QPatch applications on iPSC-derived cardiomyocytes and neurons

Precision Medicin & Ion Channel Retreat 2016
Guangzhou, China
November 11, 2016

Kazuya Tsurudome  Ph.D.
Senior Application Scientist, Biolin Scientific K.K.
1. Introduction – Automated patch clamp and iPS-derived cells
2. iPS-derived cardiomyocytes
3. iPS-derived neurons
4. Conclusion
The global ion channel modulators market is forecast to reach a value of USD 21.4bn by end-2018, driven largely by deeper understanding of channelopathies and ongoing advances in electrophysiology, particularly automated electrophysiology.
Whole cell patch clamp system

- Need to keep cells/animals alive in good condition
- Whole cell patch clamp configuration by manipulator and syringes
- Run voltage protocols and apply drugs manually
- Every step performed by skillful researcher

Time consuming experiments
QPatch 16/48 channel automated patch clamp system

- Automated cell preparation
- Whole cell patch clamp configuration by pressure system
- Programmed assays are conducted by robot and amplifiers
- Precise auto drug application
- 100% Unattended system after ‘start’
QPlate; single-hole technology

Single-hole per well

Inlet to intracellular flow channel
Inlet to extracellular flow channel
Cell positioned on patch clamp site
Waste reservoir
Capillary stop

Intracellular flow channel
Silicon chip
Surplus cell attached to flow channel floor

MEAS

REF
Other features: Temperature control with voltage clamp

• Currents were recorded from hERG expressing CHO cells. The experiments were performed at 22°C (orange) or 34°C (green).

• I-V plots showed a shift of activation curve. There was about -15mV shift of $V_{0.5}$ at 34°C (purple) compared to 22°C (pink).
Other features: Current clamp

- Membrane potentials were recorded from hERG expressing CHO cells. The experiments were performed at 22°C or higher temperature up to 34°C.

- The pulse frequency was 0.16pulse/sec at 22 °C and it increased up to 0.76pulse/sec at 34°C.
Automated systems with cultured cells

- Scalable
- Stable cell condition
- Cell lines stably expressing specific ion channels
- Suitable cells (such as CHO or HEK293 cells) for planar patch

Nowadays, we have a demand of testing iPSC-derived cells such as cardiomyocytes or neurons on automated patch clamp system
Induced Pluripotent Stem Cell (iPSC)

Discoveries

2012 Nobel Prize in Physiology or Medicine

Commercially available

iPSC-derived cells (cardiomyocytes/neurons etc.)

Skin cells → ES-like Cells → induced Pluripotent Stem (iPS) Cell

Mouse 2006 Human 2007

From Dr. Yamanaka’s Novel prize lecture
iPSCs for research and application

From Dr. Yamanaka’s Novel prize lecture
Cardiomyocytes

- iCell Cardiomyocytes by Cellular Dynamics
- Cor.At, Cor.4U by Axiogenesis AG

Neurons

- Dopa.4U by Axiogenesis AG
- ReproNeuro by ReproCELL
Cardiomyocytes

- iCell Cardiomyocytes by Cellular Dynamics
- Cor.At, Cor.4U by Axiogenesis AG
Seals were obtained from 53% of cells. Whole cell configuration rate was 22%.

The currents observed: $\text{Na}^+ > \text{K}^+ > \text{Ca}^{2+}$

Detachment: Trypsin
Cell suspension media: EX-Cell ACF CHO media

### iCell from CDI

<table>
<thead>
<tr>
<th>Seal</th>
<th>Single-hole</th>
<th>Multi-hole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seals</td>
<td>53 % (±12, n=9)</td>
<td></td>
</tr>
<tr>
<td>Whole-cell</td>
<td>22 % (±13, n=9)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Single-hole</th>
<th>Multi-hole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell size</strong></td>
<td>29 pF (±15, n=41)</td>
<td>N.D</td>
</tr>
<tr>
<td><strong>Cells expressing $I_{Na}$</strong></td>
<td>91 % (±16, n=40)*</td>
<td>N.D</td>
</tr>
<tr>
<td><strong>Cells expressing $I_{Ca}$</strong></td>
<td>41 % (±39, n=58)*</td>
<td>N.D</td>
</tr>
<tr>
<td><strong>Cells expressing $I_{K}$</strong></td>
<td>80 % (±35, n=17)*</td>
<td>N.D</td>
</tr>
<tr>
<td><strong>Usable $I_{Na}$ data/QPlate</strong></td>
<td>16 % (±10, n=224)</td>
<td>58 % (±22, n=80)</td>
</tr>
<tr>
<td><strong>$I_{Nav}$ amplitude</strong></td>
<td>-3.5 nA (±2.2, n=24)</td>
<td>-6.4 nA (±42., n=46)</td>
</tr>
<tr>
<td><strong>IC$<em>{50}$ TTX for $I</em>{Na}$</strong></td>
<td>10.3 μM (±0.5, n=6)</td>
<td>6.3 μM (±4.5, n=12)</td>
</tr>
<tr>
<td><strong>Tau for $I_{Na}$ inactivation</strong>***</td>
<td>0.85 ms (±0.29, n=22)</td>
<td>0.99 ms (±0.16, n=20)</td>
</tr>
</tbody>
</table>
Ringer solution used for current recording

- **Extracellular solution for $I_{Na}$ and $I_{Ca}$ (in mM)**
  - 120 NaCl, 5 KCl, 3.6 CaCl2, 1 MgCl2, 20 TEA-Cl, 10 HEPES. pH7.4
- **Intracellular solution for $I_{Na}$ and $I_{Ca}$ (in mM)**
  - 120 CsCl, 3 MgCl2, 10 EGTA, 5 HEPES, 5 MgATP. pH7.3

- **Extracellular solution for $I_{K}$ (in mM)**
  - 15 NaCl, 140 KCl, 1.2 CaCl2, 1 MgCl2, 10 HEPES. pH7.4
- **Intracellular solution for $I_{K}$ (in mM)**
  - 5.374 CaCl2, 1.75 MgCl2, 3.125/10 KOH/EGTA, 120 KCl. pH7.2
iCell from CDI

- IC$_{50}$ values of TTX, Nifedipine were in range of those values for Na$_V$1.5, Ca$_V$1.2 channels

Current sweeps

Dose response curve (Hill fit)

I-V plot

- Na$^+$
  - 10µM TTX
  - Saline

- Ca$^{2+}$
  - 1µM Nifedipine
  - Saline

- TTX
  - IC$_{50}$=10.3µM

- Nifedipine
  - IC$_{50}$=95.3nM

V(I$_{\text{max}}$)=0 to 10mV
iCell from CDI

- Half activated voltage step \((V_{0.5})\) of tail \(K^+\) current was similar to literature value (-23.1mV vs -21.5mV*)

\[
\frac{[K^+]_o}{[K^+]_i} = \frac{140}{123} \text{[mM]}
\]

\(E_K = 3.3\text{mV}\)

* Sanginetti and Jurkiewicz 1990
### Cor.At (ESC derived mouse cardiomyocyte) from Axiogenesis

**Detachment:** Trypsin  
**Cell suspension media:** EX-Cell ACF CHO media

<table>
<thead>
<tr>
<th>Subject</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viability after harvest procedure (%)</td>
<td>87 ± 3, n = 4</td>
</tr>
<tr>
<td>Size of cell (pF)</td>
<td>17 ± 7, n = 112</td>
</tr>
<tr>
<td>Peak $I_{Na}$ (pA) (at -30 mV)</td>
<td>1842 ± 2521, n = 52</td>
</tr>
<tr>
<td>Current density $I_{Na}$ (pA/pF)</td>
<td>104 ± 129, n = 52</td>
</tr>
<tr>
<td>Cells with recordable $I_{Na}$ amplitude (%)</td>
<td>68 (15/22)</td>
</tr>
<tr>
<td>Peak $I_{Ca}$ (pA) (at +10 mV)</td>
<td>35 ± 27, n = 32</td>
</tr>
<tr>
<td>Current density $I_{Ca}$ (pA/pF)</td>
<td>2 ± 1.5, n = 32</td>
</tr>
<tr>
<td>Cells with recordable $I_{Ca}$ amplitude (%)</td>
<td>55 (12/22)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Single-hole</th>
<th>Seal</th>
<th>60.9% (±17, n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-cell</td>
<td></td>
<td>50% (±11, n=4)</td>
</tr>
</tbody>
</table>
Cor.At (ESC or iPSC derived mouse cardiomyocyte) from Axiogenesis

\[ \text{Na}^+ \]

\[ V_{0.5} = -62 mV \pm 13 \]
\[ n=7 \]

\[ \text{TTX} \]

\[ \text{Ca}^+ \]

\[ \text{nifedipine} \]
\[ 10 \mu M \]

\[ \text{isoproterenol} \]
\[ 1 \mu M \]
\[ 10 \mu M \]
Cor.4U from Axiogenesis

Detachment: TrypLE Express
Cell suspension: manually suspended with EC

<table>
<thead>
<tr>
<th>Solution</th>
<th>Whole cell method</th>
<th>R seal [MΩ]</th>
<th>R whole cell [MΩ]</th>
<th>Success rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological</td>
<td>Suction</td>
<td>712</td>
<td>485</td>
<td>18.7</td>
</tr>
<tr>
<td>Physiological</td>
<td>Perforated</td>
<td>1255</td>
<td>561</td>
<td>18.8</td>
</tr>
<tr>
<td>KF</td>
<td>Suction</td>
<td>511</td>
<td>613</td>
<td>27.3</td>
</tr>
<tr>
<td>KF</td>
<td>Perforated</td>
<td>469</td>
<td>886</td>
<td>57.1</td>
</tr>
</tbody>
</table>

Extracellular solution:
EC000: 145 NaCl, 4 KCl, 1 MgCl₂, 2 CaCl₂, 10 HEPES, 10 Glucose (mM), pH7.4

Physiological intracellular solution:
IC000: 120 KCl, 1.75 MgCl₂, 5.374 CaCl₂, 31.25/10 KOH/EGTA, 10 HEPES, 4 Na₂-ATP (mM), pH7.2

KF containing intracellular solution:
IC700: 120 KF, 20 KCl, 10 HEPES, 10 EGTA (mM), pH7.2
Cor.4U from Axiogenesis

Reference: 1μM FPL 64176

Membrane potentials (Current clamp recording)

Tetracaine
IC$_{50}$ = 1.95μM
## Cor.4U from Axiogenesis

<table>
<thead>
<tr>
<th>Current types</th>
<th>Current</th>
<th>Membrane potential</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transient inward/outward</strong></td>
<td><a href="#">Graph</a></td>
<td><a href="#">Graph</a></td>
</tr>
<tr>
<td><strong>Transient inward</strong></td>
<td><a href="#">Graph</a></td>
<td><a href="#">Graph</a></td>
</tr>
<tr>
<td><strong>Sustained outward</strong></td>
<td><a href="#">Graph</a></td>
<td><a href="#">Graph</a></td>
</tr>
<tr>
<td><strong>Inward tail</strong></td>
<td><a href="#">Graph</a></td>
<td><a href="#">Graph</a></td>
</tr>
<tr>
<td><strong>Sustained outward</strong></td>
<td><a href="#">Graph</a></td>
<td><a href="#">Graph</a></td>
</tr>
</tbody>
</table>
Neurons

- Dopa.4U by Axiogenesis AG
- ReproNeuro by ReproCELL
Dopa.4U from Axiogenesis

<table>
<thead>
<tr>
<th></th>
<th>attached cells</th>
<th>compl. experiment</th>
<th>seal</th>
<th>whole cell with good Rs</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of cells</td>
<td>380</td>
<td>209</td>
<td>217</td>
<td>125</td>
</tr>
<tr>
<td>Success rate</td>
<td>67%</td>
<td>55%</td>
<td>57%</td>
<td>33%</td>
</tr>
</tbody>
</table>

Detachment: Accutase, Detachin or Trypsin
Cell suspension: manually suspended with EC
Ringer solution used for current recording from neurons

• Extracellular solution (in mM)
  – 145 NaCl, 4 KCl, 1 MgCl$_2$, 2 CaCl$_2$, 10 HEPES, 10 Glucose, pH7.4

• Intracellular solution (in mM)
  – 120 KF, 20 KCl, 10 HEPES, 10 EGTA, pH7.2
Dopa.4U from Axiogenesis

<table>
<thead>
<tr>
<th>A-type $K_v$</th>
<th>Na$_v$</th>
<th>delayed rectifier $K_v$</th>
<th>mix $K_v$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-AP</td>
<td>TTX</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$IC_{50}=0.4\pm1.3$ mM

$IC_{50}=2.9\pm0.01$ nM
ReproNeuro from ReproCELL

Detachment: TrypLE
Cell suspension media: EX-Cell ACF CHO media

<table>
<thead>
<tr>
<th></th>
<th>Single-hole</th>
<th>Currents</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell attached</td>
<td>45% (±12, n=19)</td>
<td>Inward</td>
<td>39</td>
</tr>
<tr>
<td>Whole-cell</td>
<td>26% (±11, n=19)</td>
<td>Outward</td>
<td>61</td>
</tr>
<tr>
<td>WC per cell attach</td>
<td>57% (±19, n=19)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>R seal</th>
<th>R whole cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;100MΩ</td>
<td>117 (98.3 %)</td>
<td>119 (100%)</td>
</tr>
<tr>
<td>&gt;500MΩ</td>
<td>52 (43.7 %)</td>
<td>88 (73.9%)</td>
</tr>
<tr>
<td>&gt;1000MΩ</td>
<td>36 (30.3 %)</td>
<td>69 (58.0%)</td>
</tr>
<tr>
<td>Average [MΩ]</td>
<td>1049.6</td>
<td>1285.0</td>
</tr>
</tbody>
</table>
ReproNeuro from ReproCELL

Inward current

Saline 100nM TTX

Outward current

A-type Kv
+ delayed rectifier Kv

A-type Kv

Saline 100 mM TEA

V(I_{\text{min}})=-15\text{mV}
Summary

- **Success rates** were 50-60% at sealing state and then dropped to 20-30% at W.C. Those success rates were strongly affected by the harvesting method and whole cell method.

- **Low cell density:** The cell attached success rate did not achieve near 100%, which is probably because of the low cell density. (the whole cell configurations were successfully obtained over 50% of the attached cells)

- **Excitability:** The recorded membrane currents and membrane potentials were consistent with the channels expressed in each cell types. However, the channel expression was not even and the excitability strongly depends on the current expression pattern.
Acknowledgement

Sophion A/S
- Rikke S Perrier
- Denise Franz
- Hervør L Olsen

NMI TT Pharmaservices
- Timm Danker

Thank you!!