SCALEABLE BLM ARRAYS FOR PARALLEL ION CHANNEL RECORDING

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ABSTRACT
This paper describes a compact, scalable and high throughput bilayer ion channel recording platform with micro-cavities and custom-made compact electronic readout circuitry using ASICs (Application-Specific Integrated Circuits). Each chip holds four separately addressable bilayers, and is able to support BLMs (bilayer lipid membranes) that are stable for up to 10 days.

KEYWORDS
Bilayer lipid membrane (BLM), Ion channel, Parallel platform, Micro cavity, Dry film resists

INTRODUCTION
Ion channel proteins play an important role in physiological processes [1,2]. They are used as drug targets and have received considerable interest from the pharmaceutical industry. Electrophysiological analysis of these proteins provides detailed information about the function of ion channels and their modulation by pharmaceutical drugs. The artificial bilayer lipid membrane is one the most convenient methods for performing electrophysiology [3], since data can be recorded at the single ion channel level and does not require live cells. Due to inherent problems with membrane stability and ion channel insertion, it is desirable to have multiple bilayers in parallel to increase the yield and throughput of the system. A parallel array approach would also enable highly multiplexed screening of compounds. Array platforms for ion-channel recording have been reported previously, with devices fabricated using for example stereolithography [4,5]. In order to reduce the size and increase integration, arrays of micro-cavities have been implemented using photolithography [6,7]. However, these discrete arrays require an electrical connection to multiple external amplifiers [6] and electrophysiological recording time can be limited due to the small size of the recording electrode [7].

We have designed a chip that holds four separate BLMs, each with its own integrated Ag/AgCl electrode and a single common electrode, as shown in Fig. 1. The BLMs are made across apertures that are manufactured on the chip using dry-film resist. The bottom cavity houses an electrode with a diameter of 150-200 μm and a depth of around 55 μm. A second layer of laminate covers this cavity to create an aperture for the suspended bilayer (20-100 μm diameter). The large electrode provides the capability for long term electrophysiological recording without electrode degradation, unlike other systems [7]. Each bilayer chip contains four separate bilayers and a common reference electrode and the chip is interfaced with a PCB edge connector. The miniaturized current recording amplifiers sit directly next to the chip, which is plugged in to the circuit. Therefore no separate wires are required for electrical connection. This makes the system much more compact and reduces the noise compared to off-the-shelf recording systems [4-7].

Fig. 1: (a) Schematic diagram of the micro-electrode cavity showing the large bottom electrode and the aperture for the BLM (not to scale) (b) A chip with four separate BLMs and a counter electrode, with contacts pad for electrical recording with an ASIC.

FABRICATION OF MICRO-ELECTRODE CAVITIES
A four-mask process was used to fabricate the device; the sequence is shown in Fig. 2. A 700 μm thick glass wafer was cleaned in nitric acid and deionized (DI) water and dried in an oven at 210 °C. A 200 nm thick gold layer (Au) was evaporated on the wafer. AZ 9260 resist was used to define the electrodes and contact pads, and the metal layers were wet etched. A second layer of AZ 9260 (~10 μm) was spin coated and patterned by photolithography to make openings for silver plating. The electrodes were electroplated with silver using AgNO₃ solution (0.1 M AgNO₃ and 0.5 M NH₃) to
a thickness of 5-6 µm, and then chlorinated with FeCl₃ solution. After fabricating the electrodes, the first layer of dry film resist TMMF S 2055 (55 µm thickness) was laminated using a roller at 80 °C and patterned by photolithography to create the bottom compartment [8]. A second layer of TMMF S 2030 (30 µm) was laminated and patterned over this first layer to define the BLM aperture. The lamination temperature was kept low (45 °C) to prevent the resist from sagging into the bottom compartments due to melting. The chip was hard baked at 170 °C for 30 min., where a 10 minute ramping up and down step was used to make sure that no stress develops in the TMMF film. Finally the chip was exposed to CF₄ plasma to make the surface hydrophobic. The full chip and a scanning electron microscope (SEM) image of a bilayer aperture are shown in Fig. 2.

**ARCHITECTURE OF THE FOUR-CHANNEL ASIC**

A photograph of the signal acquisition platform is shown in Fig. 3. Each of the four BLM chamber chips is interfaced via a PCB edge connector (a) with a custom-made current amplifier and ADC (Analog to Digital Converter), both embedded into a single custom-designed ASIC in a CMOS 0.35 µm technology (asic1-4 in Fig. 3a). The custom-made ASIC has four different acquisition bandwidths, from 625 Hz to 10 kHz, with two different current ranges, ±200 pA and ±20 nA. The voltage stimulus is generated in the ASIC, with a range of ±450 mV. The ASICs are individually programmable through a Serial Peripheral Interface (SPI). The motherboard (b) has three slots for four-acquisition channel PCBs, giving a total of 12 channels. Digital data filtering and system control are performed with an FPGA embedded on the motherboard (b), and the data are sent to a PC through a USB interface. A Graphical User Interface (GUI) in Visual Basic displays and stores data in real time, and also allows the setting of the acquisition bandwidth, voltage stimulus and the current range.

**Fig. 2:** (a) Schematic diagram of the fabrication sequence, which begins with the manufacture of the bottom Ag/AgCl electrodes (5 µm thick), followed by lamination of two layers of dry-film resist. The thickness of the bottom and top laminate layers are 55 µm and 30 µm respectively. (b) The complete chip. (c) SEM image of a 75 µm aperture and of a four-aperture array with 1×2 mm spacing (inset).

**Fig. 3:** (a) The four-channel recording PCB with the edge connector to the microfluidic chip. (b) Assembled system with provision of three microfluidic chips, each having four bilayers. (c) Graphical User Interface for data display and storage.
RESULTS AND DISCUSSION
Membranes were made as follows: initially, the top compartment was filled with buffer (1 M KCl, 10 mM HEPES, pH 7.4) and the chip was placed in a desiccator under mild vacuum to draw the buffer into the bottom compartment. The chip was then inserted into the edge connecter of the current amplifier as shown in Fig. 3b. Bilayers were made by painting, using 1,2-diphytanoyl-sn-glycero-3-phosphocholine (DPhPC) in decane at 20 mg/ml. With a 20 μm aperture in the dry film resist, bilayers were stable for up to 10 days as evaluated by continuous recording of capacitance with a 100 mV potential across the bilayer. Ion channel recording with gramicidin A was demonstrated following insertion via the top compartment at a concentration of 50 pg/ml. Current traces from multiple bilayers are shown in Fig. 4. The diameter of each bilayer aperture was 75 μm and a 100 mV potential was applied across the bilayers. The oversampling frequency was 1.25 MHz and data were filtered at 625 Hz using the FPGA built-in digital filter before applying a 100 Hz low-pass filter. Current steps at 100 mV (1 M KCl) are ~2.5 pA, matching literature values for the conductance of gramicidin A [3].

![Fig.4: Gramicidin A traces at 100 mV recorded simultaneously. \( f_s = 1.25 \text{ MHz}, f_c = 100 \text{ Hz.} \) The BLM aperture size 75 μm.](image)

CONCLUSIONS
We describe a parallel scalable bilayer platform for ion channel recording from multiple bilayers. Disposable glass chips were made using laminated dry film resist to produce micro-cavities over which bilayers are made. Custom-made ASICs sit adjacent to the bilayer chips, reducing both the electrical noise and the overall size of the recording platform. The system can easily be scaled in multiples of four channels.

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