A LAB-USE MICROFLUIDIC PLANAR PATCH-CLAMP SYSTEM

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ABSTRACT

This work presents a microfluidic planar patch-clamp system suitable for laboratory usage without high entry threshold as the traditional pipette technique. Our previously developed hourglass-shaped aperture integrated with simple microfluidic system available for whole-cell recording on HIT-T15, CHO-K1, and HEK-293T cell lines. Endogenous outward rectifier and A-type potassium currents were measured from HEK-293T. Extracellular solution are exchangeable up to six different solutions for ion channel interrogation. The fastest extracellular solution exchange rate demonstrated on HEK cells was within 500ms. The lab-use microfluidic planar patch-clamp system is believed to serve as a practical microdevice for general laboratory usage.

KEYWORDS: Planar patch-clamp, microfluidics, fluorescence

INTRODUCTION

Many studies on planar patch-clamp chip has been proposed to lessen exquisite manipulation from traditional pipette patch-clamp. Various current approaches have been investigated on planar patch-clamp chip integrating with microfluidics for single-cell analysis. However, those demonstrations were limited in using only one type of cell line[1-2]. Besides, low gigaseal formation[1] or required sophisticated fabrication process[1-2] was inevitable. Moreover, since the chips should be disposable, most microfluidic-coupled chips were one-time usage only[1-2]

Our previous work[3-4] demonstrated an hourglass-shaped aperture applicable for electrophysiological study and featured cost-effective, ease of fabrication, and high yield of gigaseal formation. In order to be applicable for general laboratory usage, the planar patch-clamp system should comprise of the following features: first, ease of fabrication in both device and patch-clamp chip; second, high yield of gigaseal formation for various cell lines; third, ease of fluidic exchange and chip replacement; and fourth, low cost.

EXPERIMENTAL

Figure 1 shows the integrated lab-use system of planar patch-clamp chip and microfluidics. The PDMS fluidic channels were first constructed via replica molding on a patterned PMMA structure. Then, without hydrophilic bonding, the channels were directly formed through innate adhesion of the contact between PDMS and glass chip facilitating the replacement of patch-clamp chip, as shown in Fig. 1C. The device photo presented in Fig. 1D visualizes corresponding chip size and the connection of electrode and tubing. Whole fabrication processes were operated in ambient environment without the use of cleanroom, and the device is also accessible to rapid intra- and extracellular solution exchange and optical detection.

RESULTS AND DISCUSSION

One threshold for planar patch-clamp suitable for general lab usage is capable of channel measurement for various types of cell lines. To demonstrate this prerequisite, HIT-T15, CHO-K1, and HEK-293T cells were tested in whole-cell configuration. Representative results of endogenous channel recording are presented in Fig. 2. Typical voltage-gated outward-rectified potassium channels measured in HIT-T15 cell with depolarizing traces from -60 to +100 mV, with 20 mV increment, at holding potential -70 mV, as shown in Fig. 2A. CHO-K1 cell inherently expressed volume-regulated chloride channels were measured in hypotonic solution of 240 mOsm (Fig. 2B). Depolarizing potential was performed from -70
mV with 10 mV increment, and dynamic channel activity observed when potential became positive. Aside from measuring individual endogenous channel in HIT-T15 and CHO-K1 cells, we have also found two types of potassium channel in HEK-293T cells. Fig. 2C shows A-type endogenous potassium currents featuring quick strong channel activation at the beginning and following with a quick inactivation. Fig. 2D displays intrinsic outward rectified potassium channels.

Figure 3. Demonstration of extracellular solution exchange and long-term recording on CHO-K1. (A) Endogenous volume-regulated chloride channels exhibited weak activity in normal isotonic solution but increased the open probability in hypotonic solution, and finally washed with 40 mM DIDS to examine the efficiency of channel blockage. I-V curves were the average of each trace in last 200 ms illustrating the representative current amplitude with respect to membrane potentials, as shown in Fig. 3B. Up to four solution used as extracellular solution exchange reagent displaying the applicability of solution change and stable recording around 30 minutes. (Fig. 3C)

Figure 2. Representative results of endogenous channel recording in whole-cell configuration measured from three types of cell lines. (A) Typical voltage-gated potassium channels measured from HIT-T15 beta cell. (B) CHO-K1 cell inherently expressed volume-regulated chloride channels. (C) A-type potassium currents of HEK-293T cell featured quick strong channel activation at the beginning and following with a quick inactivation. (D) Endogenously expressed outward rectified potassium channels of HEK-293T.

Figure 3 demonstrates whole-cell measurement on the integrated microfluidic planar patch-clamp device during extracellular solution exchange. The access resistance of the planar glass chip was 1.3 MΩ, and 1.05 GΩ seal was achieved within three seconds after negative suction pulse applied, and cell membrane ruptured by 50 ms voltage zap in whole-cell configuration. Voltage steps were elicited by 200 ms depolarization of membrane potential between -70 to +70 mV in 10 mV interval from holding potential of -70 mV. In Fig. 3A, endogenous volume-regulated chloride channels exhibited weak activity in normal isotonic solution but increased the open probability in hypotonic solution, and finally washed with 40 mM DIDS to examine the efficiency of channel blockage. I-V curves were the average of each trace in last 200 ms illustrating the representative current amplitude with respect to membrane potentials, as shown in Fig. 3B. Up to four solution used as extracellular solution exchange reagent displaying the applicability of solution change and stable recording around 30 minutes.

Similar extracellular solution exchange recording using up to six different solutions to interrogate endogenous potassium channel on HEK-293T is presented. The depolarizing voltage traces were elicited from -70 to +70 mV in 10 mV interval from holding potential of -70 mV. Whole-cell currents (Fig. 4A) and I-V curves (Fig. 4B) show significant channel blockage by 140mM TEA. Fig. 1C discloses the microfluidic integration amenable to investigate various compounds, six concentrations of extracellular KCl from 3 (control), 35, 70, 105, 140, and 3 mM were applied sequentially, and finally exchanged with 140 mM TEA to block potassium currents. The results showed the usefulness and stability of the whole-cell recordings. Figure 4D displays time-course relationship for a complete solution exchange. HEK-293T cell was used and holding potential of -70 mV. By exchanging extracellular KCl of 140 mM, an effective solution exchange could achieve within 500ms. The desired exchange rate could be attained by varying the demanded fluid delivery velocity.
Figure 4. Demonstration of various extracellular solution exchanged and rapid solution delivery on HEK-293T. (A)(B) Endogenous potassium channels in whole-cell configuration blocked by 140mM potassium channel blocker of TEA. I-V curves show significant channel blockage. (C) A serial extracellular solution exchange confirming the microfluidic integration amenable to rapidly interrogate various compounds. (D) Solution exchanged within 500ms.

CONCLUSION
We have successfully demonstrated a lab-use microfluidic planar patch-clamp system. It is believed to serve as a practical microdevice for general laboratory usage.

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REFERENCES

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