Rapid Production of Cells for Screening Voltage-Gated Ion Channels in Automated Electrophysiology Assays Using the MaxCyte® STX™ Scalable Transfection System

Introduction

Comprising a family with hundreds of members, ion channels represent one of the largest classes of drug targets. Recent advances in automated electrophysiology instrumentation have led to the routine implementation of high throughput assays for screening ion channel modulators. However, despite improvements to analytical throughput, hurdles to the development of drugs for ion channel targets still remain.

One of the biggest bottlenecks in ion channel screening stems from a wide spread reliance on stable cell lines. In addition to the labor, time and cost constraints that are associated with stable cell line development for any type of drug target, creating cell lines for ion channel screening poses additional challenges. For example, many ion channels are heteromeric structures with multiple pore forming and modulatory subunits, which means that cells must be exposed to multiple, harsh selection compounds that can impair their health and proliferation. Furthermore, ion channels are often toxic when expressed at high levels, creating a need for inducible promoters, which add an extra layer of complexity to the cell line development process.

The MaxCyte STX transfection system, based on MaxCyte's proprietary, scalable electroporation technology, overcomes the challenges that were previously associated with generating cells for ion channel assays. Single or multiple plasmids encoding different ion channel subunits can be transfected simultaneously into as few as $5x10^5$ cells in a matter of seconds using static electroporation or in up to $1x10^{10}$ cells in less than thirty minutes via flow electroporation. Optimized electroporation protocols enable high efficiency transfection of a wide variety of cell lines and physiologically relevant primary cells with DNA, mRNA, siRNA, proteins or small molecules while maintaining high levels of cell viability. Transfected cells are suitable for immediate use in cell-based assays or they can be aliquoted and cryopreserved for future screening applications.

Here we present examples of ion channel screening using CHO K1 cells that were transiently transfected with plasmids encoding the voltage gated potassium channels, $K_v1.3$ or $K_v1.5$. Transfected cells were assayed on the IonWorks® Quattro™, a widely used high throughput electrophysiology platform. The transiently transfected cells showed strong, consistent current tracings in response to the application of standard voltage step protocols, demonstrating their suitability for drug screening applications.

Materials

CHO K1 cells (ATCC CCL-61)
Ham's F12 (Mediatech 10-080)
Fetal Bovine Serum (FBS) (Mediatech 30-002-CI)
Penicillin-streptomycin (pen/strep) (Mediatech 30-002-CI)
Phosphate buffered saline (PBS)(Lonza 17-516F)
HBSS (Lonza; 10-508Q)
0.25% Trypsin (Mediatech 25-053-CI)
Accutase (Millipore SCR005)
KCNA3($K_v1.3$)-GFP expression plasmid (Origene RG207223)
KCNA5($K_v1.5$) expression plasmid (Origene SC123694)
DNase I (Sigma D4263)

Methods

Cell culture and electroporation:
CHO cells were cultured at 37°C with 5% CO_2 in Ham's F12 supplemented with 10% FBS, 1% pen/strep. Cells were passaged every 2-3 days, and they were split one day prior to electroporation to ensure that they were healthy and in log phase growth at the time of transfection.

Passage 12 cells were harvested with trypsin, pelleted at 250 X g for 10 minutes, rinsed in MaxCyte electroporation buffer (2-5X final electroporation volume), pelleted again, and then suspended in MaxCyte buffer at a density of $1x10^8$ cells/mL.

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Cells ($4x10^7$ per transfection) were then mixed with plasmid DNA (dissolved in sterile, distilled water) and transferred to OC-400 processing assemblies (PAs). Immediately after transfection using the preset "CHO" protocol, cells were transferred to one well of a 24-well plate, DNase solution (200 U/mL) was added to remove DNA that did not completely enter the cells, and the cells were incubated for 20 minutes at 37°C in a 5% CO_2 incubator. The cells were then diluted into 20 ml of cell culture medium and counted in the presence of trypan blue prior to replating.

Ion Channel Assays:
Twenty-four hours after transfection, the cells were washed twice with HBSS, treated with Accutase for 45 minutes to lift them from the plate, and resuspended in HEPES-buffered physiological saline. The $K_v1.3$ and $K_v1.5$ currents were measured with IonWorks Quattro in the single hole or HT mode. Currents were elicited with 500-msec test voltage pulses to 0 and +40 mV from a holding potential of -80 mV. For each transfection condition, cells were plated in 128 wells of the recording plate.

Results

CHO K1 cells transfected via static electroporation with increasing concentrations of either a $K_v1.5$ expression plasmid or a plasmid encoding a $K_v1.3$-GFP fusion protein were assayed on the IonWorks Quattro in single hole or HT mode 24 hours after transfection. Figure 1A shows representative current tracings from $K_v1.5$ transfected cells that demonstrate strong, voltage-induced outward currents. Cells transfected with all three concentrations of DNA exhibited currents that were significantly higher than currents generated by control cells. In Figure 1B, pictures of transfected cells expressing the $K_v1.3$-GFP fusion protein reveal fluorescence that is concentrated on the cell surface, indicating correct intracellular trafficking and membrane localization of the transiently expressed ion channels. As with the $K_v1.5$ transfections, all three concentrations of $K_v1.3$-GFP plasmid DNA elicited strong, voltage responsive membrane currents.

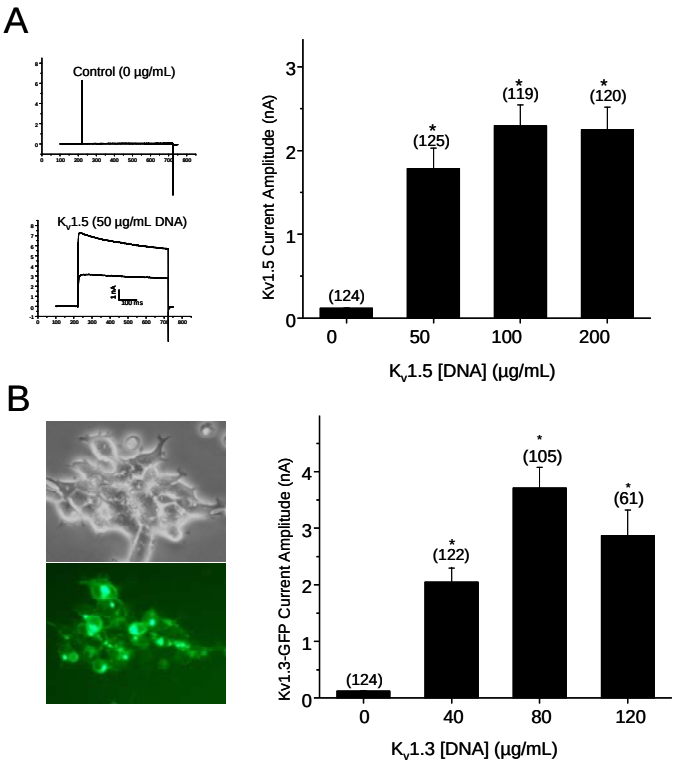

Figure 1. (A) Analysis of $K_v1.5$ expressing cells. (B) Analysis of cells transfected with $K_v1.3$-GFP plasmid DNA. Representative current tracings from $K_v1.5$ expressing cells are shown in the left panel of A. Bright field and fluorescence photographs of $K_v1.3$-GFP transfected cells 24 hrs post transfection are shown on the left in B. The right panels in A and B show histograms of average peak current amplitude recorded from cells transfected with different amounts of $K_v1.5$ and $K_v1.3$-GFP plasmid DNAs, respectively. Recordings were obtained from the number of cells indicated in parentheses above each bar. The data are presented as mean current +/- standard error. The asterisk (*) indicates a significant difference in mean amplitude from the control ($p<0.05$).

Summary

- ✓ The MaxCyte STX system enables rapid production of cells for high throughput ion channel assays.
- ✓ Ion channels exhibit membrane-localized expression in transiently transfected cells.
- ✓ Cells transfected with the MaxCyte STX exhibit good membrane integrity and show consistent current tracings when assayed on automated electrophysiology screening platforms.

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MaxCyte, Inc. • 22 Firstfield Road, Suite 110 • Gaithersburg, MD 20878
Tel: (301) 944-1700 or info@maxcyte.com