Sensors and Actuators B 176 (2013) 605–610

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Monitoring living cell assays with bio-impedance sensors

Paula Daza[a], Alberto Olmo[b], Daniel Cañete[b], Alberto Yúfera[b,*]

[a] Dto. of Cell Biology, Faculty of Biology, University of Sevilla, Av. Reina Mercedes sn. 41012, Sevilla, Spain
[b] Sevilla Microelectronic Institute (IMS), Microelectronics National Center (CNM-CSIC), University of Sevilla, Av. Américo Vespucio sn. 41092, Sevilla, Spain

ARTICLE INFO

Article history:
Received 12 July 2012
Received in revised form
17 September 2012
Accepted 24 September 2012
Available online 2 October 2012

Keywords:
Electrical bio-impedance
Biometric circuits
Sensory systems
Microelectrode
ECIS
Cell culture

ABSTRACT

This work proposes a cell–microelectrode model to be used on cell culture assays as an alternative to end-point protocols employed in cell growth and cell biometry applications. The microelectrode model proposed is based on the area overlap between the microelectrode sensor and the living cells as main parameter. This model can be applied to cell size identification, cell count, and their extension to cell growth, motility and dosimetry protocols. A procedure to fit the proposed model to microelectrode electrical performance is presented, enabling the decoding of empirical measurements and its interpretation in terms of number of cells. This fitting procedure depends on three parameters: microelectrode geometry, gap resistance between substrate attached cells and microelectrode and, mainly, on microelectrode area covered by cells. The model has been implemented employing Analog Hardware Descriptions Language (AHDL) to be incorporated also to mixed-mode simulation processes during circuit design flow.

Experiments performed with commercial electrodes are described, illustrating a procedure to obtain cell number in real time in both, growth and dosimetry assays, employing an established cell line (AA8). The results are displayed in the form of growth curves (cells were growing during a week), as well as dosimetric response after treatment with MG132, a proteasome inhibitor. The results agree with the expected performances, with errors around 10–20% in the number of cells measured, therefore we think that these results are promising.

1. Introduction

Many biological parameters and processes can be sensed and monitored using its impedance as marker [1–5], with the advantage of being a non-invasive and relatively non expensive technique. Cell growth, cell activity, changes in cell composition and shape, or in cell location are examples of processes which can be detected with microelectrode–cell impedance sensors [6–10]. Among impedance spectroscopy (IS) techniques, electrical cell-substrate impedance spectroscopy (ECIS) [7], based on two-electrode setups, allows the measure of cell-culture impedance and the definition of the biological nature (material, internal activity, motility and size) of a kind of cell and its relationship with the environment [11]. One of the main drawbacks of ECIS technique is the need of infering efficient models to decode the electrical performance of the full system composed by the microelectrodes, medium and cells. Several works have been developed in this field. In [8], magnitude and phase impedance are deduced from a first order RC model, using the electric field equation solution at the cell–electrode interface. It is derived a three parameter based model: h, the cell–electrode distance, R_b, barrier resistance between cells and r_{cell}, cell radius. In [11,12], finite element simulations (FEM) are executed for solving electrical field considering the whole structure. This method gives one parameter model (R_{gap}) for describing the gap or cell–electrode region resistance. In both, the derived model considers the cell confluent phase [7] or a fixed area covered by cells [12]. The latest was extended in [11] to several cell sizes, allowing to define the cell–electrode covered area as the main model parameter.

This work considers a modification of the model proposed by Huang et al. [12], to incorporate the cell–microelectrode area overlap [11]. Impedance sensor sensitivity curves based on the cell size and density will be presented and applied to measure the growth rate in cell cultures. Our aim was also to describe and analyse the toxic effect of the chemical MG132 on the cited AA8 cells. Section 2 describes the assays protocol followed. Moreover, the electrode solution model useful for cell–electrode characterization and the process to extract useful cell–microelectrode models are included in this section, illustrating the simulations on a simplified system for cell size detection. Section 3 relies on experiments performed on modelling commercial electrodes, and its application to real time cell culture monitoring and dosimetry experiments. Conclusions and discussion will be highlighted in Section 4.

* Corresponding author. Tel.: +34 955446666; fax: +34 955446600.
E-mail addresses: pdaza@us.es (P. Daza), aolmo@dte.us.es (A. Olmo), dani@zariweyo.es (D. Cañete), yufera@imse-cnm.csic.es (A. Yúfera).

0925-4005/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2012.09.083

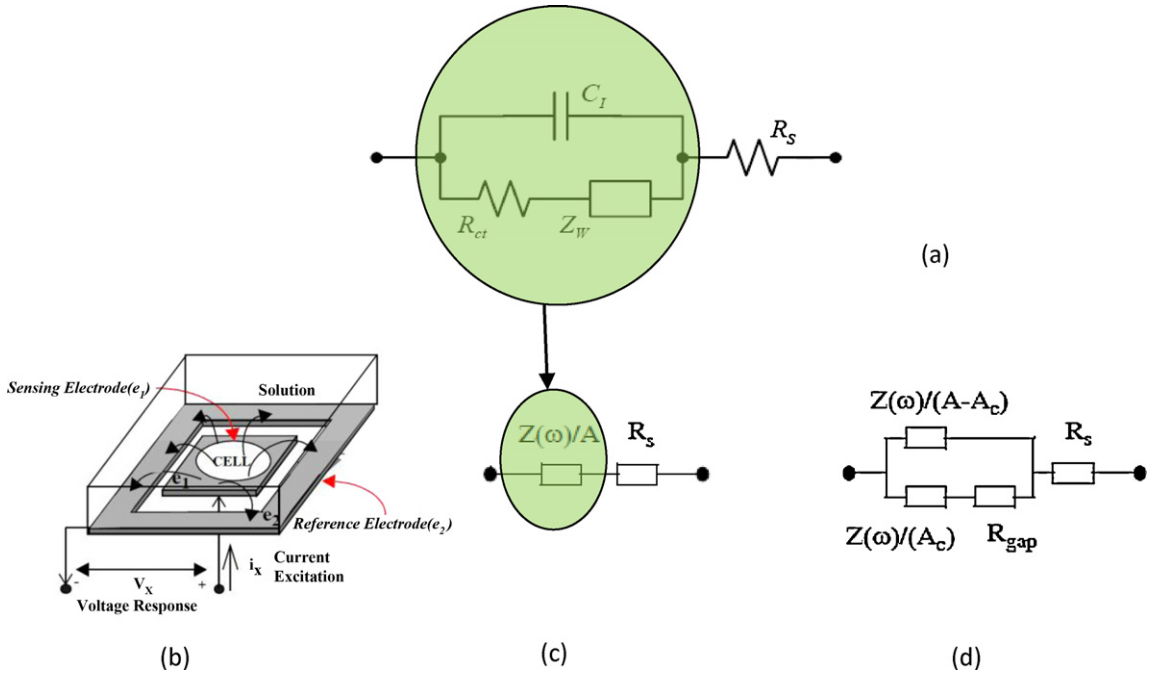

Fig. 1. (a) Circuit for the electrode–solution interface. C_I is the double layer capacitance; Faradic impedance includes Z_W, the Warburg impedance and R_{ct}, the charge–transfer resistance. The R_s is the spreading resistance. (b) Two electrodes for ECIS: e_1 (sensing) and e_2 (reference). AC current i_x is injected between e_1–e_2, and voltage response V_x is measured. Proposed model for an electrode–solution–cell model with area A, uncovered with cells (c) and covered area A_c (d).

2. Material and methods

2.1. Reagents

All chemicals were purchase from SIGMA. The drug MG132 is a reversible proteasome inhibitor.

2.2. Cell line and culture conditions

The study was carried out on Chinese hamster ovary fibroblast cell line, AA8 (American Type Culture Collection). AA8 cells were cultured in McCoy's medium supplemented with 10% (v/v) foetal calf serum, 2 mM L-glutamine, 50 μg/ml streptomycin, and 50 U/ml penicillin. The cell line was maintained at 37 °C in a humidified atmosphere with 5% CO_2. Cells were routinely sub-cultured and they were always in exponentially growth phase when they were used for experiments. Each experiment was independently performed twice.

2.3. Commercial electrodes

For cell culture assays, commercial electrodes 8W10E, from Applied Biophysics [13] (http://www.biophysics.com/) were employed. The multi-well is composed of eight separated wells; each one contains ten circular gold microelectrodes of 250 μm diameter.

2.4. Cell growth assay

Two experiments are reported in this work. Firstly, we performed a simple growth assay (without treatment) and cells were maintained in the incubator for one week. Cells were plated in a density of 5000 cells/0.8 cm^2 in multiwells delivered by Applied Biophysics [13]. Secondly, cells were allowed to adhere for three days and then they were treated with the different doses of MG132 (200 nM, 500 nM, 1 μM, 5 μM, 10 μM and 50 μM) during 96 hours more. Before treatment, cells were washed twice with PBS (Phosphate Buffer Solution) and fresh medium was added.

2.5. Electrode–electrolyte model

The impedance of electrodes in ionic liquids has been extensively investigated. An excellent review can be found at [6]. The main components describing the electrical performance of an electrode metal inside a solution are four, as it is illustrated in Fig. 1a. (1) C_I, the double layer capacitance, (2) R_{ct}, the transfer resistance, caused by the electron transfer at the electrode surface, generating a the current flowing through the electrified interface, (3) Z_W, the Warburg impedance, due to limited mass diffusion from the electrode surface to the solution, which has a $\omega^{-1/2}$ frequency dependence. The electron transfer resistance R_{ct} is in series with the mass diffusion limited impedance Z_W. Finally, (4) R_s, the spreading resistance, due to current travelling across the bulk solution. The electrode based setup has a solution conductivity, determined by this series resistance. These four components depend on the technology, medium and geometry, and require also applying small AC voltage amplitudes in experiments to be valid.

2.6. Cell–electrode model

A practical setup for ECIS technique is illustrated in Fig. 1b using a two-electrode impedance sensor: e_1 is the sensing electrode and e_2 the reference one. Electrodes can be manufactured in CMOS process with metal layers [12] or using post-processing steps [14]. The cell location and size on e_1 top must be detected.

The models in Fig. 1c and d consider that the sensing surface of e_1 could be total or partially filled by cells respectively. For the two-electrode sensor in Fig. 1b, e_1 has the sensing area A, and $Z(\omega)$ is the impedance by unit area of the empty electrode (without cells on top). When e_1 is partially covered by cells in a surface A_c, $Z(\omega)/(A - A_c)$ is the electrode impedance associated to non-covered area by cells, and $Z(\omega)/A_c$ is the impedance of the covered area. The resistance R_{gap} models the current flowing laterally through the