Biosensor Array Microsystem on a CMOS Amperometric Readout Chip

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***Abstract*—A single-chip integrated microsystem with a CMOS amperometric circuit, on-chip electrodes, a protein-based biointerface, and packaging for biosensor arrays is presented. A 2×2 gold electrode array was fabricated on the surface of the CMOS instrumentation chip. An all-parylene packaging scheme was developed for compatibility with liquid test environments as well as a harsh piranha electrode cleaning process. Proper operation of the chip was demonstrated with electrochemical and biological cyclic voltammetry experiments. A cytochrome PpcA protein interface was implemented to measure concentrations of Fe-NTA using the integrated amperometric microsystem.**

***Index Terms*—biosensor, cyclic voltammetry, on-CMOS chip.**

I. Introduction

Miniaturized sensor arrays are capable of parallel analysis of multiple parameters. Because of the distinct advantages of microsystem platforms, there has been a trend to integrate sensor arrays onto the surface of silicon chips and perform measurement using on-chip CMOS electronics [1-3]. At the same time, there is a great opportunity to expand lab-on-chip solutions that replace bulky bench top sample analysis tools with simple, low power, portable systems. The fabrication compatibility between many bio/chemical sensor interfaces and CMOS technology makes a CMOS circuit an outstanding candidate for a silicon-based lab-on-chip solution [4].

Analyte detection and quantification in liquid samples play major roles in a variety of biomedical applications**.** Many techniques have been developed to measure analyte concentration in solutions including electrochemical methods, optical imaging, thermal detection, spectrometry [5] and magnetic biosensors [6]. Among these, electrochemical methods can readily be adapted to CMOS instrumentation, and many CMOS amperometric readout circuits have been introduced with various functionality and performance to meet different applications demands. Notable examples include a wireless implanted potentiostat using current-to-frequency conversion of sensing current [7], a fully differential potentiostat that enables detection of a wide range of analytes [8], and amperometric readout circuits with temperature control to continuously monitor blood gases [9]. However, none of the reported amperometric circuits provide a complete solution to the challenges of integrating on-chip biosensor for use in a liquid environment.

This paper reports a fully integrated electrochemical biosensor array microsystem based on a broad current range amperometric readout circuit (ARC) that includes a 2×4 array of single-ended current readout circuits and two independent potentiostats. Furthermore, this paper introduces a chip-scale integration scheme for protein-based biosensors and electrochemical circuitry utilizing post-CMOS microfabrication of an on-chip microelectrode array and packaging for liquid environments. The single-chip microsystem has demonstrated electrochemical performance comparable to commercial benchtop instruments, and protein-based biosensors integrated on the chip surface have been functionally demonstrated. Compared to our prior related publications [10, 11] this paper introduces a new readout chip and reports for the first time the fully integrated protein-based biosensor microsystem operating in a liquid environment.

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II. System Architecture

Combining CMOS instrumentation circuits with miniaturized electrode arrays fabricated on CMOS chips introduces the opportunity for a monolithic measurement system. Fig. 1 illustrates the protein-based electrochemical biosensor array microsystem that serves as the conceptual model for the work described in this paper. A silicon chip containing CMOS electrochemical instrumentation circuitry serves as the substrate of the microsystem. An array of gold electrodes fabricated post-CMOS on the surface of the chip is connected through overglass contact openings to the underlying CMOS electronics. These electrodes serve as the interface to protein-based biosensors formed on the surface. A top passivation layer insulates CMOS surface metal routing, defines size-adjustable openings over individual electrodes, and provides an interface to a variety of possible fluid handling schemes, including microfluidics or a simple liquid reservoir illustrated in Fig. 1.

III. Amperometric Circuit Design

For an ARC, a potentiostat is required to control the potential of electrodes and a highly sensitive readout circuit is needed to measure the current resulting from the stimulus voltage. Fig. 2 illustrates this arrangement of components and serves as the system diagram for the ARC.

A. Potentiostat Array

The basic function of a potentiostat circuit is to control and maintain the voltage between the working electrode (WE) and

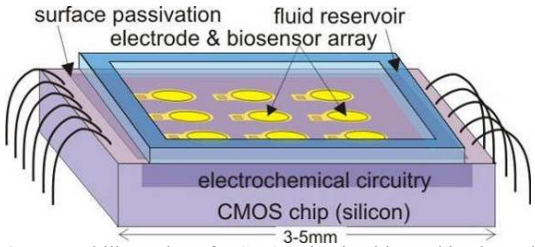

Fig. 1. Conceptual illustration of a CMOS circuit with on-chip electrode array and packaging for use in a liquid environment.

reference electrode (RE) under varying measurement conditions. A schematic diagram of the designed 3-electrode single-ended potentiostats is shown in Fig. 2. The stimulus control signal *Vsrc* sets the potential applied across the electrochemical cell through OP1. The output of OP2, which is buffered through negative feedback using OP3, is connected to the counter electrode (CE). The input of OP3 is connected to the RE, and ideally no current should flow through the RE. A cascode input stage structure was used in OP3 to reduce the input leakage current. Thus the output current resulting from the input stimulus at CE will flow only between CE and WE. OP2 was designed to provide a large current drive to support a variety of biosensor interfaces.

B. Amperometric Readout Array

To measure the electrochemical response current within the amperometric readout circuit chip, a current readout circuit was developed. Because the response current of the biosensor could be very small (in sub-pA range), the noise performance of the readout circuit would significantly affect measurement sensitivity. For most electrochemical applications, the stimulus frequency is less than 100 Hz, and within this operating frequency the circuit noise will be dominated by the 1/f noise rather than thermal noise. Based on our prior work [12], a switched capacitor current readout circuit was designed using correlated double sampling (CDS) to reduce the 1/f noise and amplifier DC offset.

Several improvements have been implemented in the new readout circuit to increase measurement sensitivity. In the CDS technique, the noise reduction factor is typically determined by

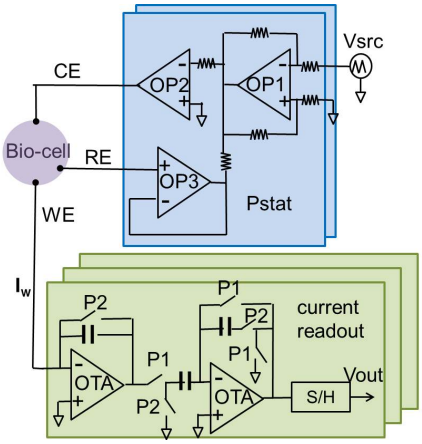

Fig. 2. Block diagram of the ARC chip that includes a 2×4 array of single-ended current readout circuits and two independent potentiostats.

device matching, timing errors, charge injection and clock feedthrough. To curtail charge injection, all switches were realized with minimum size transistors. To reduce clock feedthrough errors, the NMOS switches were replaced by a structure with one NMOS switch and two NMOS dummy transistors. An on-chip clock generator was developed to provide precise time sequence control for switch activation.

Noise in the amplifier also directly affects the sensitivity of the readout circuit. A folded cascode amplifier was chosen because it provides high DC gain and wide dynamic range. According to the noise analysis in [12], the size of the input transistors is critical. Increasing the value of WL and W/L can reduce the 1/f noise and thermal noise. However, larger input transistors will lead to larger input bias current, which limits the sensitivity of the readout circuit. An input bias current of hundreds of fA at 25ºC was reported for an input NMOS of W/L = 150µm/1.8µm [12]. To optimize the tradeoff between noise, speed and input bias current, this design sets W/L = 72µm /1.8µm for the NMOS inputs transistors. To further improve noise performance, all of the analog circuits were surrounded by capacitors between VDD and GND to minimize power supply noise, and the analog and digital power routings were separated over the whole chip to minimize digital clock noise coupling onto analog signals. Through these improvements, the sensitivity was increased by a factor of six compared to prior work [12].

IV. On-CMOS Electrodes and Package

Post CMOS processing of the ARC chip is required to form an array of on-chip electrochemical sensor sites with gold electrodes suitable for formation of protein biointerfaces. A unique chip-in-package fabrication process with parylene was developed to provide bio-compatibility and chemical resistance including to piranha cleaning solution.

To enable electrochemical measurement, each sensor site of the on-chip array includes a WE, CE and RE. Development of a reliable planar reference electrode remains a research challenge, and a pseudo RE was chosen so that all electrodes could be formed using gold. The electrode pattern was designed to maximize the electrochemical response current and realize uniform ion flow by arranging the electrodes concentrically. The distance between WE and CE is kept small to minimize errors due to potential drop in the solution.

The die-level post-CMOS fabrication process begins with formation of the electrode array on the CMOS ARC chip. We refer to this as die-level processing because we receive from the foundry and process individual die, but the same processes can be applied at a wafer scale. The electrodes are formed by PVD of titanium/gold (50Å/1000Å) and patterning by wet etching. Polyimide is then spin coated on the CMOS chip surface and patterned to insulate electrode routing and define the electrode area. The die-level process steps are illustrated in Fig. 3. At the end of this process, the electrode array was formed and the chip surface was passivated everywhere except the desired electrode areas and the wire bond pads.

Following the die-level processing, the ARC chip was wire bonded to a ceramic dual in-line package. The packaged chip

was then coated with a 5µm layer of parylene using PVD (PDS 2035CR, Specialty Coating Systems) (Fig. 4a). This process covered all surfaces within the package, including bond wires, package contact pads and the electrode array chip. Parylene is generally etched by reactive ion etching (RIE) using oxygen gas with photoresist or another solid layer deposited and patterned to form a masking layer. However, in this complex three-dimensional structure, a customized, non-traditional process was developed to overcome this challenge. First, a hole punch was used to create a cylinder of PDMS sized to match the area of the chip's surface from which parylene would be removed, ~1.5mm diameter in this case. A silicon chip of slightly smaller diameter was also cut from a wafer using a dicing saw. The cylinder was then attached, on one side, to the silicon chip using oxygen plasma assisted bonding and, the other side, to a glass slide. The silicon chip was included to eliminate direct contact of PDMS with parylene, which was observed to leave unwanted particle contaminants that were difficult to remove. The glass slide was then clamped to the parylene-coated chip-in-package with the silicon chip pressed down over the electrode area. Crystal adhesive (Crystalbond™ 590, SPI Supplies, Inc.) was then inserted into the cavity beneath the slides and melted at 120˚C to fill the cavity except where the PDMS/silicon cylinder was held (Fig. 4b). Later, the slide/PDMS/silicon assembly was removed leaving parylene exposed only over the on-chip electrodes. Parylene was then etched using RIE, with 300W RF power and 500sccm oxygen flow rate, to expose the desired electrode surfaces (Fig. 4c). Once the crystal adhesive was removed using acetone, the final package has all surfaces coated with parylene except the exposed electrode areas (Fig. 4d).

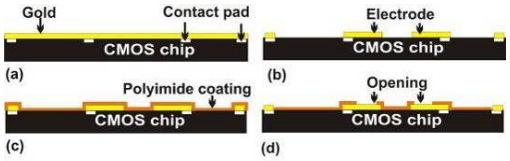

Fig. 3. Process flow for post-CMOS electrode fabrication: Ti/Au is deposited and patterned (a-b), Polyimide is spin coated and patterned (c-d).

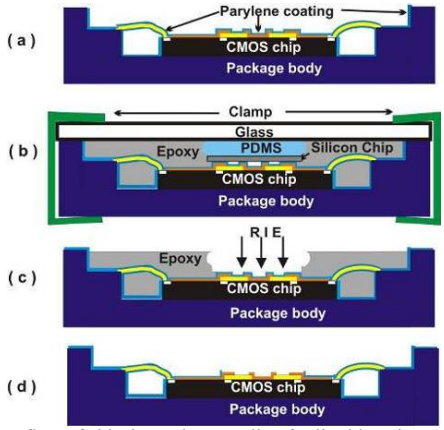

Fig. 4. Process flow of chip-in-package sealing for liquid environment. (a) Chip and package are coated by 5µm parylene. (b) A PDMS cylinder and silicon chip are pressed to the package to cover the center of the CMOS chip before crystal adhesive is melted to fill the cavity. (c) Glass slide is detached and parylene is etched away by oxygen RIE. (d) Crystal adhesive is removed to form final package

V. Measurement Results

The 8-channel amperometric readout ARC chip shown in Fig. 6 was fabricated in 0.5µm CMOS. A 2×2 electrode array was fabricated on the chip and connected to circuitry by routing gold traces to surface contact openings. The final packaged CMOS chip with electrode array is shown in Fig. 6.

To verify electrochemical measurement capability of the integrated system, a test setup composed of an ARC chip, a packaged electrode array, a PC with a DAQ card and a LabVIEW user interface was prepared. Cyclic voltammetry (CV) measurements were performed in an electrolyte solution containing 1M potassium chloride and 0.5mM potassium ferricyanide at 25°C using an on-chip WE, a commercial liquid junction Ag/AgCl RE and a platinum CE. Fig. 7a shows the results from both a commercial potentiostat (CHI760C, CH Instruments Inc.) and the reported CMOS amperometric system at scan rates of 100mV/s and 200mV/s. The peak shape, amplitude, and location measured with the ARC chip compared extremely well to those measured with the commercial system even at different scan rates. In another experiment, CV was performed in a 1mM potassium ferricyanide solution. Fig. 7b shows results from both the commercial potentiostat and the reported CMOS amperometric microsystem at two electrolyte concentrations. As expected, the peak current increased with electrolyte concentration, and again the peak locations and amplitudes of the CMOS system compared extremely well with the commercial system.

To demonstrate functionality of the microsystem as an electrochemical biosensor, a cytochrome protein biointerface was formed on the on-chip electrodes of the CMOS microsystem. Cytochrome PpcA from *Geobacter sulfurreducens* was expressed heterogeneously in E. coli and purified using cation exchange chromatography. The

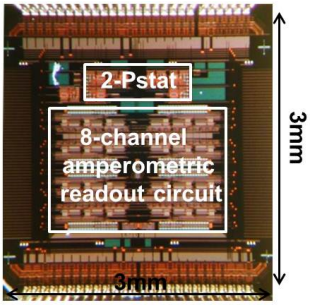

Fig. 5. The 3×3mm2 CMOS amperometric readout chip with 8-channel amperometric readout and 2 potentiostat array.

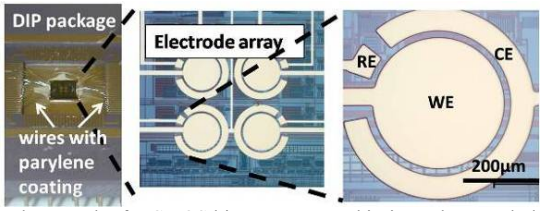

Fig. 6. Photograph of a CMOS biosensor array chip-in-package and close up views of the post-CMOS surface electrode array.

biointerface was prepared by cleaning the WE with piranha solution and contacting it with ethanolic solution of 5 mM 11-mercaptoundecanoic acid and 11-mercaptoundecanol (1:3 ratio) to form a self-assembled monolayer. Then, cytochrome PpcA was immobilized on the self-assembled monolayer by covalent bonding using 50mM (N-(3-dimethylaminopropyl) -N'-ethylcarbodiimide) and 5mM (N-hydroxysuccinimide) solution. Unbound cytochrome PpcA was rinsed away using 20mM phosphate buffer. CV electrochemical characterization was performed using an on-chip WE, a commercial liquid junction Ag/AgCl RE and a platinum CE. The cytochrome PpcA results for both the CHI760 instrument and the reported amperometric circuit are shown in Fig. 8. To determine the ability of the immobilized cytochrome biosensor interface, to detect dissolved iron, different concentrations of Fe-NTA (Ferric nitrilotriacetate) in phosphate buffer were placed in a reservoir formed on top of the CMOS chip. A dose-dependent increase in peak reduction current with increasing iron concentration confirmed suitability of the PpcA interface as a biosensor. Very good agreement was observed between the commercial instrument and the CMOS microsystem.

VI. Conclusion

A CMOS amperometric microsystem with on-chip electrode array and packaging for biosensor arrays has been developed. The ARC chip contains two potentiostats and an 8-channel current readout circuit. A post-CMOS fabrication process was developed to form on-chip electrode arrays and parylene packaging suitable for protein-based biosensors operated in a liquid environment. Functionality of the single-chip electrochemical system was verified by performing CV in a potassium ferricyanide solution, and a cytochrome PpcA protein biointerface was formed on the CMOS chip. Both systems performed well, exhibiting the expected dose-dependent response to the electrochemically active analytes. The reported instrumentation circuitry and post-CMOS fabrication processes are suitable for electrochemical microsystems using a wide range of biological and chemical sensor interfaces.

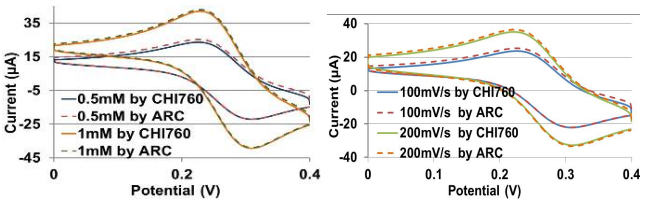

Fig. 7. CV measurement for both CHI 760 commercial instrument and ARC chip of (a) 0.5mM potassium ferricyanide at 100mV/s and 200mV/s and (b) potassium ferricyanide at 0.5mM and 1mM.

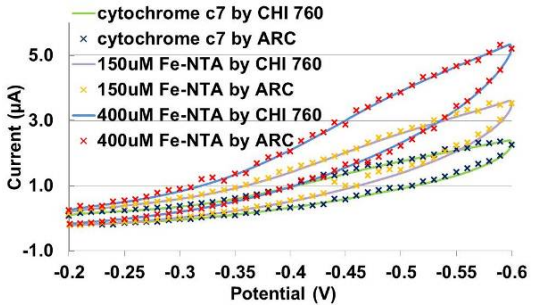

Fig. 8. CV measurement of cytochrome PpcA interface in 150μM and 400μM Fe-NTA for both CHI 760 commercial instrument and ARC chip.

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