Comparison of microelectrode sensing configurations for impedimetric cell monitoring

Caviglia, Claudia; Heiskanen, Arto; Andresen, Thomas Lars; Emnéus, Jenny; Carminati, M.; Vergani, M.; Ferrari, G.; Sampietro, M.

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Comparison of Microelectrode Sensing Configurations for Impedimetric Cell Monitoring

C. Caviglia, A. Heiskanen, T. L. Andresen, J. Emnéus
Department of Micro- and Nanotechnology
Technical University of Denmark
Lyngby, Denmark
Claudia.Caviglia@nanotech.dtu.dk

M. Carminati, M. Vergani, G. Ferrari, M. Sampietro
Dipartimento di Elettronica e Informazione
Politecnico di Milano
Milano, Italy
carminati@elet.polimi.it

***Abstract*—A theoretical and experimental comparison between vertical and coplanar interdigitated sensing configurations for impedimetric cell growth tracking is presented. For the first time, these widely-adopted approaches are quantitatively compared on the same cell populations and on the same 10µm interdigitated microelectrodes using a versatile custom-made monitoring platform including a 24-channel miniaturized potentiostat. As expected, characterization of bare microelectrodes in buffer and tracking experiments with HeLa cells over 16 hours demonstrate that the coplanar configuration provides a higher sensitivity to cell adhesion and spreading (Cell Index = 1.6 vs. 0.4) albeit at a higher frequency of maximum sensitivity (100kHz vs. 24 kHz).**

***Index Terms*— impedance spectroscopy; interdigitated electrodes; cell tracking.**

I. Introduction

The use of impedance spectroscopy, consolidated in other electrochemical applications such as corrosion studies or characterization of batteries, is becoming a routine tool also in the biomedical field, in particular for monitoring the growth and response to chemical stimulation of cell populations cultivated on planar microelectrodes. In fact, since the pioneering work by Giaever and Keese [1], a huge volume of research has flourished and various custom-made and commercial systems are available for biological investigation [2]. The success of this technique, based on the increase of impedance due to the insulating barrier represented by the cells filling the volume at a height of 10-20nm above the electrodes, relies on its simplicity enabling label-free and automatic analysis. However, as highlighted by Orazem and Tribollet [3], as impedance is not specific by itself and is affected by any variation of the interface properties, the interpretation of impedance data is particularly delicate. Elaborate equivalent models, though providing good fitting, can lead to the loss of the physical meaning of their parameters.

In this work we present a theoretical and experimental comparison of two alternative sensing configurations, i) the standard "vertical" configuration (a single working electrode (WE) versus a large, distant counter electrode (CE), and ii) the interdigitated configuration (WEa comb versus WEb comb). The latter was introduced for cellular monitoring 15 years ago [4] and is now becoming commonly adopted due to its good performance [5] even in commercial systems, such as *exCELLigence* by Roche. The major novelty of our investigation is that for the first time a quantitative comparison is performed exactly on the same cells, alternating the two configurations when measuring impedance spectra on the same couples of electrodes, as enabled by an original and versatile monitoring platform we have recently designed [6].

II. Theory and Modeling

The theoretical spectral characteristics of the two alternative configurations, illustrated in Fig. 1 along with their simplified lumped-parameter equivalent impedance models, will be discussed. Design rules, issues on the instrumentation side and expressions to estimate the equivalent parameters will be illustrated. It will be highlighted how the coplanar configuration results in a higher corner frequency. This frequency roughly corresponds to the optimal operating frequency, where the spectral sensitivity to cell adhesion is maximum.

For small initial cell densities (i.e. small fractions of covered electrode area, far from confluence) it is expected that the coplanar detection is more sensitive to the presence of cells in the volume between the fingers, with respect to the vertical condition, in which the solution resistance is set by the radius of the microelectrode (i.e. by the electrode border in a radial diffusion regime).

III. Experimental Results

A. Electrodes Characterization

Prior to the experiments with cell populations, the interfacial impedance of the bare electrodes in contact with PBS buffer has been characterized. The Au microelectrodes were fabricated with a standard lift-off process and passivated by Si_3N_4 layer, which was reactive ion etched to expose the active electrode areas. Each comb comprises 12 fingers with length 500µm, width 10µm and spacing 10µm. As the vertical penetration of the electric field between interdigitated electrodes is roughly equal to the gap, this size has been chosen in order to probe cells in the 5µm-20µm size range. The measured spectra (Fig. 2) excellently match the expected values, confirming the correct estimates of the double layer capacitance (C_{DL}) and solution resistance (R_S) of WEa in both cases (i.e. vs. CE and WEb respectively). The corner frequency shifts from ~30kHz (vertical) to ~300kHz (coplanar).

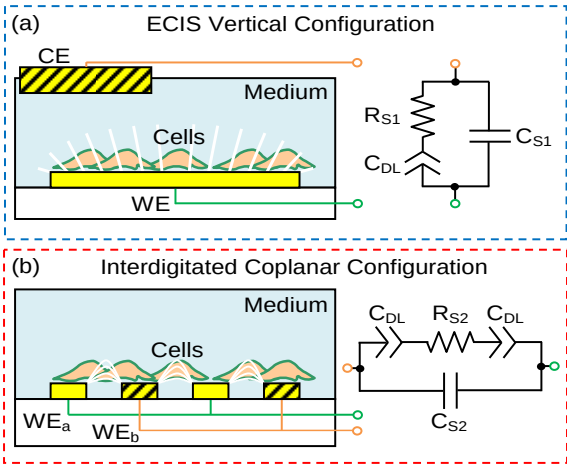

Figure 1. Compared vertical (a) and coplanar (b) sensing configurations.

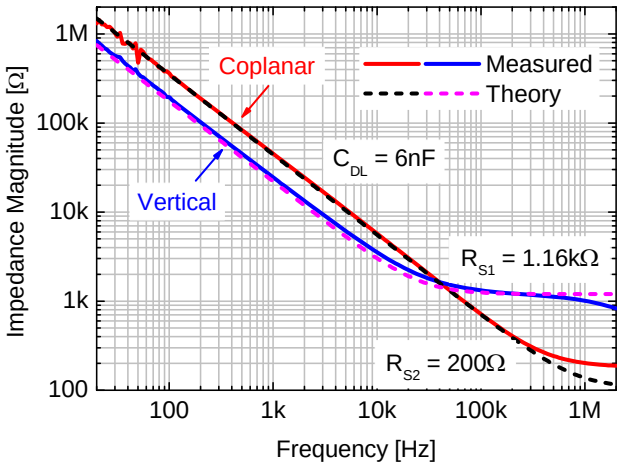

Figure 2. Compared WEa spectra measured in PBS (V_{AC} = 4mV).

B. Monitoring HeLa Cells

Electrochemical impedance spectroscopic tracking has been used as a non-invasive biophysical approach to continuously monitor adhesion and proliferation of HeLa cells. The experiments have been performed by initially seeding about $2.5 \cdot 10^5$ cells into a sterilized well containing poly-L-lysine-coated gold electrode arrays. Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin was used as culture medium. The chamber was incubated at 37° C in a humidified atmosphere having 5% CO2.

Impedance spectra were acquired from each sensor element for 16 hours: during the first 4 hours after cell seeding data were recorded every 20 minutes, then every hour. A 200µV sinusoidal perturbation potential was applied (to reduce the max. current below the 1µA safety limit) and 30 points were recorded in the frequency range between 100Hz and 100kHz with an averaging time of 2s. Data recorded from the different sensor elements were averaged and processed to derive, for each frequency and for each time point, the Cell Index (*CI*). It is defined as $CI(t,f) = (|Z(t,f)| / |Z(0,f)|) - 1$, where $|Z(0,f)|$ is the magnitude of impedance acquired when cells were not yet seeded into the well and $|Z(t,f)|$ is the magnitude of impedance acquired after cell seeding at different time points. Fig. 3A shows examples of *CI* values acquired using the ECIS vertical configuration. A peak frequency, indicating the most sensitive region of the spectra, can be determined at about 24kHz. Data recorded using the interdigitated coplanar configuration showed instead a peak frequency at 100kHz. Fig. 3B shows the *CI* tracking, over 16 hours, acquired using the two different configuration modes and plotted at the characteristic peak frequency. The maximum value of *CI*, about 1.6 ± 16% and 0.45 ± 17% for coplanar and vertical configurations, respectively, was reached in both cases 10 hours after cell seeding. This clearly demonstrates that the coplanar configuration provides the highest sensitivity for monitoring cell adhesion and proliferation.

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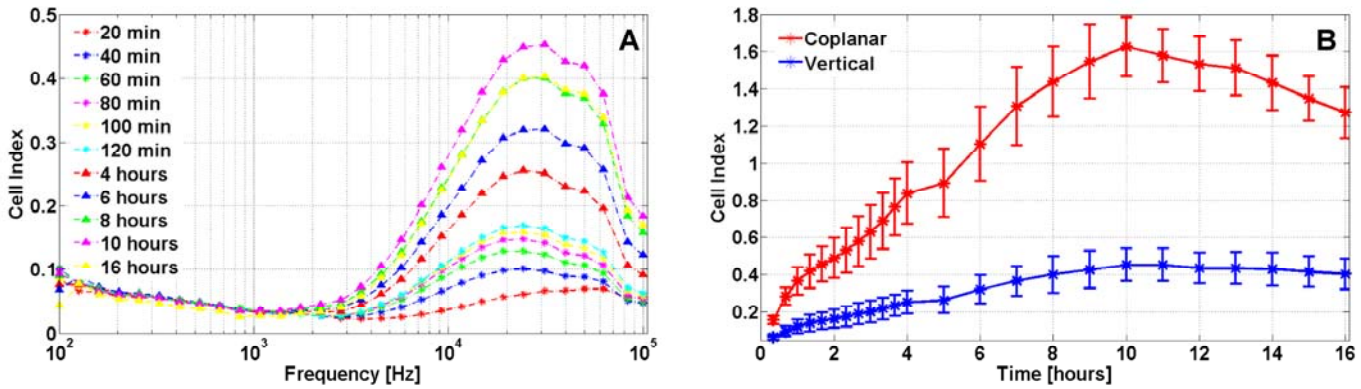

Figure 3. (a) Cell Index over frequency at different time instants after cells seeding; (b) Cell Index tracking over time at 24kHz and 100kHz respectively for vertical and coplanar configuration. V_{AC} is reduced to 200µV to limit the electromagnetic perturbation stress applied to the cell culture.