

ultrastructure in determining the spatiotemporal changes in intracellular Ca^{2+} which is central in ECC.

3365-Pos Board B470

Mechanisms Underlying Pulsed Infrared Stimulation of Cardiomyocytes

Gregory M. Dittami, Kenneth W. Spitzer, Suhud M. Rajguru, Richard A. Lasher, Robert W. Hitchcock, Richard D. Rabbitt.

In this study, we investigated the origins of the endogenous cellular mechanisms underlying IR (1862 nm, 3-4 ms/pulse, 9.1 - 11.6 J/cm²/pulse, Capella, LHM Aculight) stimulation of neonatal rat ventricular and adult rabbit ventricular cardiomyocytes, *in vitro*. Confocal imaging (FV1000, Olympus; Fluo-4 AM, 4-6 μM , Invitrogen) of neonatal cardiomyocytes revealed IR-induced transient $[\text{Ca}^{2+}]_i$ responses consisting of a rapid $[\text{Ca}^{2+}]_i$ buffering component, discernable during periods of elevated $[\text{Ca}^{2+}]_i$, followed by consistent, sub-threshold $[\text{Ca}^{2+}]_i$ rises that resulted in visible cell contractions with each IR pulse. Pharmacological block of the IR-evoked responses in neonatal cardiomyocytes with CGP-37157 (20 μM , N=12 cells) and Ruthenium Red (40 μM , N=13) suggested an integral role of the mitochondrial Ca^{2+} transporters in the IR-induced $[\text{Ca}^{2+}]_i$ cycling in neonatal cardiomyocytes. While initial results with adult cardiomyocytes during comparable IR stimulation also revealed visible contractile responses, the corresponding $[\text{Ca}^{2+}]_i$ transients were surprisingly not detected. To further investigate the response in adult cardiomyocytes, whole cell patch clamp measurements were performed to monitor sarcolemma membrane potential (V_m) changes during IR stimulation. Preliminary data revealed either depolarizing or hyperpolarizing V_m responses in the cells, the nature of which was determined by the relative timing of the IR pulse applications to threshold, electrically-induced cell depolarization. Based on these findings, additional efforts focused on resolving the extent and nature of this sarcolemmal involvement in the IR-evoked responses of both neonatal and adult cardiomyocytes.

3366-Pos Board B471

Macromolecular Crowding Facilitates Adipogenic Microenvironments for Human Mesenchymal Stem Cells

Felicia C. Loe, Krystyn Van Vliet, Michael Raghunath.

Mesenchymal stromal or stem cells (MSCs) are multipotent precursor cells in the bone marrow. The intended clinical applications of MSCs require ex vivo expansion to generate therapeutically relevant cell numbers, but extended propagation results in a loss of self-renewal capacity and multipotentiality. It is increasingly recognized that the microenvironment - including growth conditions and substrata - differ greatly from the original tissue microenvironments from which these cells are derived. The *in vivo* stem cell microenvironment is characterized by macromolecular crowding (MMC) due to the presence of extracellular macromolecules of molecular weight >50 kDa. In solution, it is known that such MMC generally accelerates macromolecular association kinetics, due to excluded volume effects. In contrast, current ex vivo culture systems are devoid of crowding. Here, we report the effects of a synthetic macromolecular crowder on the adipogenic differentiation of human MSCs. This MMC cocktail comprises a mixture of Ficoll70 and Ficoll400 with a hydrodynamic radius of ~4nm and ~13nm, respectively, resulting in a biologically relevant volume fraction occupancy of ~15%. We find that this maintenance of crowding more typical of *in vivo* environments substantially amplifies the adipogenic differentiation response, as compared to standard protocols of chemically induced differentiation. We show that this amplification was facilitated by the MMC-enhanced deposition and supramolecular assembly of extracellular matrix (ECM) components, and by more efficient lineage-specific remodeling of the ECM during differentiation. Further, decellularised ECM deposited by adipocytes under MMC drives naïve hMSCs into adipogenesis without chemical induction. This work demonstrates that *in vivo* levels of macromolecular crowding accelerate deposition of the ECM microenvironment, and that the application of MMC ex vivo can enhance hMSC differentiation potential via this matrix reciprocity.

3367-Pos Board B472

Effect of PDMS Nanopatterned Substrates on Embryonic Stem Cells Differentiation into Neuronal Lineage

Elisa Migliorini, Gianluca Grenici, Jelena Ban, Alessandro Pozzato,

Maria Elisabetta Ruaro, Massimo Tormen, Vincent Torre, Marco Lazzarino.

Embryonic stem (ES) cell differentiation in specific cell lineage is a major issue in cell biology particularly in regenerative medicine. Differentiation is usually achieved by using biochemical factors which concentration and mechanism are not completely understood and with side effects difficult to overcome. Using a substrate which mimics brain extracellular matrix it could be possible to induce ES-cells differentiation into neurons without adding any biochemical factors. Therefore, we produced patterns in polydimethylsiloxane (PDMS)

consisting of groove and pillar arrays of sub-micrometric lateral resolution as substrates for cell cultures. Neuronal precursors from ES cells were obtained using a Stromal Cell-Derived Inducing Activity protocol and we analyzed the effect of different nanostructures on differentiation into neuronal lineage. Neuronal precursors adhered on PDMS more effectively than on glass coverslips. After 48 hours of culture on PDMS pillars with a 500nm period, neuronal differentiation increased and almost doubled with respect to flat PDMS substrates. Neuronal yield was enhanced by increasing pillars height from 35 to 400 nm. With pillars, 500nm period and 360nm height, the neuronal yield reached ~80% 96 hours after plating. However the largest differentiation enhancement of pillars over flat PDMS was observed during the first 6 hours of culture. These shown results that PDMS nanopillars accelerate ES cells differentiation into neurons.

3368-Pos Board B473

Wnt-Catenin Signaling System Functions in Embryoid Bodies Aggregated from Human Embryonic Stem Cell

Xuehong Xu, MengMeng Xu, Xunzhang Chen, Odell Jones, Harry Davis, Shangen Zheng, Joseph Bryant.

As an essential molecule in Wnt/ β -catenin signaling, β -Catenin plays a crucial role in the decision making for tissue differentiation in embryogenesis and pathogenesis. The signaling was implicated in the development of skin. In mouse skin, the deferential fate of the skin stem cell depends on β -catenin, which organizes stem cells into follicular or epidermal lineages. These analyses indicate that Wnt/ β -catenin signaling should also function in the development and differentiation of human embryonic stem cell. To study the function of β -catenin in early differentiation of human stem cells, we cultured H9 stem cell and aggregated them into embryoid bodies (EB). In this study, we revealed that in early EBs some guarding cells were first differentiated from EB stem cell aggregates. These early differentiated cells for guarding epithelial cells have strongest expression of β -catenin within EB. These cells were flattened on the surface of EB, covering the surface by connections formed through protein interactions. At certain confocal sections of EB, instead of a round boundary, a polygonal boundary was observed even though the EB appeared round under conventional microscope. In these polygonal boundaries, β -catenin positive guarding epithelial cells were positioned on every corner of the polygon. In the inner portion of the EB, undifferentiated β -catenin positive cells express β -catenin in the nucleus. As the initially simple shape of EB becomes more and more intricate during development, we revealed that more β -catenin positive cells were also observed in this complex structure. Based on these results, we predicted β -catenin to play different roles while guarding epithelial cells or undifferentiated stem cells in the inner portion of EB in *in vitro* culture system. Phosphorylation of β -catenin may be a critical factor for fate determination of the human stem cell.

3369-Pos Board B474

Interfacing Three-Dimensional Curved Structures and Cellular Adhesion

Mary E. Wilson, Nithyanand Kota, Burak O. Ozdoganlar, Yadong Wang,

Donna B. Stolz, Philip R. LeDuc.

Curvature is a fundamental geometric design principle found in an array of biological systems, such as vasculature. Therefore, in studying cellular processes such as adhesion, proliferation and migration, it is important to consider the effects of curved 3D micro-topography as compared to flat 2D substrates, which are far more common. Creating these 3D curved systems requires novel approaches as well. Microfluidic devices would seem to be a good approach for this as they have often been utilized as a platform for studying cell adhesion and migration *in vitro*. However, the fabrication of curved, non-rectangular channels has been a major challenge to the field of microfluidics due to conventional fabrication methods. To overcome these limitations, we have developed a novel and robust approach using mechanical micromachining in combination with a two-step reverse polymer molding process to fabricate microfluidic channels with circular cross-sectional geometries. Here, we utilize these 3D microfluidic networks to study the effects of curvature on cell adhesion mechanics. Both fibroblast (NIH-3T3) and endothelial (HUVEC) cell lines were cultured within circular cross-section microfluidic channels and on reserve molded cylindrical curved polymers. Cell morphology on these curved versus flat substrates was then characterized via confocal and scanning electron microscopy. Furthermore, the formation of stable focal adhesions and cytoskeletal organization was analyzed by immunofluorescent confocal microscopy. We believe that this approach for fabricating bioinspired microfluidic systems provides a powerful platform for interfacing cellular interactions with curved 3D structures, which could be useful in a variety of fields from vascular biology and immune cell transmigration to cell mechanotransduction and tissue engineering.