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Bioimpedance measurements on human neural stem cells as a benchmark for the development of smart mobile biomedical applications

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Abstract. Over the past 30 years, stem cell technologies matured from an attractive option to investigate neurodegenerative diseases to a possible paradigm shift in their treatment through the development of cell-based regenerative medicine (CRM). Implantable cell replacement therapies promise to completely restore function of neural structures possibly changing how we currently perceive the onset of these conditions. One of the major clinical hurdles facing the routine implementation of stem cell therapy is the limited and inconsistent benefit observed thus far. While unclear, numerous pre-clinical and a handful of clinical cell fate imaging studies point to poor cell retention and survival. Coupling the need to better understand these mechanisms while providing scalable approaches to monitor these treatments in both pre-clinical and clinical scenarios, we show a proof of concept bioimpedance electronic platform for the Agile development of smart and mobile biomedical applications like neural implants or highly portable monitoring devices.

Keywords: Electrical Impedance Spectroscopy (EIS), Bioimpedance, Neural Stem Cells (NSCs), Proliferation, Embedded System, Mobile Technologies.

1 Introduction

Around 1905, the electric galvanometers were sensitive and rapid enough to non-invasively pick up the very small potential difference generated by heart activity (ECG), and, by 1910, Höber used bioimpedance methods to prove the existence of cell membranes and calculated how extremely thin they are (less than 0.01 micrometer) [1].

Since then, many clinical applications based upon bioimpedance have been developed, including measuring cardiac minute volume non-invasively, measuring lung respiration activity, and taking electronic biopsies for diagnosis of skin cancer among others [2]. More than 100 years later, as we enter the age of cell-based regenerative medicine (CRM), bioimpedance is more relevant than ever. Reasonable progress has been made within the field of stem cell therapy, but there is still a limited and inconsistent benefit provided by numerous pre-clinical and a handful of clinical CRM approaches. While the challenges like poor cell retention and survival or teratoma [3, 4] of pluripotent stem cells are well known effects *in vivo*, the inability to determine cell fate and survival in humans has been a significant obstacle to understanding the mechanisms of the variable efficacy. While the incorporation of cell fate imaging in clinical trials may help address these significant hurdles, live bioimpedance surveillance may be the perfect candidate to monitor those treatments. Moreover, multifaceted monitoring and control strategies will be vital in addressing these issues to enable the successful rise of cell-based regenerative medicine. Numerous studies based on impedance measurements of live biological cells enabled the technique to become widely accepted as a label free, non-invasive and quantitative analytical method to assess cell status. To show some examples, bioimpedance can be used to monitor proliferation [5], apoptosis [6], migration [7], degeneration [8], morphological changes [9] and also (neuronal) differentiation [10].

In this work, we show a proof of concept bioimpedance electronic platform for the Agile development of smart and mobile biomedical applications like neural implants or highly portable monitoring devices. Most current bioimpedance setups rely on what we will define as a traditional approach. A typical traditional approach in portable medical electronic system comprises components like analog front-ends for data acquisition, amplifiers and filters for signal conditioning, analog to digital (ADC) converters for signal and sensor data acquisition, buttons to accept user feedback, an MCU to execute algorithms, and a variety of interfaces such as an LCD display, USB port and so on. Traditional design methodologies bring together all of the needed components onto a printed circuit board (PCB) [11].

Modern system-on-chip (SoC) architectures provide a new way of designing portable medical electronic devices bringing numerous advantages. Portable medical electronics equipment of all types, like glucose meters, pulse oximeters, portable ECG devices, etc. – are already implemented using SoCs [11]. By integrating many of the peripheral components required by portable medical electronics applications, fewer components are needed resulting in simpler PCBs which take less time to prototype resulting in shorter iterations. In the case of PSoC (programmable SoC) superior reconfigurability greatly reduces the need for new prototypes for each iteration. By speeding up iterations [12] and making prototypes more flexible, PSoC development platforms enable the usage of Agile development methodologies.

To show a proof of concept of our PSoC-based bioimpedance analyzer, electrical impedance spectroscopy was performed by using both our proposed instrument in comparison to a commercial impedance analyzer to assess proliferation of the neural stem cell line hVM1 (human ventral mesencephalic neural stem cell line 1).

2 Materials and methods

2.1 Development methodology and contextual framework

We show a proof of concept for the bioimpedance measurement instrumentation to be used on the Training4CRM (European Training Network for Cell-based Regenerative Medicine [13]) optogenetic neural implant for the treatment of neurodegenerative diseases. Apart from the control logic core, the implant will roughly consist of optogenetically modified neuronal cells, a sensing fiber electrode, and an analog front-end, both still under parallel development. Interfacing biomedical and engineering technologies under parallel development is one of the major challenges. To handle this, an open Agile iterative development methodology was adopted. To overcome the restraints of traditional electronics hardware development, the Cypress PSoC (programmable system-on-chip) 32-bit Arm® Cortex®-M3 PSoC® 5LP platform was chosen. These chips include a CPU core and mixed-signal arrays of configurable integrated analog and digital peripherals. This hybrid architecture enables high flexibility for quick developing and prototyping as a tradeoff for state-of-the-art performance allowing the translation of many hardware design decisions to software development choices. The current implementation is built upon the CY8CKIT-050 PSoC® 5LP Development Kit and external passive components using concepts based on application notes suggested in Cypress online resources.

2.2 Analog and digital design

As it can be seen in the schematic in **Fig. 1**, the current implementation is based on a four-electrode configuration. However, for the sake of this work, this system was adapted to a two-electrode configuration by connecting the electrodes. The excitation source is an 8-bit current output DAC (digital to analog converter) which can source or sink current in three ranges (2040 μA, 255 μA, and 31.875 μA). For this application, the source was set at 31.875 μA.

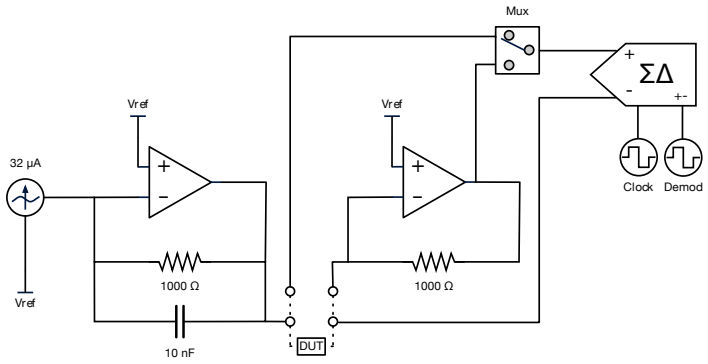

Fig. 1. Analog design schematic of PSoC-based impedance analyzer

A PWM (pulse width modulator) generates the clock to trigger the DMA (direct memory access) transactions which transfer a sine wave LUT (lookup table) to the current output DAC every quarter wave and changing the sign though source and sink control every half wave. The frequency excitation signal can be changed through the period of the PWM divider. This approach limits the number of available frequencies and the DMA transfer speed creates a bottleneck for the maximum available excitation frequency even using a low-resolution waveform. Tuning these variables through iteration, we generate 10 frequencies from 100 Hz to 100 kHz in multiples of 1, 2 and 5. Each quarter of wave table has 13 elements with bigger tables resulting in a significant slower maximum frequency due to the DMA bottleneck. Future iterations can use alternative approaches exploiting the FPGA (field-programmable gate array) abilities of the platform to overcome these limitations. However, for the purpose of establishing a proof of concept device, they are sufficient. Finally, an internal operational amplifier is used as shown in the schematic in **Fig. 1** as a low pass filtered current to voltage converter. The ΣΔ ADC (analog to digital converter) modulator input is used to dynamically control the polarity of the signal in the modulator inverting it when the input is high. This way, it can be used to modulate the input signal with an independent clock to act as a signal mixer. Two state machines control both the ADC and also generate in-phase and quadrature clock signals to be used in the signal mixer.

2.3 Embedded application

The firmware was kept as simple as possible. In its current form, the development kit allows the device to work standalone requiring external power only. Through the integrated LCD, the user is able to read the measurements for both phase and amplitude while a button cycles through the available frequencies by setting the divider of the PWM. The application itself uses a loop based architecture. In a simplified description, the routine starts by initializing the necessary hardware namely the operational amplifiers and calibrating them, and then it initializes the excitation signal generator and DMA, the ADC and finally the LCD. The measurement routine follows by getting in-phase and in-quadrature measurements for the transimpedance amplifier but also for the excitation signal selectable through two multiplexers. Phase and amplitude for impedance are calculated through synchronous detection and they are then printed in the LCD.

2.4 Cell culture of hVM1

hVM1 cell line: hVM1 is a human ventral mesencephalic neural stem cell line. Cell isolation and immortalization were described previously [14]. Briefly, human neural stem cells (hNSCs) were isolated from a 10-week-old aborted fetus (Lund University Hospital). Tissue procurement was in accordance with the Declaration of Helsinki and in agreement with the ethical guidelines of the European Network of Transplantation. The cells were immortalized by stable transfection with a retroviral vector coding for *v-myc* (LTR-vmyc-SV40p-Neo-LTR) [15]. It has been shown that neuronal differentiation results predominantly in dopaminergic neurons that exhibit electrophysiological

activity [16]. Stable transfection with the vector LTRBcl-XL-IRES-rhGFP-LTR resulted in expression of the anti-apoptotic protein Bcl-XL (basal cell lymphoma – extra-large) which increased the dopaminergic properties [17, 18].

Cells were routinely cultured in cell culture flasks pre-treated with Geltrex™ (ThermoFisher Scientific, A1413301) and grown at 37 °C and 5 % CO_2.

hVM1 growth medium: Dulbecco's modified Eagle medium/F-12 medium with Glutamax (ThermoFisher Scientific) supplemented with 0.5 % Albumax (ThermoFisher Scientific), 5 mM HEPES (ThermoFisher Scientific), 0.6 % glucose (Sigma), N2 supplement (ThermoFisher Scientific), non-essential amino acids (Ala, Asn, Asp, Glu, Pro, 40 mM each; MerckMillipore), penicillin/streptomycin, epidermal growth factor and basic fibroblast growth factor (20 ng/ml each; R&D Systems).

2.5 Carbon electrode chips

The pyrolytic carbon electrode chips were provided by Technical University of Denmark [19]. They are composed of a circular pyrolytic carbon working electrode (WE) with an area of 12.5 mm^2, surrounded by a platinum counter electrode (CE) with an area of 25.2 mm^2. The reference electrode (RE) has an area of 0.8 mm^2 and is also made from Pt, as well as the electrical contacts and leads. The chip (10 mm x 30 mm) is finally covered by an insulating SU-8 passivation layer, except for the sensing area and the contact pads (**Fig. 2**).

The electrode chips were initially treated with oxygen plasma and then assembled in a chip holder with magnetic clamping. The wells were sterilized using 0.5 M NaOH solution for 15 min, followed by washing with cell-culture-tested water. After coating of the electrode surface with Geltrex for at least 2 h, hVM1 cells were seeded at a density of 0.4 Mio cells/cm^2 and cultivated at 37 °C and 5 % CO_2.

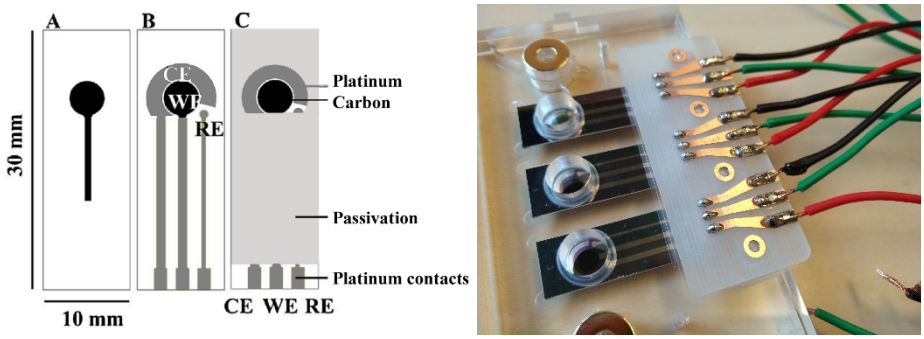

Fig. 2. Schematic drawing (left) of 2D carbon electrodes: layer of pyrolytic carbon (A), platinum electrodes, contact leads and pads (B), SU-8 passivation layer (C), modified after [19]. CE = counter electrode, WE = working electrode, RE = reference electrode. Photo of electrodes assembled in chip holder with magnetic clamping (right).

2.6 Electrical impedance spectroscopy

At this point, one of the main goals is to verify the viability of the measurement principle of the PSoC system while ensuring the sampled cells survive the excitation signal. Therefore, the system under test is solely used to measure on the first well of the triple chip system, while our reference system, a Zurich Instruments MFIA Impedance Analyzer is used on the three wells to both assess cell growth and also provide a reference to the measurements done with our proposed instrumentation. The MFIA connects to the chip holder using a couple of 1 m long RG-6 coaxial cables while our proposed instrument uses 8 cm 0.5 mm wide copper wires. For the excitation signal, the MFIA uses a sinusoidal voltage source of 45.78 µV with working currents ranging up to 200 nA in the chosen frequency range while our instrument uses a 32 mV source from the 32 µA AC source, resulting in estimated currents around 300 µA. In broad terms, both instruments use the lock-in amplifier technique. This technique has been widely used in impedance measurement applications being the gold standard in noise suppression and accuracy. Impedance spectroscopy was performed every second day to follow the proliferation of hVM1 cells for the total duration of 9 days. A two-electrode configuration was used by connecting only working and counter electrode. A medium change was performed before each measurement. Spectra from 1 kHz to 1 MHz (100 points, log) were recorded with the MFIA, while with the PSoC prototype, a spectrum from 100 Hz to 100 kHz (10 points) was analyzed. After the end of the experiment, cells were removed from the electrode surface with trypsin and an impedance spectrum was recorded of the electrode system without cells, but with the same amount of medium providing a reference. Due to electrode degradation, it was not possible to do a no cells reference measurement using the PSoC instrument.

3 Results and Discussion

3.1 Characterization of Cell proliferation by Electrical Impedance Spectroscopy (EIS)

Bioimpedance measurements to follow the proliferation of hVM1 cells have been performed to assess the performance of the PSoC-based impedance analyzer compared to a commercial instrument, the Zurich Instruments MFIA Impedance Analyzer. The resulting spectra of impedance and phase angle as a function of frequency are shown in **Fig. 3**. For the PSoC-based instrument, impedance values could only be measured up to 100 kHz due to limitations of the platform. Due to electrode deterioration, no measurement without cells was possible. From both the commercial instrument and the developed impedance analyzer, it is visible that the impedance increases with the cultivation period. An increasing number of cells leads to an increased impedance [20]. No significant change of the phase angle θ was detectable.

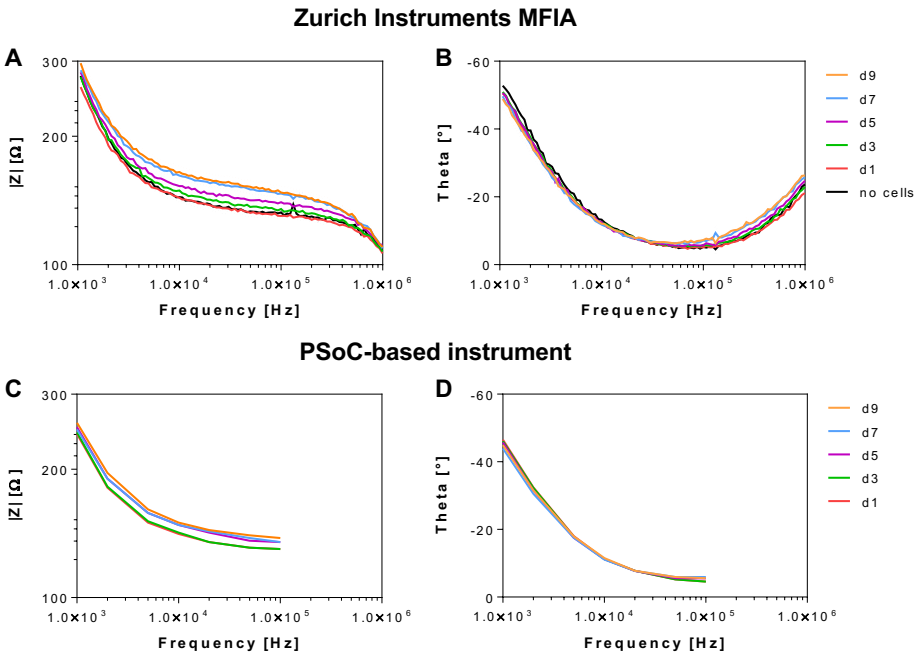

Fig. 3. EIS with Zurich Instruments MFIA (A, B) and PSoC-based Impedance Analyzer (C, D). Impedance |Z| (A, C) and phase angle θ (B, D) as a function of frequency for different days of growth of hVM1 cells (d1-d9) in comparison to the electrode system without cells (only MFIA). No cells could not be shown for PSoC due to electrode deterioration. MFIA n = 3, dotted line represents SEM. PSoC n = 1.

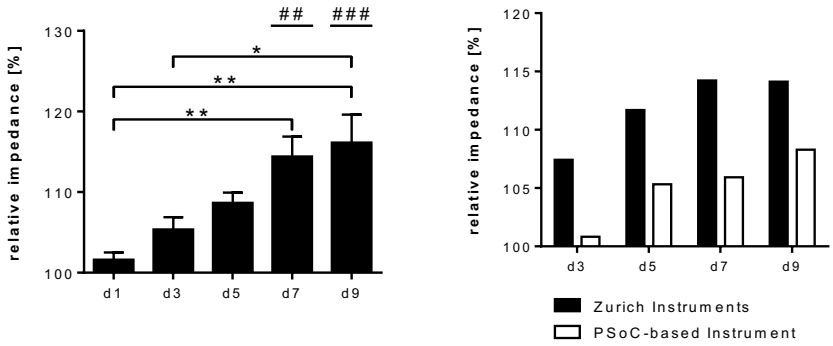

Fig. 4. Relative impedance [%] for growth of hVM1 cells normalized to electrode chip without cells (left, n = 3) or d1 due to electrode deterioration (right, n = 1) for Zurich Instruments vs. PSoC-based instrument. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. # indicates significant difference to system without cells.

The normalization of impedance values for the different days of growth to the impedance of the chip without cells provides a quantification of the change in impedance that is caused by the cells only, eliminating the influence of electrode design, cables etc. This relative impedance was calculated using equation 1 and the maximum over the frequency range is shown in **Fig. 4**.

$$\text{relative impedance} = \frac{|Z|_{\text{with cells}}}{|Z|_{\text{without cells}}} \cdot 100\ \% \tag{1}$$

The calculation of relative impedance confirms the increasing impedance values due to cell growth. From day 7, the growth of the cells resulted in a significantly higher impedance compared to the system without cells. This trend is also visible with the PSoC-based instrument. However, the measured increase in impedance is smaller for the PSoC-based instrument than for the MFIA impedance analyzer. The single well 10 point measurements done with the PSoC is not enough to assess the reason for this behavior requiring further work to understand the underlying causes.

4 Conclusions

The instrument proposed in this article provides a miniaturized, highly flexible and affordable solution in a single-chip format allowing for impedance measurements of adherent cells. A single chip fully integrated solution provides superior immunity to noise, allowing fast development of miniaturized prototypes. A PSoC integrated circuit is composed of a core, configurable analog and digital blocks, and programmable routing and interconnect. The configurable blocks in a PSoC are the biggest difference from other microcontrollers. In this work, we exploited this ability to use a ΣΔ ADC as a mixer for achieving synchronous detection. The results suggest that the applied current does not harm the cells and that the system allows for measurement of cell growth comparable to a commercial impedance analyzer. However, future work aims at improving both excitation and sensitivity systems, as well as testing our proposed instrument in bioimpedance measurements of other cellular phenomena.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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