

CELL-LAB ON A CHIP: A CMOS-BASED MICROSYSTEM FOR CULTURING AND MONITORING CELLS

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Abstract — We describe a MEMS-on-CMOS microsystem to encage, culture, and monitor cells. The system was designed to perform long-term measurements on arrays of single electrically active cells. A MEMS process flow was developed for the fabrication of closable micro-vials to contain each cell, a custom bio-amplifier CMOS chip was designed, fabricated, and tested, and the fabrication of the MEMS structures on this chip was demonstrated. In addition, bovine aortic smooth muscle cells were plated on the surface, and over the course of a week they adhered, formed processes, and reproduced, verifying the compatibility of the materials used with the cell culture.

Keywords — lab-on-a-chip, cells, polypyrrole, MEMS, CMOS

I. INTRODUCTION

Microsystems are promising tools for cell study [1, 2] because the feature sizes of MEMS structures are commensurate with those of cells. Micro-scale sensors and actuators are anticipated to facilitate rapid processing of many cells simultaneously [3], improving the speed and accuracy of assays [4]. Such microsystems are therefore being developed for use in miniature, low-cost, multi-purpose platforms having potential applications in home health care [5], drug screening [6], and environmental monitoring [7].

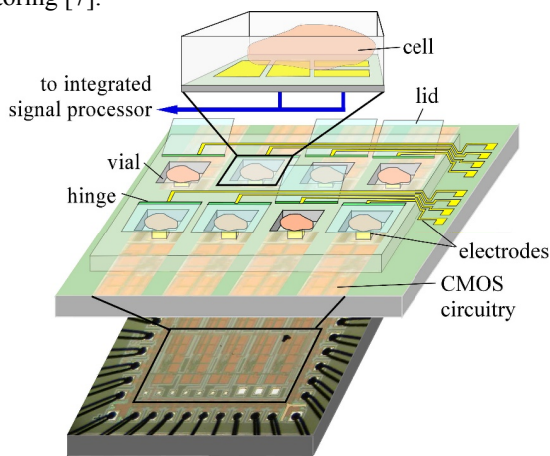


Fig. 1. Conceptual illustration of the microsystem that is being developed. An array of closable vials contains single cells, or small groups of cells. Inside each vial are electrodes or other sensors, and underlying the MEMS structures is additional CMOS circuitry.

MEMS structures can be built directly on CMOS circuitry to form labs-on-a-chip for cell study. For example, Eversmann et al. reported culturing a single neuron directly on a CMOS biosensor array and using it to record the cell's

electrical activity [8]. CMOS chips offer a variety of sensing capabilities including pH, impedance, and temperature [9-11].

In this paper, we describe an approach to fabricating a “biolab-on-a-chip” comprising microstructures for isolating and containing cells, sensors, and circuitry for on-chip signal conditioning (Fig. 1). We have developed and tested a MEMS process sequence and have fabricated the first-generation prototypes.

II. METHODOLOGY

A. Microsystem Design

The microsystem design in Fig. 1 was based on the following system requirements.

- Cells adhere to the surface and function normally (or as closely to normally as possible in culture).
- Electrical measurements to be made on many single cells simultaneously and over long times (e.g. days).
- Exploitation of CMOS-based sensors and circuitry.

Optical tweezers [12] and dielectrophoresis [10] are well established techniques for trapping cells suspended in liquid. Many cells, however, must adhere to a solid surface before they can grow and reproduce [13, 14]. Therefore, we decided to employ mechanical structures, i.e. vials with lids, to form enclosed environments for encaging cells. Since many cells, such as neurons, are mobile [15], mechanical confinement will ensure that the cell remains over the electrode throughout the measurement period.

In order to measure many cells independently and simultaneously, an array of vials can be formed that are made of insulating material. The vials need to be loaded with cells, and then closed during measurement to prevent electrical interference. One successful approach has been PDMS-based microfluidic structures actuated externally [16]. In order to further miniaturize the system, we chose to explore an option that will allow on-chip electrical actuation for loading and isolation. The microactuators should thus

- operate in a cell culturing medium (an electrolyte),
- operate at low voltage and consume little power, and
- undergo large motion with sufficient force.

Polypyrrole/gold bilayer actuators meet these requirements [17, 18]. Polypyrrole (PPy) is an electroactive polymer that changes volume under low-voltage electrochemical stimulation when it is immersed in an electrolyte, and it is biocompatible [19].

The MEMS components need to be fabricated by low-temperature steps because high temperature processing would threaten the integrity of the CMOS sensors on the

chip surface, and the structures need to be surface micromachined, rather than bulk micromachined, because the sensors must be placed inside the vials.

The circuit is required to measure weak signals from the cells [20]. A continuous measurement is necessary in order to follow a complete cell cycle, whose typical duration is 24 hours [21, 22]. All of the microsystem materials should thus have good corrosion-resistance and biocompatibility.

The system in Fig. 1 satisfies these constraints. A custom bio-amplifier chip (1.5 mm × 1.5 mm) was manufactured in a commercial CMOS technology to record action potentials between sensing and ground electrodes from electrically active cells and to amplify the voltage. The CMOS Al electrodes were covered with Au to prevent corrosion and improve the electrode/cell interface. The MEMS structures comprised an array of vials that could be opened and closed by rotating a lid over the rim of the vial using a bilayer PPy/Au actuator. Au was also used for the electrodes, and all the Au deposition steps (electroless plating and thermal evaporation) were low-temperature. Vials were defined on the surface using photopatternable SU8, and polypyrrole was electrodeposited.

The following sections outline the fabrication steps for the MEMS structures. The CMOS chip design has been previously described elsewhere [23].

B. Electroless Au Plating

The CMOS chip surface was a layer of glass (silicon oxide) with glass cuts 1 μm down to the aluminum electrode layer. However, Al is not a suitable material for use as a sensing electrode in an electrolyte due to its poor corrosion resistance [24]. Therefore, Au was deposited over the Al by electroless plating in a series of commercially available solutions (Technic Inc.). This is a three-step process because it is not possible to plate Au directly on Al: first a thin layer of Zn thick is deposited that next allows a layer of Ni 5 μm thick to be coated, onto which the Au can finally be plated. The chips were immersed in each of the heated solutions in turn, and the Au was plated to a thickness of approximately 10 μm.

C. Handling the CMOS Chip

Since the chips were quite small, and thus difficult to handle during the fabrication processes, we employed a handle wafer. Photoresist (Shipley® 1813) was spun onto a piece of silicon (approx. 20 mm × 20 mm), and a chip was placed onto this surface and tapped gently at the edges to ensure flatness and uniform adhesion. The handle wafer was baked on a hotplate at 90 °C for 1 minute to harden the resist glue. After baking, the chip adhered strongly to the holder, and the combination could be handled as a regular sample.

D. MEMS Fabrication

Fig. 2 illustrates the microstructure fabrication process, which was developed and test-run on oxidized wafers prior

to transitioning it to individual CMOS dice. The first step was to deposit 40 Å Cr and 300 Å Au layers by thermal evaporation (Fig. 2a). The Cr and Au were patterned to form openings for “differential adhesion,” a release method described in [25]. To remove the residual Cr etchant, the sample was subsequently immersed in 10% HNO₃ for 1 minute, then rinsed with DI water.

A 1500 Å Au structural layer was thermally evaporated and patterned to define the electrodes, hinges, and lids (Fig. 2b). This layer covered the openings in the first Cr/Au layer on all sides.

Photoresist was spun and patterned to form a template into which PPy doped with dodecylbenzenesulfonate, PPy(DBS), was electropolymerized (Fig. 2c, conditions given in [18]). The PPy deposited only on the exposed metal surfaces.

After removing the resist from the previous step in ethanol, an SU8 layer was deposited and patterned to create the lids (150 μm × 150 μm), vials (150 μm × 120 μm × 10 μm), and insulation for the wires (Fig. 2d). SU8 has been broadly used in bioMEMS [26] because it allows high-aspect ratio, insulating structures to be defined at low temperatures. This is critical because the PPy should not be exposed to temperatures above 120 °C.

The Au layer was etched in the final step to release the hinges and lids (Fig. 2f). The other areas on the sample were protected by photoresist during the Au etching.

After the hinges were released, they were able to bend away from the substrate because Au does not adhere to silicon oxide. Note that the hinges were anchored to the substrate over those areas covered by Cr.

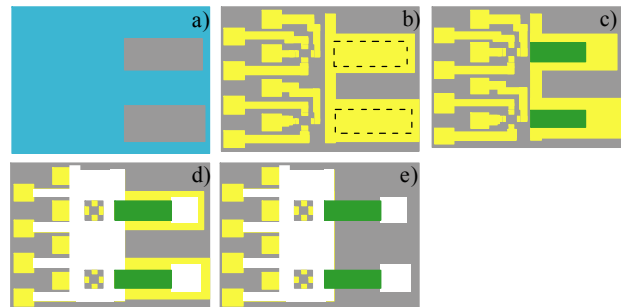


Fig. 2. Schematic top view of MEMS fabrication process: a) Deposit and pattern Cr/Au; b) evaporate and pattern structural Au layer; c) selectively electrodeposit PPy; d) deposit and pattern SU8; e) etch Au to release bilayer hinges and lids.

III. RESULTS AND DISCUSSION

A. MEMS Testing

The feasibility of fabricating the MEMS structures using this process on a CMOS chip was tested using a prototype chip not specifically designed to be integrated. The fabrication sequence was as successful as previously demonstrated on plain oxidized silicon wafers. The function of the bilayer actuators was tested by placing the chip into

an electrolyte solution, with electrical connection made to the chip surface through a micromanipulator probe, and voltages between 0 and -1 V vs. Ag/AgCl were applied to the sample using an external potentiostat (see [18] for details). Fig. 3 shows a rotating lid. Note that the lid, although it moved, did not close; the hinge design still required further optimization (see below). The next generation of devices, including both modified CMOS chip and MEMS structures, has already been designed and is being fabricated.

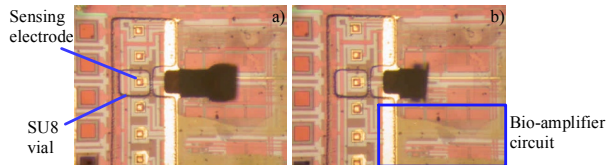


Fig. 3. The MEMS process was tested by fabricating prototype microstructures on a prototype bio-amplifier chip. A vial was positioned around an Au-plated sensing electrode, visible as a small square. The PPy/Au bilayer hinge was successfully actuated, as shown by the rotating lid in these images. The area of the chip with the amplifier is indicated.

B. Cell Cultivation

We are currently using bovine aortic smooth muscle cells (Cell Applications, Inc.), which generate action potentials spontaneously. The cells are cultured in a humidified incubator at 37°C with 5% CO₂. The cells have proliferated through multiple generations.

C. Cell Recordings Using the Bio-Amplifier Chip

Fig. 4a illustrates the function of the circuit. The chip was fabricated in a commercially available 0.5 μm, 3-metal, 2-poly CMOS technology. On the first-generation bio-amplifier chips, there was an array of ten sensing electrodes. These circuits were comparable in size to the lidded vials.

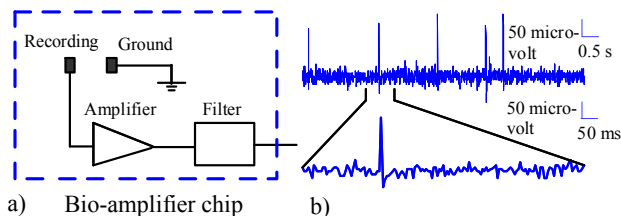


Fig. 4. a) Schematic showing the function of the bio-amplifier chip. b) Electrical signals from cells plated on the surface were recorded by electrodes, amplified by the amplifier, processed by the filter, and output to a digital oscilloscope.

To test the circuitry, bovine aortic smooth muscle cells were plated onto a packaged chip with electroless plated Au electrodes (but without the MEMS structures), and voltage signals were measured between a sensing electrode and a ground electrode. The voltages were amplified ($\times 100$ gain) on chip, buffered by an off chip amplifier configured for unity voltage gain, and then displayed on a digitizing oscilloscope. The recorded signals from the many cells on

the surface are shown in Fig. 4b, verifying that the cells are active on the chip surface and that the circuitry is able to pick up these signals.

D. Optimization of Hinge Design and Fabrication

In order to close the vials, the hinges must rotate the lids 180° so that they can lie flat on the vial surface and fully cover the vials. Fig. 5a shows an unsatisfactory hinge. To close properly, the hinge bending diameter should equal the sum of the height of the vial h and the thickness of the lid t (Fig. 5b). The curvature is determined by both hinge geometric parameters and component layer mechanical properties. However, simple models of cantilever beam bending, such as the Timoshenko equation [27], cannot be used to optimize the PPy/Au hinge design because: 1) there is a strain gradient in the PPy arising from the electrodeposition process [18]; 2) during actuation, the stress, strain, and Young's modulus of PPy vary with applied potential and have been only measured on thick macroscopic specimens, and 3) the radius of curvature is small. Therefore, we need experimental data to develop and validate a PPy/Au microbilayer bending model. Arrays of hinges with different design parameters (layer thicknesses, aspect ratios, and hinge configurations) have been fabricated, and we are measuring the bending curvature and force as a function of these variables.

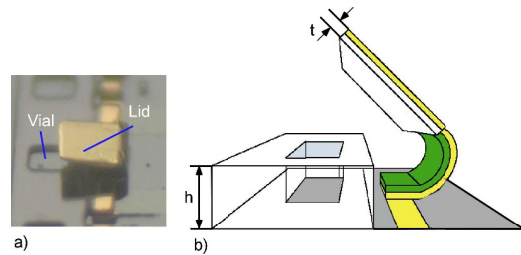


Fig. 5. A lid will lie flat over a vial when the hinge bending diameter equals the sum of the height of the vial h and the thickness of the lid t . a) A side-view photo of an actual hinge bending and b) a schematic of hinge bending.

E. Biocompatibility Testing

A prerequisite of investigating cells with the microsystem is that cells stay alive on the surface. MEMS structures on an oxidized silicon wafer were used to test the compatibility of the cells with these materials: SiO₂, Au, SU8, and PPy. The samples were cleaned with ethanol and rinsed in DI water, then covered with the cell culture medium, Hank's balanced salt solution (Biowhittaker Inc.). The bovine aortic smooth muscle cells were stained with neutral red, manually plated onto the surface, and cultured overnight in the incubator. Fig. 6 shows that the cells adhered and formed processes not only on the bottom of the vial, a silicon oxide surface, but also on the surrounding SU8 and Au. The cells were continuously cultured on samples for one week, during which they stayed alive and

reproduced, verifying that the microstructures were not toxic to the cells. We shall soon begin testing methods such as optical tweezers and dielectrophoresis to plate single cells into the vials, and then close the vials with the lids for culturing.

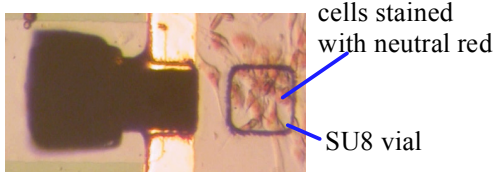


Fig. 6. This was a sample only for testing the compatibility of cells and microstructures. After overnight culturing, stained bovine aortic smooth muscle cells adhered to the microstructures and spread out.

IV. CONCLUSIONS

We have designed a microsystem for long-term electrical studies of single cells. A MEMS process has been developed to fabricate closable vials on a custom bio-amplifier CMOS chip. We have cultured bovine aortic smooth muscle cells on the microstructures to test biocompatibility and on the bio-amplifier circuit to verify that cell electrical signals can be recorded. Now that each of the components has been demonstrated, the next step is to fabricate a fully-functional prototype.

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