the target species. A significant limitation of previous low-penetration methods arises from the very character that provides their utility: the low penetration depth also means they can only probe molecular events very close to the substrate surface. We have fabricated vertical silicon dioxide nanopillars which, at a height of up to one micron, carry that low penetration depth up into the cell environment where the relevant molecular processes occur. The pillars can also be specifically functionalized with molecules of interest for either delivery into the local environment or study while tethered in the observation volume. With single molecule detection at biologically-relevant concentrations and biologically-applicable locations, these nanopillars provide a template on which to study a multitude of biological processes in a controlled, dynamic, and localized fashion.

2085-Pos

Evaluation of Photophysical Properties of Methylene Blue Incorporated within ZMOF Framework

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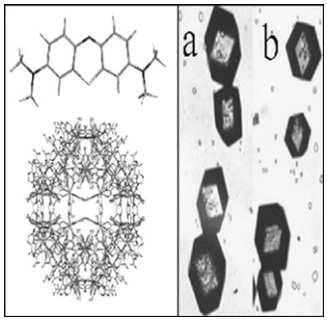
Zeolite-like metal organic frameworks (ZMOF) are of particular interest due to their application as gas storage material, drug delivery vehicles, and sensors. Stability, relatively straightforward synthesis and large internal cavities allow us to explore the possibility using of ZMOF material as a nanoreactors for various chemical and photochemical reactions. Here we present a synthesis and photophysical characterization of the ZMOF framework functionalized by encapsulation of a photosensitizer, methylene blue. Our data show that encapsulation of methylene blue within the ZMOF framework facilitates fluorophore self-aggregation as evident from the red-shift of methylene blue emission spectra, anisotropy increase, and decrease of the fluorescence lifetime. Interestingly, the fluorescent properties of methylene blue incorporated within zeolite-like methyl organic framework differ significantly from those reported previously for methylene blue aggregates in aqueous medium indicating strong interactions between the fluorophore and the framework.



Biotechnology & Bioengineering I

2086-Pos

Intracellular Effects of Nanosecond, High Field Electrical Pulses

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Mitochondria, which play a crucial role in apoptosis, release several apoptosis-inducing factors into the cytoplasm, presumably through the mitochondrial permeability transition pore (MTP). Under certain conditions, nanoelectropulses can manipulate mitochondrial structure and permeabilize the mitochondrial membrane without permanent damage to the plasma membrane. In this work we investigate the effects of nanoelectropulses on mitochondrial membrane permeabilization, assess changes in mitochondrial transmembrane potential, and monitor plasma membrane integrity under the same pulse conditions. 4 ns electrical pulses were applied to living human Jurkat T lymphoblasts in an electrode microchamber on a microscope slide. Changes in mitochondrial transmembrane potential were evaluated with rhodamine 123 (R123), a lipophilic cationic fluorescent dye that is accumulated within mitochondria. For assessing MTP opening, a calcein-cobalt quenching method was used. Calcein-AM is an anionic fluorochrome that enters cells freely and labels cytoplasmic as well as mitochondrial regions following esterase removal of the acetoxymethyl group. Because cobalt ions do not readily pass through mitochondrial membrane, mitochondria can be specifically identified by the cobalt quenching of cytoplasmic, not mitochondrial, calcein fluorescence, and MTP opening can be recognized by the decrease of calcein fluorescence within mitochondria. Finally, cell membrane integrity was evaluated with propidium iodide (PI), which is excluded from the cell interior by intact cell membranes. When the cell membrane is permeabilized, PI enters the cell, binds to double-stranded nucleic-acid molecules, and exhibits red fluorescence. The effects of different pulse amplitudes and pulse numbers on mitochondrial membrane permeability will be reported, providing a framework for an analysis of pulse doses and exposure conditions which lead to mitochondrial modifications while minimizing effects on the plasma membrane. We will also discuss the interpretation of data from fluorescence microscopic imaging analysis using R123 and cobalt-quenched intracellular calcein fluorescence intensity and the influx of PI.

2087-Pos

Hydrodynamic Trap for Single Cells and Particles

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Particle trapping and micromanipulation techniques have revolutionized biological sciences during the last two decades. Proteins, enzymes and cells have been studied extensively through manipulation methods based on optical, magnetic and electric fields. In this work, we present an alternative trapping method called the hydrodynamic trap which is based solely on hydrodynamic forces generated in a microfluidic device. The hydrodynamic trap is based on a purely extensional flow field created at the junction of two perpendicular microchannels where opposing laminar flow streams converge. The flow field in the vicinity of the microchannel junction can be described as a potential flow with a semi-stable potential well and a stagnation point. We implement an automated feedback-control mechanism to adjust the location of the stagnation point, thereby actively trapping arbitrary particles in free solution. Using the hydrodynamic trap, we successfully demonstrate trapping and manipulation of single cells and single particles with micron and sub-micron dimensions for arbitrarily long observation times. Brownian dynamics simulations show that the trap stiffness is comparable to alternative trapping techniques including magnetic traps. Overall, this new technique offers a venue for observation of biological materials without surface immobilization, eliminates potentially perturbative optical, magnetic and electric fields, and enables the ability to vary the surrounding medium conditions of the trapped object in real-time.

2088-Pos

Atomic and Photonic Force Microscope: from Nanonewton to Piconewton

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Cell differentiation and organization is influenced from chemical and mechanical characteristics of the extracellular matrix which determine its fate. Cellular compartmentalization can be explained as mechanical equilibrium of tensed and compressed cables which is continuously changing during cell motility, intracellular transport and cell division. Chemical composition of subcellular compartments determine the function they accomplish in the cell. Change in the chemical composition of subcellular structure produce not only change in their function but create also a change in their mechanical properties.

Dynamic behavior of the cell is obtained by continuous modulation of chemical composition and local recruitment of molecules in cell compartments: cell membrane vary its stiffness during endocytosis and exocytosis, cell refractive index change during cell division, actin and tubulin persistence length is modulate by assembly proteins during cell protrusions formation. Moreover single molecule mechanical characterization is becoming an important tool to study the molecule properties in different condition.

On the other side during pathologies cell mechanical characteristics change too: Brownian motion of trapped healthy cell is different from malignant one, membrane elasticity is changed in cell presenting abnormal organization of cytoskeleton.

Cell mechanics is becoming an emerging field to understand cell organization in healthy state and represent an additional way to analyze the onset of pathologies. Therefore we are developing a setup which combine AFM and Photonic force microscope to apply force spectroscopy measurement either in the piconewton and nanonewton range.

2089-Pos

Mechanotransductive Engineering of Neural Stem Cell Behavior

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Neural stem cells (NSCs) play important roles in learning and memory in the adult mammalian brain and may also serve as a source of cells in cell

replacement therapies to treat neurodegenerative diseases. Therefore, investigating how NSC behavior is regulated is crucial to understanding the fundamental biology of the brain as well as in engineering biomedical therapies. Towards these ends, an increasing wealth of knowledge in the NSC field describes a complex picture of biochemical and genetic regulation of NSC self-renewal and differentiation. However, little is known about the biophysical control of NSC behavior by the extracellular matrix (ECM). Here we demonstrate that ECM-derived mechanical signals can act with Rho GTPases to regulate NSC stiffness and differentiation. Culturing NSCs on increasingly stiff ECMs suppresses neurogenesis and enhances gliogenesis, even in the absence of exogenous differentiating agents. This shift is accompanied by enhanced RhoA and Cdc42 activation and increased cellular stiffness. Direct manipulation of RhoA and Cdc42 activity disrupts the ability of NSCs to sense ECM stiffness and tips the balance between neurogenesis and gliogenesis in the presence and absence of exogenous differentiation cues. Inhibitors of a downstream effector of RhoA, Rho kinase, as well as inhibition of myosin II contractility rescues neuronal differentiation of NSCs cultured on stiff substrates as well as for NSCs expressing CA RhoA and CA Cdc42, suggesting that NSC stiffness/contractility regulates NSC differentiation. These results establish Rho GTPase-based mechanotransduction and cellular stiffness as novel regulators of NSC behavior.

2090-Pos

Biophysical and Biochemical Tunable Environments for Controlled Cell Adhesion and Differentiation

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In the present study, we used a nanoengineered gold particle array on elastic polyethylene glycol (PEG) hydrogels and biofunctionalized the particles with different peptides. This experimental setup was used to investigate neural cell adhesion, neurite outgrowth, and cell binding in co-culture systems.
Nanostructured hydrogels were generated with interparticle distances of 50 nm and 100 nm measured by cryo-sem. In order to quantify the mechanical properties of PEG-DA hydrogels (Young's modulus EY) we performed AFM indentation measurements based on the Hertz model and adjusted for conical-shaped tips with a semi-vertical opening angle α. PEG-hydrogels were used in this work with EY $<$1kPa to 6MPa.
Cell adhesion on nanostructured gels were visualized and analyzed with cryo-sem and static adhesion assays. On substrates with 50 nm interparticle distances, cell adhesion was observed for up to two weeks for neural cell lines as well as for fibroblasts. Fibrolast cell lines (REF-52 and NIH3T3) adhere around two times better to RGD PEG-hydrogels in mono-cell culture when compared to neuroblastoma cell lines. For IKVAV decorated PEG-hydrogels neuroblastoma cell adhesion was increased to a comparable level of fibroblast adhesion on similar substrates. In co-culture systems a significant lower amount of fibroblast cells adhere to IKVAV substrates and vice versa a higher number of N2a cells (2,5fold) were detected on the surface. Lower elasticity ($<$1kPa) increased the neural cell number to around 5fold over fibroblasts. Neurite length was increased on substrates with lower elasticities independently from functionalization. Neurite initiation was independent from substrate elasticity but 4fold more cells with neurites were observed on IKVAV functionalized hydrogels.
In conclusion, neural cell adhesion and neurite formation depends on substrate elasticity as well as biofunctionalization and particle density. Substrates can be tuned to direct the adhesion of specific cell types.

2091-Pos

Control of Cardiomyocyte Adhesion and Organization by Microscale Topographical Cues

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Regeneration of myocardial tissue through the use of synthetic scaffolds requires strategies to promote cardiomyocyte attachment and organization. Our previous studies and others have demonstrated that a synthetic platform consisting of an array of microscale polydimethylsiloxane (PDMS)-based pillars (''micropegs'') can accomplish this, yet the mechanism through which this occurs remains a mystery. Here we test the hypothesis that the micropegs serve as organizational centers for cardiomyocytes, enhancing adhesion and clustering of cells via cell-ECM and cell-cell junction proteins. Our studies utilize HL-1 cardiomyocytes, a continuous cell line of atrial origin that retains several defining molecular markers and functional properties of primary cardiomyocytes. We show that ECM-coated PDMS surfaces can support the growth of HL-1 cardiomyocytes and that these cells maintain the ability to beat and express cardiac-specific myosin. Furthermore, adhesion of cardiomyocytes to micropegs alters nuclear positioning within the cell, as well as expression of several cell-ECM and cell-cell junction proteins. Interestingly, adhesion to micropegs does not appear to significantly alter cellular compliance as measured by atomic force microscopy. These findings support a model in which micropegs act as topological and spatial cues for the cardiomyocytes, and suggest potential value in incorporating such cues into myocardial tissue engineering scaffolds.

2092-Pos

Monitoring Gene Expression Via Novel Nucleic Acid and Delivery Methods

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Application of single-molecule and high-resolution fluorescence methods to monitor gene expression in living cells increase the demand on novel probes and delivery methods. They require fluorophores with high photostability and quantum yield and highly-efficient delivery methods that ensure the minimum interference with cell processes such as metabolism and signal transduction.
Here, we use a novel class of dendrimers (Parekh et al., Bioorgan Med Chem 2006, 14: 4775) with varying generations and branching factors and different number of positive charges due to different moieties and functional groups. These different properties were tested for their efficiency to transfect eukaryotic cell lines with oligodeoxynucleotides (ODNs) labelled with fluorophores. Different parameters (temperature, concentration of dendrimers, ratio of dendrimers and ODNs) were evaluated and optimised. We utilised these established optimal conditions to deliver a modified concept of SmartProbes (Stöhr et al., Anal Chem 2005, 77 (22):7195) to mammalian cells targeting endogenous mRNAs involved in signal pathways. We tested different mRNA targets and we optimised the fluorescence signal by varying a range of parameters, namely the fluorescent label and the intrinsic properties of the SmartProbe (length of the loop and stem, conformation and number of guanosines). In the near future, we plan to use these probes for monitoring gene expression levels using Diffusion Imaging Microscopy (DIFIM).

2093-Pos

Raster Image Correlation Spectroscopy for Anti-Cancer Drug Screening Based on the Identification of Molecular Dynamics

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Analysis of protein-protein or protein-DNA interaction in cells is indispensable for current basic cancer research and anti-cancer drug screening. However, it usually performed by conventional biochemical approaches, which require long process time and a large amount of samples. In this presentation, we will show the new application of Raster image Correlation spectroscopy (RICS) that can detect protein-protein, or protein-DNA interactions directly without the time-consuming biochemical process. As a result, this technique significantly reduces the analysis time from a few days to a few hours. As a proof of the concept, we investigated the effects of anti-cancer drugs, cisplatin and etoposide, on tumor-suppressor p53 protein dynamics in Hela cells. We measured the fast diffusion of GFP-tagged p53 in living Hela cells treated or untreated with each anti-cancer drug by RICS. After the drug treatment, the significant reductions of p53 mobility were observed compared to the one without drug treatment. Both cisplatin and etoposide induce DNA damage, and it has been shown that DNA damage stabilizes and activates p53, resulting in the formation of the DNA-p53 complex. Therefore, data obtained by RICS perfectly explain the status of p53 inside the cells. Together, these results suggest that RICS approach is a powerful tool to measure protein-protein or protein-DNA interactions in living cells. Since the small molecules disrupt specific protein-protein interactions are considered as promising drugs for targeted cancer therapy, our novel RICS system may serve as a powerful tool for future drug screening.