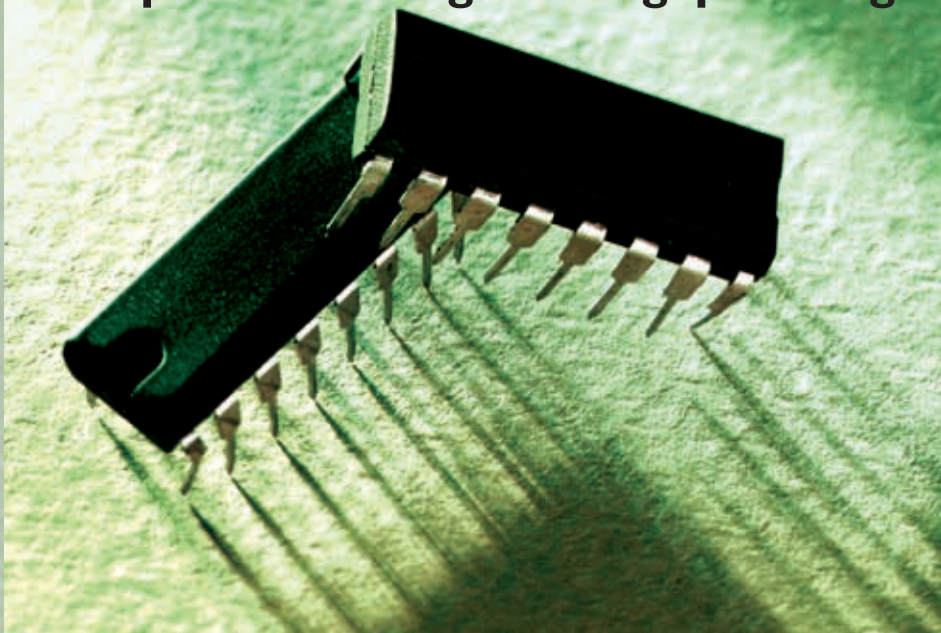


In-silico Cell Electrophysiology

A Step Closer to High Throughput Drug Screening



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The transport of ions across biological membranes is important in drug development, as large numbers of new drugs work by modulating ion channels, pumps or carriers. Therefore, drug discovery is limited by the specificity, reliability, and reproducibility of measurements of ion activity in cells and membranes. New devices like the Cell Electrophysiology Lab-on-a-Chip promise to leapfrog technology bottlenecks in drug discovery by monitoring multiple cells with multiple ion-specific sensors.

The phospholipid cell membrane contains thousands of ion channels necessary for the transfer of essential electrolytes and into and out of the cell. They serve many other functions such as signaling, chemical transduction, maintenance of pH, and regulation of ion concentrations. In mammalian systems the most important ions we need to consider include H^+ , Na^+ , K^+ , Ca^{2+} and Cl^- . Functional abnormalities in ion channels causes many diseases called channelopathies [1] such as cystic fibrosis [2], central core storage disease [3] and certain forms of epilepsy. Drugs used for treating ion channel related diseases are usually ion channel modulators. Many other drugs which target non ion channel related diseases also do it through ion channel modulation [1]. New discoveries in these drugs are limited by the technology available to experimentally measure ion channel activity, and now High Throughput Screening (HTS) assay technologies [1] are required to screen the library of potential pharmacological agents.

Currently Available Technologies and Limitations

Some of the available technologies that have been used to monitor ion channels in screening assays include radioactive uptake assays, atomic absorbance spectroscopy, fluorescent ion sensitive dyes and some membrane fluorescent dyes. All of these approaches lack adequate resolution as compared to traditional electrophysiology and the patch clamp technique developed in the 1970's [4]. The technique can monitor the activity of single isolated channels, excised from a cell membrane by measuring currents or voltages associated with ion transport. Patch clamping is traditionally done by a dedicated, highly skilled scientist, using a single electrode to study individual single cells, one at a time. There has been attempts to develop microfabricated planar patch clamp microchip devices which would theoretically have a higher throughput. However, conversion of the highly complicated procedures associated with the technique has not been



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translated into an automated microdevice. Even if successful the automated patch clamp microdevice would still have major limitations. It is invasive, and causes injury to the cell, which limits the potential for subsequent analysis of the cells using other techniques. It also lacks ion specificity in the measurements, given the technique is based on electrical signals associated with membrane potential

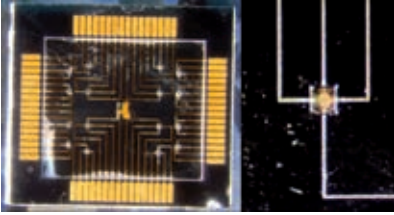


Fig. 1: (Left) Full view of the CEL-C biochip. (Right) A single celled fern spore placed in one pore of the biochip.

(voltage) or membrane transport (current). Because of this lack of ion-specificity the technique requires ion-replacement control experiments to determine what ion is responsible for the measured electrical signal. This means that large numbers of parallel control experiments must be done, and this ultimately limits the throughput of this approach.

CEL-C Biochip: Single Cell Electrophysiology

The Purdue University Physiological Sensing Facility (PSF) is developing new technologies, enabling real-time single cell electrophysiology experiments. This approach also enables non-invasive measurements to be made of specific ion transport activity. This culminated in the development of the Cell Electrophysiology Lab-on-a-chip, commonly referred to as the CEL-C Biochip [5] shown in figure. 1. This is a logical extension of the self referencing ion selective electrode technique [6]. The device is manufactured using silicon microfabrication, and consists of 16 pores on a silicon substrate each with four separate electrodes. An SU-8 layer (a UV cross linked epoxy) is used to form an electrically insulating layer and an encapsulated culture chamber. The electrodes are coated with an ion selective membrane for Ca^{2+} selectivity, based on ionophores which can be substituted to provide specificity for other

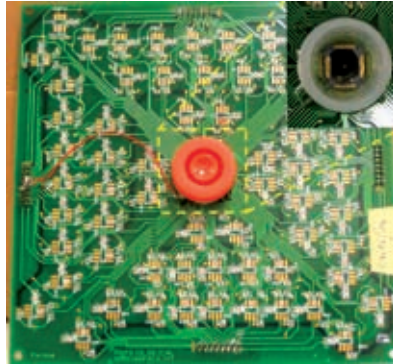


Fig. 2: The CEL-C biochip (inset) mounted on the custom built printed circuit board for signal interface.

ions. The final configuration creates 16 cellular chambers, each capable of measuring Ca^{2+} activity at four different points around a single cell. The CEL-C biochip (9 x 11 mm) is wire bonded to a printed circuit board (fig. 2) for amplification and signal conditioning. This provides an interface to a high density data acquisition system, controlled by a user friendly interface [7], which can be configured with minimal training.

Non Invasive, Ion Specific Screening

The two most important benefits of the CEL-C biochip are the use of ion-specific sensors, and non-invasive monitoring of living cells. The use of ion-selective sensors eliminates ion-replacement controls. Since all fabrication materials are biocompatible, mammalian cells can be cultured on chip and monitored at different intervals during the culture. This all ensures that the signals are devoid of misleading artifacts associated with disruption of the cell membrane in patch clamped cells. The first biological recordings on the chip were done using single cell spores of the fern *Ceratopteris richardii*. These were germinated on the biochip and we measured (fig. 3) calcium signals associated

with normal germination and germination when exposed to a Ca^{2+} channel blocker. This illustrates how the CEL-C biochip could be used to measure ion-transport activity for drug screening applications. Furthermore these experiments were conducted over a period of 24 hours emphasizing long term, non-invasive, ion-specific monitoring of cells.

The Future

We describe the technology as an advanced throughput platform, but conceive that future work will result in high throughput capabilities. The current CEL-C biochip is selective for measuring Ca^{2+} but can be adapted for other ions by changing the selectivity of the sensor membrane. Cells are now loaded manually but design of an automated system will reduce prep time and increase throughput. The chip design is also being modified to incorporate microfluidics/temperature control for on-chip cell culture, and to miniaturize the data acquisition system onto a hand held device. We believe that these capabilities will enable drug screening, by eliminating the need for highly skilled personnel. The non-invasive nature of the CEL-C biochip, fast analysis times, potential multi analyte detection capabilities and efficient data management and storage are very promising for high throughput ion-channel drug screening assays.

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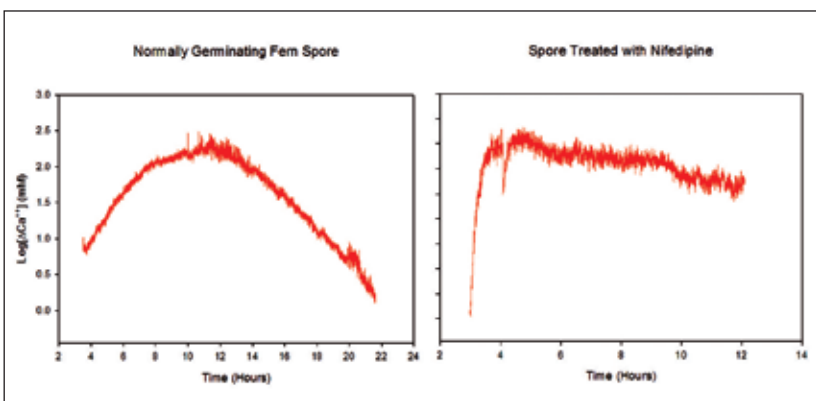


Fig 3: Transcellular Ca^{2+} current across a normal fern spore and one subjected to Nifedipine, a Ca^{2+} channel blocker.