Enabling “Plate Reader” Simplicity in High Throughput Electrophysiology through Well Plate Integration with Microfluidics

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Abstract

While automated electrophysiology systems have been developed to record from individual cells and groups of cells, both approaches have limitations. Platforms that record from individual cells suffer from relatively low recording success rates, which typically hover around 50%. Coupled with the need to get a sufficient number of data points for statistically relevant results, the resulting experimental throughput is compromised. Systems that record from groups of cells provide an inherent averaging mechanism that increases recording success. However, available systems for recording from groups of cells do not allow continuous recording during compound application, preventing the system’s use with fast-acting ligand-gated ion channels.

To address these limitations, Fluxion Biosciences has developed the IonFlux microfluidic electrophysiology system. The IonFlux system integrates microfluidic flow channels and cell trapping sites into SBS-standard 384-well plates, allowing for cell/compound dispensing using standard liquid handlers. Because each compound channel is individually controlled via pneumatic pressure, the plate-reader like instrument can apply any compound to any number of cell groups simultaneously, with an ‘on’ time below 100ms.

Materials and Methods

Instrument: The IonFlux system includes the following novel features:

- Well-plate integration: In a departure from traditional automated electrophysiology systems, the IonFlux instrument is more similar to plate readers in both footprint and workflow; after loading, IonFlux plates are ‘read’ by the benchtop system without the requirement for external liquid addition or removal steps at any point during the recording protocol. Plates can be filled by standard liquid handlers.
- Fast compound addition: The well plates include integrated flow channels, allowing the rapid addition of multiple compounds (<100ms), and the ability to perform wash sequences between compound applications.
- “Single Head” integration: Electrodes are directly integrated with a pneumatic flow control interface. There is no scheduling overhead limiting compound application while the liquid handler is serving a different group of cells.
- Continuous recording: Continuous recording is important for monitoring current stability, use dependence, and recording of ligand-gated channels

Throughput in the IonFlux system is maximized by the use of multiple amplifier channels and cell recording from groups of cells to increase overall recording success rate. Systems are available with either 16 or 64 multiplexed amplifiers. To facilitate high-throughput experimentation, each group of cells can be addressed by up to 8 different compounds or concentrations, so that each trapping/recording protocol generates as many as 8 data points.

Recording plates. IonFlux plates were used for cell trapping and patch clamp recording. Groups of 20 cells were trapped in a parallel array, with each cell trapping channel having dimensions of 1.5um wide x 2um high. Experiments were run on the IonFlux 100 system, which interfaces to the IonFlux plates and provides pressure and electrical connectivity to run experiments automatically.

Cells. For ion channel recording experiments, freshly suspended mammalian cells expressing human voltage and ligand gated ion channels were used. Cells were maintained at 37ºC in DMEM with 10% fetal bovine serum. Before the start of experiments, adherent cells were trypsinized, spun down at 1000 rpm, and resuspended in extracellular electrolyte solution at a concentration $5x10^6$ cells/ml.

Procedure. Devices were first primed using positive pressure (~3 in Hg) to fill all of the channels. Trapping well/channels were filled with electrolyte and compound introduction channels with the compound of interest. The main channel was filled last, establishing electrical connectivity and allowing for the measurement of access resistance.

System Description

Figure 1. The IonFlux system is designed to operate much like a plate reader. The entire recording protocol, including cell trapping, whole cell formation, compound perfusion, and ion channel recording is managed automatically via software. Plates are preloaded with cells, compounds, and reagents using conventional liquid handlers. Once filled, plates are fed automatically into the IonFlux system. All fluidic control necessary to trap cells and deliver compounds is handled within the instrument, and integrated electrodes record currents continuously.

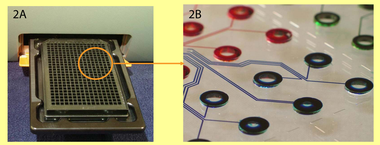

Figure 2. The IonFlux plates are based on the 384 well SBS-standard format. Figure 2A shows the microfluidic network attached to the bottom of a well plate. Figure 2B shows a magnification of the parallel trapping region. Each cell trapping junction is approximately 1.5 X 2 um. In a typical trapping ensemble, 20 cells are connected in parallel to a common amplifier. Cells are recorded in “whole cell” configuration. Trapping and whole cell protocols are configured and controlled via software control.

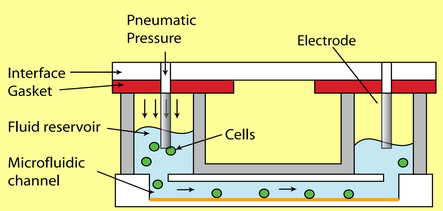

Figure 3. The interface incorporates pneumatic pressure control to enable rapid and precise fluid delivery. A gasketed interface seals to an entire 384 well plate after it loads in the instrument. The interface includes pneumatic connections to each well, with positive and negative pressure regulation from computer-controlled electro-pneumatic regulators. Precise control of compound delivery allows the system to resolve fast-activating ligand-gated ion channels. Compound exchange times are less than 100 msec.

Ag/AgCl electrodes are integrated in the interface. The IonFlux system includes up to 64 multiplexed amplifiers. Each 384-well plate includes 32 experimental zones, each containing 8 compound wells and 2 multi-cell trapping regions. This allows the entire plate, with a total of 64 trapping ensembles, to be recorded at one time. Each plate can deliver up to 512 data points. Throughputs in excess of 1,000 data points per hour can be achieved.

Recording of hERG and $GABA_A$ Receptor-Mediated Currents

GABAergic signaling is the major mediator of CNS inhibition, and is important for understanding and treating conditions such as anxiety, schizophrenia and possibly neuropathic pain. The hERG channel is the most important target for asessing cardic liability. Using IonFlux, we have characterized the Cl- current conducted by $hGABA_A$ receptor/channels expressed in LTK cells, and hERG current expressed in HEK 293 cells.

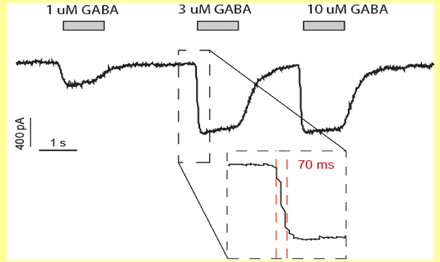

Figure 4. Inward Cl- current during exposure to a group of LTK cells expressing hGABAA receptor/channels to 1, 3, and 10 uM GABA. Inset: same current on expanded timescale showing the rapid onset (10%-90% time 70 ms). Typical GABA-evoked inward Cl- currents increased to 90% of maximum in 90 +/- 20 ms (N=10). The decay of current due to desensitization in the continued presence of GABA was slower, taking 500 +/- 100 ms (N=10). These recordings indicate clearly that IonFlux can resolve hGABAA receptor currents.

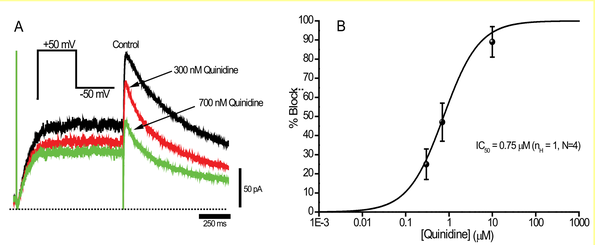

Figure 5. (A) hERG potassium currents before and during exposure to quinidine (300 and 700nM). (B) Dose response curve for quinidine block, fitted with the hill equation. We found the IC50 value to be 0.75 um, in good agreement with literature values.

Recording of $K_V2.1$ potassium currents

The Kv2.1 channel is a slowly inactivating voltage-gated potassium channel. In these experiments, current recordings as a function of voltage were generated. A comparison to “gold standard” manual patch clamp recordings shows good agreement between the two methods.

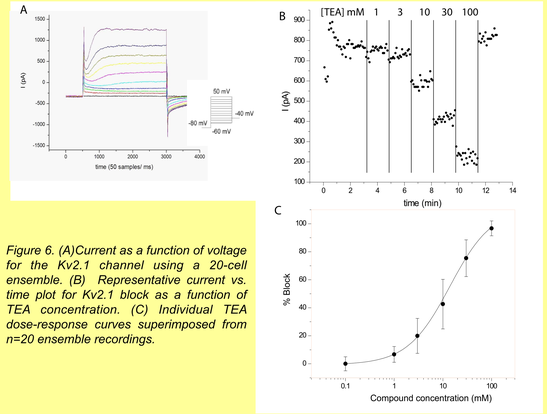

Figure 6. (A)Current as a function of voltage for the Kv2.1 channel using a 20-cell ensemble. (B) Representative current vs. time plot for Kv2.1 block as a function of TEA concentration. (C) Individual TEA dose-response curves superimposed from n=20 ensemble recordings.

Conclusions

- The IonFlux multi-cell microfluidic automated patch-clamp system provides the ability to record from an ensemble of 20 cells simultaneously, with good agreement to manual patch recordings.
- The IonFlux recording plate microfluidics integrate to SBS-standard well plates for compound and cell loading, facilitating plate prep by standard liquid handlers.
- The system rapidly generates dose response curves by dosing the same group of cells at multiple concentrations in rapid succession.
- The use of a “plate reader” format and 64 multiplexed amplifiers provides sample throughputs to 1,000 data points per hour.
- The ability to apply compounds in less than 100ms enables recording of ligand-gated channels.

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