DRUG SCREENING ON FAST KINETICS LIGAND GATED ION-CHANNELS

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ABSTRACT

To overcome limitations for current automated patch-clamp systems, we have used a microfluidic approach to achieve rapid solution exchange around patch-clamped cells. A fully automated system has been developed, which provides all necessary plate, liquid and cell handling throughout an experiment. We have achieved 10-90% response times to glycine on glycine receptor expressing cells below 30ms. Results on several cell- and ion channel receptor types show good correspondence with reference measurements and literature for pharmaceutical parameters such as EC50.

KEYWORDS: Microfluidics, Ion-Channels, Drug Screening, Automated System

INTRODUCTION

Existing automated patch clamp systems for screening on ion channel drug targets have a limitation in measuring fast kinetics ligand gated ion channels.[1] Fast ligand gated ion channels can open in the ten millisecond range and then locks down in closed states for hundreds of milliseconds or seconds, depending on exposure time.[2] Using pipetting in an open well system, or simple (micro) fluidics is not feasible since uncertain mixing conditions occur either through turbulence or diffusional mixing, and thence the time for total change of liquid environment is far beyond the time for deactivation of the ion channel.

MICROFLUIDIC CONSUMABLE

A microfluidic consumable has been design to provide a complete system to capture cells in suspension at specific points connected to patch-clamp amplifier channels, and then repeatedly and controlled expose them to different compounds and/or concentrations. (Figure 1) The microfluidic chip is designed without active components on chip. Electrical connections for ion channel current recordings and flow controlling vacuum pressures are connected through external metal pipes sealed against conical wells of each chip.

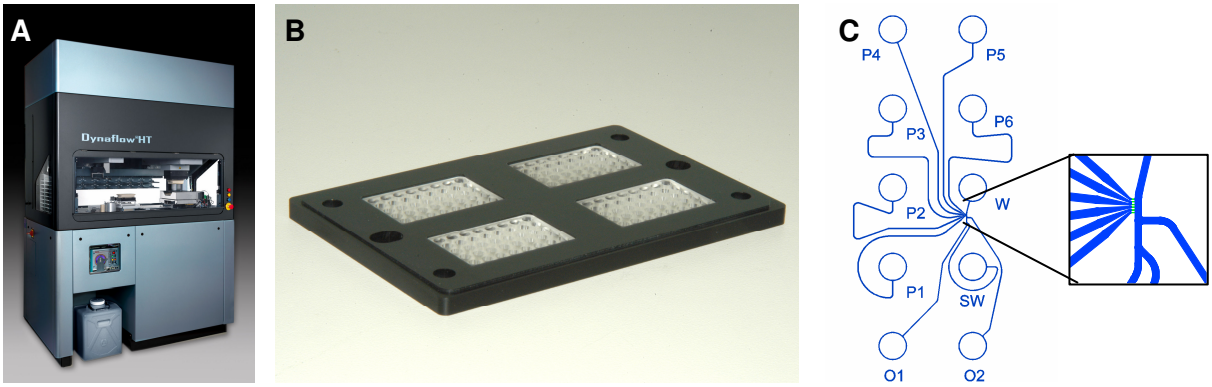

Figure 1: A) Photo of the system. B) Photo of the microfluidic consumable plate. C) Sketch of a single microfluidic unit with six patch-clamp sites. Blue denominates the main fluidic layer with channel sizes appropriate for cells and liquid delivery. Green denominates a 2.1µm layer defining the patch channels. The dimensions are small enough to capture a single cell and form a high resistance electrical seal to the cell membrane without deforming the cell by sucking it through the channel.

Each microfluidic plate is divided into four separate microfluidic chips, each of which consists of four subunits that make its own microfluidic unit. Each of these units has six patch-sites for cell capturing and recording, each an individual amplifier channel. The microfluidic unit has two open wells without interface pipes to provide access for exchanging liquids during the experiment. With a common ground and waste well and a fluidics control well to control exposure between the two open wells, this sums up to 10 wells for each microfluidic unit. The consumable is made from Poly(DiMethylSiloxane) bonded to glass. It is compliant with the SBS microplate standards to enable integration with automated plate/liquid handling.

SYSTEM

The system is built around a recording module consisting of a pneumatic system for flow control and “Richmond” a 96 channel patch-clamp amplifier developed by Tecella. A Tecan Evo platform provides liquid handling, such as priming the consumable plates with buffers, and feeding substances into the open wells of the consumable plate during an experiment. Subsystems for cell culturing and a plasma chamber for patch-clamp seal improvement by surface treatment of the PDMS are also integrated. The hardware is controlled by a customized software designed for high flexibility to

permit a wide set of cell types and applications. The system is capable of standalone runs comprising of 12 DF-HT plates in four hours.

EXPERIMENTS AND RESULTS

Fluidics evaluation by fluorescent probes and actual ion-channel response to ligand has been performed. (Figure 2) Cell capture capabilities were evaluated by optical inspection and resistance measurement.

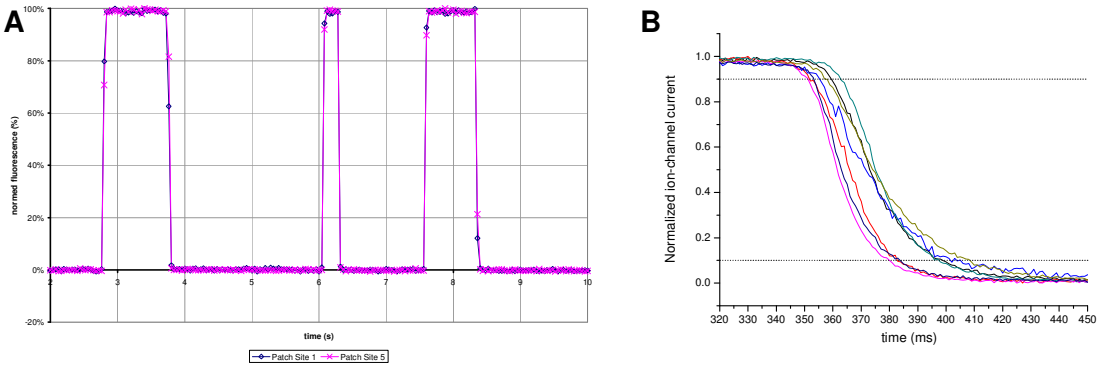

Figure 2: Characterization of the microfluidic switch. A) Switching performance evaluated by switching from buffer to 100µM fluorescein and measuring the fluorescence in arbitrary units in ROIs with positioning and size as a real captured cell in the system. B) Switching performance evaluated by switching from PBS buffer to 3mM glycine on a system with captured cells, measuring the ion channel current (Which is negative and thus the normalized current has a baseline at 1 and peak amplitude at 0). In A) the resulting on/off switching times are both <40ms and for B) <30ms.

Electrophysiological measurements on a number of different cell types and ion channels have been performed with several model compounds relevant for drug screening. Results from the glycine receptor, a fast ligand gated ion-channels can be seen in figure 3. We have confirmed that the system works with voltage gated ion channels, with results comparable to literature.

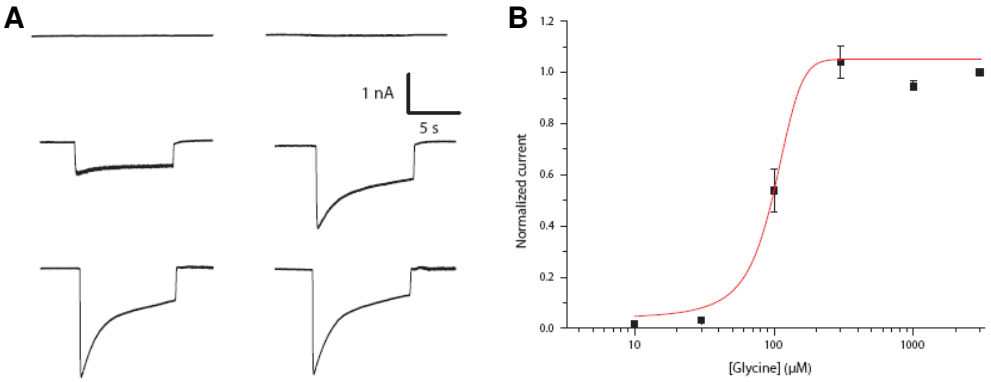

Figure 3: Dose response measurements of Glycine on Glycine receptors. A) Current traces for a single cell on six Gly concentrations. B) Normalized dose response where the points represent averaged data from 42 cells with standard deviation error bars, and the line is a Hill fit corresponding to an EC50 of 99µM.

CONCLUSIONS

We have developed a microfluidics based system for drug screening on ligand/ and voltage gated ion channels. The system is proven to provide fluidic switch fast enough for ligand gated ion channel recordings, as well as an electrophysiological recording situation good enough for voltage gated ion channels.

REFERENCES

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