Nanion Technologies

Complementary HTS Technologies towards a more Rigorous Safety Screening Paradigm

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Nanion Technologies Inc.
Agenda

• Company Background
  ➢ Brief history
  ➢ Technologies

• Comprehensive In Vitro Pro-arrhythmia assay
  ➢ Goals
  ➢ Relevant ion channels

• Recording platforms
  ➢ Patchliner
  ➢ CardioExcyte
  ➢ Syncropatch 384/768
Company History & Development
Continuous organic & dynamic growth since 2002

Dedicated staff

First APC instrument

Versatile automation

Entering HTS

Final scale-up to HTS

It's a people's business

Seed investment

Long experience & exceptional customer service

Company profitable since 2003

Management buyout

Dedicated staff with long experience for best customer service

Buyout
Nanion:

The SURFE®
Catch the wave for transporters.
- In-depth analysis of transporter protein activity and function
- Compatible with diverse membrane sources
- Multiple targets investigated with one sensor

The CardioExcyte 96
Bump up your safety screening.
- Ultra-precise impedance measurements
- Real time access to beating parameters
- Quick experiments and long term observations

The Port-a-Patch
The world’s smallest patch clamp rig.
- Fast access to high quality patch clamp data
- Quick evaluation of cells and compounds
- Novel experimental possibilities

The Patchliner
Because quality does matter.
- Unlimited experimental freedom
- Best of all worlds: throughput, performance and versatility
- Press one button and walk away: - 48 cells in one run

The Vesicle Prep Pro
Liposomes made easy.
- Quick and easy formation of GUVs
- Temperature control
- Stable bilayers for ephys recordings

The SyncroPatch 96
Get more throughput.
- Cost-efficient ion channel screening
- Ligand- and voltage-gated channels
- High throughput and high data quality

The Orbit 16
Instant bilayer – just add protein.
- 16 parallel bilayers
- Low noise, high bandwidth recordings
- Compatible with your existing amplifier

13 years of patch clamping - and more to come

Measure More Membrane
Smart tools for ion channel and transporter research and screening
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Ongoing HESI/FDA initiative: CiPA
(Comprehensive *In Vitro* Pro-arrhythmia assay)

CiPA

- A proposal to evaluate the pro-arrhythmic risk of compounds based on mechanistic electrophysiological understanding of this risk
Ongoing HESI/FDA initiative: CiPA
(Comprehensive In Vitro Pro-arrhythmia assay)

CiPA
• A proposal to evaluate the pro-arrhythmic risk of compounds based on mechanistic electrophysiological understanding of this risk

Goal
• Move the analysis and evaluation of pro-arrhythmia risk earlier in the discovery/development process
• Increase efficiency of development pathway
• Enhance accuracy of current or future drugs labeling
• Increase the number of compounds in development
• Revise ICH S7B guideline, and remove TQT study described in ICH E14 guideline
  – Proposed timelines: abandon E14 by July 2015, and revise S7B by July 2016
HTS on the CIPA stipulated ion channels

*(hERG, Nav1.5, Cav1.2 and KvLQT1, Kir2.1)*

**Voltage gated channels:**
BK, Cav1.2, Cav2.2, Cav3.1, Cav3.2, Cav3.3, hERG, hEAG, KCa1.1, Kv1.3, Kv1.5, KCNQ1, Nav1.1, Nav1.2, hNav1.5, Nav1.7, hNav1.8, Shaker I, Shaker II

**Ligand gated:**
5-HT3, ASIC, CNG, GABA, hGlyRa1, HCN, hNACHRa7, NACHRa3β4, NMDA, P2X2/3, P2X7, TRPA1, TRPC1, TRPC3, TRPC5, TRPM2, TRPM3, TRPM7, TRPM8, TRPV1, TRPV3, TRPV4

**Others:**
Kir1.1, Kir7.1, Kir2.1, rGIRK, kNBCs-1 (NBCe1-A), ROMK, TPCN2

**Bilayers:**
Alamethicin, Bacterial Cytolysin, Connexins, Gramicidin, IP3, KcsA, Kv1.2, McsL, NaChBac, OmpC, OmpF

**Cell lines:**
1321 N1, BHK, HEK293, CHO, COS, HeLa, IMR-32, Jurkat, L-tk, ND7-23, NG108-15, PC-12, RBL, S2, S9, SHS5Y5

**Primary cells:**
BY2 Protoplasts, DRG neurons, erythrocytes, hippocampal granule cells, human corneal endothelial cells, human sanoviocytes, human T-lymphocytes, human neutrophils, human vasacular smooth muscle cells, lysosomes, lymphoblasts, mesophyll protoplasts, mitochondria, mitoplasts, rat astrocytes

**Stem cells:**
hES (undifferentiated), hESC-derived cardiomyocytes (Axiogenesis, Cellectis, CDI, Geron/GE Health Care), mESC-derived cardiomyocytes (Axiogenesis), Primary neuronal stem cells

Measured on Nanion APC
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Is your safety program up-to-date?
Nanion’s approach to CiPA....

CardioExcyte 96
Non-invasive impedance system for beating cardiac networks

Patchliner
High quality whole cell patch clamp recordings

SyncroPatch 384/768
High quality patch clamp & 100% HTS
Patchliner

Automated patch clamp for safety screening
Rapid solution exchange

Heatable pipette

Physiological temperature

Efficient CRC generation

Internal exchange

Current clamp recordings

Patchliner®. Unlimited experimental freedom.

+ accurate pharmacology!
Pharmacology can be altered at physiological temperature.

Erythromycin is 10X more potent at physiological temperature vs RT.

Patchliner offers all required features for screening of stem cell-derived cells

- **Current clamp** – automated current clamp recordings
- **GigaOhm seals even with primary/stem cells**
- **Temperature control** – stable physiological temperatures or temperature jumps (<70°C)
- **Minimized cell usage** – higher cost efficiency
- **Internal solution exchange** – allows modulation of the ion channels on the cytosolic membrane side
- **Fast solution exchange** - >20 ms solution switch time
- **Brief compound exposure** – allows short compound exposure times down to 500 ms

Action potential are shortened by the presence of nifedipine, an L-type calcium channel blocker.
CardioExcyte 96

label-free cardiac safety screening
CardioExcyte96
CardioExcyte96

- Combined *Impedance* and MEA-like extracellular field potential (*EFP*) measurements non-invasive

- 96-well format, fully parallel readout from stem cell derived cardiomyocytes

- Enables cost efficient safety pharmacology and contractility assays

- Efficient measurements inside the incubator or with climate control chamber
Replacing the Incubator – CardioExcyte

- Incubation chamber

- Temperature control up to 40°C
- 5% CO₂
- 95% humidity

→ Minimal space requirements
→ Easy experimental setup for assessment of temperature-dependent effects
Impedance and Extracellular Field Potential (EFP) measurements recorded in a combined mode

A. Human ventricular action potential

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

C. Surface electrocardiogram (ECG).

Extended Applications: cell adhesion and initiation of rhythmic activity

Base Impedance, Amplitude and Beat Rate during the attachment/growth of cardiac cells (iCell® Cardiomyocytes) (T = 0: cell seeding, dotted lines: Medium exchange).
Combined impedance and EFP recordings

CardioExcyte96
Cardiac Pharmacology: Dofetilide – single point addition

**Baseline**

- **Impedance**
  - Time (s): 0.0, 2.5, 5.0, 7.5, 10.0
  - Impedance (Ω): 0, 2, 4, 6

- **EFP**
  - Time (s): 0.0, 2.5, 5.0, 7.5, 10.0
  - Voltage (µV): 0, -20, -40, -60

**100 nM Dofetilide**

- **Impedance**
  - Time (s): 0.0, 2.5, 5.0, 7.5, 10.0
  - Impedance (Ω): 0, 2, 4, 6

- **EFP**
  - Time (s): 0.0, 2.5, 5.0, 7.5, 10.0
  - Voltage (µV): 0, -50, -100
Detection and quantification of secondary beats

Impedance recordings

- Compound addition
- 3nM Dofetilide
- Automatic detection of secondary beats
Cardiac Pharmacology: Nifedipine

Impedance

EFP

Raw data

Dose-response

- Impedance graphs showing raw data for different concentrations of nifedipine (0 nM, 10 nM, 30 nM, 100 nM, 300 nM, 1000 nM).
- EFP graphs showing voltage change over time for different concentrations.
- Dose-response graphs showing change in various parameters (amplitude, beat rate, pulse width 50%, beat rate regularity index) across different concentrations.
## Compound profiles

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Impedance</th>
<th>EFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dofetilide</td>
<td>100 nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisapride</td>
<td>1 µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-4031</td>
<td>1 nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sotalol</td>
<td>1 µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astemizole</td>
<td>1 nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinidine</td>
<td>30 µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terfenadine</td>
<td>1 µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>30 µM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td>300 nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAYK8644</td>
<td>1 nM</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Impedance
- **Amp**: Amplification
- **Rate**: Rate of change
- **PW50**: P50 of response
- **BRRI**: Baseline response ratio

### EFP
- **Amp**: Amplification
- **Rate**: Rate of change
- **FPD**: Focal point density
- **BRRI**: Baseline response ratio

### Effects
- **Red**: Increase
- **Blue**: Decrease

- **hERG blocker**: hERG channel blocker
- **Ca,Na blocker**: Ca and Na channel blocker
- **hERG,Na blocker**: hERG and Na channel blocker
- **Ca blocker**: Ca channel blocker
- **Ca activator**: Ca channel activator
- **β-adrenergic agonist**: β-adrenergic agonist
Nanion Technologies

SyncroPatch 384 & 768 PE
The PatchEngine – 100 % integration in HTS environment:

- Fits into commercially available liquid handler
- Up to two PE’s per robot
- Open design allows integrations into fully robotic environments
- Used successfully with Beckman Coulter’s Biomek Cybio’s Felix

The PatchEngine – the core of the SyncroPatch 384PE
Current-Voltage (I/V) Relationship of hERG (CHO)
Success rates of hERG expressed in CHO cells

Counts

C slow [pF]

<5  5-10  10-15  15-20  20-25  >25

Counts

R series [MΩ]

<5  5-10  10-15  15-20  20-25

Counts

R seal [MΩ]

disabled (no catch)  <100  100 - 500  >500

Start of experiment
End of experiment

nan]i[on
Simultaneous Assessment of CiPA Stipulated Ion Channels on the SyncroPatch® 384PE

The electrophysiology team at Nanion Technologies GmbH, Munich. Cells kindly provided by ChanTest.
Summary

The cardiac action potential is defined by multiple voltage-dependent ion channels (see Fig. 1). A drug candidate’s capacity to interact with the ion channels involved in the depolarization or repolarization phases of the cardiac action potential is important for drug safety assessment. Until now, safety testing has focussed on interaction with the hERG channel and QT prolongation which can lead to potentially fatal torsades de pointes (TdP). Although this approach has been largely successful in preventing new drugs reaching the market with unexpected potential to cause TdP, it is also possible that potentially valuable therapeutics have failed due to this early screening. A new paradigm, the Comprehensive In-vitro Proarrhythmia Assay (CiPA), was introduced in 2013 to provide a more complete assessment of proarrhythmic risk. An assessment of a multitude of cardiac ion channels, in addition to hERG, should provide a more accurate prediction of the proarrhythmic risk of a compound compared with testing on hERG alone.

Results

A combined voltage step-ramp protocol (Fig. 2) was applied simultaneously to HEK or CHO cells expressing different cardiac ion channels. The first segment was used to elicit Na,1.5 or Ca,1.2 (1) followed by a classical hERG-like segment to activate K,4.3, K,11.1 and K,7.1 (2) and finally a ramp protocol to elicit inwardly rectifying K,2.1 (3).

Figure 1: Cardiac action potential and underlying currents (reproduced from Ref. 2).

Figure 2: A voltage protocol was used to activate 6 distinct cardiac ion channels. The top panel shows the voltage protocol used on all wells to simultaneously activate the 6 distinct cardiac ion channel currents shown in the bottom panels.
Figure 3: Six different cardiac channels recorded simultaneously on the SyncroPatch® 384PE. Shown is a screenshot of the data acquisition and analysis software used on the SyncroPatch® 384PE showing an experiment recording six different cardiac channels at once. A representative image of the current recording for each ion channel is shown at the top.
Ongoing project: HTS screening of CDI/Cor.4U cells!

- Cor.4U iPSC Derived Human Cardiomyocytes
- Low Cell consumption: ~300 cells per well
- One T-75 Flask with 1.5 Mio cells is sufficient for 10 x 384-patch clamp plates
Summary: HTS on the CIPA stipulated ion channels

- Cav1.2
- hERG
- Nav1.5
- Kir2.1
- KvLTQ

- Cor.4U iPSC Derived Human Cardiomyocytes
- All 6 targets recorded with high success rate
- We have cooperations with Millipore, ChanTest and Anaxon – choose your cell line!
Thank you!
• Company intro
• Overall platforms
• CIPA intro
• PL
• CE
• Syncro
• Relate to CIPA
The objective of the CIPA initiative is to facilitate the adoption of a new paradigm for assessment of clinical potential of TdP that is not measured exclusively by potency of HERG block and not at all by QT prolongation. The new CIPA paradigm will be driven by a suite of mechanistically based in vitro assays coupled to in silico reconstructions of cellular cardiac electrophysiologic activity, with verification of completeness through comparison of predicted and observed responses in human-derived cardiac myocytes. It is envisioned that the CIPA initiative will ultimately require the modification or replacement of the existing ICH S7a/b guidelines and elimination of E14 guidelines, although progress can be made in the short term under the existing regulatory construct. Read more about the proposal.

Anticipated Final CIPA will eliminate negative dataset by profiling in silico.

New data Cardiomyos CIPA panel user meeting

CIPA partners:
US FDA, HESI, CIPA

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