IMPROVED ASSAY OF TRANSIENTLY TRANSFECTED ION CHANNELS ON AN AUTOMATED ELECTROPHYSIOLOGY SYSTEM

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Abstract

At present, the applications of automated electro-physiology systems have been limited primarily to the assays of stably transfected recombinant cell lines or native cells endogenously expressing ion channels of interest. Despite their popularity, the construction and optimization of stable cell lines are both time consuming and costly. In this study, we evaluated an alternative approach where we used a high-efficiency electroporation-based gene delivery instrument, MaxCyte STX system, to transiently transfect HEK-293 parental cells with either a potassium channel (K_V1.3) or a chloride channel (TMEM16A) gene. Twenty-four to forty-eight hours after transfection, the cells were harvested and assayed on the PatchXpress® 7000A automated parallel patch clamp system. With some optimization of the protocol, the transfection efficiency, as measured by the expression of green fluorescence protein tagged to the ion channel, reaches ~80%. In addition, using PatchXpress 7000A system we were able to achieve ~50 - 65% Gigaohm seal rate with either transfected cells, comparable to that for stable cell lines. More importantly, currents mediated by either channel can be reliably recorded from the transfected cells at qualities equivalent to manual patch clamp. In conclusion, the combination of this high-efficiency transient transfection system with the PatchXpress 7000A automated parallel patch clamp system can provide ion channel investigators with an improved tool that is faster, cheaper and more versatile than stably transfected cell lines.

Materials

Cells

- **Human Embryonic Kidney (HEK293) cells from ATCC (Catalog # CRL-1573)**

Plasmids

- **Mouse TMEM16A full length cDNA was purchased from Open Biosystems (Huntsville, AL) and was cloned into pCMV-SPORT6 (clone ID: 3591765)**
- **Human K_V1.3 cDNA with C-terminal GFP tag was provided by MaxCyte, Inc and was originally purchased from OriGene (Rockville, MD)**

Reagents and Protocols

- **<u>For recording of K_V1.3 channel</u>**

1) **External Solution (in mM): 138 NaCl, 0.5 $MgCl_2$, 0.9 $CaCl_2$, 2.7 KCl, 1.5 KH_2PO_4 and 8.1 Na_2HPO_4, pH 7.4**
2) **Internal Solution (in mM): 140 KCl, 2 $MgCl_2$, 5 EGTA, and 10 HEPES, pH 7.2**
3) **Voltage protocol: I-V relation of K_V1.3 channel is measured by a series of incremental voltage steps from -80 mV to +60 mV. V_h = -60 mV.**

- **<u>For recording of mTMEM16A channel</u>**

1) **External Solution (in mM): 140 NMDG-Cl, 1 $MgCl_2$, 2 $CaCl_2$, 5 KCl and10 NMDG-HEPES, pH 7.2 with NMDG**
2) **Internal Solution (in mM): 130 CsCl, 1 $MgCl_2$, 10 EGTA, 10 HEPES, 8 $CaCl_2$ and 1 Mg-ATP, pH 7.3. The calculated free Ca^{2+} concentration of this internal solution is 600 nM.**
3) **Voltage protocol: I-V relation of mTMEM16A channel is measured by a series of incremental voltage steps from -100 mV to +100 mV. V_h = 0 mV.**

- **All electrophysiology data were stored and analyzed with DataXpress 2.0**

Fig 1. **MaxCyte® STX™ Scalable Transfection System**

Key features of the system:

- Ease and flexibility of use
- Works with cell lines, primary cells, stem cells
- Works with DNA, RNA, proteins, small molecules
- >90% viability for many cell types
- >90% loading & transfection efficiency for many cell types.
- ~5E5 cells in <1minute to 1E10 cells in <30 minutes.

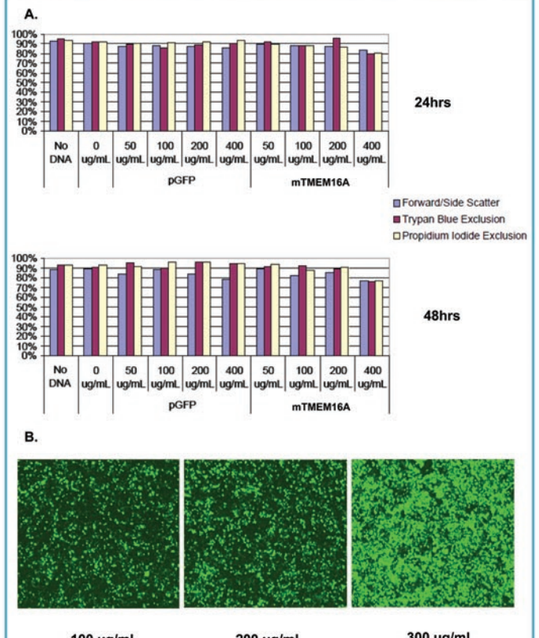

Fig 2. **Optimization of transfection protocol using MaxCyte STX system.** HEK-293 parental cells were grown in T175 culture flasks following standard procedures. Cells were split 24 hrs prior to transfection and were seeded at appropriate density so they reached ~70% confluence at the time of transfection. All transfections were performed following the standard procedure provided by MaxCyte. **A)** Viability of HEK-293 cells post transfection with GFP or mTMEM16A plasmid DNA. For either DNA four different concentrations were used for transfection. The viability of transfected cells at 24 or 48hrs as assayed using three different methods: Fluorescence Activated Cell Sorting (FACS), trypan blue exclusion and propidium iodide exclusion; **B)** Concentration-dependent expression of K_V1.3 channels measured by the green fluorescence protein signal tagged to the channel. The assays were performed 36hrs post transfection using IsoCyte DL laser scanner (Molecular Devices, Sunnyvale, CA). As shown in the right panel, when transfected with 300 µg/ml K_V1.3-GFP DNA, ~80% of the cells are GFP-positive.

Fig 3. **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
- Script control of the system for online data analysis and decision making

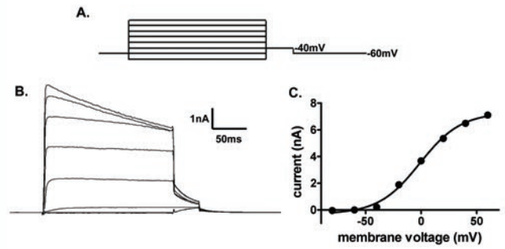

Fig 4. **Recording of K_V1.3 currents in HEK-293 cells transfected with K_V1.3-GFP plasmid DNA.** HEK-293 cells were transfected with 300 µg/ml DNA and plated on 35mm culture dishes. Thirty-six to forty-eight hours post transfection, cells were trypsinized and resuspended immediately before they were assayed on the PatchXpress 7000A system. **A)** Voltage protocol; **B)** Sample K_V1.3 currents recorded from a transfected cell; **C)** Current-voltage relationship of K_V1.3 channel extracted from the same cell as in B; each data point represents the peak outward current elicited by its respective voltage step .

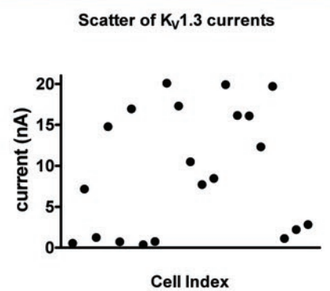

Fig 5. **High transfection efficiency of K_V1.3 channel in HEK-293 cells.** In this study, two experiments were performed on the PatchXpress 7000A system using the same batch of transfected cells. Out of a total of 32 chambers (16 x 2), K_V1.3 currents were successfully recorded from 21 cells. As shown in the scatter plot, the amplitudes of K_V1.3 currents (measured as the peak outward currents elicited at +60 mV) varied among cells, indicating a distribution of expression rate of K_V1.3 channels. This variability can be reduced through further optimization.

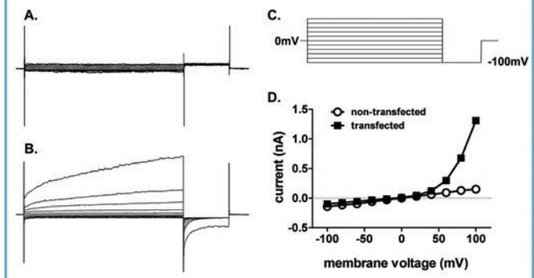

Fig 6. **Recording of whole-cell currents in HEK-293 cells transfected with mTMEM16A plasmid DNA.** HEK-293 cells were transfected with 300 µg/ml DNA and plated on 35mm culture dishes. Thirty-six to forty-eight hours post transfection, cells were trypsinized and resuspended immediately before they were assayed on PatchXpress 7000A system. **A)** Sample recording from a HEK-293 parental cell showing only the leak currents; **B)** Sample recording of TMEM16A currents from a transfected cell; **C)** Voltage protocol ;**D)** Current-voltage relationships extracted from the same non-transfected parental cell as in A (open circles) , and transfected cell as in B (closed circles). Note significant outward rectification of current at depolarized potentials, a known characteristics of TMEM16A channel.

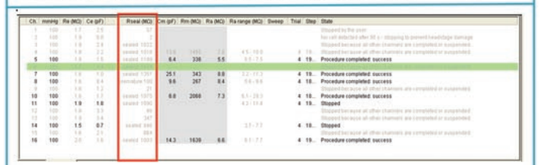

Fig 7. **Transfected cells can be recorded on PatchXpress 7000A system at the same Giga-seal quality as manual patch clamp but at higher throughput.** HEK-293 cells transfected with 300 µg/ml mTMEM16A DNA were assayed on PatchXpress 7000A thirty-six hours post transfection. The screenshot of a sample experiment shows 8 cells out of a possible 16 achieved Giga-seals (highlighted in the red box).

Summary

- MaxCyte STX system transfects ion channels into HEK-293 cells with good viability and high efficiency, ~80% as measured by the GFP signal tagged to the channel. Further optimization might be necessary to reduce the variability in membrane expression.
- PatchXpress 7000A is capable of achieving ~50-65% Giga-seal rate and whole cells access using transfected cells. The quality of recording is equivalent to that obtained from conventional patch clamp method.
- The high transfection efficiency provided by MaxCyte STX, combined with the high recording quality and throughput provided by PatchXpress 7000A system, allows ion channel researchers to collect data and make decisions more quickly. This improved and versatile method will be generally applicable to other channels.

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Presented at Biophysical Society 54th Annual Meeting
San Francisco, CA, February 20-24, 2010

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