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High-density CMOS Microelectrode Array System for Impedance Spectroscopy and Imaging of Biological Cells

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Abstract

A monolithic measurement platform was implemented to enable label-free in-vitro electrical impedance spectroscopy measurements of cells on multi-functional CMOS microelectrode array. The array includes 59,760 platinum microelectrodes, densely packed within a 4.5 mm × 2.5 mm sensing region at a pitch of 13.5 μm. The 32 on-chip lock-in amplifiers can be used to measure the impedance of any arbitrarily chosen electrodes on the array by applying a sinusoidal voltage, generated by an on-chip waveform generator with a frequency range from 1 Hz to 1 MHz, and measuring the respective current. Proof-of-concept measurements of impedance sensing and imaging are shown in this paper. Correlations between cell detection through optical microscopy and electrochemical impedance scanning were established.

Keywords

CMOS Microelectrode Array (MEA); Electrochemical Impedance Spectroscopy; Electrophysiology; Lock-in Amplifier

I Introduction

Electrochemical impedance spectroscopy (EIS) is a popular method for quantitative and qualitative monitoring of processes that occur in cells and other biological units. The main advantages of EIS are its label-free and noninvasive nature, and its real-time detection capability. Several integrated circuits, based on CMOS processes, for impedance sensing have been demonstrated in recent years. Applications included electroanalysis and biosensing: small impedance changes that occur at an electrode-electrolyte interface have been detected in real time and have been correlated with the presence of target analytes [1]; label-free impedimetric immunosensing has been demonstrated for diagnosis and prognosis of cancers, such as brain cancers [2–3], for studying neurodegenerative diseases [4], and for capturing complex cellular responses during drug or chemical administrations [5]. In practice, a 2-dimensional (2D) impedance mapping is highly useful in characterizing the cell location, tissue structure, and cell attachment to the surface. Ideally, one would want to monitor the impedance of multiple cells simultaneously at high spatial density and signal quality. To realize such features requires a low-noise impedance measurement system, which can perform many measurements in parallel and acquire all the data.

This paper presents work on impedance measurement units, which have been integrated in a multi-functional microelectrode array (MEA) system featuring 59,760 microelectrodes [6]. Simultaneous electrical recording and impedance spectroscopic measurements on a high-density microelectrode array will enable us to study presence, morphology and electrophysiology of specific biological preparations.

II System Design

A block diagram of the CMOS MEA chip is shown in Fig. 1. The 59,760 working electrodes were arranged in 180 rows and 332 columns. To enable measurements with different types of cells and multiple cells at the same time, the electrodes were designed to have a size of $7.5 \mu\text{m} \times 3.0 \mu\text{m}$ with a pitch of $13.5 \mu\text{m}$. The reference electrode was placed at the periphery of the array. The chip provides all circuitry needed for conducting the impedance experiments. There are 32 lock-in amplifiers to sense the magnitude and phase of the electrode impedance over a wide frequency range. Any electrode from the microelectrode array can be connected to these lock-in amplifiers. To measure the impedance, on-chip generated sinusoidal voltages were applied between the reference electrode and the arbitrarily selectable electrodes. The in-phase (I) and quadrature (Q) signals were also generated within the wave generator. The output signals of the lock-in amplifiers were first low-pass filtered to remove the higher frequency mixing signals, multiplexed and then digitized with delta-sigma converters.

A serial peripheral interface (SPI) bus was implemented to communicate with and configure the chip. A custom program was developed in C# that generated different commands and sent them through an SPI protocol to the chip. The output data from the chip were acquired using an NI PXIe-6544 DAQ card. The bit streams of the delta-sigma converters were decimated using cascaded integrator-comb (CIC) filters, implemented in a LabVIEW program. Magnitude and phase of the impedance were then extracted from the V_I and V_Q data as follows:

$$\text{Magnitude} = \sqrt{V_I^2 + V_Q^2}, \text{Phase} = \tan^{-1}(V_Q/V_I)$$

III Fabrication

The chip was fabricated in a 6M1P $0.18 \mu\text{m}$ CMOS process. The die size is $12 \times 8.9 \text{ mm}^2$. Platinum electrodes were post-processed on wafer level by using ion-beam deposition and etching. A platinum resistor was fabricated next to the sensor array in order to monitor temperature during experiments. The silicon area of one individual impedance measurement unit is about 0.1 mm^2 . As shown in Figs. 2A, 2B, the chip was packaged on a custom PCB inside a plastic epoxy well, in which we could culture primary neurons or brain slices. For electrical testing and characterization, bright Pt electrodes of four different sizes ($8 \times 8 \mu\text{m}^2$, $4 \times 4 \mu\text{m}^2$, $2 \times 2 \mu\text{m}^2$, $1 \times 1 \mu\text{m}^2$) were fabricated on the CMOS chip (Fig. 2C).

IV Measurement Results

First, the electrical characterization of the chip was carried out. Impedance measurements of the electrodes were performed in a phosphate-buffered saline solution (PBS), which had similar electrical properties as a physiological solution. The electrode-electrolyte interface impedance showed a $1/f$ roll-off behavior, which evidenced the dominance of the double-layer capacitance as expected in this frequency range (Fig. 2D). The phase at low frequency ranged around 60-80 degrees, which is indicative of predominantly capacitive behavior of the electrodes. At higher frequencies, the spreading resistance started to dominate the impedance, and the phase tended towards zero. Pt-black was electroplated on the surface of the array and its effect on the impedance is shown in Fig. 2E. The impedance was reduced 30-40 times as compared to that of the corresponding bright Pt electrodes.

For the impedance imaging experiment, mouse embryonic stem cells (mESC) were cultured in hanging drops to form three-dimensional multicellular aggregates, called embryoid bodies (EBs). The cardiac differentiation of EBs was monitored by using impedance and electrophysiological sensing. In the first 5 days, the EBs were differentiated in the suspension culture. After 5 days, the EBs were plated on the electrode-array surface, where they could adhere and outgrow (Fig. 3A). The pictures in Fig. 3 were taken 5 days after the beginning of EB formation and on the first day of EB adhesion. The position of the cells, their adhesion and growth can be clearly seen through the impedance change, both in magnitude and phase (Fig 3B, 3C). After 5 days of adhesion, electrophysiological activity of the differentiated EB can be recorded by using the integrated electrophysiology channels (Fig. 3D).

Conclusion

In this paper we presented a CMOS chip that can perform EIS along with electrophysiology recordings on 59,760 electrodes at a $13.5\ \mu\text{m}$ spatial resolution. We demonstrated the system's capability to investigate and monitor position, adhesion and the electrical activity of cardiac embryoid bodies. The spatial resolution is higher than that of commercially available systems like the xCELLigence® RTCA CardioECR system [7]. The possibility to perform high-resolution impedance sensing in combination with electrophysiology recording makes the presented system a versatile tool for indepth studies of cellular behavior and dynamics.

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References

- [1]. Manickam A, Chevalier A, McDermott M, Ellington AD, Hassibi A. A CMOS electrochemical impedance spectroscopy (EIS) biosensor array. *IEEE Trans Biomed Circuits Syst.* 2010; 4(6): 379–390. PART 1. [PubMed: 23850755]

- [2]. Chen Y, Wong CC, Pui TS, Nadipalli R, Weerasekera R, Chandran J, Yu H, Rahman ARA. CMOS high density electrical impedance biosensor array for tumor cell detection. *Sensors Actuators, B Chem.* 2012; 173:903–907.
- [3]. Elshafey R, Tlili C, Abulrob A, Tavares AC, Zourob M. Label-free impedimetric immunosensor for ultrasensitive detection of cancer marker Murine double minute 2 in brain tissue. *Biosens Bioelectron.* 2013; 39(1):220–225. [PubMed: 22898660]
- [4]. Guo J, Ng W, Yuan J, Li S, Chan M. A 200-Channel Area-Power-Efficient Chemical and Electrical Dual-Mode Acquisition IC for the Study of Neurodegenerative Diseases. *IEEE Trans Biomed Circuits Syst.* 2015:1–12. [PubMed: 24760945]
- [5]. Chi T, Park JS, Butts JC, Hookway TA, Su A, Zhu C, Styczynski MP, McDevitt TC, Wang H. A Multi-Modality CMOS Sensor Array for Cell-Based Assay and Drug Screening. *IEEE Trans Biomed Circuits Syst.* 2015 Dec; 9(6):801–814. [PubMed: 26812735]
- [6]. Viswam, V., Dragas, J., Shadmani, A., Chen, Y., Stettler, A., Mueller, J., Hierlemann, A. Multi-functional microelectrode array system featuring 59,760 electrodes, 2048 electrophysiology channels, impedance and neurotransmitter measurement units. 2016 IEEE International Solid-State Circuits Conference (ISSCC); 2016. p. 394-396.
- [7]. Nguemo, F., Semmler, J., Hescheler, J. Label-Free Impedance Measurements for Profiling Drug-Induced Cardiotoxicity. *Label-Free Biosensor Methods in Drug Discovery.* Springer; 2015. p. 283-305.

Fig. 1.

The CMOS chip provides all circuitry units needed for conducting impedance and electrophysiology experiments.

Fig. 2.

A) Measurement setup. B) CMOS chip packaged on a PCB featuring an epoxy well. C). Pt-electrodes of different sizes: el1: 8 × 8 μm², el2: 4 × 4 μm², el3: 2 × 2 μm², el4: 1 × 1 μm². (D) Magnitude and phase of the bright Pt-electrode impedance in a frequency range from 1 Hz to 1 MHz. E) Magnitude of bright Pt and Pt-black electrode impedances measured at 1 kHz.

Fig. 3.

Embryoid bodies were placed onto the chip surface, and EIS measurements were performed before and after the cell placement. A) Picture taken 5 days after the beginning of EB formation and on the first day of EB placement on the chip. B) The impedance changes of the electrodes (3200 microelectrodes out of all 59,760 electrodes in 100 electrode configurations) upon cell attachment was measured. The impedance difference after EB adhesion was plotted. C) Corresponding phase response of the electrodes upon cell

adhesion. D) Electrophysiological recordings (cardiac beating) of the differentiated EB after 5 days of adhesion and culturing on the chip.

Table 1

Characteristic features of the MEA and impedance spectroscopy

Technology	0.18 μ m CMOS
Sensing area	4.5 mm $\times$ 2.5 mm
Number of electrodes	59,760
Standard electrode size	7.5 μ m $\times$ 3.0 μ m, Pt
Frequency range	1 Hz – 1 MHz
Power (per channel)	412 μ W (including ADC)
Impedance range	10 k Ω -10 G Ω