Innovations for cell monitoring in safety and toxicity assays

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Senior Scientist / Sales Manager at Nanion Technologies
Agenda

Cell Monitoring Approaches:
- Cell proliferation monitoring
- Hepatotoxicity monitoring
- Cancer growth monitoring

Cardiomyocyte attachment and toxicity monitoring via impedance

SyncroPatch 384/768 PE
ion channels in cell lines and iPS CMs

The SyncroPatch 384/768 PE with Optical Stimulation
Long-term and short-term monitoring for chronic and acute effects

- Controlled environment
- Long-term stable consumables
- Dual readout from the SAME cells

Impedance readings

- Reference electrode
- Impedance / EFP electrode

Graph showing:
- Time (days)
- Base Impedance
- Drug application
- Cell attachment and network formation
- Pharmacological response

Chronic effects vs. Acute effects
Cell proliferation monitored via impedance on CardioExcyte 96

**Cell growth**

CHO cells proliferation monitoring (72h)

**Cell death**

Effects of Escin and Triton X on CHO cells (>100h)
Hepatotoxicity monitoring via impedance on CardioExcyte 96

Effects of increasing concentrations of paracetamol on base impedance of monocyte-derived hepatocyte-like (MH) cells (>80h monitoring)
Investigating therapy response/resistance of mammary carcinoma cell lines in vitro

Orthotopic Tumor Model

Murine H8N8 mammary carcinoma cells exhibit a plastic epithelial or mesenchymal phenotype, validated by in vitro immunofluorescence stainings.

Experimental workflow

<table>
<thead>
<tr>
<th>Animal</th>
<th>Tumor</th>
<th>Cell Line</th>
<th>Shortcut</th>
</tr>
</thead>
<tbody>
<tr>
<td>1124</td>
<td>6163</td>
<td>pH8N8T1.1</td>
<td>T1.1</td>
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<tr>
<td>1194</td>
<td>6164</td>
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<td>1195</td>
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<td>1099</td>
<td>6172</td>
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<td>T2.1</td>
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<td>6174</td>
<td>pH8N8T2.2</td>
<td>T2.2</td>
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<tr>
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<td>6173</td>
<td>pH8N8T2.3</td>
<td>T2.3</td>
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<tr>
<td></td>
<td></td>
<td>Insufficient cell isolation</td>
<td></td>
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<td>1098</td>
<td>6223</td>
<td>pH8N8T2.1</td>
<td>T3.1</td>
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<td>6230</td>
<td>pH8N8T3.2</td>
<td>T3.2</td>
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<tr>
<td>1106</td>
<td>6229</td>
<td>pH8N8T3.3</td>
<td>T3.3</td>
</tr>
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<td>1177</td>
<td>6231</td>
<td>pH8N8T3.4</td>
<td>T3.4</td>
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</tbody>
</table>

G1 - Untreated: Untreated Control; dissected after tumor growth to 0.5 cm³
G2 - Residual: Treated with CAF; dissected at minimal tumor volume
G3 - Relapse: Treated with CAF; dissected after tumor regrowth to 0.5 cm³

<table>
<thead>
<tr>
<th>Group</th>
<th>CAF Chemotherapy (i.p.)</th>
<th>Dissection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Untreated Control</td>
<td>dissection</td>
</tr>
<tr>
<td>Group 2</td>
<td>Treated with CAF</td>
<td>dissection</td>
</tr>
<tr>
<td>Group 3</td>
<td>Treated with CAF</td>
<td>dissection</td>
</tr>
</tbody>
</table>

Therapeutic schedule to analyse the effect of chemotherapy on endogenously induced animals?

- Implantation: ca. 6 days
- V = 0.5 cm³
- CAF: 100 mg/kg Cyclophosphamide, 5 mg/kg Doxorubicin (Adriamycin), 100 mg/kg 5-Fluouracil
Investigating therapy response/resistance of mammary carcinoma cell lines \textit{in vitro}

Murine H8N8 cells: immortal mammary carcinoma cell line with tumor stem cell properties.

H8N8 T3.2 cells represent a recurrent tumor variant. H8N8 T3.2 cells were established from a solid breast tumor that received a CAF clinical regimen in vivo.

\textbf{Poster P374}: `In Vitro Investigations of Adjuvant Chemotherapy for Prevention of Breast Cancer Tumor Recurrence' on \textbf{Tuesday March 13th}. Data courtesy of Dr. Oliver Reinhardt, Dr. Frauke Alves, Translational Molecular Imaging Group, Max-Planck Institute of Experimental Medicine, Göttingen, Germany.
Cancer growth monitoring via impedance on CardioExcyte 96

Impedance readout (CardioExcyte 96)

Image-based live-cell analysis readout

Normalized Impedance

Confluence (%)

Time / h

Time (h)

00:00 100:00 200:00

0 25 50 75 100

1 2 3 4 5

H8N8 H8N8T3.2

Neg. Control H8N8 H8N8T3.2

tumor cell doubling time (h)

tumor cell doubling time (h) +/- SEM

H8N8 untreated residual relapse

0.0 20.0 40.0 60.0

0.0 20.0 40.0 60.0

0.0 20.0 40.0 60.0
Investigating therapy response/resistance of mammary carcinoma cell lines in vitro

Impedance readout (CardioExcyte 96)

Image-based live-cell analysis readout

CAF mix applied:
10 µg/ml Cyclophosphamide;
0.05 µg/ml Doxorubicin; 10 µg/ml 5-Fluouracil

CAF mix applied:
0.64 µg/ml Cyclophosphamide;
0.032 µg/ml Doxorubicin; 0.64 µg/ml 5-Fluouracil
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SyncroPatch 384/768 PE
ion channels in cell lines and iPS CMs

The SyncroPatch 384/768 PE with Optical Stimulation
CardioExcyte 96 Hybrid Measurements: Mode of Operation

**controled environment**

<table>
<thead>
<tr>
<th>Start up</th>
<th>EFP</th>
<th>switch</th>
<th>Impedance</th>
</tr>
</thead>
</table>

- Independent data files for each recording mode.
- EFP and IMP recordings of **the same cells**.

- long-term stable consumables
- dual readout from the **SAME** cells

Impedance signals

EFP signals
Cardiomyocyte attachment and toxicity monitored via impedance on CardioExcyte 96

Development of beat rate and EFP amplitude of iPSC CMs

Chronic effect of Nifedipine (>24 h)
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Cardiomyocyte attachment and toxicity monitoring via impedance

SyncroPatch 384/768 PE for ion channels in cell lines and iPSC CMs

The SyncroPatch 384/768 PE with Optical Stimulation
Automated patch clamp technology
from manual work to high throughput state-of-the-art automation

- **Port-a-Patch**
  - 50 data points / day
  - 6-20 compounds / day

- **Patchliner**
  - 600 data points / day
  - 75-300 compounds / day

- **SyncroPatch 384/768PE**
  - 20,000 / 40,000 data points / day
  - 2.5k-10k / 5k-20k compounds / day
The SyncroPatch 384/768 PE
the 4th generation APC

Giga-seal patch clamping
384 or 768 recording channels
Up to 20,000/40,000 data points per day
Unattended operation 4-6 hours
€ 0.16 - 0.65 per data point

Ultra flexible: Can be applied in ALL phases of drug discovery

SyncroPatch 384 PE – consumables NPC-384
Variable hole number and size
Partial run (any 32 wells per run)
Partial plates can be run without using the whole chip
One protocol takes care of many ion channels

$\text{Na}_v 1.5$ ("$I_{Na}$")

$\text{K}_v 11.1$ (hERG – "$I_{Kr}$")

$\text{K}_v 4.3$-$\text{K}_{\text{Chip}} 2.2$ ("$I_{to}$")

$\text{Ca}_v 1.2/b2/a2d$ ("$I_{Ca, L}$")

$\text{K}_v 7.1$-$\text{KCNE1}$ ("$I_{Ks}$")

$\text{K}_i 2.1$ ("$I_{K1}$")
Ion Channels of the CIPA Initiative – on ONE plate
Different (iPS CM) cells on one 384-well plate

<table>
<thead>
<tr>
<th>Vendor iPSC-CMs</th>
<th>Ncardia Cor.4U</th>
<th>CHO Nav1.7</th>
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</thead>
<tbody>
<tr>
<td>GE</td>
<td>400M</td>
<td>350M</td>
</tr>
<tr>
<td>IF</td>
<td>250M</td>
<td>270M</td>
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<tr>
<td>IF</td>
<td>150M</td>
<td>150M</td>
</tr>
<tr>
<td>FC</td>
<td>400M</td>
<td>350M</td>
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<td>IF</td>
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<tr>
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<td>150M</td>
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<tr>
<td>IF</td>
<td>150M</td>
<td>150M</td>
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</tbody>
</table>

The SyncroPatch 384/768 PE the 4th generation APC

Made in Germany by nanion
Inter-platform and Inter-site Comparison

- CiPA cpds were tested on medium and high throughput APC devices
- 3 Nanion sites contribute IC50 data on 6 ion channels

Exemplary results for 3 cpds on hERG and Nav1.5

<table>
<thead>
<tr>
<th>Channel</th>
<th>Cpd</th>
<th>IC50 Literature** [µM]</th>
<th>Highest test conc. [µM]</th>
<th>Ambient temp. IC50 [µM]</th>
<th>Physiol. temp. IC50 [µM]</th>
<th>Ambient temp. IC50 [µM]</th>
<th>Physiol. temp. IC50 [µM]</th>
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<tbody>
<tr>
<td>hERG</td>
<td>Sotalol*</td>
<td>111</td>
<td>300</td>
<td>160</td>
<td>151</td>
<td>192</td>
<td>217</td>
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<tr>
<td></td>
<td>Diltiazem</td>
<td>13.2</td>
<td>100</td>
<td>13.9</td>
<td>15.6</td>
<td>4.1</td>
<td>8.4</td>
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<tr>
<td></td>
<td>Quinidine*</td>
<td>0.72</td>
<td>10</td>
<td>1.02</td>
<td>0.92</td>
<td>1.54</td>
<td>1.02</td>
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<tr>
<td>Nav1.5</td>
<td>Sotalol*</td>
<td>1180</td>
<td>300</td>
<td>&gt;300</td>
<td>&gt;300</td>
<td>&gt;300</td>
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<td>Diltiazem</td>
<td>22.4</td>
<td>100</td>
<td>44.4</td>
<td>44.5</td>
<td>25.2</td>
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<td></td>
<td>Quinidine*</td>
<td>14.6</td>
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<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>

* Risk of TdP
** Chantest

➢ Strong consistency across platforms and sites
HTS pharmacology: current-clamp on iPSC Cardiomyocytes

Online Analysis APD 90

Online Analysis APD 90 and APD50

AP raw trace: CTR vs Nifedipine

Made in Germany by nangion
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SyncroPatch 384/768 PE ion channels in cell lines and iPS CMs

The SyncroPatch 384/768 PE with Optical Stimulation
The SyncroPatch 384/768 PE with **Optical Stimulation**

- First Prototype:
  - 96 blue LEDs (470 nm) below the Chip
  - Constant stimulation at selected intensity possible
  - Stimulation at selected frequency (e.g. 1 Hz) possible
The SyncroPatch 384/768 PE with Optical Stimulation

Depolarization Via ChR2: Nav1.5-ChR2

- Proof of Concept:
  - CHO cells expressing ChR2 and Nav1.5 were used
  - Cells were measured in Current Clamp (CC)
  - -90 mV were achieved by LFVC (Low Frequency Voltage Clamp)
  - 1 ms Light Pulse at 2Hz (470 nM) was used for stimulation

Cells: ChR2 + Nav1.5 in CHO provided by nanJi[on]
Thank you!

SyncroPatch 384/768PE.

The CardioExcyte 96.

Nanion PE team

Nanion CE team

Visit our posters:

- **P183**: ‘Assessment Of Drug Effects On Cardiomyocyte Function: Comprehensive In Vitro Proarrhythmia Assay (CIPA) Results’ on **Monday March 12th**
- **P374**: ‘In Vitro Investigations of Adjuvant Chemotherapy for Prevention of Breast Cancer Tumor Recurrence’ on **Tuesday March 13th**

Nanion Booth #534