Next level toxicity screening: From single channel to overall cell behavior

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Abstract

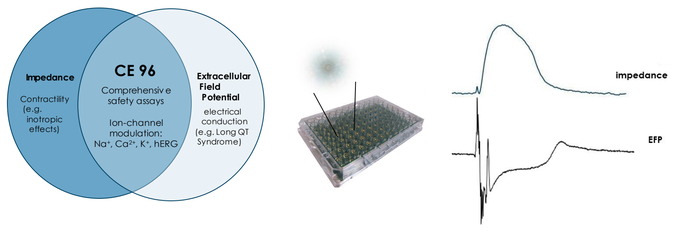
Many lead compounds fail in the late stages of the drug development process mainly by inflicting drug induced injury on liver, heart or other organs. Therefore, devices for detecting possible cell toxicity in early stages of the development process are highly demanded. Especially ion channels represent an important class of drug targets for in vitro pharmacological profiling.

High throughput screening (HTS) assays such as automated electrophysiological patch clamp and impedance based assays allow for the determination of drug effects on a whole cell level whereas artificial bilayers provide a robust environment for the assessment of single ion channel molecules.

We here present the CardioExcyte 96, a system providing a combination of Electric Impedance Spectroscopy (EIS) as well as Electric Field Potential (EFP) readout for a network of diverse cells like iPS cardiomyocytes or hepatocyte-like cells which is exemplified by toxicity effects utilizing reference compounds such as Dofetilide (on iPS cardiomyocytes) or Paracetamol (on hepatocyte-like cells).

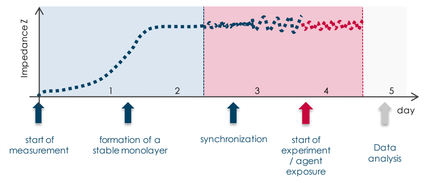
Furthermore we present the temperature dependent activation or deactivation of different Transient Receptor Potential (TRP) channels by means of planar patch clamping on our HTS platforms Patchliner and SyncroPatch 384PE as well as with highest resolution on a single channel level on our recently introduced Orbit mini setup.

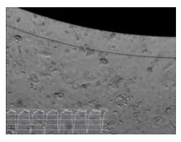
CardioExcyte 96 Sensor Technology



The CardioExcyte 96 combines impedance and EFP to measure effects on contractility and ion channel modulation (left). The NSP-96 plate contains gold electrodes embedded in each well (middle). The impedance (top) and EFP (bottom) signals are recorded from the same cell, an example is shown (right).

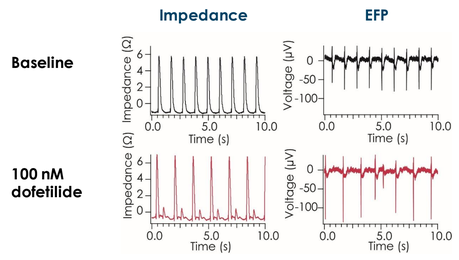
Development of the Impedance Signal





1. Intrinsic sensor impedance is measured
2. Cells adhere forming a monolayer, impedance increases
3. Differential impedance changes detectable
4. Beating pattern stabilizes
5. Pharmacology experiment and data analysis

CardioExcyte 96 Compound Profiling



Dofetilide induces a decrease in beat rate and EADs in impedance (left) and EFP (right) modes.

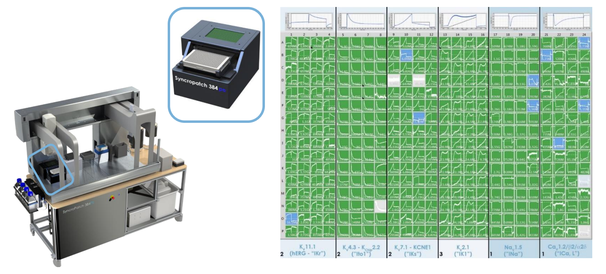
Compound		Impedance Amp	Impedance Rate	Impedance PW 50	Impedance BRR	EFP Amp	EFP Rate	EFP FPD	EFP BRR	Effect
Dofetilide	100nM									hERG blocker
Cisapride	1 µM									hERG blocker
E-4031	1 nM									hERG blocker
Sotalol	1 µM									hERG blocker
Astemizole	1 nM									hERG blocker
Quinidine	30 µM									hERG,K_v,Ca_v,Na_v blocker
Terfenadine	1 µM									hERG,Na_v blocker
Isoproterenol	30 µM									β-adrenergic agonist
Nifedipine	300 nM									Ca_v blocker
BAYK8644	1 nM									Ca_v activator

increase
decrease

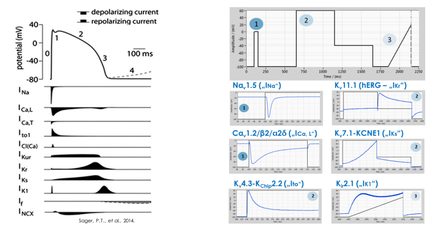
Ten different compounds were tested on Cor.4U cells in impedance and EFP modes. The results are summarized in this table.

Cor.4U Cardiomyocytes, Axiogenesis AG

Complementary Experiments Using the SyncroPatch 384PE



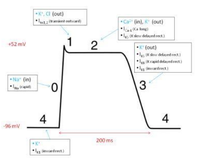
The SyncroPatch® 384PE is a patch clamp module that can be used with a state-of-the-art pipetting robot, e.g. Biomek FX (Beckman Coulter) shown here (left). Six different cardiac channels recorded simultaneously on the SyncroPatch 384PE. Screenshot shows data acquisition and analysis software performing recordings from HEK or CHO cells expressing the CiPA stipulated ion channels (ChanTest, a Charles River company) activated within one single experiment on the SyncroPatch 384PE.



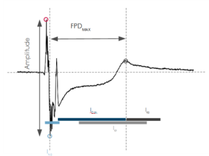
The ion channel currents mediating the cardiac myocyte action potential (AP; left). The voltage clamp protocol and examples of the 6 different currents recorded on the SyncroPatch 384PE.

AP, EFP and ECG – How They Correlate

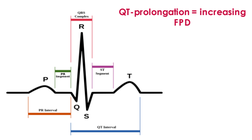
A. Human ventricular action potential [1]



B. Extracellular electric field potential (EFP) and cardiac ion channel currents that contribute to iPSCM potentials.

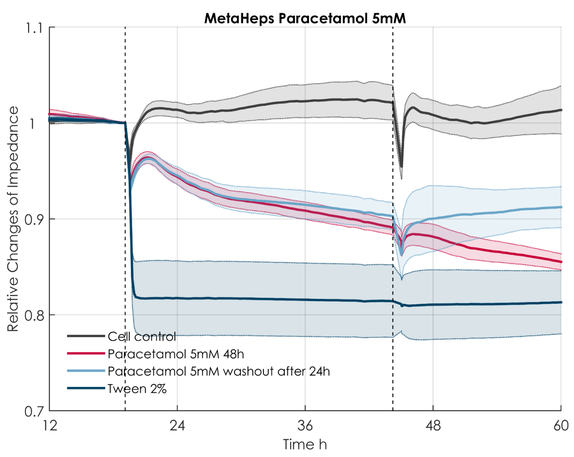


C. Surface electrocardiogram (ECG)



1. Sherwood, L. (2012). Human Physiology, From Cells to Systems (8th [revised] ed.). Cengage Learning. ISBN 9781111577438.

Toxicology Screening on Hepatocyte-like Cells



Besides Cardiomyocytes, MetaHeps cells that exhibit hepatocyte-like characteristics have been studied with the CardioExcyte96. Application of 5 mM Paracetamol (APAP) led to a considerable decrease in overall impedance indicating severe cell damage within 24 hour of incubation (indicated by the dashed lines). Of note, removal of APAP after 24 hours resulted in detectable cell regeneration (light blue graph) while incubation with 5 mM APAP for 48 hours led to further decrease in impedance (pink graph).

MetaHeps®

Conclusions

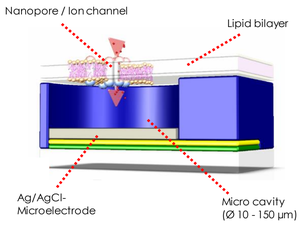
- ***CardioExcyte* 96** is a hybrid system combining impedance and EFP recordings from the same cell.
- Recordings are made at physiological temperature in an incubator or using the Environmental Chamber.
- The ***CardioExcyte* 96** can be used in cardiac safety screening to measure effects of CiPA relevant compounds such as E4031.
- The ***CardioExcyte* 96** can be utilized for toxicology studies on e.g. hepatocytes or cell quality assessment.
- The ***CardioExcyte* 96** provides complementary data to other assays such as APC.

The Orbit mini with Temperature Control

- Including eFOUR 4 channel amplifier (Elements S.R.L., Cesena, I)
- Smallest footprint, USB powered
- Max. bandwidth: 100 kHz

elements
enabling technologies for Life Science

- Optional temperature control
- Active heating and cooling
- Temperature range : 5-50° C
- Software controlled



- Introducing unique MECA chip technology: micro electrode cavity array (Ionera GmbH, Freiburg, D)
- Enabling recordings at highest bandwidth with excellent RMS noise behavior

Ionera

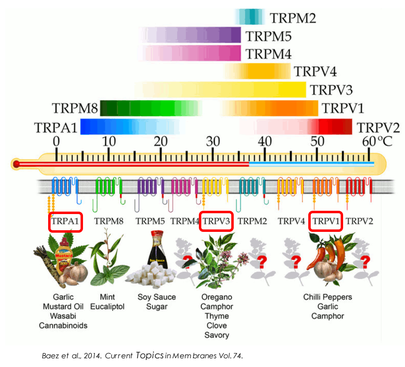
Typical Noise figures of a bilayer on:

Orbit mini	Axon 200B amplifier
1.2 pA	950 fA – 5.5 pA (10 KHz)
7.0 pA	> 20 pA at (100 KHz)

(depending on the bilayer setup used)

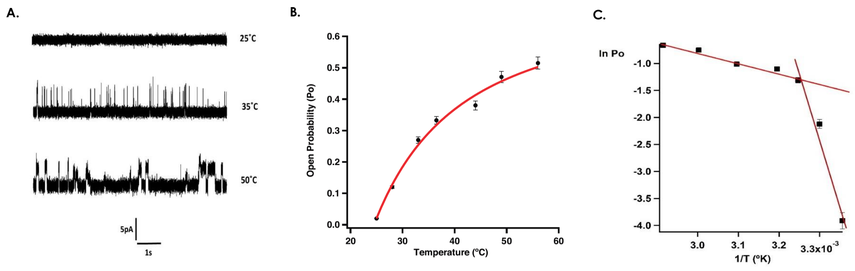
Thermo-sensitive TRP Channels

Thermal Transient Receptor Potential (TRP) channels are an important class of receptors found widely distributed throughout the mammalian central and peripheral nervous systems. They have been shown to be directly activated by heat or cold in physiologically relevant temperature ranges but are also activated by mechano-stimulation and various ligands. Understanding the mechanisms of temperature activation could lead to the discovery of novel compounds with differing effects on ligand activation and temperature activation for the treatment of pain and other disease states, with fewer side effects.



Baez et al., 2014, Current Topics in Membranes Vol.74.

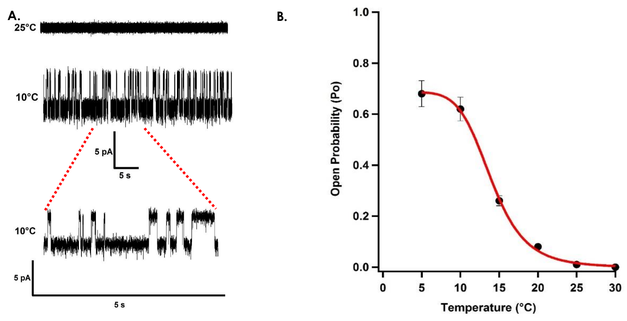
Heat Activated TRPV1



The effect of temperature on Purified TRPV1 reconstituted into lipid bilayers on the Orbit Mini.

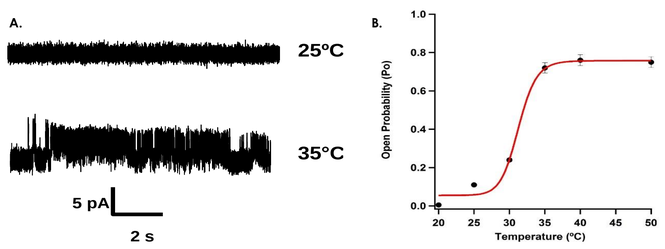
A. Evoking activity of TRPV1 in bilayers by elevated temperature; B. The effect of elevated temperatures on the open probability (Po) of TRPV1; C. Corresponding Arrhenius plot of the same data resulting in a **Q10 of 72** (Literature: Q10 ~18 to >100).

Cold Activated TRPA1



TRPA1 on the Orbit Mini. A. Effect of temperature on TRPA1 activity; B. the open probability (Po) versus the temperature and fitted with Boltzmann equation (EC_{50} was found at 14°C). The Arrhenius plot of the same data resulted in a **Q10 of 46** (Literature: Q10 ~ 40).

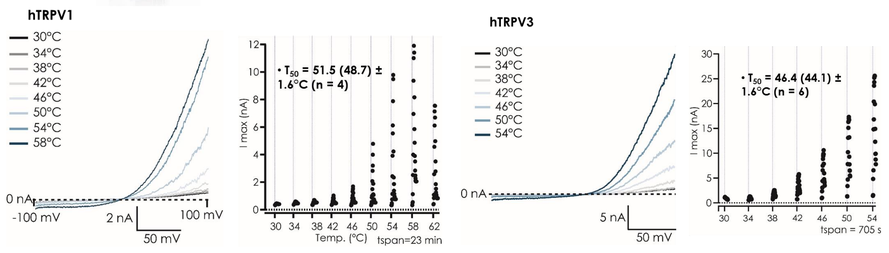
Heat Activated TRPV3



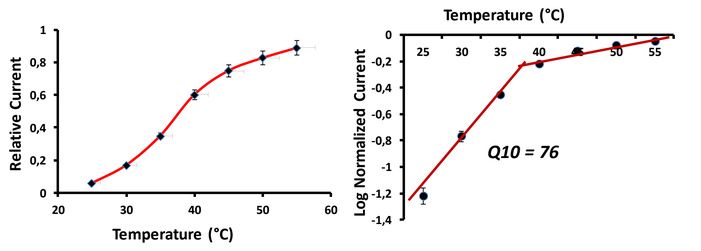
Purified TRPV3 reconstituted into bilayers on the Orbit Mini. A. Effect of temperature on TRPV3 activity; B. Effect of elevated temperature on the open probability (Po). Fitting the data with the Boltzmann equation yields an EC_{50} of 31.67°C. The Arrhenius plot of the same data results in a **Q10 of 22.2** (Literature: Q10 ~17-56).

Literature values of TRP channels taken from: 1. Mandadi, S., Roufogalis, B.D. 2008. ThermoTRP Channels in Nociceptors: Taking a lead from Capsaicin receptor TRPV1. Curr. Neuropharmacol. 6:21-38. **2.** Gavva, N.R., Treanor, J.J., et al. 2008. Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans. Pain. 136: 202-210. **3.** Damien S.K. Samways and Terrance M. Egan. Calcium dependent decrease in the single channel conductance of TRPV1. Pflugers Arch. 2011 Nov ; 462(5): 681–691.

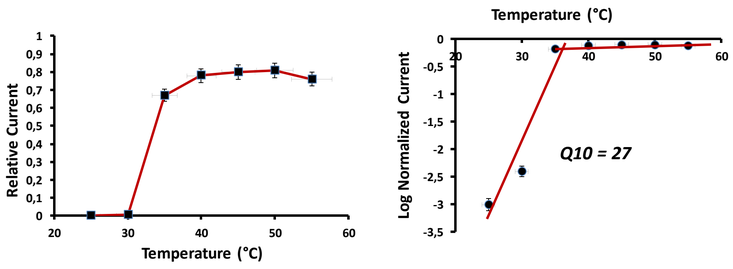
Correlation of Results Obtained via Automated Patch Clamp and via Bilayer Recordings with Temperature control



Response of TRPV1 and TRPV3 to an increasing temperature application in whole cell recordings on Nanion's Patchliner system. The T50 values were determined at 51.5 °C and 46.4 °C respectively. Note that the overall conductance of the channels increases with the temperature.
Inducible TRPV1 CHO cell line kindly provided by Pfizer / TRPV3 HEK cell line kindly provided by Millipore



Thermodynamic analysis of TRPV1 relative current upon heat activation, Left: Current of TRPV1 obtained during a temperature increase. Right: temperature dependence presented in log(Po) versus temperature plot. The data resulted in a Q10 of 76 (lipid bilayers experiments Q10 = 72 and Literature: Q10 ~18 to >100).



Thermodynamic analysis of TRPV3 relative current upon heat activation, Left: Current of TRPV3 obtained during a temperature increase. Right: temperature dependence presented in log(Po) versus temperature plot. The data resulted in a Q10 of 27 (lipid bilayers experiments Q10 = 22 and Literature: Q10 ~17-56).