Neonatal ventricular myocytes (CM) have long been used as an *in vitro* model for hypertrophy studies. In conventional 2D culture, CM lack axial orientation and rhythmic electrical stimulation. Micropatterned cultures can restrict cell attachment to narrow stripes, leading to enhanced axial orientation, particularly in spontaneously contracting CM (Rohr et. al., Circ Res, 1991). In this study, we investigated the effect of continous electrical field stimulation (CES) on micropatterned CM and examined their response to hypertrophic stimulation. Rat CM plated in serum-containing media selectively attached to stripes (100 μm x 10 mm) of fibronectin (FN) that were microcontact-printed onto coverslips. CM cultures were subjected to CES (1 Hz, 5 V/cm) for 48 hrs, with the current applied parallel or perpendicular to FN stripes. To induce a hypertrophic response, micropatterned CM were incubated for 48 hrs in serum-free medium with the α_1 adrenoceptor agonist phenylephrine (PE, together with timolol). We determined that the size, minor/major axis ratio and angles relative to FN stripes of DAPI-stained nuclei can be used as surrogate measures of CM size, elongation and alignment, respectively. Compared to unpaced CM, parallel CES increased nuclear size (1028 ± 121 vs. 798 ± 87 μm^2, $P<0.001$), elongation (minor/major axis: 0.76 ± 0.10 vs. 0.84 ± 0.08, $P<0.001$) and alignment ($P<0.001$, Mardia-Watson-Wheeler circular statistics). Perpendicular CES caused similar but significantly less pronounced changes. PE stimulation increased nuclear size (809 ± 93 vs. 682 ± 99 μm^2, $P<0.05$), but did not increase elongation or alignment with or without CES. In conclusion, CES can be used to enhance the degree of differentiation of micropatterned CM due to continuous electrical activation and/or contractions and does not interfere with their hypertrophic response. Continuously paced micropatterned CM represent an advanced model for the investigation of hypertrophic responses and mechanisms and may be suitable for other applications.

3134-Pos

Epithelial Coating Mechanisms by Semi-Solid Materials: Application to Microbicide Gels

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Many epithelial surfaces have natural coating by polymeric materials, e.g. mucus. Foreign materials may be introduced for coating, e.g. for lubrication or drug delivery: examples are vaginal gels delivering mucosal antigens or topical microbicides. We present here a next generation biophysical vaginal coating model, which supersedes our previous work. The model characterizes the vagina as an elastic tube with a flattened lumen. The walls have porous surfaces through which natural vaginal fluid transudates, contacting and diffusing into a gel coating layer within the lumen. Spreading of the gel layer is driven by gravity and other trans-luminal pressure gradients, and wall elasticity. Gel rheology is characterized by the Carreau constitutive equation, including the presence of a yield stress. The model determines the local dilution of gel as water is transported into it, which is linked to local dilution and time-dependent rheological properties. This association is obtained experimentally. Gel coating flow is computed, accounting for variable properties at each spatial location and time step. A set of current and prototype microbicide gels is being evaluated. Results show the predominance of yield stress at later times during flow; the flow ceases when remaining vaginal wall distension is insufficient to develop shear stresses that exceed the yield stress. Dilution is most important near the vaginal walls and the leading edge of the spreading bolus. It is there that dilution proceeds most quickly, where the local viscosity of the gel drops most, and where spreading accelerates most. For the test gels, there are tradeoffs amongst the dilution-dependent yield stress, limiting low shear viscosity, and rate of shear thinning, in rates of epithelial coating. Practically, these provide flexibility in optimizing gel compositions for target rates of epithelial coating. [Supported by NIH AI48103, CHRP ID07-B-135]

3135-Pos

Mussel-Inspired Self-Healing Hydrogels

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The strength of the coordinate bonds in metal-ligand coordination complexes combined with their capacity to reform after breaking has been proposed as a source of the high toughness and potential self-healing of certain natural materials. Several studies have aimed at testing the mechanical properties of solid-state materials crosslinked with tris-catechol-Fe^{3+} complexes. However, due to the low solubility of Fe^{3+} at high pH, these studies have been performed at low pH favoring mono-catechol-Fe^{3+} complexes and at Fe:catechol ratios >> 1/3, in disagreement with the stoichiometry of tris-catechol-Fe^{3+} complexes. The tough outer cuticle of mussel holdfast threads has recently been shown to be crosslinked by tris-catechol-Fe^{3+} complexes, in agreement with the alkaline pH of seawater (pH 8). Inspired by the likely pH changes in the secretory pathway of mussels we demonstrate that a concentrated solution of a simple polymer modified with catechol and mixed with Fe^{3+} at a Fe:catechol ratio of 1/3 at pH 3 instantly gels via tris-catechol-Fe^{3+} crosslinking upon raising the pH 9. The resulting gels have strengths comparable to covalently cross-linked gels (~ 10^3-10^4 Pa) but with an order of magnitude higher energy dissipation as well as the capacity to self-heal.

3136-Pos

Hydrogel for *in Situ* Encapsulation of Multiple Black Lipid Membranes

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Hydrogels are hydrophilic, porous polymer networks that can absorb water up to thousands of times their own weight. They have many applications, one of which is the encapsulation of free-standing black lipid membranes (BLMs) for novel separation technologies or biosensor applications. We investigated gels for *in situ* encapsulation of multiple black lipid membranes across apertures in a hydrophobic ethylene tetrafluoroethylene (ETFE) support. These gels consisted of networks of poly(ethylene glycol)-dimethacrylate or poly(ethylene glycol)-diacrylate polymerized using either a chemical initiator or a photoinitiator. The hydrogels were studied with regard to their material properties such as chemical resistance, swelling behaviour, water permeability and porosity. We found that lifetimes of membranes in gel precursor solutions were short compared to lifetimes in buffer. However, crosslinking the gel stabilized the membranes and increased BLM longevity substantially over lifetimes in buffer. Optical images of the membranes and incorporation of the transmembrane peptide gramicidin A showed that the lipid membranes retained their integrity after encapsulation with hydrogel.

3137-Pos

Fabricating 3d Ordered Cell Culture Matrix by Microfluidic Device

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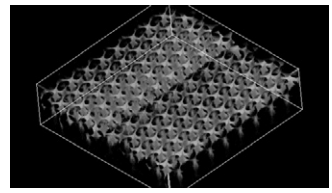
We create a novel 3D matrix with uniform and ordered pores by microfluidic technique. We can vary the pore size and the interconnection between the pores andmeasure the elastic modulus of the matrix. We culture cells inside and observe their morphology by confocal microscopy. Our matrix allows 3D cell cultures in a uniform environment.



3138-Pos

Affinity Baits and the Interior Environment of Hydrogel Particles

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Low molecular weight peptides and proteins can provide biomarkers that are diagnostic for diseases such as cancer. Unfor les can be difficult to isolate and analyze, hampering the identification of suitable biomarkers for many diseases. Thermoresponsive hydrogel particles based on a cross-linked poly(N-isopropylacrylamide), pNIPAm, architecture can be used to harvest biomarkers from biological fluids. The hydrogel particles sequester and concentrate low abundance low molecular weight analytes that can be subsequently analyzed using methods such as mass spectrometry. Introduction of monomers such as acrylic acid and allylamine into the pNIPAm skeleton allows the particles to preferentially attract and concentrate analytes based on charge. Moreover, affinity dyes, such as Cibacron Blue F3G-A, have been added to enhance the harvesting capabilities of particles.[1]

Particles based on pNIPAm shrink and swell in response to changes in environmental conditions, such as temperature, pH and salt concentration. As the pNIPAm particles shrink, their interior environment becomes more hydrophobic, which likely impacts their binding, sequestration and release properties. Here, a phenolphtahlein uptake assay has been used to monitor the interior environment of pNIPAm-based hydrogel particles, and how it changes in response to alterations in the exterior environment. The study focuses on particle responses to changes in the concentration of salts that have been shown to impact particle size, such as guanidinium chloride and ammonium sulfate.

The obtained uptake data has been correlated with particle composition and changes in size, allowing for comparisons among affinity baits and their effect on the interior environment of pNIPAm particles.

Citations:
[1] Longo C, Patanarut A, George T, Bishop B, Zhou W, et al. (2009) Core-Shell Hydrogel Particles Harvest, Concentrate and Preserve Labile Low Abundance Biomarkers. PLoS ONE 4(3): e4763. doi:10.1371/journal.pone.0004763

3139-Pos

UV Laser Patterning for Biocompatibility Control of Polystyrene

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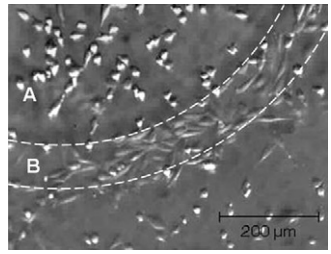
The control of cell adhesion at polymer surfaces is of great interest for applications in medicine and biotechnology research.

We present a method of polystyrene (PS) surface treatment by UV laser irradiation (laser wavelength λ=193 nm) for the improvement of adhesion of Chinese hamster ovary (CHO) cells.

We irradiated the PS foils with a total fluence of 200 mJ/cm2 in a circular spot. The irradiation led to formation of specific micro pattern at the surface (assessed by AFM and SEM). Depending on the position at the spot, the pattern structure varies. There is a specific ring area (B in figure), where seeded CHO cells show effective adhesion and pronounced spreading. The cells display here a preferential alignment along the ring. The topography of this area has a height variation of 5 to 10 nm, while the surface region irradiated with higher laser intensity (A in figure) has a higher roughness of hundreds of nm. This area is less favorable for CHO cells adhesion as indicated by the round cell morphology, similar to the flat area outside the ring. (Supported by the Austrian NANO Initiative in the projects NSI_NBPF and NSI_PolyModEUV.



3140-Pos

A Microfluidic Device for Generating Titration Curves of Biomolecular Interactions

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We present a microfluidic device for measuring affinities of biomolecular interactions. Previous work in our group has led to the development of microfluidic mixing devices, a method for biophysically characterizing molecular interactions, a platform for in situ protein expression from arrays of DNA templates, and the ability to independently address rows of the array. By combining these techniques, we can generate titration curves for a single species of a fluorescently labeled molecule against as many as 48 interaction partners in a single experiment. This approach consumes less than a picomole of the labeled molecule per titration curve, of particular use when the material is precious and of low abundance.

3141-Pos

Using Magnetic Fluids as a Versatile Method for Manipulating and Sorting Unlabeled Nonmagnetic Particles in a Flow

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Sorting and detection technologies have become an important part of industrial and medical practice. Recently, innovation in lab-on-a-chip technologies promises smaller, less expensive, and more portable devices for these applications. Labeling efficiency, specificity and throughput are challenges that must be overcome in developing such technologies. We introduce a continuous-flow magnetic flow focusing, sorting and detection scheme for unlabeled particles on the size order of cells. Unlabeled particles are focused and sorted by size in the apparatus using the magnetophoretic force in a specially crafted high-gradient magnetic field. The magnetic scheme is orthogonal to other sorting techniques, allowing other physical properties to be explored. We demonstrate using the light pressure force from a laser to actively sort a focused stream of flowing particles and use the balance between the light pressure force and the magnetic force as an additional physical axis on which particles can be sorted. We show that the positions and distribution of the particles conform to their theoretical expectations, and use the theory to explore the limitations of this technology in practice.

3142-Pos

Detection and Localization of Specific Sequences on Single Microfluidically Trapped DNA Molecules using PNA Probes

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We propose a single molecule fluorescence-based approach to rapidly locate specific sequences on DNA. Using the roughly 50,000 base pair lambda-DNA as a model molecule, we demonstrate that patterns of targeted sequences can be detected using peptide nucleic acid (PNA)-based probes. These bisPNAs, modified with biotin and Tamra on opposing ends, bind to target sequences on double-stranded lambda-DNA. While PNA probes were chosen for their specificity and versatility, they are prone to bind to non-target sites that differ from the target site by one terminal base pair. PNA binding to these single-end mismatch (SEMM) sites can be minimized by a moderate amount of additional heating following the binding reaction and this step must be optimized to achieve the requisite specificity.

Here we demonstrate the single-molecule analysis of the binding of two PNAs, with three and two target sites on lambda-DNA, respectively. Neutravidin-coated 40 nm fluorescent polymer spheres are attached to the DNA-bound biotinylated PNAs and the DNA is fluorescently stained. The locations of the bound beads along single DNA molecules are determined by stretching the DNA on slides and by trapping and stretching single DNA molecules in a microfluidic cross-slot, utilizing a stagnation-point extensional flow. In comparing to previously published results, we find that the end modifications have substantial effects on binding conditions. Furthermore, the effects of additional heating on individual target sites and mismatched sites were quantified.

3143-Pos

Suppressing Non-Specific Interactions Between Solid Surfaces used for Single-Molecule Force Measurements

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Magnetic tweezers (MT) are broadly used for investigating interactions involving nanometer-sized molecular complexes, using micrometer sized super paramagnetic beads. In contrast to other force techniques such as optical tweezers and atomic force microscopy, magnetic tweezers offer key advantages: (1) MT allows for recording of hundreds of single-molecule events in parallel with a single measurement; (2) magnetic forces are orthogonal to most biological interactions, eliminating perturbation of the sample properties during the MT measurements; (3) MT requires relatively low energy - thereby sample overheating is not an underlying problem for MT measurements; (4) the capability to conduct MT measurements at constant force eliminates the need for considering loading rates (*dF/dt*). Due to prevalent forces resultant from non-specific interactions between probe interfaces at nanometer separation, the utilization of single-molecule force techniques in proteomics remains largely unexplored. Employing surface-engineering methodologies, developed in our laboratory (*Ann. Biomed. Eng.***2009**, *37*, 1190-1205), we aim to establish the utility of MT for proteomics research by suppressing the non-specific interactions between superparamagnetic microbeads and flat substrates. Surfaces coated with synthetic polymers, providing entropic repulsion, allow for desorption of the beads from the flat surfaces with quantitative yields. Covalent attachment of to such interfaces does not compromise the functionality of enzymes and other proteins.

3144-Pos

Excitation of Microtubules Using a Double Slit Ultrasound Device

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Microtubules (MTs) are a major part of the cytoskeleton of all eukaryotic cells and directly contribute to the process of cell division by forming mitotic spindles and providing force for the segregation of chromosomes. In this work first we have analytically solved the problem of the vibrational dynamics of a MT that is attached at its two ends (which is relevant for MTs during the mitosis) inside a viscous solution, driven by an ultrasound plane wave. We have shown that with using ultrasound plane waves, the resonance only happens at high values of the harmonic mode number. However, due to the small amplitudes of those modes we cannot have both frequency control and energy transfer to the MT at the same time. Having a large enough amplitude for the resonant vibration effect is crucial in order to maximize the bending moment of a MT. In order to overcome this difficulty, we propose to excite the MT using an ultrasound generation device using a double slit design that allows for both the frequency control and optimized energy transfer to the MT.