Patch-clamp Array with On-chip Electronics, Optics, Flow Control and Mechanical Actuation
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ABSTRACT
Fast and quantitative analysis of cellular activity, signaling and responses to external stimuli is a crucial capability and it has been the goal of several projects focusing on patch clamp measurements [1-3]. To provide the maximum functionality and measurement options, we have developed a patch clamp array device that incorporates on-chip electronics, mechanical, optical and microfluidic coupling as well as cell localization through fluid flow. The preliminary design, which integrated microfluidics, electrodes and optical access, was fabricated and tested. In addition, new designs which further combine mechanical actuation, on-chip electronics and various electrode materials with the previous designs are currently being fabricated.

Keywords: cell manipulation, patch clamp, multi-modal measurement platform

INTRODUCTION / INITIAL DESIGNS
We have been developing micromachined tools for manipulation and analysis of cells and particles (beads) [4]. The basic cell capture device configuration is shown in Fig.1. Microfluidic channels are formed from silicon nitride (2-6μm in height) and cell localization is accomplished by applying vacuum to ports that connect to the channels with holes of varying diameter (3-5μm). These ports are also used for reagent delivery and sample extraction, which allows precise delivery of test compounds to the cells. Electrodes are positioned around these cell localization points to allow multiple, simultaneous measurements on a single or group of cells. The PDMS module (50 to 100μm high) is formed by molding and is assembled on top of the silicon module to handle larger fluid volumes and to prevent evaporation. The silicon substrate behind the cell localization area is removed to allow through-device optical access.

Figure 1. (a) Cross section of the patch clamp array and the cell localization structure with a PDMS cage to hold the sample. (b) Four cell capture regions are visible in this design with two polysilicon electrodes below each hole in the center. Two larger holes (5μm diameter) are for fluid delivery/extraction from the cell localization structure.
Preliminary device testing has shown the ability to localize cells and couple with them optically (Fig.2). Impedance measurements using the electrodes around the cell localization ports have demonstrated the electrical access and characterization capability in this structure. Further testing is underway with different cell types, chemical delivery, and electrical manipulation options.

![Figure 2. (a) Delivery of red blood cells into the device and subsequent capture on the holes with flow out of the lower left port. The silicon substrate behind the cell localization region has been etched to provide through-device optical access. (b) Fluorescein loaded fluid is delivered into the structure which is imaged by an epifluorescent microscope. Brighter section in the center is due to the light source which is positioned behind the device to illustrate the through-device microscopy capability.](image)

**NEW DESIGNS**

**Mechanical / optical / microfluidic coupling**

Some of the new capabilities in the next generation devices are shown in Figure 3. Mechanical coupling to the cells is accomplished through probes that extend into the cell localization area. These polysilicon probes are driven by electrostatic or thermal actuators and can be locked in place to perform force measurements (as deduced from deflection and displacement of components) on cells such as cardiac myocytes. The actuators provide up to 10μm of displacement in either direction. Probes are located 2μm above the nitride layer which has the cell localization ports. Large (40μm by 5μm) coating ports connected to individual channels are also fabricated close to the mechanical probes to allow localized coating of the probe tip with proteins and other chemicals of interest. As in the previous designs, silicon behind the cell localization area is removed to provide through-device optical access.

To confine the fluid sample, a molded PDMS module is assembled over the cell localization region. A polysilicon ring above the silicon nitride layer also surrounds this region that serves as an assembly guide and sealing surface.
Figure 3. Physical coupling to the cells is established by the mechanical probe and actuators – probes can move in and out 10μm. Each cell capture port is individually accessible through separate flow channels and there are two electrodes underneath each cell capture port. Larger fluid delivery/coating ports are located close to the mechanical probes.

On-chip Electronics

New designs also incorporate on-chip electronics to perform pre-amplification and processing of electrical signals, which is necessary to perform very low level current/charge measurements. The processed signals are then taken off-chip, which overcomes many of the problems associated with parasitics and noise. The electronic functionality, provided by nMOS transistors, is integrated into these designs with minimal changes in the process flow since the polysilicon layers that serve as mechanical layers also serve as gate electrodes of the transistors and the dopant source for the source/drain regions. Gate oxide growth is an additional process step over the standard MEMS process (Fig. 4).

Figure 4. General process flow for generating the on-chip electronics (SFETs-SUMMiTT™ FETs). Contact openings are filled with in-situ doped LPCVD poly, which serves as the source/drain dopant source. These process steps are only slightly different from the MEMS-only flow.
The differential pair shown in Fig.5 forms the input stage for the charge sensing and stimulation circuitry that is connected to the electrodes around the cell localization ports. Preliminary characterization of the test devices fabricated show sub-pA level current detection capability with such stages. In addition, on-chip mux/demux circuitry allows signal routing without the need for a large number of wire bonds.

CONCLUSIONS

In order to carry out complex, precise and multiplexed measurements on cells and/or tissue, highly functional and integrated microsystems are needed. Devices presented here and others currently being developed are expected to serve as the primary components in our microsystems toolbox which provide the flexibility and sensitivity that is required to carry out the most demanding and biologically significant cell and tissue based measurements.

Sandia is a multiprogram laboratory operated by Sandia Corporation, a Lockheed Martin Company, for the United States Department of Energy’s National Nuclear Security Administration under contract DE-AC04-94AL85000.

References