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An electrical method to measure low-frequency collective and synchronized cell activity using extracellular electrodes

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ABSTRACT

An electrical method to measure extracellular bioelectrical activity *in vitro* is presented. This method exploits the Helmholtz capacitive double-layer established at the electrode surface. Small extracellular voltage variations in the order of μVs induce through the double-layer capacitor a displacement current that is measured. This current is then enhanced by a gain factor proportional to the electrode capacitance. In addition, when measurements are carried out at low frequencies in current mode the electrode contribution to the noise can be minimized. The performance of the electrodes and the method is demonstrated using zebrafish hearts and glioma cell cultures. We propose that this electrical method is an ideal tool to measure *in vitro* slow and temporally synchronized events that are often involved in long range intracellular signaling.

1. Introduction

Microelectrode arrays (MEAs) are substrate-integrated extracellular electrode matrices kept in contact with cells in culture. MEA-based cell electronic interfaces enable the study of neuronal network processes, the evaluation of the effects of drugs and the electrophysiological mechanisms related to pathological conditions [1]. Continuing key improvement of this platform are the spatial resolution and the electrical coupling between the cell and the sensing device [2–6]. The spatial resolution has been improved by increasing the density and the number of the electrodes. The signal-to-noise ratio (SNR) has been enhanced by minimizing the impedance of the sensing electrode through the use of suitable materials, such as conducting polymers [7–12].

Voltage sensing readout followed by voltage amplifiers with appropriate filters to select particular events is frequently used to measure cell activity, particularly action potentials generated by neurons [13, 14] in the kHz range. Here, we propose and demonstrate a methodology to measure weak and low-frequency biological signals using extracellular electrodes. Our results show that it is advantageous to measure the cell signals in current mode using a trans-impedance amplifier and sensing electrodes with high capacitance and relatively high resistance. This methodology is not suitable for measuring signals in the kHz range. At these frequencies the high electrode capacitance acts as a low-pass filter, which degrades the trans-impedance amplifier frequency response. However, for low frequencies ($f < 10$ Hz), it is possible to benefit from the high-performing characteristics of current amplifiers. Furthermore, this method takes advantage of the high capacitance region, viz. Debye-Helmholtz layer, established at the electrode/electrolyte interface, to improve the signal-to-noise ratio (SNR).

The proposed measuring methodology makes use of large area electrodes and therefore, is particularly suitable for recording low frequency cooperative cell activity. There are a number of cooperative low-frequency cell signals that have an important role in brain activity, including spatial exploration and memory. Changes in low-frequency neuronal oscillations have been associated with brain disorders such as schizophrenia or epilepsy [15,16]. New tools able to record these cooperative low frequency signals are essential for understanding their role in brain function.

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The understanding of extracellular measurements in current mode requires the knowledge of three aspects; (i) the role of the electrode design and impedance in shaping the native cell signal, (ii) the relationship between the voltage and current signal shapes and, (iii) how electrode impedance contributes to the SNR. These aspects will be addressed in this article.

The paper is structured as follows: in Section 2 we first present the basic measurement system. Section 2.1 introduces the equivalent circuit model that describes the displacement current and how the voltage signal is related with the shape of the signal when measured in current. We evaluate how the electrode impedance affects the signal shape and adds noise to the measurement setup. In Section 3.1 the proposed current measurement methodology is validated using zebrafish heart demonstrating state-of-the-art transduction results. In Section 3.2 it is shown that this methodology combined with the use of relative large area electrodes is adequate to probe glioma cell cultures that engage into cooperative activity. Therefore the method provides a tool to study a variety of low-frequency biological signals, which remain yet to be explored using *in vitro* experiments.

2. Material and methods

2.1. Measurement set-up and cells

This section presents the measurement set-up. The sensing electrodes used consist of two co-planar, parallel conductors on the upper surface of an insulating substrate. Gold electrodes were deposited by thermal evaporation on glass substrates. The electrode shapes and dimensions used are outlined in Table 1. Two different designs were used, finger type and round electrodes as shown in Fig. 1. Finger type electrodes were used to measure glioma cells and the round electrodes were used to measure zebrafish hearts. Fig. 1(c) shows a photograph of a zebrafish heart and Fig. 1. (d) a photograph of a confluent culture of glioma cells.

The patterned electrodes were fitted at the bottom of a standard Petri dish (SARSTEDT®) and loosely covered with a lid to prevent evaporation of the medium. After filling the compartment with the cell suspension the system was placed in an incubator (HERACell®150).

All electrical measurements were performed with a Stanford low-noise current amplifier (SRS 570), or alternatively in voltage mode using the voltage amplifier (SRS 560), connected to a dynamic signal analyser (Agilent 35670A). The low noise pre-amplifiers operated with internal batteries. Small-signal-impedance measurements were carried out by a Fluke PM 6306 impedance meter. All electrical measurements were carried out inside of a thick iron based Faraday cage to shield low frequency interferences and the entire system is mechanically decoupled from external vibrations.

To validate our approach, small zebrafish hearts with were used. The hearts from adult fish were chirurgical extracted and placed in Krebs' solution. These hearts can beat for as long as one day. They are perfectly suitable to use as bioelectronic signal generators to demonstrate the proposed signal detection methodology. The heart has a size of about 1 mm and beats at a rate of about two times per second. The zebrafish cardiac system is one of the most well-known systems often used in cardiac research [17–19]. Fish care and experimentation complied with the national legislation for the use of laboratory animals under a Group-1 license issued by the Veterinary General Directorate of the Ministry of Agriculture, Rural Development and Fisheries of Portugal. Fish were anesthetized (in water containing 200 $mg \cdot l^{-1}$ ethyl-3-aminobenzoate methanesulfonate salt, MS222) and then killed by decapitation.

Table 1
Shapes and dimensions of the electrodes used in this work.

Electrode type	Electrode length (W) (μm)	Inter-electrode distance (L) (μm)	Electrode depth (D) (μm)
(A) finger shape	10.000	20	15
(B) finger shape	3.520	325	100
	Diameter (D) (μm)	L	
(C) Round shape	3.400	7.000	

2.2. Circuit model and noise analysis

The sensor comprises two parallel electrodes of depth D and length W, separated by a gap distance L (see Table 1). One of the electrodes acts as measuring electrode and the other as counter-electrode. The electrodes are connected to a trans-impedance amplifier or alternatively to a voltage amplifier. The impedance of these electrodes has an important role in the system performance, since it determines not only the electrical coupling of the extracellular signal to the sensing electrode but also the electrode contribution to noise.

The interface between cells and microelectrodes *in vitro* has been described using an electrical equivalent circuit. Both point/area contact circuit models, that assume a tight seal between the cell and the electrode and circuit models that consider the detection of the electrical activity of cells that are not in tight contact with the electrode, have been considered [20–27]. These circuit models are helpful to interpret how charge fluctuations generated by a cell are coupled into the sensing electrode and how they are measured either as voltage or as current signal. Charges passing through the channel pores at the cell membrane create regions of charge excess/depletion giving rise to potentials that can be detected at different points. A simplified point/area circuit model based is shown in Fig. 2. R_D and C_D are the resistance and capacitance, respectively, of a simplified model of the electric double-layer that forms at the electrode-electrolyte interface. This circuit is a reduction of the more complex model, consisting of a constant-phase-angle impedance and charge-transfer resistance. For the sake of simplicity these elements are not included in the model of Fig. 2. Without cells, when we look across the two electrodes the high impedance double layers appears effectively in series with the low impedance electrolyte layer described by a resistance R_S and a capacitance C_S. When cells are in contact with the measuring electrode, the signal loss between cell and the measuring electrode is modeled by the resistance R_C. It is important that $R_C \ll Rs$, this ensures that the extracellular signal is essentially coupled into the measuring electrode.

The electrolyte impedance in series with the counter electrode double-layer impedance represents what is called the seal impedance Z_{Seal}. The seal resistance is usually defined as the resistance between the cell and the surrounding solution (ground).

In our measurement setup we use a trans-impedance amplifier, whose output voltage is given by

$$v_o(t) = -R_F i_S(t) \tag{1}$$

where R_F is the feedback resistance and $i_S(t)$ the current flowing through the measuring electrode impedance. It is important to understand how this current is related with $v_S(t)$, the voltage signal generated by the cell. Fig. 3 shows the circuit used to derive the relation between $v_S(t)$ and $i_S(t)$ which is a simplified version of the circuit in Fig. 2. As discussed above we assume that Z_{Seal} is high and the cell generated current signal $i_{CS}(t)$ is effectively coupled into the measuring electrode ($i_{CS} \approx i_S$).

First we express $i_S(t)$ in function of $v_C(t)$, the voltage across the double-layer capacitor,

$$i_S(t) = \frac{v_C(t)}{R_D} + C_D \frac{dv_C(t)}{dt} \tag{2}$$