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.