current noise realized with glass substrates. Femtosecond laser ablation is used to fabricate a pair of micropores in close proximity to each other (distance < 5 μm) in glass, while the access channels to these pores are kept separate. This configuration makes it possible to use one pore for automated positioning of a cell by suction, while the other pore is kept clean by applying positive pressure. Once the cell is pre-positioned onto the first pore, suction may be applied to the second pore to establish a high quality seal that could be used to interrogate individual transmembrane transporter proteins and single ion channel proteins. In addition, this dual micropore configuration may enable the first automated gap junction recordings, bypassing the difficulties of manually positioning two micropipettes.

3360-Pos Board B465
A Device to Measure Ligand- or Voltage-Gated Channels Simultanously in 384 Wells
Xin Jiang, Trisha Tutana, David Yamane, Yuri Osipchuk, Edward Verdonk, **James Costantin**.
We have developed patch clamping instrumentation to measure ligand-gated or voltage-gated ion channels (LGICs or VGIC) simultaneously in a 384-well microplate. The apparatus combines a 384-well pipettor with 384 amplifier and digitizers for parallel recording in all wells at once, no multiplexing is performed. We use the perforated patch clamp technique on a polyimide substrate and are able to record stable currents over tens of minutes. Currents are measured using either a single hole at each recording site or an array of 64 holes at each site (Population Patch Clamp or PPC, Finkel et al. 2006). PPC measures the ensemble current through all 64 cells in an individual well using a single pair of electrodes. We present here data from LGICs including GABA chloride channels, acid sensing ion channels (ASIC), nicotinic acetylcholine (α1 nACh) receptors, and VGICs including Na_V, K_V and hERG channels. Data will be presented on 10 - 90% exchange rates for individual wells using high K^+ solutions. Pharmacological data will be presented on the blockade of several channel types listed above to validate this type of apparatus as being appropriate for screening ion channel targets in a drug discovery setting. Ref: Finkel, A. et al. (2006). J Biomol Screen **11**(5): 488-96.

3361-Pos Board B466
Development of a GPCR Based Electrophysiological Biosensor
Masato Suzuki, Shigeki Kiyonaka, Tomohiro Numata, Ken Shimono, Hiroaki Oka, Yasuo Mori.
G-protein coupled receptors (GPCRs), which can recognize various extracellular compounds, including hormones, neurotransmitters and chemical molecules such as odors and flavors, represent the largest superfamily of cell surface receptors. Moreover, it was estimated the 25 % of the 100 top-selling drugs targeted GPCRs. For these reasons, it is desirable to develop a robust, reliable, and cost-effective functional screening technique for compounds related to the GPCR in pharmaceutical industry.
We have developed a new platform to detect chemical compounds binding to a GPCR by using a hybrid G-proteins which could transmit the signal from the Gs/olf-coupled GPCRs to the inward rectifier potassium channels (Kir 3). To improve the receptor coupling profile, fifteen kinds of the hybrid G-proteins were designed by swapping some domains of Gαi protein to corresponding amino acids of Gαolf protein.
For evaluation of signal transduction from GPCR to ion channel, each hybrid G-protein was heterogeneously expressed in HEK-293T cells with mutated potassium channel (Kir 3.1 (F137S)) and β1 adrenergic receptor, which could not activate Kir 3. By utilizing of a hybrid G-protein, which was replaced with 94 amino acids from C-terminal, dramatically change of inwardly rectifying K^+ current was occurred in the presence of β1 agonist (30 nM isoproterenol). Moreover, the lower detection limit was possible to detect even 30 pM isoptoterenol (EC50 = 1 nM).
Thus, this new sensing platform based on a hybrid G-protein might provide a powerful method to screen drug compounds targeting for GPCR.

3362-Pos Board B467
Lowering Series Resistance in Whole-Cell Patch Clamp Experiments using the Pushpen Patch Clamp Electrode
Samsoon Inayat, Lawrence H. Pinto, John B. Troy.
Series resistance introduces voltage clamp errors in whole-cell patch clamp experiments and reduces the bandwidth of recordings by forming a low-pass filter with the cell membrane capacitance. The bulk of the series resistance is located at the pipette tip. During an experiment, cellular debris or membrane fragments can clog the tip and result in an increase in resistance. This effect is most notable when recording from small neural structures, as the pipette tip used is smaller than usual then. An increase in series resistance seriously limits the duration of recording and the reliability of the data recorded. The current procedure to lower series resistance is to apply gradual pulses of suction to clear the pipette tip of cellular debris or membrane, but this usually results in disruption of the gigaseal and ends the experiment. We introduce a new procedure for clearing clogged tips using our Pushpen patch clamp electrode.
The Pushpen patch clamp electrode is a novel electrode for whole-cell patch clamp experiments, in which a linear motor moves a conical tungsten wire wound with Ag/AgCl wire, linearly inside the pipette. The tungsten wire tip can protrude from the pipette tip like a push pen, the procedure we call the "pushpen operation". Using the pushpen operation, we obtain whole-cell configuration from cell-attached without applying suction but by impaling the cell membrane with the tungsten wire tip, while maintaining gigaseal. Currently we are working to demonstrate that we can lower series resistance during an experiment by using the pushpen operation to clear a clogged pipette tip and thus prolong the lifetime of experiments.

3363-Pos Board B468
Limitations of Equivalent Circuit Models in Data-Driven Simulation of the Neuron-Electrode Interface
Vaibhav Thakore, Peter Molnar, James J. Hickman.
Noninvasive neuroelectronic interfacing has important applications in the fields of neural prosthetics, biological computation and biosensors. Traditionally, neuron-electrode interfaces have been modeled as linear point or area contact equivalent circuits but such models have not been able to explain the shapes and magnitudes of the experimentally recorded extracellular signals. Also, previous work with Volterra-Wiener characterization of the planar neuron-microelectrode junction using bandlimited Gaussian white noise had shown that the mechanism of signal transduction across the nanoscale neuron-microelectrode cleft region might be nonlinear. In this optimization based study we compared and contrasted experimental and simulation data for point contact models of the extracellular 'on-cell' neuron-patch electrode and the planar neuron-microelectrode interface. The nonlinear contributions of the neurons to the dynamics of the equivalent circuit representation of the interfacial medium were systematically isolated by an independent estimation of the ion-channel parameters through a fitting of the simulated intracellular signals to the experimentally recorded voltage and current clamp signals. These ion-channel parameters were then employed in the optimization of the cell-electrode interface parameters based on extracellular recordings obtained from a neuron simultaneously interfaced to the 'on-cell' patch-electrode and the planar microelectrode using sub- and supra-threshold stimuli. An examination of the optimized model parameters for the experimental extracellular recordings from sub- and supra-threshold stimulations of the neuron-electrode junctions allowed us to draw important distinctions between the 'on-cell' neuron-patch electrode and neuron-microelectrode interfaces that could be attributed to the presence of electric double layer (EDL) and ionic electrodiffusion effects. Based on these results, we then discuss and point out the limitations of the equivalent circuit models in their failure to take account of the nonlinear EDL and ionic electrodiffusion effects occurring during the process of signal transduction at the neuron-electrode interface.

3364-Pos Board B469
Cardiac Excitation-Contraction Coupling Proteins: A 3D Spatial Analysis
Evan I. Blumgart, Isuru D. Jayasinghe, Michael J. O'Sullivan, Christian Soeller, Cameron G. Walker, Vijay Rajagopal.
The vital heart beat that pumps blood around the body is caused by millions of cardiac muscle cells exciting and contracting in concert. Cellular contraction is inextricably linked to the sarcoplasmic reticulum (SR) release event and subsequent binding of Ca^{2+} to myofibril binding sites. An integral protein in the release event is the SR membrane bound Ryanodine Receptor (RyR). Ca^{2+} enters the narrow spaces between transverse tubules and the termini of the SR via voltage-gated L-type Ca^{2+} channels. It then binds to RyR's to evoke the release of more Ca^{2+} from the SR, into the cytosol. Mathematical models examining this mechanism of excitation-contraction coupling (ECC) have thus far lacked anatomically realistic parameters for representing the ultrastructure and the distributions of key proteins in these myocytes. Our work has focused on producing an anatomically correct 3D model of a healthy cardiomyocyte that represents the spatial organization of the different cellular components involved in ECC. To this end, this study seeks to develop and perform statistical analyses on the 3D spatial distribution of RyR's and other ECC proteins in the healthy cell. Using immunofluorescence confocal microscopy images of rat ventricular muscle, we have carried out 3D spatial analyses which have enabled us to investigate the anisotropy, clustering, randomness and scatter of RyR's. We have also examined aspects of the spatial relationship between RyR's and myofibrils at Z-disks via the implementation of computational models. We have thus developed a novel anatomically based model of cardiomyocyte RyR distribution. This combined with models of other ECC component proteins, will enable us to gain new insights into the role of cardiomyocyte

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, Suhrud 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/cm^2 /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 $[Ca^{2+}]_i$ responses consisting of a rapid $[Ca^{2+}]_i$ buffering component, discernable during periods of elevated $[Ca^{2+}]_i$, followed by consistent, subthreshold $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ cycling in neonatal cardiomyocytes. While initial results with adult cardiomyocytes during comparable IR stimulation also revealed visible contractile responses, the corresponding $[Ca^{2+}]_i$ 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 Grenci, 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 sides 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.