 3D scaffolds will be discussed, especially its potential for tissue engineering.

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Last MinutePoster

Plasma polymerized surface chemistries influence adhesion, morphology & positioning of cells

P. Thomsen, C. Berendsen, S. Bouaidat, H.K Lilja-Fischer, S.G Petersen

Scandinavian Micro Biodevices A/S, Gammelgaardsvej 87C, DK-3520 Farum, Denmark.

INTRODUCTION: A major focus is maintained on producing biocompatible surfaces suitable for preventing adhesion of proteins and cells. Both topography and chemistry is manipulated to obtain these surfaces. However, none of the present available nonfouling surfaces are complete in their ability to prevent protein and cell binding.

METHODS: HeLa cells were grown in DMEM with 10% FCS. The cells were observed on glass slides coated with the surface of interest. Using a Zeiss 200m microscope and a 20x lens the cells were counted at various time points. The cells were scored as round (no adhesion) or with extensions (adhesion).

RESULTS: We used a new low energy plasma polymerization technique called SoftPlasma to produce various chemical surfaces. The low energy results in an intact functionality of the monomer which is polymerized onto the surfaces of interest (figure 1). This has been used to generate surface chemistries ranging from nonfouling to facilitators of protein adhesion and cell adhesion. These aspects has been studied using HeLa cells and imaging microscopy to characterize cellular adhesion and morphology (figure 2). In the present study three chemistries are demonstrated: PEO (Poly Ethylene Oxide) Fluor coatings and PVP (Poly Vinyl Pyrolidone). Furthermore, sub functionalities of the chemistries have been achieved by adjusting three parameters: Pressure, time and energy (figure 3). Thereby full control of positions and dimensions of cells can be achieved. The various degrees of nonfouling characteristics render the surfaces more or less resistant to protein and cell adhesion. However, full control of cells and proteins is achieved when the different surfaces are combined. Lithography processes were used to produce micro-structured surfaces. Resolutions down to 2 µm were achieved and various patterns of fouling and nonfouling surfaces were tested (figure 4). Due to the ability of SoftPlasma to bind covalently to most surfaces both glass and silicon wafers were used for the experiments. In addition the effects observed can be isolated to chemistry and not topography because the thickness of the surfaces was in the range of 10 to 50 nm.

DISCUSSION: The applications of nonfouling surfaces and micro structuring are many with respect to implants, bio-compatibility, cell arrays, cell handling, drug delivery and sensor technology. An example may be the facilitation of wound healing in one region of an implanted biosensor due to one surface and the prevention of cell adhesion in other regions due to a second surface. Covalent attachment of specific proteins onto SoftPlasma generated surfaces may further improve the functionality of biodevices.

CONCLUSIONS: In conclusion we have shown that a nonfouling covalently bound plasma polymerized surface (PEO) can be micro structured and used as a guiding tool for cells. Furthermore, intermediates of PEO, fluor coatings and PVP were produced to control cell attachment on a time scale.

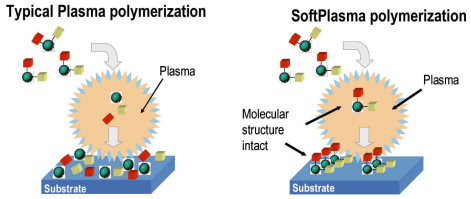

Fig.1. Functionality of SoftPlasma. The low energy SoftPlasma leave the molecular structure intact.

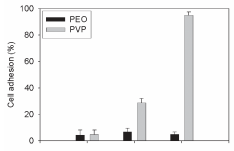

Fig.2. Cell adhesion on SoftPlasma polymerized PEO and PVP. PEO is nonfouling and PVP is a facilitator of cell adhesion.

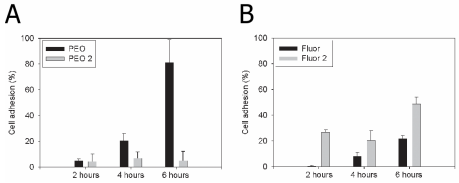

Fig. 3. Regulation of specific chemistries. PEO (panel A) and fluor coatings (panel B) by SoftPlasma settings. The polymerization process influence the functionality.

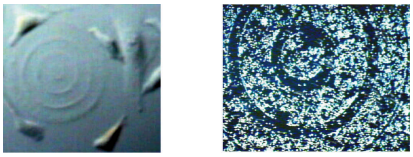

Fig.4. Micro structuring of PEO surfaces. Lithography processes were used to generate patterns of various surfaces preventing or facilitating cell adhesion.