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Patch-clamp array neurochips: value in interrogating simple neuronal networks with high resolution

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“Simultaneous pre- and post-synaptic recordings on a patch-clamp chip have tremendous value as a model to investigate synaptic function and for use as an assay to advance drug development.”

Understanding brain function, how it is affected by disease and how therapeutics can protect or restore it requires an understanding of neuronal communication from the network level down to individual synapses and ion channels. In fact, a common harbinger of many neurodegenerative diseases is impaired synaptic function, which manifests as disruption of neural network activity. The search for novel therapeutic targets and the need for reliably predictive assessments of potential therapeutics are driving an urgent demand for improved screening technologies against these parameters. Simple, but physiologically relevant, *in vitro* preparations capable of modeling key aspects of brain function, interfaced with high-resolution functional interrogation that permits high-throughput assessment at reasonable cost, must be developed to address this demand.

The patch-clamp method developed by Nobel laureates Neher and Sakmann [1] has proven to be the technique of choice for high-resolution interrogation of electrophysiological activity. A glass pipette with a micron-sized aperture at its apex is filled with a physiological saline and carefully manipulated to contact a cell and isolate a ‘patch’ of its membrane by forming a high resistance seal between the membrane and the

perimeter of the aperture (cell-attached configuration). Activity from individual ion channels spanning this membrane patch is subsequently recorded as discrete current jumps. Electrical access to ion channels populating the entire cell membrane can be achieved by rupturing this membrane patch, while maintaining the membrane–pipette aperture seal (whole-cell configuration). Measurement of current flow (ion channel activity) is accomplished by controlling, or ‘clamping’, pipette voltage. Alternatively, clamping current and monitoring voltage allows the highest resolution measurement of spontaneous or stimulated electrophysiological activity and discrete events, such as action potentials. Despite the eloquence of this technique, conventional patch-clamp requires highly qualified personnel and is labor intensive. This makes it slow and expensive, which limits its potential as a high-resolution interrogation tool for drug development assays.

Planar patch-clamp chips have been developed and refined over the last decade to specifically address this limitation. The apex of the pipette is replaced by a micron-sized aperture through a surface separating an upper cell suspension chamber from an underlying ‘pipette’ chamber [2]. This clever design makes the mechanized delivery of isolated cells

in suspension to the aperture possible and, in combination with programmed seal detection and stimulation-recording routines, has led to the automation of patch-clamp. An alternative approach developed to increase the throughput of direct ion channel interrogation is the application of dual sharp-electrode voltage-clamp systems to record currents from ion channels, overexpressed in large and robust oocytes [3]. In contrast to the patch-clamp method, sharp intracellular electrodes are used to measure and control the voltage and current. This process is also amenable to automation by using robotically controlled electrodes and assay chambers. Automated patch-clamp and dual electrode voltage-clamp systems have been commercialized by several companies for high-throughput screening [4], and now complement fluorometric imaging plate reader assays [5,201] for high-throughput drug screening. While these systems certainly improve data throughput, they have limitations:

- Reduced signal-to-noise ratio due to lower quality cell-to-aperture seals;
- Lower recording success rates than conventional patch-clamp; restricted application to non-neuronal cell lines with over-expression of specific ion channels, resulting in models of questionable relevance to physiological function;
- They are restricted to isolated cells lacking synaptic communication.

“We are developing planar patch-clamp array technology in an attempt to combine key benefits of both conventional patch-clamp and MEAs on a chip.”

Multielectrode arrays (MEAs) allow interrogation of activity in communicating networks of neurons using cell culture, brain slice or *in vivo* preparations [6] by sensing extracellular field potentials from proximal cells. Commercially available MEAs incorporate as many as 256 electrodes, while custom MEAs may be as large as 4096 electrodes, permitting the recording of passive and stimulated electrophysiological activity from various sized windows within a neuronal network, with varying spatial resolution. More recently, complementary metal-oxide semiconductor technology has allowed the integration of up to tens of thousands of probes with subcellular spatial resolution [7], although this technology is not yet commercially feasible and data analyses are daunting. Furthermore, progress in nanotechnology has allowed the development of lower impedance electrodes, resulting in remarkable improvements in signal-to-noise ratio [8,9]. Despite these advances, voltage-clamp of cells is not possible using MEA technology. Consequently, specific information pertaining to ion channel activity cannot be extracted from the complex signals obtained. This represents a serious impediment to expanding future MEA applications for drug discovery.

We are developing planar patch-clamp array technology in an attempt to combine key benefits of both conventional patch-clamp and MEAs on a chip [10,101]. The concept enables simultaneous

high-resolution patch-clamp interrogation of individual cultured neurons at multiple sites in communicating neuronal networks, where individual neurons are probed through apertures that connect to dedicated subterranean microfluidic channels. Neurons are first aligned to these apertures by stamped chemical adhesion or guidance cues [11], and can subsequently form synaptic connections. Two novel designs using different materials, fabrication strategies and architecture have been developed, and proof-of-concept has been demonstrated for both using Molluscan neurons. The first design is constructed from a silicon wafer containing a single aperture in a suspended silicon nitride/silicon dioxide film stack, which allows single-site recording [12]. The second design, constructed by laminating a polyimide film on a silicone plastic substrate, contains two apertures connected to independent subterranean microfluidic channels [13]. These neurochips are suitable for use with primary neurons cultured directly on the chip surface, meaning that, for the first time, interrogation of synaptically communicating neurons on a patch-clamp chip is possible. We have recently demonstrated its application to the study of synaptic communication between two neurons at the resolution of whole-cell patch-clamp [MARTINA M, LUK C, PY C *ET AL.* INTERROGATION OF CULTURED NEURONS USING PATCH-CLAMP CHIPS. MANUSCRIPT SUBMITTED]. The design is readily scalable to multiple (up to eight) recording sites. Practical limitations imposed by the subterranean microfluidic channels, such as space and chip-to-world interfacing, would require a different design to expand beyond that number. However, for many assessment studies it is of greater importance to be able to patch-clamp several connected neurons than it is to monitor extracellular field potentials from hundreds or thousands of neurons. Simultaneous pre- and post-synaptic recordings on a patch-clamp chip have tremendous value as a model to investigate synaptic function and for use as an assay to advance drug development. Furthermore, fundamental aspects of complex network activity, resulting in specific functions, such as the acquisition of memory, can be reduced to modeling and studying interactions between just three neurons [14–16]. This makes the application of multiple-site patch-clamp array chips to cultures of communicating neurons an important and promising technology to advance functional assay development. While the challenges of applying this technology to mammalian cells should not be underestimated, preliminary whole-cell patch-clamp recordings from rat cortical cultures [17] suggest that this is an achievable goal well worth pursuing. Finally, it is important to recognize the potential for application of multiple-site patch-clamp array chips to other biological disciplines where high-resolution assessment of cell-to-cell communication is required, including cardiology [18].

Financial & competing interests disclosure

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References

- 1 Neher E, Sakmann B. Single-channel currents recorded from membrane of denervated frog muscle fibers. *Nature* 260, 799–802 (1976).
- 2 Behrends JC, Fertig N. Planar Patch-Clamp. In: *Neuromethods. Second Edition*. Walz W (Ed.). Springer Press, NY, USA, 14 (2007).
- 3 Schnizler K, Kuster M, Methfessel C. The roboocyte: automated cDNA/mRNA injection and subsequent TEVC recording on xenopus oocytes in 96-Well microtiter plates. *Recept. Channels* 9, 41–48 (2003).
- 4 Dunlop J, Bowlby M, Peri R, Vasilyev D, Arias R. High-throughput electrophysiology: an emerging paradigm for ion-channel screening and physiology. *Nat. Rev. Drug Discov.* 7(4), 358–368 (2008).
- 5 Allenby G, Dodgson K, Harper P *et al.* Automated high throughput screening of fluorescent imaging plate reader (FLIPR) assays using assay platform (TM) integrated with activity base for 'on the fly' compound reconfirmation. *JALA* 6(5), 48–50 (2001).
- 6 Boven K-H, Fejtl M, Möller A, Nisch W, Stett A. *Advances in Network Electrophysiology: Using Multi-Electrode Arrays*. Taketani M, Baudry M (Eds). Springer Press, NY, USA (2006).
- 7 Herz P. Neuroelectronic Interfacing: Semiconductor chips with ion channels, brain cells, and nerves. In: *Nanoelectronics and Information Technology*. Waser R (Ed.). Wiley-VCH Verlag, Weinheim, Germany (2003).
- 8 Ben-Jacob E, Hanein Y. Carbon nanotube micro-electrodes for neuronal interfacing. *J. Mater. Chem.* 18(43), 5181–5186 (2008).
- 9 Huys R, Braeken D, van Meerbergen B *et al.* Novel concepts for improved communication between nerve cells and silicon electronic devices. *Solid State Electron* 52(4), 533–539 (2008).
- 10 Py C, Mealing G, Denhoff M *et al.* A multiple recording patch clamp chip with integrated subterranean microfluidic channels for cultured neuronal networks. Presented at: *Micro Total Analysis Systems*. CA, USA, October 12–17 (2008).
- 11 Charrier A, Martinez D, Monette R *et al.* Cell placement and guidance on substrates for neurochip interfaces. *Biotechnol. Bioeng.* 105(2), 368–373 (2010).
- 12 Py C, Denhoff M, Martina M *et al.* A novel silicon patch-clamp chip permits high-fidelity recording of ion channel activity from functionally defined neurons. *Biotechnol. Bioeng.* 107(4), 593–600 (2010).
- 13 Martinez D, Py C, Denhoff M *et al.* High-fidelity patch-clamp recordings from neurons cultured on a polymer microchip. *Biomed. Microdevices.* 12(6), 977 (2010).
- 14 Milo R, Shen-Orr S, Itzkovitz S *et al.* Network motifs: simple building blocks of complex networks. *Science* 298(5594), 824–827 (2002).
- 15 Song S, Sjöström PJ, Reigl M, Nelson S, Chklovskii DB. Highly nonrandom features of synaptic connectivity in local cortical circuits. *PLoS Biol.* 3(3), e68 (2005).
- 16 Lukowiak K, Syed NI. Learning, memory and a respiratory central pattern generator. *Comp. Biochem. Physiol. Mol. Integr. Physiol.* 124(3), 265–274 (1999).
- 17 Martinez D, Martina M, Kremer L *et al.* Development of patch-clamp chips for mammalian cell applications. *Micro Nano Systems* (2010) (In press).
- 18 Witchel H. Emerging trends in ion channel-based assays for predicting the cardiac safety of drugs. *IDrugs* 13(2), 90–96 (2010).

Patent

- 101 Mealing G, Py C, Denhoff M *et al.* PCT/CA2005/000682 (2005).

Website

- 201 Molecular Device Corporation
www.moleculardevices.com/Products/
Instruments/FLIPR-Systems.html