sub-sarcolemmal caveolae and caveolar structure with pressure load, and conduction slowing was attenuated. Cardiomyocyte caveolar localization and conformation change with increased ventricular load, and this alteration appears to play an important role in load-induced conduction slowing, possibly by contributing to changes in effective membrane capacitance.

2762-Pos Board B532

Ionic Mechanism for the Formation of Excitable Scroll Wave Filaments

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Scroll waves revolving at high frequency in the heart are responsible for fatal arrhythmias. Some mechanisms of arrhythmias require scroll waves to revolve at high frequency around an excitable filament. Recent ventricular cell models including calcium dynamics cannot reproduce this phenomenon. We address this problem revising sodium current kinetics and key phase II inward currents. We perform a nonlinear analysis of the sodium current gathered in canine cardiac myocytes. Despite an extensive data set, our nonlinear analysis shows that several Hodgkin-Huxley formalisms, i.e., a model family, reproduce the voltage clamp data. We incorporate formalisms taken from this family in the latest version of the Luo and Rudy cell model (LRd) and explore the parameter space for scroll wave dynamics. The simulations are performed on a monolayer of cells (3cm x 3cm) and portions (about 2/3) of the left ventricular free wall with realistic representation of the microanatomy. The Bidomain equations are solved at a resolution of 100um in space and 100us in time and are carried out on a supercomputer of the Texas Advanced Computer Center.
Our bifurcation analysis shows that sodium current formalisms associated with higher threshold and slightly slower rate of rise during early depolarization may be more realistic. When combined with relatively minor revision of phase II plateau inward currents, the LRd model can produce scroll waves revolving around an excitable filament. The revised model exhibits a rotation period significantly briefer than the original one, and even briefer than the refractory period measured on a plane wave. This mode of excitation allows us to study mechanisms of cardiac death.
In conclusion, revision of sodium current kinetics and phase II inward currents of the LRd model allows to realistically reproduce scroll wave revolving around unexcited filament.

2763-Pos Board B533

Computational Predictions of Tissue-Specific Consequences of Long QT Mutations: Role of the Purkinje Fiber

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The long QT syndrome (LQTS) is a heritable cardiac disorder that leads to prolongation of ventricular repolarization, episodes of ventricular arrhythmia, and sudden cardiac death. Mounting evidence has implicated the Purkinje fiber (PF) conduction system in the genesis of ventricular arrhythmias. This study assesses for tissue-specific consequences of the biophysical alterations induced by LQTS-related mutations, using computational models of ventricular and PF cells. Mutations causing LQT1 and LQT2 (in KCNQ1 and HERG, respectively) are first simulated by reducing density of the respective components of the delayed rectifier current. These mutations prolong action potential duration (APD) in ventricular myocytes more than in their PF counterparts, due to differences in the conductance and activation of plateau potassium currents between the two cell types. Next, the canonical LQT3 mutation delKPQ (in the cardiac NaV 1.5 sodium channel, encoded by SCN5A) is modeled in both tissue types (as described previously, by increasing entry into a bursting mode of channel gating, producing substantial late non-inactivating inward current). Marked APD prolongation is confirmed in both tissue types, exacerbated by slow stimulation rates or pauses in pacing. Simulation of another SCN5A mutation, F1473C, which clinically produces severe QT prolongation and heavy arrhythmia burden, is shown to have a markedly larger effect on PF cells than ventricular myocytes (including a propensity for repetitive early afterdepolarizations), owing to a depolarizing shift in the mutant channel availability and lower plateau potential in PF. Finally, the interactions between PF and ventricular cells at the tissue level are investigated in the context of these mutations in a cable model representing a section of ventricular wall. In conclusion, the biophysical alterations induced by LQT mutations may have significant tissue-specific consequences, with important implications for arrhythmia and its therapy.

2764-Pos Board B534

Optical Screening of Electrical, Mechanical, and Signaling Function on Adult Cardiac Myocytes as Alternative QT-Screen

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QT interval prolongation is a biomarker for ventricular tachyarrhythmias and a risk factor for sudden death. It is well known that some drugs can prolong the QT interval. QT-interval screens are recommended by FDA for nearly all new molecular entities. Preclinical QT screens include animal experiments and patch-clamp based methods solely screening for alterations in heterologously expressed hERG channel conductance in cell lines. The aim of the present study was to explore the recording conditions and define settings which allow the optical screening of multiple electrophysiological parameters in isolated cardiac ventricular myocytes as an alternative to traditional QT-screens. Here we has show that modern calcium and voltage fluorescent probes allow for high temporal resolution (under 3 ms) of cytoplasmic calcium and membrane potential transients in beating adult cardiac myocytes. A novel approach allowing for simultaneous measurements of intracellular calcium and cell contractions using fast line scanning mode of laser confocal microscope is described. Concomitant ratiometric detection of voltage sensitive probes fluorescence allowing for motion artifacts correction is also discussed.

2765-Pos Board B535

Impact of Multiple Ionic Changes in Arrhythmic Risk Biomarkers in Human Ventricular Electrophysiology

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Electrophysiological variability represents an important challenge in evaluating cardiac response to disease and drug action. Our goal is to quantitatively evaluate the sensitivity of biomarkers to the simultaneous alteration of several ionic current in human cardiomyocytes. Computer simulations of human ventricular electrophysiology are conducted using an action potential (AP) model proposed by Carro et al.
Ionic current conductances and kinetics were simultaneously varied by +/-30%. Model output was evaluated by quantifying biomarkers including AP duration (APD) and triangulation. Statistical techniques based on experiments design along with a second order response surface model were used.
Results reveal that simultaneous alteration of several ionic properties results in nonlinear effects in biomarkers response. APD is more sensitive than triangulation to changes in ionic properties. 30% changes in sodium/potassium pump and in calcium inactivation result in APD values outside the physiological range (287-350ms; Fig. 1A).
In contrast, 30% changes in most ionic properties result in triangulation within physiological range (70-86ms). However, simultaneous reduction of GKr and GCaL by 30% significantly increases triangulation (Fig. 1B).

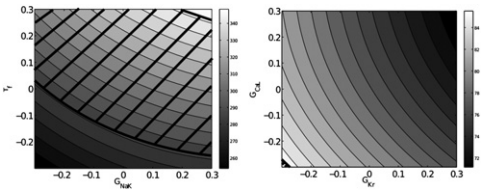

Figure 1. A) AP duration, B) AP triangulation. Marked area indicates physiological range

2766-Pos Board B536

Action Potential Duration Maps in Homogeneous Cardiac Ventricular Tissue Slices are Independent of Changes in Stimulation Site

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Cardiac slices are an increasingly popular model system for cardiac electrophysiology research, as they combine ease of handling with patho-physiologically relevant cell-type representation and distribution. The revival of the cardiac slice technique, first established in the 1980's[1] has been linked to improved techniques for preparation and monitoring. It now offers a well-posed target for computational modelling of cardiac structure-function interrelations.
This study used slices from the New Zealand white rabbits (female, ~1kg, n=12), cut tangentially to the left ventricular wall surface (350um thick, ~1x2cm large), offering a simplified 'pseudo-2D' experimental model. Slices, loaded with Di-4-ANBDQPQ (voltage sensitive dye; 10uM) to optically monitor action potential duration (APD), were stimulated at four different sites with concentric-bipolar point-electrodes, using a biphasic rectangular pulse 50% above activation threshold and, for comparison, with field stimulation.
APD at 50% and 80% repolarization (APD50 and APD80, respectively), was used to establish APD-maps in each slice. Locally-resolved average APD patterns were obtained for pacing frequencies of 1-4Hz. In addition, at each slice location the

largest (and smallest) APD-values were selected to create a maximum (and minimum) APD-map. This illustrated the variability-range per slice which, as an example for APD80 at 2Hz, was 196.37ms ± 6.59ms (maximum; mean ± SD), 187.00ms ± 6.57ms (minimum), and 191.69ms ± 5.44ms (average). Interestingly, even though the spread of activation differed significantly for the different stimulus sites, resulting APD-maps were comparable for all pacing rates in homogeneous slices (i.e. in the absence of pronounced source-sink mismatches).
Thus, APD heterogeneity in cardiac tissue slices shows a reproducible intrinsic pattern that prevails regardless of acute changes in stimulation site and frequency.
Claycomb. J Biol Chem. 1976/251:P6082-P6089.

2767-Pos Board B537
Pluripotent Cell-Derived Cardiomyocytes: One Cell Type as a Predictive Tool For the Assessment of Drug Efficacy and Safety on a Variety of Cardiac Targets
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The lack of a reliable and standardized in vitro cardiac myocyte cell culture model hampers the development of new cardiac drugs and leads to costly late stage failures of potential drug candidates.
Pluripotent stem cell-derived cardiomyocytes have the capacity to bridge this gap. The standarized production process, the availability of large frozen stocks, and the long-term stability in culture make them an attractive in vitro model.
Now cardiomyocytes originated from various species and different kinds of stem cells are available. These cells are only as good as the predictivity, as measured against human clinical data, of the assays that have been developed for them. Genetically selected mouse embryonic stem cell-derived cardiomyocytes were successfully used in a variety of electrophysiological assay systems including high throughput FLIPR fluo-4 and membrane potential assays (up to 1536 well format) as well as rubidium flux assays. The cells were also used in high content assay systems like automated voltage and current clamp and microelectrode array recordings and long-term recording in impedance-based assay systems. Each technique has their advantages and particular application. We can show high predictivity in their application in the assessment of drug efficacy and safety for many cardiac targets.
Furthermore, we have established and validated a cardiac cytotoxicity assay based on pluripotent stem cell-derived cardiomyocytes to predict drug-induced cardiac-specific cytotoxicity of different compound classes including anthracyclines as well as tyrosine kinase inhibitors.
Taken together, these assays can be implemented early in the drug optimization and development process and will reduce costly in vivo studies and will help to prevent late stage drug candidate failures.

2768-Pos Board B538
Exploring Stem Cell-Derived Cardiomyocytes with Automated Patch Clamp Techniques
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There is increasing interest for cardiomyocytes as models for studying cardiac cellular physiology and preclinical drug safety testing. Stem cell-derived cardiomyocytes have the potential for such a model and have the possibility for modeling human diseases. The present investigation is the first to describe current properties from stem cell-derived cardiomyocytes using multi-hole recordings with planar automated patch clamp technology.
In our study pluripotent stem cell-derived cardiomyocytes were biophysically and pharmacologically characterized. The cells are differentiated in large numbers and cryo-preserved, which make them suitable for automated patch clamping and facilitate their use in drug screening. We tested the cells in two different recording modes; single-hole and multi-hole, respectively. For multi-hole recordings up to ten cells are patched at the same time and the total current is measured per site. This recording mode can be useful for small currents (e.g. endogenous) and typically increases the success rate for useful data. For all experiments the whole-cell configuration was used and three different types of currents were studied; Na^+, Ca^{2+} and K^+. Using specific voltage protocols biophysical characteristics of each current was described and compared from single-hole and multi-hole experiments. We showed that currents recorded from these pluripotent stem cell-derived cardiomyocytes are similar to human cardiomyocytes and the response to known pharmacology is as expected. The $V_{0.5}$ values, I-V relationships, current kinetics and IC_{50} values determined for known blockers (TTX, nifedipine and cisapride) were comparable for the two recording modes. Clearly the success rate for usable data per measurement plate was significantly increased with the multi-hole technology. This is the first time current properties of stem cell-derived cardiomyocytes have been described from multi-hole recordings with planar automated patch clamp. Our study has shown that automated patch clamp is ready for stem cell-derived exploration.

2769-Pos Board B539
Individual Cell Electrophysiology (ICE) of Cardiac Myocytes _ Using Innovative 'Onion-Peeling' Technique to Study the ICE of Excitable Cells
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The action potential (AP) of a cardiac cell is a finely choreographed dance involving many ion channels and transporters interacting with one another via membrane potential and intracellular Ca^{2+}. The ionic mechanisms that cause arrhythmogenic APs (early or delayed afterdepolarizations, short or long AP duration) are still incompletely understood. Traditional voltage-clamp experiments measured one current from one cell and in most cases without Ca^{2+} transients. Data averaged from many cells were then used to build a canonical AP model. However, the canonical AP may be very different from the AP of any particular cell due to cell-to-cell variations. Recently, we developed an innovative *'Onion-Peeling' (O-P)* technique to achieve (***a***) a direct recording of ionic current under *in situ* condition (during AP, with Ca^{2+} cycling, in a physiological milieu, undergoing contraction) and (***b***) recording of *multiple* currents from a single cell to study how different currents interact and integrate to shape AP. Here we report the results of using *O-P* to investigate: (1) recording of key ionic currents (I_{Ks}, I_{Kr}, I_{K1}, $I_{Ca,L}$, I_{NCX} , $I_{Na\text{-}Late}$) during AP in the guinea pig ventricular myocyte; (2) effects of the Ca^{2+} transient on modulating these currents during AP; (3) changes of multiple currents under beta-adrenergic stimulation and how concerted changes in currents integrate to reshape AP. The ability to measure many currents in a single cell has also revealed two hitherto unknown characteristics of the currents in cardiac cells: coordination of currents within a cell and large variations of currents between cells. Hence, our *O-P* technique presents a significant step beyond the traditional way of measuring only one current in any one cell, and provides a powerful new tool for studying the individual cell electrophysiology.

2770-Pos Board B540
Electrophsiological Tools to Study Cardiomyocytes from Single Cell to Organ Level
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The electrophysiological properties of cardiomyocytes are fascinating yet complex. A finely tuned composition of different ion channel classes is necessary for the precise execution of an action potential. Modification of channel subsets by pharmacological substances or by genetic mutations can disturb the timing of electrical excitation cumulating in life threatening arrhythmias of the entire heart. Heart pathologies can be studied in different sets from cellular to organ level. Here we are going to discuss the advantages and limitations of different preparations and techniques applied in our lab, including 1) patch clamp analysis of adult cardiomyocytes cultivated for up to 7 days without showing major ion channel dedifferentiation, 2) microelectrode array (MEA) recordings of electrically coupled embryonic cardiomyocytes to study cardiac excitation, signal propagation and antiarrhythmic drug effects on a multicellular level, 3) Human stem cell derived cardiomyocyte technology and their potential for safety pharmacology screening, 4) acute and cultivated ventricular heart slices from different species including humans as test platform for drug screening in intact tissue and 5) Flex-MEA technology to perform extracellular recordings in intact beating hearts in a Langendorff preparation.
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2771-Pos Board B541
Quasi-*In Vivo* Electrocardiogram Measurement using Convolution of Field Potential Propagation in the *On-Chip* Cardiomyocytes Network Circuit
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Understanding the importance of spatial and temporal regulation of cellular community size and shape are keys to resolve mechanisms in highly complex heart system by new quasi-*in vivo* assay, which can predict the phenomenon *in vivo* using *in vitro* assay. For that, an *on-chip* cell network cultivation system has been