

Integrating Conjugated Polymer Microactuators with CMOS Sensing Circuitry for Studying Living Cells

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ABSTRACT

We present the use of electroactive polymer actuators as components of a biolab-on-a-chip, which has potential applications in cell-based sensing. This technology takes full advantage of the properties of polypyrrole actuators as well as the wide range of CMOS sensors that can be created. System integration becomes an important issue when developing real applications of EAP technologies. The requirements of the application and the constraints imposed by the various components must be considered in the context of the whole system, along with any opportunities that present themselves. In this paper, we discuss some of these challenges, including actuator design, the use of complementary actuation techniques, miniaturization, and packaging.

Keywords: lab-on-a-chip, cells, polypyrrole, conjugated polymers, MEMS, CMOS, potentiostat, dielectrophoresis, packaging

1. INTRODUCTION

We have been working for a number of years to realize “cell clinics” [1-5], which are lidded vials containing sensors for measurements on single cells or small groups of cells. This paper discusses the next step in that effort: the integration of microelectromechanical systems (MEMS) with complementary metal oxide semiconductor (CMOS) circuitry to form a complete biolab-on-a-chip. This system offers the possibility of sensing and analysis without the need for a cell biology laboratory, with automated measurements that take place continuously and in real time, and with data obtained at the same length scales as the physical dimensions of the cells themselves.

A schematic diagram of the biolab-on-chip that is being developed is shown in Figure 1. It will consist of an array of lidded vials fabricated over CMOS sensors in which single cells or small groups of cells are cultivated. The lids for the vials will be opened and closed using polypyrrole/gold (PPy/Au) bilayer hinges. Individual vials in the array could contain different types of sensors, different types of cells, or both, as required by the application. The final system would be required to keep the cells alive for a period of days to weeks and would also require a means for loading cells into the vials.

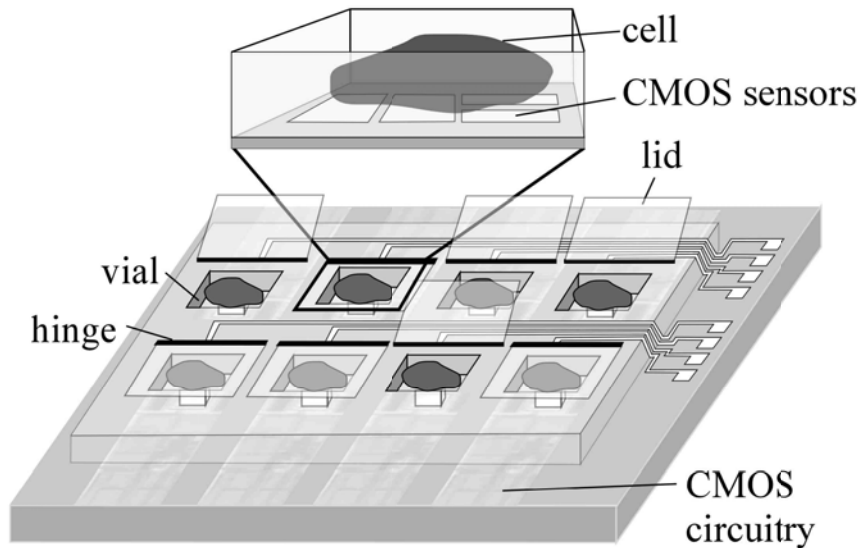


Figure 1. Illustration of the microsystem under development.

One potential application of this technology is a “bionic nose” based on olfactory neurons, which convert chemical signals into electrical signals. In biological systems, this transduction is done with high selectivity and sensitivity. Cells that respond to different odorants would be placed into each vial, and every vial would contain a CMOS amplifier to magnify the electrical signals generated by the cells. Since neurons are mobile [6, 7] it is critical to confine them. In addition, the closed lids help to electrically isolate the signals generated in each vial from those generated in other vials. Other potential applications of biolabs-on-a-chip are in homeland security for the detection of pathogens, in pharmaceutical screening for drug discovery and personalized medicine [8], and in the real-time study of biochemical pathways in cells.

The advantages of PPy for actuation in biomedical applications have been previously reviewed [9]. They include biocompatibility, operation in neutral pH media and a range of biofluids, and low actuation voltage. The latter is recognized as being important from a safety standpoint, but it is also an advantage for cell clinics because there is negligible electrolysis of water near the cells. In addition, in the biolab-on-a-chip system, the low voltage means that the actuators can be driven by ordinary CMOS circuitry, rather than by a special high-voltage technology, which gives one more flexibility in the sensors that can be created.

The MEMS vials and other microstructures are built over custom CMOS dice that have been fabricated in a foundry. The CMOS circuitry covers the entire surface of the chip, because it includes not only the sensors, but also signal processing, communication, etc. Therefore, for this microsystem in particular, PPy actuators are also attractive because they are fabricated using low-temperature surface micromachining steps, and because they have a small footprint on the substrate. Surface micromachining must be used to create the vials since the cells have to be positioned directly on top of the sensors on the surface. Low-temperature processing is required because the CMOS circuitry fails at elevated temperatures. Above the circuitry is an insulating layer onto which the MEMS devices are added by post-processing. The structures include not only the vials and lids, but also counter and reference electrodes, as well as interconnecting leads. The small area taken up by the EAP actuators (in comparison with MEMS electrostatic comb drives, for example) enables such an array of sensors to be created.

The advantages gained over prior work [5] by combining the cell clinics with CMOS are substantial. In the case of the bio-nose application, we have already shown that action potentials from electrically active cells can be readily detected with our bio-amplifier chip, with signal amplitudes that are well above the noise [10].

It also allows one to employ other types of sensors, such as for pH, ion concentration, and temperature [11-14].

We have also created a chip with an array of capacitance sensors that is capable of long-term monitoring of the interaction between cells and the chip surface [15]. When cells are first plated onto a surface, it takes some time (approximately 24 hours in the case of bovine aortic smooth muscle cells) for the cells to firmly attach to the surface, which is necessary for them to function normally. Cell proximity to the surface and the strength of their attachment can be sensed through capacitive coupling. Capacitances measured by the sensors increased during the attachment phase and then stabilized; when cells died the capacitance decreased rapidly. This is an important monitoring capability for the biolab-on-a-chip.

In addition to electrical sensors, optical sensors can also be produced with CMOS technology. A contact imager, similar to a CCD camera, has already been fabricated, as have fluorescence sensors. A contact imager consists of an array of pixels comparable in size to that of cells. We have used this chip to image cells on the surface [16]. Placing optical sensors inside the vials would significantly enhance the capability of the biolab, since fluorescence sensing is one of the most commonly used sensing modalities in biology.

In the remainder of this paper, we discuss some of the challenges that face the realization of the biolab-on-a-chip system. These include design and fabrication of PPy actuators that can effectively close the vials, contain the cells, and operate in a cell culture medium at cell culture temperatures; loading individual cells into the vials; miniaturization of the system; and packaging.

2. SYSTEM DESIGN

2.1 Polypyrrole bilayer actuators

First-generation lidded microvials have been previously described [4]. However, in these the lids were not closed during the impedance measurements performed on the cells [5]. The design and fabrication of lids that properly cover the vials to confine and electrically isolate the cells is a challenge. Figure 2a shows an example of a lid that is rotated, but that does not seal the vial because the radius of curvature is too large and the bending angle too small.

The first requirement of the bilayer actuators is that they have a radius of curvature r equal to the thickness of the vial (Figure 2b). At the same time, the length of the hinges must be such that the actuator bends 180° and the lid lays flat when it meets the vial. This is particularly critical in the case of non-adherent cells, for example red blood cells, that do not form confluent layers but that remain suspended in the medium. Since existing models do not correctly predict microactuator bending angles [17], parameters must be determined experimentally. Initial results of bending angle and force studies are presented elsewhere [18] in these proceedings. A complete characterization will lead to better models that can later be used in the design process.

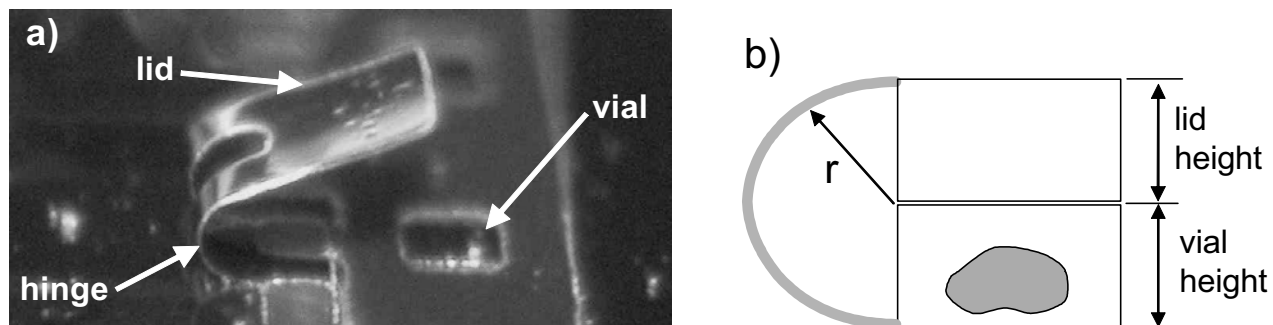


Figure 2. a) Oblique view of a PPy/Au bilayer hinge with a lid that does not seal the vial. (The dimensions of the vial are $130 \times 130 \mu\text{m}^2$.) b) It is required that the PPy/Au bilayer rotates the lid 180° and places it flat on top of the vial.

The second requirement is that the actuators close with sufficient force to confine the cells, because neural cells in particular have been observed to “escape” from on-chip containers. The force exerted by a heart muscle cell is $12 \mu\text{N}$ [19], and if the vial contains a small colony of cells, then the force required to contain them is even greater. Furthermore, if additional forces are applied to seal the lids against the vials in the closed position, the actuators must exert a sufficient counter-acting force to open. It is therefore important to quantify the force exerted by the bilayers both when bending and straightening so that a sufficient thickness of PPy is deposited.

A third requirement is that the actuators perform as expected in various cell culture media. Different types of cells require different mixtures and concentrations of salts, but ions of different size and valence produce varying magnitudes of actuation strain [20]. A systematic study of actuation in electrolytes with various cations (in the case of PPy(DBS)) is therefore needed, particularly those found in physiological media such as Hanks balanced salt solution (HBSS). To complicate matters, if there are small anions in the electrolyte, they are also exchanged, and this reduces the strain [21]. Thus, the effect of these anions must also be quantified. Finally, in order to design the actuators for a particular cell type, their behavior in a given mixture of many different anions and cations must be predictable. Our basic studies of ion transport in PPy will be of value for these issues [22, 23].

A fourth requirement is that the actuators have a reasonable lifetime. In NaDBS solutions, PPy(DBS) loses electroactivity after 20,000-50,000 cycles, which is sufficient. However, delamination can occur much sooner. On smooth gold surfaces, delamination can occur as early as the first cycle or after many as 20,000, but the lifetime is unpredictable. We are therefore characterizing the effects of various Au surface morphologies on the delamination lifetime [24]. A $1 \mu\text{m}$ thick electroplated layer of Au ensures a lifetime of at least 40,000 cycles, but $1 \mu\text{m}$ is too thick to yield the small radius of curvature required for the cell clinics, and further research is necessary.

In addition to these requirements, it is desirable to have a high-yield MEMS fabrication process if the biolabs-on-a-chip are ever to be produced commercially. All of the bilayer fabrication steps must be optimized to give uniform, reproducible results, and to consistently yield functional devices that have the same behavior.

2.2 Complementary actuation mechanisms

One of the opportunities of working in an electrolyte is that of exploiting the complementary actuation mechanisms that have been developed in the microfluidics field, such as dielectrophoresis (DEP), which has been widely applied for cell steering in lab-on-a-chip applications. The successful implementation of DEP in conjunction with PPy actuators was demonstrated by Park et al. [25]. These researchers used PPy valves in a microchannel to hold embryo cells stationary in a fluid flow, and then reoriented the cells using electro-rotation (a form of dielectrophoresis). The techniques complement each other nicely because the force fields created by DEP can move the cells in ways not possible using PPy actuators alone, and PPy actuators can modify the geometry of the environment in ways that DEP cannot. Both work in neutral pH electrolytes without adversely affecting live cells.

In the biolab-on-a-chip, we will use three-dimensional DEP to load the cells into the vials, maneuvering them over the CMOS and MEMS topography of dielectrics, semiconductors, and conductors. We are currently modeling the electric fields numerically using FEMLAB (Comsol Co.) and validating the results with basic experiments.

2.3 Miniaturization

To achieve overall system miniaturization, the actuators must be completely integrated on-chip, eliminating the external potentiostat, counter electrode, and reference electrode. We have fabricated a first-generation CMOS potentiostat (a single-ended amperometric potentiostat based on that presented in [26]). The CMOS circuitry, and therefore the working, counter, and reference electrodes, are made of Al, which is neither biocompatible nor electrochemically inert. For system integration these electrode pads must be coated with a material that is, such as gold. We used electroless plating, which involved depositing intermediate layers of Zn and Ni, in order to coat all of the exposed electrodes simultaneously, avoiding the requirement to electrically address each one [10].

To test the first-generation potentiostat, we used it to actuate an off-chip PPy(DBS) film in 0.1 M NaDBS solution (PPy deposition has been described elsewhere [27]). The on-chip potentiostat was connected to an external Ag/AgCl reference electrode and an external graphite counter electrode. The desired potential between the reference and working electrodes was input by a voltage source. The chip supplied the necessary current between the counter and working electrodes to achieve the desired voltage difference between the working and reference electrodes. No current flowed through the reference electrode. PPy is electrochromic, so thin films deposited on Au change color during oxidation and reduction. (This is due to addition and removal of states in the conjugated polymer's bandgap.) The PPy film was 40 nm thick, and it turned from light brown to transparent as the desired voltage was switched from 0 to -1 V, indicating that the PPy film was indeed oxidized and reduced. The next-generation chip with on-chip counters and reference electrodes is being developed.

On-chip counter and Ag/AgCl reference electrodes have already been demonstrated for cycling microfabricated bilayer actuators [28]. However, such Ag/AgCl electrodes are not stable in cell media or other electrolytes with relatively low Cl concentrations. Pseudo-reference Au or Pt electrodes will be used.

The use of a flat working electrode presents additional challenges aside from miniaturization. For example, its position with respect to the counter electrode will influence the electric field lines during the

electrochemical reaction. We need to model and optimize the dimensions, shape, and position of all electrodes.

2.4 Packaging

The biolab requires a package that allows the cells and the cell medium to contact the part of the chip surface containing the sensors, but not the wires bonded to the chip (Figure 3a and b): if these were immersed, all of the circuitry would be shorted. This is a reoccurring theme in many applications of wet-medium electroactive polymers: electrical wiring has to be isolated from the conducting medium in which the polymer operates.

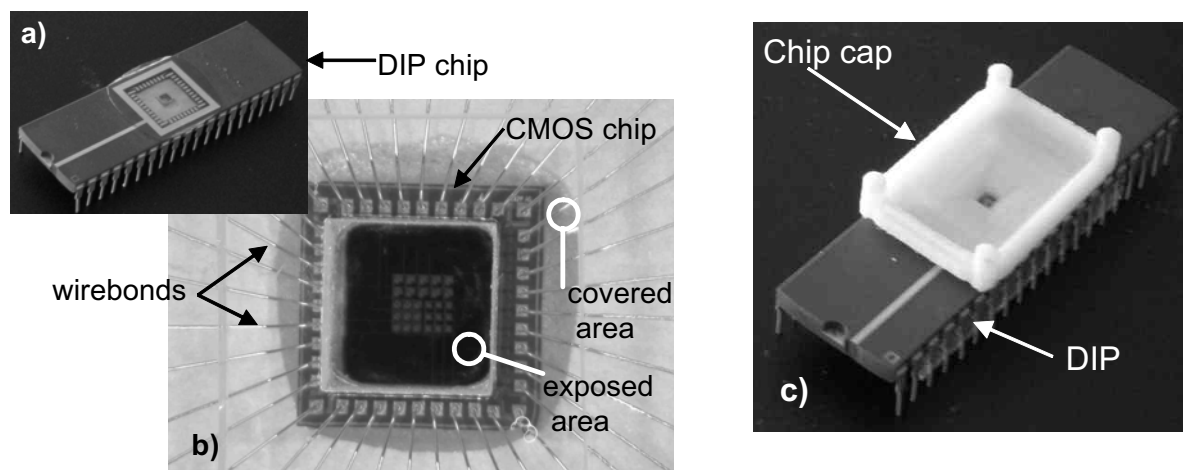


Figure 3. (a) The DIP 40 package used in this work has a window exposing the chip and the wirebonds. To cover the bond wires but leave the central part of the chip with the sensors exposed, we used (b) a UV-curable epoxy patterned photolithographically and (c) a plastic chip cap supported at the edge of the active area and backfilled with epoxy.

A temporary packaging solution we used during device prototyping was UV-curable Loctite adhesives patterned over the wires, leaving the active area exposed (Figure 3b) [15, 16]. We are also pursuing a different solution in which a plastic cap (fabricated using selective laser sintering) is glued to the CMOS chip, physically covering the wirebonds (Figure 3c). The space under the cap with the wires is backfilled with an epoxy for further protection. A window in the plastic cap gives access to the active area of the CMOS chip. Longer-term packaging solutions must be co-developed with the biolab as it evolves.

Since one of the requirements of the overall system is to monitor and maintain the viability of cells over a period of days to weeks, all of the components of the system must be biocompatible. This is also true of the packaging materials. One of the Loctite adhesives (3340), while providing excellent pattern definition and good protection of the wires from the electrolyte, killed the cells. We are comparing the biocompatibility and patternability of different UV curable adhesives to find an appropriate material.

3. CONCLUSIONS

Over the last decade, conjugated polymer based actuators have received increasing attention from the scientific community. Several groups are exploring applications in which their biocompatibility and ability to work in a wet environment are significant advantages over existing technologies. Nevertheless, integration of EAP actuators into complete systems has just recently begun. In this paper we have presented a few of the challenges involved in the integration of polypyrrole micro-actuators into a lab-on-a-chip device.

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