**Ca<sup>2+</sup>** loss from these intracellular stores, not from the extracellular space, we developed a flexible in vitro 3D skin equivalent model that allows intra-organellar monitoring of calcium levels by genetically encoded sensors. These new findings suggest that long-held hypotheses addressing Ca<sup>2+</sup> control of differentiation and barrier formation require revision.

### 3124-Pos Board B279

**Store-Operated Ca<sup>2+</sup> Entry is a Tonic and Phasic Signal in Skeletal Muscle**

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Store-operated Ca<sup>2+</sup> entry (SOC) is activated very rapidly in skeletal muscle upon depletion of [Ca<sup>2+</sup>]<sub>SR</sub> below an activation threshold due to a prepositioning of ORAI1 and STIM1L uniformly throughout the junctional membranes (Edwards et al 2010, Cell Calcium, Darbellay et al, 2011, J Cell Biol). Its physiology role appears to be signalling, not refilling the sarcoplasmic reticulum (SR) (Launikonis et al 2010, Pflügers Arch). If this is the case then the rate, amplitude and frequency of SOC during the release of SR Ca<sup>2+</sup> is important. To examine SOCE kinetics during SR Ca<sup>2+</sup> release we imaged cytoplasmic rhod-2 with fluo-5N inside t-system or SR of skinned fibres from mouse fast-twitch muscle, to measure Ca<sup>2+</sup> release with [Ca<sup>2+</sup>]<sub>SR</sub> or [Ca<sup>2+</sup>]<sub>cyt</sub>, respectively. Ca<sup>2+</sup> release was induced by lowering [Mg<sup>2+</sup>]<sub>SR</sub> causing a slow Ca<sup>2+</sup> release. Step changes in -d[Ca<sup>2+</sup>]<sub>SR</sub>/dt and -d[Ca<sup>2+</sup>]<sub>cyt</sub>/dt during cell-wide Ca<sup>2+</sup> transients and waves were observed. Imaging of SR fluo-5N indicated three different states of SR Ca<sup>2+</sup>-buffering, dropping in a stepwise manner because the SR Ca<sup>2+</sup>-buffer calsequestrin (CSQ) reduces its aggregation state and affinity for Ca<sup>2+</sup> with the progressively lowered total SR calcium, allowing [Ca<sup>2+</sup>]<sub>SR</sub> to change more freely with each step (Launikonis et al 2006, PNAS). SOCE was active in the phase of reduced CSQ aggregation. SOCE was active in the two latter phases and its rate was always proportional to -d[Ca<sup>2+</sup>]<sub>SR</sub>/dt. There was a tonic response during the cell-wide transient, then a fast, phasic SOCE response during repetitive Ca<sup>2+</sup> waves. Phasic SOCE signal amplitude could vary by a factor of 10 but was always proportional to depletion of [Ca<sup>2+</sup>]<sub>SR</sub>. Our results show that the kinetics of SOC signals are tightly regulated by [Ca<sup>2+</sup>]<sub>SR</sub> and we predict that these signals can be decoded by the muscle for gene expression.

### 3125-Pos Board B280

**A Method to Characterize Calcium Activity in Stimulated Cultures of Cardiac Myocytes**

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Cardiac function and disease constitutes a complex physiological process involving several biophysical scales, from the stochastic dynamics of a single calcium release channel to the calcium signal of a single myocyte and the activation of contractions across a cardiac tissue. Integration of signals from several of these scales requires specific tools to study their interdependence. We present a method for automatic detection of calcium signals in cultured cardiomyocytes subjected to external field stimulation. The method is applied to a sequence of confocal fluorescence images, and provides information on both the calcium activity in individual myocytes, the global calcium signal of the imaged field, as well as the propagation of the calcium signal across the cell culture. The approach first segments each cell in the culture and computes its average calcium activity. An automatic classification method then identifies the response of each cell among six different dynamical regimes a) uniform response, b) alternating response, c) irregular response, d) calcium waves, e) phase-lock (conduction block) or f) inactive. The system computes the area, the full duration and the amplitude of the calcium waves. An automatic classification method then identifies the response of each cell among six different dynamical regimes a) uniform response, b) alternating response, c) irregular response, d) calcium waves, e) phase-lock (conduction block) or f) inactive. The system computes the area, the full duration and the amplitude of the calcium waves.

### 3126-Pos Board B281

**Improved Protocol to Record Sarcoplasmic Reticulum Calcium Concentrations in Mouse Cardiomyocytes**

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In cardiomyocytes, the sarcoplasmic reticulum (SR) plays a very important role as the Ca<sup>2+</sup> store. During excitation-contraction coupling Ca<sup>2+</sup> is released from the SR through ryosynadine receptors (RyRs) to the cytosol to activate the contraction. Deregulations in the SR-Ca<sup>2+</sup>-release mechanism can be associated with cardiopathologies (e.g. arrhythmias, CPVT, heart failure). Alterations of the SR Ca<sup>2+</sup> concentration ([Ca<sup>2+</sup>]<sub>SR</sub>) can be explained by dysfunctions of elements that participate in the Ca<sup>2+</sup> uptake/release balance (SERCA and RyR). Recently, the interest to measure [Ca<sup>2+</sup>]<sub>SR</sub> directly has led to the application of low affinity Ca<sup>2+</sup> indicators (mag-fluo-4, fluo-5N) to quantify changes of [Ca<sup>2+</sup>]<sub>SR</sub> in dog and rabbit cardiomyocytes. However, direct measurement of [Ca<sup>2+</sup>]<sub>SR</sub> have not been achieved in freshly isolated mouse cardiomyocytes. Here, we show a new protocol optimized to measure [Ca<sup>2+</sup>]<sub>SR</sub> in mouse cardiomyocytes. We used electrophysiological and confocal imaging techniques to simultaneously acquire cytosolic (high-affinity Ca<sup>2+</sup> indicator rhod-2) and SR (fluo-5N AM) Ca<sup>2+</sup> signals in patch-clamped cardiomyocytes during Ca<sup>2+</sup> currents and SR Ca<sup>2+</sup> release. Dialysis via patch-clamp electrode, and reversible permeabilization with streptolysin-O, were sometimes applied in combination to remove contaminations of fluo-5N entrapped in the cytosol. We also recorded spontaneous cytosolic and intra-SR Ca<sup>2+</sup> waves in permeabilized mouse cardiomyocytes.

The application of this protocol to measure [Ca<sup>2+</sup>]<sub>SR</sub> in mouse myocytes will find applications in various experimental studies, for example in mouse models of disease or in transgenic mice exhibiting mutants of RyR or other Ca<sup>2+</sup> signaling proteins. The technique can help to understand how these diseases and mutations affect [Ca<sup>2+</sup>]<sub>SR</sub>. Since RyRs are regulated by cytosolic and luminal Ca<sup>2+</sup> and mutations in RyRs could modify the sensibility to Ca<sup>2+</sup>, this new protocol will be useful to study how changes in the [Ca<sup>2+</sup>]<sub>SR</sub> regulate the RyR activity. Supported by SNF.

### 3127-Pos Board B282

**Identification of Novel Hydroxyl-Benzoquinones as Redox Switchable Calcium Chelators and Potent Biological Antioxidants**

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Quinones have important functions in many vital biological processes. We have previously reported novel hydroxylated-quinone structures with a high Ca<sup>2+</sup>-binding affinity and a putatively high antioxidant capability. However, the molecular mechanisms of the chemical reactions and the physiological significance were not resolved. Here, we analyze the physiologically relevant 2,6-dimethoxy-1,4-benzoquinone (BQ), which is found in wheat and thus part of a everyday diet, but also a precursor in coenzyme Q biosynthesis. We show that BQ can be transformed into novel hydroxyl-benzoquinone forms (OHBQ) and unmask the molecular nature of the chemical reactions. Importantly, the novel OHBQ forms are very stable under physiological conditions and scavenge superoxide radicals formed by primary human monocytes with very high efficiency. Its antioxidant and Ca<sup>2+</sup>-binding properties can be switched in a redox-dependent manner. The insights into the molecular mechanism of the quinone transformation should increase their usage as powerful antioxidants and facilitate their pharmacological potential.

### Membrane Receptors & Signal Transduction II

**3128-Pos Board B283**

**Clusters of Arp2/3 Activators Mimic Pathogenic Actin Comets and Pedestals**

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We investigate RTK signaling to the actin cytoskeleton through the Nck adaptor protein. The SH2 domain of Nck binds phosphorylated tyrosine residues and the SH3 domains of Nck bind and activate N-WASP. Activation of N-WASP involves release of the VCA domain. Simultaneous binding of G-actin monomers and actin nucleation factor Arp2/3 to the VCA domain initiates nucleation of a new actin branch. Pathogenic microorganisms induce this pathway to exploit actin polymerization machinery of the host. Experimental aggregation of a fusion protein containing the Nck SH3 domains on the plasma membrane results in the formation of dynamic actin comet tails. The VCA domain of N-WASP is directly responsible for Arp2/3 activation, and aggregation of VCA can bypass the need for adaptor proteins such as Nck to initiate actin polymerization. We used image analysis to characterize the differences between Nck- and VCA-induced actin structures. Morphometric analysis demonstrates that aggregation of VCA domains on the membrane produced thicker, denser and less elongated actin structures. Particle tracking showed that motile Nck comets move faster than VCA-induced actin structures. Our data indicates that Nck comets reproduce the behavior of Vaccinia comets and VCA clusters resemble EPEC/HEC actin pedestals. As a consequence of the experimental design, VCA membrane clusters have higher VCA density.