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CMOS high density electrical impedance biosensor array for tumor cell detection

***Yu Chen^{a,*}, Chee Chung Wong^a, Tze Sian Pui^a, Revanth Nadipalli^b,
Roshan Weerasekera^a, Jegatha Chandran^a, Hao Yu^b, Abdur R.A.
Rahman^a***

*^a Institute of Microelectronics, A*STAR (Agency for Science, Technology and Research), 11
Science Park Road, Singapore 117685, Singapore*

*^b School of Electrical and Electronic Engineering, Nanyang Technological University, S1-
B1-C-85, 50 Nanyang Avenue, Singapore 639798, Singapore*

* Corresponding author.

E-mail address: chenyl@ime.a-star.edu.sg (Y. Chen).

Abstract:

We present a high-throughput label-free electrical-impedance spectroscopy (EIS) platform composed of a 96 x 96 microelectrode array for counting and analysis of breast tumor cells (MCF-7). These Au-electrodes are densely packed within a 3.5 mm x 3.5 mm interrogation region. Each Au-electrode has an edge-length of 25 μm designed to detect single tumor cell by electrical impedance spectroscopy. The detected signal is read out via an integrated circuit (IC) of addressable electrode-selection-switch array manufactured with 0.18 μm CMOS technology. The independently addressable microelectrode array is integrated onto a printed circuit board (PCB) containing associated circuitry for electrode switching and readout. MCF-7 cells were dispensed onto the chip surface and EIS measurements were recorded before and after the cell dispensation. On an average 20% increase in impedance was found associated with cell occupancy on the electrode surface. A positive correlation was observed between cell detection with optical microscopy and with electrical scanning.

1. Introduction

Cancer represents a major health challenge worldwide. In 2008, an estimated 12.4 million new cases of cancer were diagnosed with 7.6 million deaths worldwide. Most mortality from cancers such as breast, lung, prostate, and colorectal cancer are caused by the hematogenous spread of tumor cells into distant organs and its subsequent growth into evident metastases. Such metastases are initiated by the invasion of tumor cells into the circulation through normal blood vasculature or through tumor induced angiogenesis

[1]. Cells in the peripheral blood that possess the cancer phenotype are referred to as circulating tumor cell (CTCs) and may represent the tumor population that is most likely to develop overt metastases. The presence of CTCs provides a useful surrogate through which the tumor genetic and phenotypic status can be evaluated through a relatively non-invasive procedure [2]. The detection, quantification, and characterization of CTCs in peripheral blood have several potential applications in cancer medicine. Molecular and functional analysis of circulating stem cells may provide insights into the biology of cancer metastasis and identification of new therapeutic targets in the treatment and prevention of disseminated disease [3,4].

However, the detection of CTCs is very challenging. The frequency of CTCs in blood is calculated to be approximately one per 10^7 – 10^9 blood cells, demanding highly sensitive tools for their reliable capturing and analysis [5]. It is estimated that CTCs could be as rare as 1 cell per 5 billion red blood cells (RBCs) and 10 million white blood cells (WBCs) per ml of blood [6]. Numerous approaches are available for detection of CTCs in blood including some describing their molecular characterization [7–9]. The US Food and Drug Administration (FDA) approved method of enumerating tumor cells is based on optical/fluorescence microscopy which requires a trained operator to interpret the data. This method is beset with challenges such as staining requirements, high cost of microscope instruments, and over-reliance on subjective analysis. Another alternative method is to array the cells on electrodes and measure changes in electrical impedance accordingly [10]. Traditionally, large area electrodes are used to measure populations of cells which may not be useful for CTCs since 5 CTCs can make a difference in prognosis. Hence single cell detection limit is required. Jiang et al. proposed a passive electrode array for single cell detection for up to 200 electrodes [11]. However, passive electrodes are not scalable to accommodate enumeration of wide dynamic range of cells, such as CTCs, which may be as few as 1 to as many as a few hundred to thousand. Hence an active electrode array which can enumerate CTCs with single cell detection limit and a wide dynamic detection range is necessary [12].

In this paper, a novel high density electrical-impedance spectroscopy (EIS) biosensor array has been developed and characterized for CTC testing. The main objective of this work is to show that fabricated CMOS electrodes can be successfully utilized to enumerate cancer cells with single cell resolution. CMOS electrode based biosensor array can be deployed for impedance [13–16] or capacitance [17] change of cell. The biosensor array developed in this paper is impedance-based similar to the work from reference [13]. The significant distinct features of our device are as follows. Firstly, high electrode density enables a large dynamic range in detection. More than 9000 electrodes are compactly packed in an area of 3.5 mm x 3.5 mm. Secondly, small electrode size enables high counting accuracy down to the scale of single-cell detection. Each of the electrodes has a size comparable to a typical single tumor cell at 11–17 μ m [6]. Thirdly, CMOS

based biosensor array enable high throughput [14,15] when integrated with other components to form a system. Instead of being one isolated large working electrode device, the sensing area in this paper is consisted of an array of densely packed microelectrodes as the working electrode. As a result, cell counting is accomplished by summing the response of the individually addressable microelectrodes. Herein, the developed biosensor array system can detect a single cell and precisely count the total number of circulating cells from a few to thousands.

2. Materials and methods

2.1. System overview

A CMOS biosensor array was designed to accommodate ~9000 electrodes on a 3.5 mm × 3.5 mm silicon microchip. The number of microelectrodes was designed to enable counting of tumor cells in a big dynamic range for patients at different stages. An electronic demultiplexing was implemented to address the individual electrodes (Fig. 1). All other circuits essential for electrical impedance measurements were integrated on a printed circuit board (PCB).

2.2. CMOS post-processing for device integration

The eight-inch wafer with integrated microelectrode array and readout circuit was fabricated in Global Foundries in a 0.18 μm 1P 6 M standard CMOS technology with a supply voltage of 1.8 V. A 96 × 96 microelectrode array was fabricated on Al. The switches and the other circuitry were also built for high density electrode array (Fig. 1). The physical design (layout) consists of an array of 17 μm × 17 μm pixels with a pitch of 30 μm on top of the CMOS chip. Aluminum microelectrodes oxidize readily when exposed to air and degrades easily in solution which is not suitable as an interface for biological application. Therefore the exposed microelectrodes were capped with 1 μm - thick gold (Au) layer for biocompatibility and stability under electrochemical analysis. The detailed fabrication process is illustrated in Reference [18].

2.3. EIS system integration

The post-processed CMOS wafers were diced and the silicon chips with high density microelectrode arrays were attached to a ceramic pin grid array (PGA) board using silver epoxy (H20E, Epoxy Technology, INC.). Electrical connections to the chip were made through Au wire bonding. The PGA board was connected to a microcontroller based system which enables external EIS measurement to be conducted on individual electrode sequentially and simultaneously. Silicone glob top was dispensed over the Au wire to protect the fragile wire during the biological experiment. During testing, a uniform vertical electric field is generated between the working Au microelectrodes and a cap

indium tin oxide (ITO) coated glass. ITO serves as a counter/reference electrode for a two-electrode measurement system and also facilitates optical imaging of cells during the experiment.

2.4. Cell culture

Breast cancer cell, MCF-7 (American Type Culture Collection, MD, USA) was cultured in Dulbecco's modified Eagle's medium (DMEM, GIBCO Labs) supplemented with 10% fetal bovine serum (FBS, Hyclone) and grown at 37°C under a 5% CO₂ atmosphere in T75 flask.

2.5. EIS system testing

The impedance spectrum of the high density electrodes array was measured by HP 4284A LCR meter and controlled by a computer via the universal serial bus (USB) interface. The measurements were performed between the frequencies ranging from 100 Hz to 1 MHz. An alternating current (AC) of 25 mV peak to peak was used to excite each microelectrode in a sequential manner. Low amplitude of excitation voltage provides noninvasive analysis of the cells.

Fig. 2A shows the impedance spectrum change of three electrodes at different KCl concentrations. A clear increase of the impedance was observed when the salt concentration decreased. A fixed frequency was used to scan all the electrodes in a single chip. The impedance variability of electrodes across the chip depends on factors such as post processing of the capping gold electrode, vertical displacement of the counter electrode, and the effect of surface modification (Fig. 2B). With the salt concentration of the buffer changed, both capacitance and resistance of the buffer and also double layer capacitance at the interface between electrodes and the buffer are affected. Fig. 2C and D show the impedance contour of 1600 electrodes at KCl concentration of 30 mM and 300 mM and the impedance variance of the microelectrodes are 197 ± 20 k Ω at 30 mM and 117 ± 9 k Ω at 300 mM, respectively. These variances show a good uniformity of the fabrication and post processing of our microelectrode biosensor array.

3. Measurement result and discussion

Fig. 3A shows a micrograph of MCF-7 cells adhere on the electrode. The electrodes are either fully or partially covered by cells. The impedance of the electrode system can be considered as the equivalent circuit composed of the spreading resistance, cell impedance and electrode impedance [9]. The inset in Fig. 3C shows the total of impedance system when electrode is immersed into the electrolyte. R_s is defined as the spreading resistance of the bulk solution. $Z(\omega)/A$ is the equivalent impedance of

electrode–electrolyte interface that is frequency and electrode size dependent. The measured impedance is modeled as a parallel circuit which consists of a resistance (R_d) and a parallel capacitance (C_d). When electrode is partially covered by cells in a surface A_c , the impedance of the electrode can be divided into two parts in parallel, $Z(\omega)/(A - A_c)$ and $Z(\omega)/A_c$. To model the gap between the cells and the electrode surface, we introduce a serial resistance R_g , the equivalent circuits of the system with cell attached is depicted in inset of Fig. 3D. The impedance change can be estimated based on the coverage of cell on the electrode. In general, when the coverage is high (say coverage is 100% if the cell covered the whole electrode), higher impedance change is obtained. For a fully exposed electrode (coverage is 0%), the impedance should be similar to that of the measurement prior to cells seeding into the chamber.

Electrode impedance measurements were performed with the Au electrodes and ITO coated glass electrode immersed in the PBS (phosphate buffered saline). Fig. 3C shows the impedance spectrum of a typical electrode without cell binding in PBS (phosphate buffered saline) buffer and PBS spiked with breast cancer tumor cells (MCF7), the typical size of which is 17 μm . Fig. 3D shows the impedance spectrum of a typical electrode with the cell binding in the PBS buffer and in the buffer spiked with MCF7 cells. The impedance spectrum of the electrode can be fitted using the model described above and we got the spreading resistance R_s for electrode with cell to be 64.5 k Ω and that without cell to be 41.3 k Ω . The gap resistance between the cell and the electrode is fit to be 0.32 M Ω . The resistance (R_d) is 28 k Ω . C_d is a constant phase element related to the double layer capacitance of the electrode/electrolyte interface. It is given by $1/(j\omega C_{\text{coeff}})^n$, where C_{coeff} is 132×10^{12} and n is 0.975. The spectrum of the electrode without cells show negligible change compared with 15–20% change of the electrode with cells at the frequency around 100 kHz. This clear difference of the impedance change for the electrodes with cell attached and that without cell attached allow us to distinguish them and thus make it possible for cell counting. Fig. 3B shows the statistical impedance change of the electrodes with and without cell attachment under two different frequencies, respectively.

The largest impedance change ($\Delta Z/Z_0$) for electrodes after cell binding was 20% as captured from frequencies between 10 and 200 kHz. The slight discrepancies can be further improved from several aspects: uniformity of the size of the electrodes, the surface condition, and the characteristics of buffer solution. The later includes the salt concentration, and the distance between the micro electrodes array to the counter electrode. The percentage of impedance change is proportional to the cell surface coverage of the electrode. Therefore, smaller electrodes can achieve enhancement in sensitivity.

4. Conclusions

Based on CMOS-process compatible high-density microelectrode biosensor array, a high-throughput label-free electrical impedance spectroscopy (EIS) platform is demonstrated in this paper for the detection and counting of breast tumor cells with single cell resolution. The advantage of this platform is its large array size with densely packed microelectrodes, which are tailored to the approximate size of one single tumor cell. As a result, the EIS platform for the single cell detection potentially enables a precise tumor cell counting from patient blood sample. The impedance change of the electrodes before and after cell binding was measured and was found to be four times larger than that of environmental noise. This impedance change indicated the existence of the tumor cell and also proved the feasibility of applying this high density CMOS EIS platform for cancer diagnosis and prognosis.

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Biographies

Yu Chen received his M.Sc. in Physics in 2002 from Fudan University, China, and Ph.D. degree in Physics in 2008 from Boston University of United States. He is currently a Scientist at Institute of Microelectronics, A*STAR, Singapore. His research interests include design and fabrication of nano-scale biosensor, design and characterization of high through put cell based biosensor array.

Chee Chung Wong received his Bachelor of Engineering (Mechanical) and the Ph.D. degree in Bioengineering from Nanyang Technological University (NTU), Singapore in 2005 and 2011, respectively. Since 2010, he has joined the Bioelectronics program at the Institute of Microelectronics (IME), A*STAR, Singapore as a Research Scientist. The focus of his research activities is on integrating silicon based micro/nano-electronics with biological cells for molecular detection and analysis. He is also developing microfluidic devices to isolate, manipulate and lysis of biological cells. He has co-authored 5 publications and a patent.

Tze S. Pui received his M.Sc. in Medical Physics in 2004 from the University of Malaya, Malaysia and Ph.D. degree in Biomedical Engineering in 2011 from the Nanyang Technological University, Singapore. He is currently a Scientist at Institute of Microelectronics, A*STAR, Singapore. His research interests include high throughput, high content screening systems for biological analysis, and electrical/electrochemical assays for cell based biosensing.

Revanth Nadipalli is a Research Associate with VIRTUS, IC Design Centre of Excellence in Nanyang Technological University, Singapore. He received his MS from the Technical University of Munich and Nanyang Technological University. His current work focuses on CMOS Biosensors and low power analog circuits.

Roshan Weerasekera is a Scientist in the Institute of Microelectronics (IME) since March 2011. He received the B.Sc.Eng. degree in Electrical and Electronics from the University of Peradeniya, Sri Lanka, in 1998 and the M.Sc. and Ph.D. degrees in Electronic and Computer System Design from the Royal Institute of Technology (KTH), Sweden, in 2002 and 2008, respectively. His major research interests are: the design, modeling and analysis of on-chip and off-chip interconnects; 2.5D and 3D IC design and modeling; and CMOS biosensors.

Jegatha Chandran received her Bachelor of Technology (Chemical and Electro-chemical) from Central ElectroChemical Research Institute (CECRI), India in 2004. She obtained her Integrative Ph.D. from Korea Institute of Science and Technology (KIST), South Korea in 2011. Since 2011, she is serving as Scientist I in BioElectronics Program at the Institute of Microelectronics (IME), A*STAR, Singapore. Her research interests

include the development of Micro-Nano Systems for bio-sensor applications, Microfluidic Cell sorting and analysis.

Hao Yu is an Assistant Professor in the School of Electrical and Electronic Engineering, Nanyang Technological University, Singapore. He received his Ph.D. from the UCLA in 2007. He is the Associate Editor and technical committee member of several journals and conferences in integrated circuit (IC) design. His research interest includes 3D IC sensor design platform for bio-imaging. He won the best paper award in ACM Transaction for 3D IC design in 2010.

Abdur R.A. Rahman is a Principal Investigator in the Bioelectronics Department at the Institute of Microelectronics. He obtained his Ph.D. from University of South Florida in 2007. Prior to pursuing his Ph.D., he served as a MEMS Process Development Engineer at Formfactor Inc. and Standard MEMS Inc., between the years 2000 and 2002. He received his M.Sc. degree in MEMS from the University of Cincinnati in 2000. His interests are in development and commercialization of in vitro clinical diagnostic systems.

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- Fig. 2 (A) The impedance spectrum of three different electrodes at four different KCl concentrations. (B) The impedance variation of 1600 electrodes at different KCl concentration at a fixed frequency of 100 kHz. (C) and (D) Impedance contour of 1600 electrodes at KCl concentration of 30 mM (C) and 300 mM (D), respectively.
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Fig. 1

Fig. 2

Fig. 3