Receptor tyrosine kinases (RTKs) are cell membrane receptors with intrinsic tyrosine kinase activity that trigger signal transduction. RTKs are known key regulators of essential cellular processes and have a critical role in the development of many types of cancer. Here we introduce an assay using micro-patterned surfaces in combination with TIRF microscopy for the analysis of EGFR-receptor (EGFR) and insulin/insulin-like growth factor-receptor (IR/IGFR) downstream signaling. In addition, we applied the technique to validate the efficacy of medically relevant RTK modulators. We used micro-patterned to force bim-EGFR/IR/IGFR molecules into microscopic domains on the surface of living cells, while monitoring co-recruitment of a fluorescent intracellular prely protein (Grb2/IRS), respectively. First, micropatterning of HeLa cells expressing the pre Grb2 enabled us to quantify the EGFR-Grb2 interaction, and we could show that Grb2 was recruited to the EGFR in a phosphorylation dependent manner. Pretreatment with pharmacologically active ingredients used for the treatment of human cancers significantly reduced the induction of the signaling system. Based on these results we set up a dose-response relationship in a live cell context. Second, we performed fluorescence recovery after photobleaching (FRAP) experiments with RTK class II receptors (IR and IGFR) and different cytosolic insulin receptor substrate (IRS) proteins. Our results indicate prominent differences in the interaction strength of IRS1 and IRS2 to the IR/IGF1-R, compared to the one of IRS3. Taken together the results approve the power of the micropatterning technique to study the interaction properties of plasma-membrane localized receptors. This method enables a fast analysis of the samples with high sensitivity in a live cell context. We could quantify the molecular effects of different RTK modulators and we will use the system in the near future to study further active pharmaceutical ingredients.

### 3644-Pos Board B372

**Resolving the brassinosteroids signal transduction mechanisms by single-molecule assays**

Song Song1, Haijiao Wang2, Xue-Lu Wang2, Yan-Wen Tan2

1Physics, Fudan University, Shanghai, China, 2School of Life Sciences, Fudan University, Shanghai, China.

Brassinosteroids (BRs) are the sixth class of plant hormones that involved in numerous plant development processes such as leaf expansion, shoot elongation and pollen tube formation. Once the signal transduction is initiated by the membrane receptor kinase BRI1 (brassinosteroid insensitive 1), the signal transmits from the cytoplasm to the nucleus and a number of genes will be regulated. The downstream signaling pathway is realized by three proteins: BIN2 (brassinosteroid insensitive 2), BES1 (BRI1 ems suppressor) and a kind of 14-3-3s protein. BRs signaling pathway have been extensively studied via genetics, proteomics, genomics and cell biology techniques. However, these bulk methods can’t follow the transduction process in situ or resolve molecular details at a rate matching the true signaling time-scale. Here we use a single molecule assay based on Total-Internally Reflected Fluorescence (TIRF) microscopy to observe the interaction of these three proteins. The result shows that BIN2 can phosphorylate BES1 in the order of seconds, and the dimeric 14-3-3s can only bind with BES1 in its phosphorylated form. In addition, we have, for the first time, found that the interaction between BIN2 and BES1 is oxygen dependent. This result may have implications on BRs signaling pathway’s involvement of stress acclimation in plants.

### 3645-Pos Board B373

**Fast and local mechanotransduction control via magnetic nanoparticles:**

Mechanical stimulation of auditory cells

Michael Levy1, Jae-Hyun Lee2, Albert Kao1, Ji-wook Kim1, Seung-hyun Noh2, Yung Ji Choi2, Dolores Bozovic2, Jinwoo Cheon2

1Department of Physics and Astronomy, UCLA, Los Angeles, CA, USA, 2Department of Chemistry, Yonsei University, Seoul, Korea, Republic of.

Converting a mechanical force into an electrochemical signal is a fundamental physiological process that underlies a number of senses, including touch, balance, proprioception, and hearing. Mechanosensitive ion channels play a crucial role in this process, switching into the open state when subjected to a mechanical stimulus which can take the form of stretch, pressure, or twist, and thus allowing an influx of ions into the cell. Although there has been much interest in understanding and controlling the mechanotransduction process, the development of appropriate tools with precise spatiotemporal control has posed challenges. We develop a technique to actuate mechanosensitive cells in a fast, reversible, and localized fashion and we test this method on auditory hair cells from the bullfrog’s sacculus. Cube-shaped magnetic nanoparticles are conjugated to Concanaevalin A to bind to the hair bundle’s surface. An electromagnetic probe then applies a calibrated magnetic force on pN scale on the particles, inducing a mechanical entrainment of the hair bundle at frequencies up to 10kHz. Such mechanical stimulation triggers the switching of ion channels from open to closed state, with the concomitant Calcium influx into the cell during the open state. Such influx is observed using fluorescent Calcium indicators. Moreover, the magnetic force stimulus can be designed to be complex and non-periodic, in order to probe specific nonlinear properties of the biological system. Our technique is applicable not only to the auditory system but to a broad range of sensory systems with mechanosensitive channels. The utilization of a controlled magnetic field, to which biological tissue is transparent, can be beneficial for remote and non-invasive stimulation of a wide range of biological targets.

### 3646-Pos Board B374

**Role of Calcium signaling in endothelial barrier function**

Judith A. Stovaljik1, Christian W. Renken1, Mohamed Trebak1, Eric J. Toth1, 2College of Nanoscale Science and Engineering, University at Albany, State University of New York, Albany, NY, USA, 2Applied Biophysics Inc., Troy, NY, USA.

Intact endothelia lining the vasculature play a crucial role in tissue homeostasis and organ function. Various blood borne and tissue released mediators influence endothelial barrier function under physiological conditions and become critical under critical conditions such as inflammation and sepsis, known to be associated with increased vascular leakage. Prominent inflammatory mediators such as Thrombin and Histamine transiently disrupt the endothelial barrier via activation of G-protein coupled receptors (GPRCs), while other GPCR agonists, including Sphingosine-1-phosphate (SIP), enhance endothelial barrier function. The barrier disruptive activities of Thrombin and Histamine were repeatedly proposed to be associated with these agonists ability to increase intracellular Calcium (Ca$^{2+}$) concentrations ([Ca$^{2+}$]$_{i}$) via activation of Gq-associated signaling involving phospholipase C (PLC) activation, production of inositol 1,4,5 triphosphate (IP$_{3}$), Ca$^{2+}$ entry through store-operated calcium channels (SOCs) and initiation of Ca$^{2+}$-dependent endothelial contractility through myosin light chain kinase (MLCK) activation. Here, we use Electric Cell-Substrate Impedance Sensing (ECIS) to challenge this view. We determined barrier function upon stimulation with various barrier modulating agonists in primary human dermal microvascular endothelial cells (HDMECs). We noted obvious discrepancies in the effective agonist concentrations able to evoke...
either detectable changes in [Ca\(^{2+}\)], or in barrier function which pointed to a complex role of Ca\(^{2+}\) signaling, if any, in endothelial barrier regulation. Absent or inconsistent [Ca\(^{2+}\)-transients under conditions of increased cellular confluency further challenge a role of Ca\(^{2+}\)-mediated signaling in receptor-mediated disruption of barrier integrity. A thorough pharmacological examination revealed that different SOC inhibitors (e.g. lanthanides, 2-APB, BTP) completely abrogated store-operated calcium entry (SOCE) while having no effect on receptor-mediated disruption of endothelial barrier function thus suggesting that SOCE is not required for endothelial barrier regulation.

**Cardiac, Smooth, and Skeletal Muscle Electrophysiology II**

3647-Pos Board B375

*Optimizing Rate Correction of Field Potential Duration, a Biomarker for QT Risk Assessment, in Human IpsC-Cardiomyocytes*

ChanTest, Cleveland, OH, USA.

The combination of human induced pluripotent stem cell-differentiated cardiomyocytes (hiPSC-CMs) with multi-electrode array (MEA) technology allows for a medium throughput assessment of cardiovascular risk at preclinical stages of drug discovery well in advance of candidate selection. Field potential duration (FPD) is a surrogate marker for QT duration and, like QT duration, is beat rate-dependent. Correction for changes in rate is required to properly interpret direct effects on FPD. The FPD is defined as the time interval between the initiation of the fast (Na\(^+\) spike) voltage deflection due to \(I_{Na}\) and subsidence of the slow, positive deflection due to repolarizing \(I_{K}\) currents, and is a biomarker for QT duration. Our data demonstrate that FPD is highly correlated with the beat period in a largely non-linear fashion. Additionally, non-paced hiPSC-CMs are susceptible to dramatic drug-induced beat period changes. Consequently, interpretation of the effects of drugs on these parameters requires a beating rate correction. However, commonly used methods, such as ECG derived QT correction formulae (e.g. Fredericia’s, Bazet’s) prove inadequate outside a narrow range of FPD-beat period linearity. Their use may incorrectly classify QT/FPD modifying compounds. We therefore developed and qualified a set of algorithms for FPD-beat period correction based on the response of hiPSC-CMs to the \(I_{K}\) inhibitor ZD7288 and the beta adrenergic agonist isoproterenol. The resulting FPD-beat period relationship had a limited linear range and an extended non-linear range. These algorithms were compared to other common correction methods. We found that individual correction provided a statistically significantly improvement over classical correction formulae (on parameters of linearity & slope) in the non-linear range. This correction algorithm has been incorporated into our MEA assay for QT risk assessment.

3648-Pos Board B376

*Combine use of Intracellular Calcium and Sarcolemma Voltage Measurements to Distinguish Mixed Channel Effects in Human Induced Pluripotent Stem Cells Derived Cardiomyocytes (hiPSC-CMS)*

**Maria P. Hortigou-Vinagre**, Emma L. Low, Iffath A. Ghouri, Robert Wallis\(^{2}\), Francis L. Burton\(^{1,3}\), Margaret A. Craig\(^{2}\), Blake D. Anson\(^{4}\), Junko Kurokawa\(^{1}\).

1Tokyo Medical and Dental University, Tokyo, Japan, 2National Institute of Biomedical Engineering, University of California-San Diego, San Diego, CA, USA, 3Sanford Burnham Medical Research Institute, La Jolla, CA, USA, 4Cellular Dynamics International, Inc, Madison, WI, USA.

Blockade of the human ether-a-go-go-related gene (hERG) ion channel is a marker for pro-arrhythmic risk. However, the extent of hERG channel inhibition is poorly correlated with action potential duration (APD) prolongation in vitro, due to multi-channel block. Subsequently, compounds which block multiple ion channels may be overlooked by pre-clinical safety screening when measuring voltage alone, with the potential risk of late stage drug attrition and withdrawal. In this work, we propose optical measurements of voltage and intracellular calcium ([Ca\(^{2+}\)]\(_i\)) in hiPSC-CMs as an alternative to the current drug screening methods to distinguish mixed action drugs in early stages. We used hiPSC-CMs loaded with 3μM di-4-ANEPPS to record membrane potential and 1μM Fura 4-AM to measure [Ca\(^{2+}\)]\(_i\), from areas of iPSCs using Cell-IQPTIQ platform (Clyde Biosciences, Glasgow UK). Fluorescence signals were digitized at 10kHz and analyzed offline using proprietary software. Mixed ion channel blockers were mimicked by the co-administration of E4031 (hERG blocker) and Nifedipine (Ca\(^{2+}\) channel blocker). The results showed that at a critical combined concentrations (e.g. 30nM E4031 + 30nM Nifedipine) which individually prolonged and shortened APD at 75% of repolarisation (APD75) respectively, in combination did not significantly change APD75 from baseline (APD75 6.5 ± 7% of control), whereas [Ca\(^{2+}\)], was markedly reduced (by 18 ± 1.2% change, p<0.05). Low concentrations of Verapamil (30nM), an antiarrhythmic drug with known mixed hERG and L-type Ca\(^{2+}\) channel activity, had no significant effect on APD (APD75 1.5 ± 0.7%) whereas the amplitude of Ca\(^{2+}\) transient was significantly reduced (by 45 ± 8%, p<0.05). In conclusion, simultaneous measurement of [Ca\(^{2+}\)], and voltage provides a convenient indicator of mixed ion channel effects when screening for drug-induced cardio toxicity.

3649-Pos Board B377

*Multiparametric One-Color Assays for Functional Assessment of Cardiomyocytes*

**Alex Savetchenko**, Manuel Ruidiaz\(^{2}\), Evan W. Miller\(^{1}\), Wesley McKeithan\(^{1}\), Susanne Heynen-Genel\(^{1}\), Mark Mercola\(^{1}\), Carlos Obejero-Paz, Arthur M. Brown, Andrew Bruening-Wright.

1Bioengineering, University of California-San Diego, San Diego, CA, USA, 2Sanford Burnham Medical Research Institute, La Jolla, CA, USA.

Heart-related health problems remain a leading cause of mortality in developed countries. To efficiently fight these problems, cardiology needs an information-rich screening for new drug candidates. Recent achievements in stem cell research provide a long-awaited solution by producing human stem cell-derived cardiomyocytes and, thus, providing various “disease-in-a-dish” models of cardiac disorders. Two biologically distinct parameters are required to efficiently characterize the cardiomyocytes function: changes in cell membrane potential and distribution of intracellular calcium ions. Unfortunately, due to experimental limitations and lack of appropriate indicators, theoretical approach to perform such an assessment is to record one signal at a time. Here we present novel approach to perform multi-parametric assessment of cardiomyocytes while using two functionally different fluorescent indicators excited by the same wavelength of light. Specifically, using the IC200 HCS screening system (Vela Sciences), we performed a functional assessment of stem cell-derived cardiomyocytes by parallel optical recording of two physiologically distinct signals in real time: dynamic changes in cell membrane voltage as well as redistribution of calcium ions in cells. To do that, in addition to a calcium indicator Fluo4, we used a novel highly efficient voltage-sensitive fluorescent dye (VF 2.1) to detect voltage changes in cardiomyocytes. To perform data analysis, we have developed a dedicated software program that by creating spatially distinct subsets of masks is separating calcium and voltage signals. Using our multiparametric assay, we have performed several screening campaigns using benchmark compounds such as modulators of ion channels involved in generation of cardiac action potential (hERG channels, sodium and calcium voltage-gated ion channels), as well as drugs that affect the intracellular calcium handling. In summary, we have developed a multi-parametric approach for comprehensive screening of a human stem cell-derived cardiomyocytes for drug discovery and cardiotoxicity purposes.

3650-Pos Board B378

*A Novel Approach for Evaluation of Drug-Induced QT Prolongation using Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes*

**Min Li**, Yasunari Kanda\(^{1}\), Yuko Sekino\(^{1}\), Tetsushi Furukawa\(^{1}\), Junko Kurokawa\(^{1}\), Carlos Obejero-Paz, Arthur M. Brown.

1Tokyo Medical and Dental University, Tokyo, Japan, 2National Institute of Health Sciences, Tokyo, Japan.

Drug-induced cardiac arrhythmias characterized by QT prolongation have been a major reason for drug withdrawal at late stage of clinical trials. Species difference is a cause of insufficient predictability of current drug safety models. Therefore, human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) have great promise for application of cardiac drug safety testing as a human cardiac model. Our perforated patch-clamp recordings from hiPS-CMs revealed that the cells exhibited diverse shapes of spontaneous action potentials (APs) with relatively small upstroke velocity (~10V/s) and depolarized maximum diastolic potential (MDP; -50 mV), which represents rather immature forms of cardiac cells. Actually, a selective hERG blocker, E4031, depolarized significantly MDP and stopped spontaneous beating on a monolayer of hiPS-CMs, which makes it difficult to evaluate the effects on AP durations. Thus, in order to improve evaluation of risks for drug-induced QT prolongation in hiPS-CMs, we generated ventricular-like hiPS-CMs by over-expressing protein-X into hiPS-CMs and characterized the utility for evaluation of drug effects on cardiac repolarization process. The protein-X over-expression induced hiPS-CMs quiescent with hyperpolarized MDP (~67 mV), and were excitable with rapid upstroke velocity (~95 V/s) by electrical field stimulation. When E4031 was applied to hiPS-CMs overexpressing protein-X, both AP durations of single myocytes and extracellular field potential durations of cardiac sheets were prolonged in a dose dependent manner. These results suggest that hiPS-CMs engineered with gene X would be a novel...