Characterization of Ion Channels in Stem Cell-derived Cardiomyocytes on An Automated Parallel Patch Clamp System

Xin Jiang1, Jan Dolzer1, James L. Costantin1, David Yamane1, Heribert Bohlen2, Silke Schwengberg2, Ralf Kettenhofen2, Bernd Fronhoff2

Molecular Devices, Sunnyvale, California; Axiogenesis AG, Cologne, Germany

Abstract
The advent of several automated electrophysiology platforms in recent years has significantly increased the throughput of patch clamp assays. This allows pharmaceutical and biotechnological companies to employ direct electrophysiological determination of compound activity on ion channels, with much larger numbers of compounds, and at earlier stages of the drug discovery and development process. However, existing automated electrophysiology platforms rely heavily on the quality and homogeneity of cell suspension for a satisfying success rate. This requirement often limits the application of current systems to recording from cell lines stably transfected with ion channel of interest, rather than primary cells that are more biologically relevant. In a collaborative effort to overcome this challenge, we attempt to characterize the electrophysiological and pharmacological properties of endogenous ion channels in stem cell-derived mouse cardiomyocytes using an automated patch clamp system. Genetically selected from mouse embryonic stem cells, Cor.At cells are highly homogeneous cardiomyocytes with normal morphological and functional properties. More importantly, they reproduce an intact cellular environment that includes all essential cardiac ion channels. In this study we demonstrate that the PatchXpress 7000A automated parallel patch clamp system can reliably record Na, K and Ca currents from Cor.At cells with reasonable success rate. This study demonstrates that the combination of “ready-to-use” cardiomyocytes with an automated patch clamp system offers a powerful assay platform not only for faster profiling of lead compounds in a more biologically relevant system.

Materials
Cells
Cor.At "ready-to-use" pure cardiomyocytes: Embryonic cardiomyocytes derived from mouse embryonic stem (ES) cells, 99.9% pure with no fibroblast contamination
Cor.At cells were packaged and shipped in standardized frozen vials. Upon thawing, cells were plated on fibronectin coated culture dishes in appropriate media.

> 2-10 days after resuscitation, cells were trypsinized and re-suspended before being used immediately on the PatchXpress 7000A automated parallel patch clamp system

Reagents and Protocols
Recording solutions were prepared to isolate specific channels of interest (Na, K, Ca).
Voltage protocols are shown in the figures

Properties

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Cost</th>
</tr>
</thead>
</table>

Table 1. Comparison of cell lines and primary cells; table is revised from GPCR Newsletter, 2008, Vol 2

Fig 1. Cor.At cells are morphologically and functionally normal. Top: Cor.At cells are produced with a GFP-reporter gene driven by a cardiac specific promoter for easy identification. Note the elongated shape of the cells. Middle: Immunohistochemistry of β cardiac actinin (red), a specific marker for cardiomyocytes. The nuclei (blue) are stained with DAPI. Bottom: Immunostaining of gap junction protein connexin 43, anther specific marker for cardiomyocytes. In addition, Cor.At cells display autonomous contractility, indicating they are functionally similar to primary cardiomyocytes.

Fig 2. PatchXpress 7000A Automated Parallel Patch Clamp System

Key features of the system:
• 16 parallel recording chambers
• 16 parallel wash channels
• True gigaseals
• Whole-cell recording
• Access resistance compensation
• Capacitance compensation
• Disposable pipette tips
• ERobot control of the system for online data analysis and decision making

Fig 3. Recording of Na+ currents from Cor.At cells. Left panel: Sample Na current traces obtained from Cor.At cells using conventional patch clamp method. Data was collected by Axiogenesis through collaboration. Right panel: Sample Na current recorded with PatchXpress 7000A. (inset, voltage protocol).

Summary
• Cor.At cells express all essential cardiac ion channels, and they are likely to have intact signaling pathways that modulate channel functions.
• PatchXpress 7000A can record all endogenous channels in Cor.At cells, the quality of recording is equal to that of conventional patch clamp method.
• With some optimization, PatchXpress can record Cor.At cells with good success rate (~ 50% as defined by percentage of chambers reaching gigaseals).
• Open channel design of the SealChip offers easy access of larger cells for successful recording.
• The successful application of Cor.At cells on PatchXpress provides a powerful platform for assaying compounds in a more bio-relevant system.

Fig 4. Recording of K+ currents from Cor.At cells. Left panel: Sample K current traces obtained from Cor.At cells using conventional patch clamp method. Data was collected by Axiogenesis through collaboration. Right panel: Sample K current recorded with PatchXpress 7000A.

Fig 5. Recording of Ca2+ currents from Cor.At cells. Left panel: Sample Ca current traces obtained from Cor.At cells in the absence (control) and presence of sympathetic agonist epinephrine (10 μ M). The experiment was performed on PatchXpress, using Barium at charge carrier. Note the amplitude of peak inward current increased after the cell was treated with epinephrine. Right panel: Recordings of peak inward Ba current before and after the application of epinephrine. The two arrows indicates the time points currents on the left panel were recorded.

Fig 6. Sympathetic modulation of Ca channels in Cor.At cells. Left panel: Sample Ca-current traces obtained from Cor.At cells in the absence (control) and presence of sympathetic agonist epinephrine (10 μ M). The experiment was performed on PatchXpress, using Barium at charge carrier. Note the amplitude of peak inward current increased after the cell was treated with epinephrine. Right panel: Recordings of peak inward Ba current before and after the application of epinephrine. The two arrows indicates the time points currents on the left panel were recorded.