A MICROELECTRODE IMPEDANCE METHOD TO MEASURE INTERACTION OF CELLS

H. L. Gomes, R. B. Leite, R. Afonso, P. Stallinga and M. L. Cancela
University of Algarve, Molecular Biology Laboratory (CIMAR/CCMAR), Faro, Portugal, hgomes@ualg.pt

Abstract
An impedance method was developed to determine how immune system cells (hemocyte) interact with intruder cells (parasites). When the hemocyte cells interact with the parasites, they cause a defensive reaction and the parasites start to aggregate in clusters. The level of aggregation is a measure of the host-parasite interaction, and provides information about the efficiency of the immune system response. The cell aggregation is monitored using a set of microelectrodes. The impedance spectrum is measured between each individual microelectrode and a large reference electrode. As the cells starts to aggregate and settle down towards the microelectrode array the impedance of the system is changed. It is shown that the system impedance is very sensitive to the level of cell aggregation and can be used to monitor in real time the interaction between hemocyte cells and parasites.

Keywords
Microelectrode, impedance spectroscopy, biological cells.

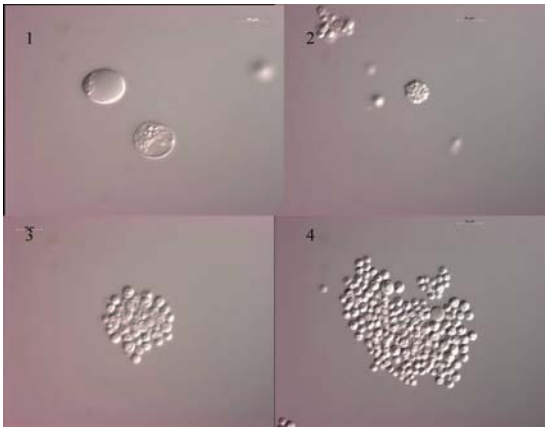
INTRODUCTION

Cell impedance sensors have been reported in the literature to measure the interaction between cells and their surroundings, for cell identification, adhesion, coverage, and even cell motility [1-8]. This work expands on the use of microelectrical impedance spectroscopy for the characterization of the interaction between different cells. The hemocyte cells used in this study were cells of bivalves (*Ruditapes decussatus*) and the parasite is the *Perkinsus atlanticus*. These cells can survive at room temperature in normal atmospheric conditions, and are therefore easy to handle and thus an ideal prototype system. In the presence of hemocytes the parasites aggregate in clusters, the level of this aggregation is a measure of the immune system response to the parasite. These studies can be extended to other types of cells and used as biosensing techniques to monitor in real time how the immune system responds to infections.

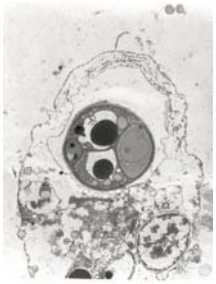
EXPERIMENTAL

Fig.1a shows phase contrast photos of individual *Perkinsus* cells (parasite) and clusters of these cells. The parasite *Perkinsus* has a typical dimension of about 5 μm The aggregation in clusters is normally stimulated by the presence of cells of the immune system (hemocyte). Fig. 1b shows an hemocyte with several parasite cells inside.

Two designs of microelectrodes were used in this study. Round interdigitated electrodes fabricated on oxidized silicon wafers and a set of parallel electrodes. Typical dimensions are 20 μm width, 2mm in length separated by a 20μm gap.



(a)



(b)

Figure 1. (a) Phase contrast photo of *Perkinsus* cells in culture. (b) Scanning electron microscopy photoshowing the phagositosys of a *Perkinsus* cell by an hemocyte.

On top of the microelectrode systems a small reservoir was built using a thin plastic ring (2mm height) as schematically represented in Fig 3. This reservoir was then filled with a medium of parasites. To study the interaction between the parasites and the immune system, hemocyte were subquently added to the medium. The AC impedance (in the range 100 Hz – 1 MHz) was measured between each individual microelectrode and a large top electrode made of a transparent conducting glass. Measurements were also

carried out between individual electrodes in a planar mode. The impedance was measured using a Fluke PM6306 impedance analyser.

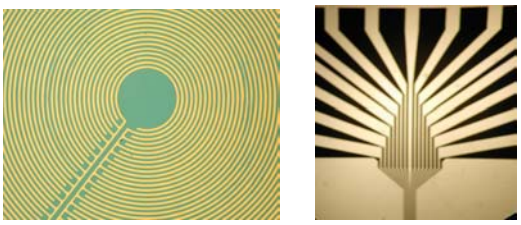
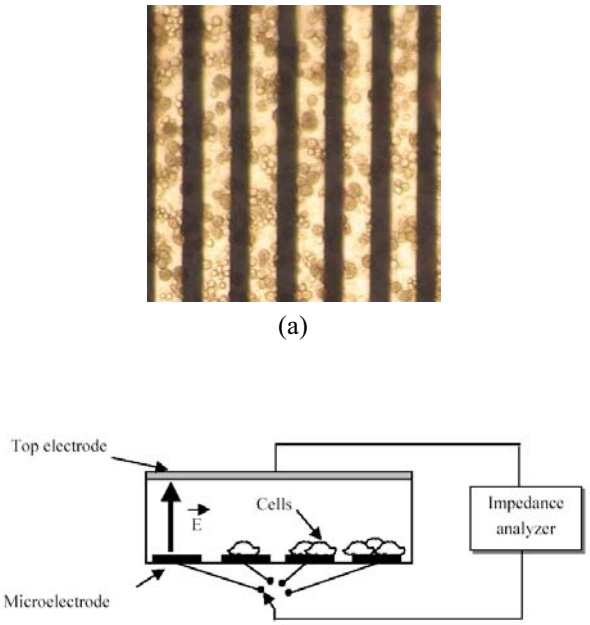


Figure 2. Typical microelectrode structures used.



(a)

(b)

Figure 3. (a) Perkinsus cells deposited on top of gold microelectrodes. (b) Scheme of a micro-electrical impedance device. Cells are injected into a small reservoir. The microelectrode array is placed at the bottom of the container. The AC impedance is measured between each individual electrode and the top large electrode.

RESULTS

Figure 4 shows the temporal evolution of the impedance spectra as the parasites cells start to aggregate and settle down on top of the microelectrodes. The measurements were carried out between one of the individual electrodes and the large top reference electrode made of a transparent conducting glass. A large change in the measured electrical impedance of the system is observed as the cells aggregate on top of the gold electrodes. A decrease in the system capacitance and a large shift to low frequencies in the main relaxation frequency is observed in a time scale of 30 minutes. This shift in the loss peak to lower frequencies is caused by the presence of the cells on top of the electrodes, which have the effect of reducing the ac conductivity of the overall system.

Using a large concentration of cells and measuring the impedance between the microelectrodes instead of measuring it in a vertical mode, a second relaxation peak appears at low frequencies as shown in Fig 5.

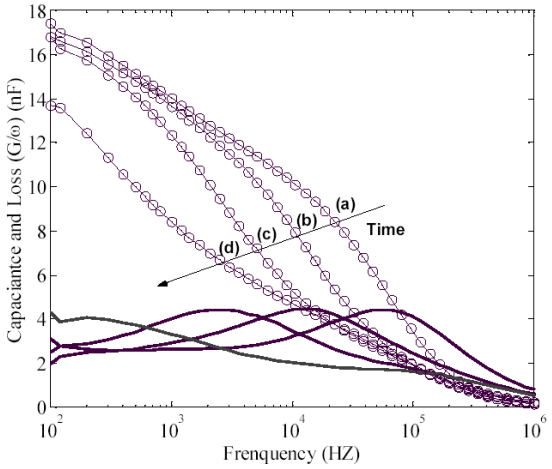


Figure 4. Time evolution of the impedance spectra as the parasites cells start to aggregate and settle down on top of the microelectrodes. (O) Capacitance and (–) Loss (Conductance/ω). (a) Soon after insertion, (b) t=18 minutes, (c) t=25 minutes, (c) t=32 minutes, and (d) t=50 minutes. Measurements were done using the configuration shown in Fig.3b.

Also the shift in frequency of the main relaxation process is relatively small and contrary to the results in Figure 4 moves slightly to the higher frequencies.

The behaviour of the system is being now modelled using an equivalent RC network that takes into account the cells aggregation and the medium. The measurements in vertical mode are quite straightforward to model using a general equivalent circuit network, which consist of the series association of two parallel RC circuits. However, the behaviour of the impedance between adjacent microelectrode is apparent more complex than expected, and it has yet not been possible to establish a clear link between circuit models and the physical system formed by the two types of cells.

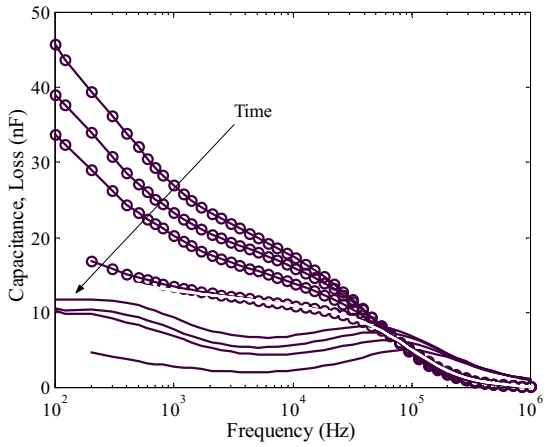


Figure 5. Time evolution of the impedance spectra as the parasites cells start to aggregate and settle down on top of the microelectrodes. (O) Capacitance and (–) Loss (Conductance/ω). Measurements done between two parallel microelectrodes, separated by 20 μm gap.

CONCLUSIONS

The use of microelectrode arrays and impedance techniques shows great promise for the measurement of the interaction between two types of cells. When measurements are done in a vertical system, the cell aggregation causes a large shift in the main relaxation frequency of the system as well as a decrease in the low frequency capacitance. However, it is not yet possible to establish a quantitative relation between the impedance changes and level of aggregation of the parasites cells. Electrodes with a size of a typical cluster and individual addressable are necessary to fabricate a quantitative sensor.

Measurements between adjacent microelectrodes revealed a more complex impedance spectra. These features are currently being modeled using equivalent circuits.

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