

FAST BIO-MICROFLUIDIC PULSEGENERATOR FOR SINGLE CELL ELECTROPHYSIOLOGY

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We recently introduced a microfluidic platform for high-throughput patch-clamp electrophysiology in which lateral cell trapping junctions enable hydrodynamic trapping of cells from a bulk cell suspension and electrophysiological recording [1]. We then developed a platform for rapid fluid exchange across the surface of a microfluidic channel at frequencies of up to 10 Hz [2]. We now introduce a device that integrates these two capabilities into a single microfluidic platform that can rapidly exchange the fluid across a trapped cell for the pharmacological profiling of its current response to various compounds during electrophysiological recording. As a result of design modifications to the pulse generator platform and the integration of a computer control system our device is now able to deliver ultra-fast, millisecond pulses across a trapped cell.

Rapidly exchanging the fluid surrounding a patch-clamped cell, or fast fluid exchange, enables experimentation on ligand-gated ion channels and has applications in neurophysiology and drug discovery. These membrane-bound proteins allow specific ions to flow in and out of the cellular interior only when a specific chemical signal (ligand) binds to their extracellular surface. This flow of ions results in changes in current across the cell-surface that can be recorded through an electrode circuit. Furthermore, the ability to rapidly wash out these chemical signals from the cell surface within milliseconds of their application is necessary if one wants to mimic the physiological timescale of ligand-gated ion channel interactions and avoid receptor desensitization to the ligand [3].

Previous studies of ligand-gated ion channels commonly relied on piezo-electric translators, which rapidly manipulated two laminar fluid streams coming out of glass pipette during electrophysiological recording [4]. While this technique ably collects ion channel kinetic data, its setup is complex and expensive and thus not widely used for studying dynamics in systems biology [5].

We have built a system that uses a complex computer controlled test pattern to deliver 20 millisecond pulses of ligand to a patch clamped cell. The device itself is easily fabricated through soft-lithography with polydimethylsiloxane (PDMS). The platform can generate arbitrary valve control waveforms with microsecond accuracy, and in turn apply brief pulses of ligand. Once a cell is trapped we expect that these millisecond ligand pulses will elicit a response and that the magnitude of this response will reflect the level of desensitization.

With this device we have performed basic electrophysiological characterization of the cell line WSS-1, which stably expresses GABA_A ligand-gated ion channel receptors [6]. In further work we will apply our entire platform to the measurement of the dose responses of the GABA_A receptor to successive millisecond pulses of its agonistic ligand, GABA (γ -aminobutyric acid).

References

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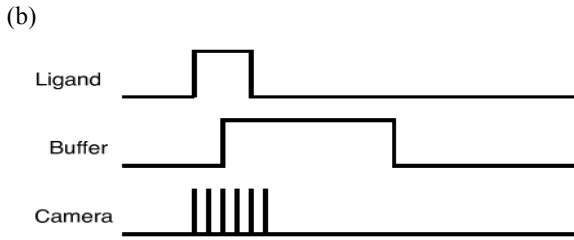
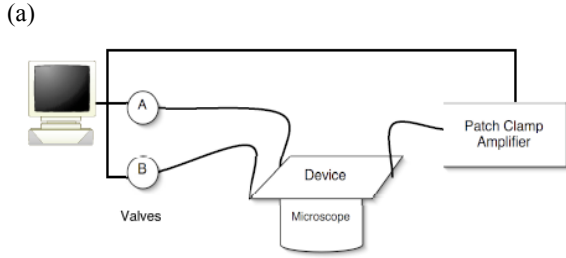


Figure 1: (a) System Overview. The microfluidic pulse electrophysiology device is multiplexed with two computer-controlled solenoid valves (ligand flows from A and buffer flows from B) and a patch clamp amplifier, which obtains electrophysiological recordings. (b) Device Control Waveforms. Each valve is controlled independently of the others, allowing arbitrary control of waveforms. This allows us to control pulse width and duration.

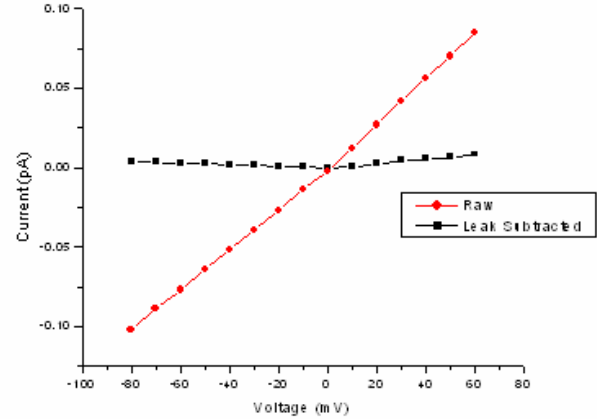


Figure 3: Steady-state current-voltage relationship of a WSS-1 cell for raw (red) and leak-subtracted current (black) in the *absence* of agonist. Since the cell line mainly expresses ligand-gated GABA_A receptor ion channels there is a very small current response to positive voltage applied across the cell when an agonist is not present.

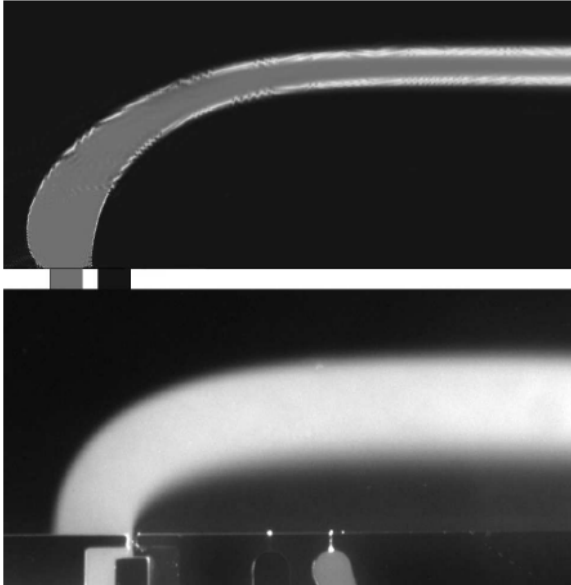


Figure 2: [Top] Femlab simulation with all valves open and ligand exiting injection channel 1, and buffer from injection channel 2. Note that if all valves are open, the downstream injected fluid pushes the upstream injected fluid away from the main channel wall. [Bot] Image of device with both valves open. Lateral cell-trapping junctions are down stream of the injected fluid flow.