ELECTRICAL-IMPEDANCE-SPECTROSCOPY CHARACTERIZATION OF INDIVIDUALLY IMMOBILIZED SINGLE PARTICLES AND YEAST CELLS

Zhen Zhu*, Olivier Frey, Niels Haandbæk, Diana Ottoz, Fabian Rudolf and Andreas Hierlemann
ETH Zürich, Switzerland

ABSTRACT

This work presents a comparison of single particles and yeast cells in a microfluidic chip using electrical impedance spectroscopy (EIS). Single particles or cells are reliably immobilized at defined trapping sites and characterized by their electrical impedance spectra, which are measured via integrated electrodes. Parallel optical monitoring allows for direct correlation between the cell morphology and the recorded electrical impedance amplitude and phase signals over a frequency range from 10 kHz to 10 MHz. The results demonstrate that EIS is able to differentiate between immobilized individual particles and cells by measuring their resistance and membrane capacitance.

KEYWORDS: Microfluidics, Electrical Impedance Spectroscopy, Single-Cell Analysis, Cell Trapping

INTRODUCTION

Electrical impedance spectroscopy (EIS) is a non-invasive and label-free method to analyze single cells according to their dielectric properties as a function of frequency [1]. Typically, EIS provides information related to the cell size or volume at lower frequencies, from ~100 kHz to ~1 MHz, the cell membrane capacitance at higher frequencies, between 2 MHz and 5 MHz, and intracellular features, such as dielectric properties of the nucleus, at even higher frequencies, close to 10 MHz [2].

In order to perform single-cell analysis with EIS, researchers have integrated EIS into microfluidic devices, which enable high-throughput continuous-flow measurements of biological samples [3]. Other groups are focusing on the EIS of trapped single cells. Results show either a simple comparison of the signal amplitude before and after trapping of a single cell [4], or the variation of recorded signals during drug treatment of cells, seeded on a metal electrode [5].

Using the approach described previously [6], single cells can be reliably immobilized at defined sites in a microfluidic chip. This array-based cell trapping method is now extended with a local EIS system that enables the precise detection of immobilized particles and cells. Monodisperse spherical polystyrene particles and budding yeast cells (*S. cerevisiae*) have been characterized in the experiments. Performing multi-frequency EIS to determine both, the resistance of single particles/cells as well as the capacitance of the cell membrane are the first steps in the direction of multi-parameter measurements of biological cells, based on their dielectric properties.

EXPERIMENTAL

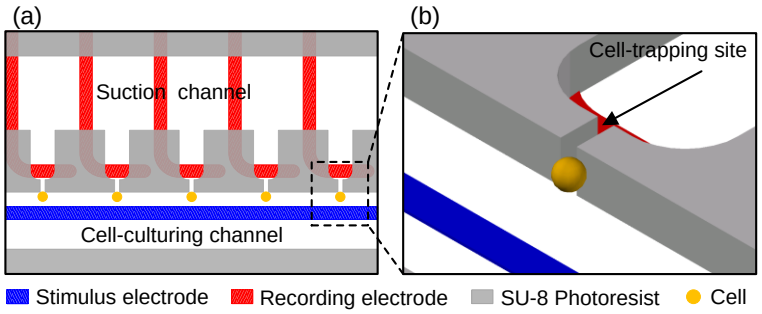

Figure 1: (a) Schematic top view of the microfluidic single-cell EIS chip without PDMS cover for better visibility and (b) 3D close-up view of a cell-trapping site with an immobilized cell.

Figure 1 shows the schematics of the microfluidic chip, which is fabricated by means of a simple hybrid glass-SU-8-PDMS process, similar to our previous work [6]. This microfluidic single-cell EIS chip consists of a cell-culturing channel, a suction channel, 10 cell-trapping sites that interconnect the cell-culturing and suction channels, a common electrode (stimulus electrode), and individual electrodes (recording electrodes), situated at the respective cell-trapping sites. In a typical experiment the suspension of particles or cells is introduced into the cell-culturing channel using a conventional syringe pump. Single particles or cells are reliably immobilized at the cell-trapping sites by applying a lower pressure to the suction channel via a precise pressure controller. Once a cell or particle is trapped, an AC signal is swept over a frequency range from 10 kHz to 10 MHz on the stimulus electrode, and the induced signal is measured by the recording electrode (HF2IS Impedance Spectroscope, Zurich Instruments, Switzerland). At the same time, a bright-field microscope photograph of the immobilized particle or cell is taken. Finally, the cell/particle is released by increasing the applied pressure. For reference, the same impedance measurement is carried out again on the empty cell-trapping site to measure the baseline, which has to be

consistent during an experimental run. This whole procedure of EIS is repeated for each immobilized particle or cell, and the results of electrical impedance measurements are correlated with optical images.

THEORY

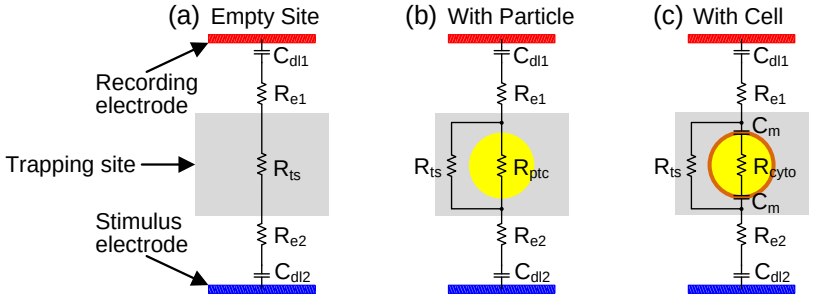

Figure 2: Schematics of equivalent circuit models of (a) an empty cell-trapping site, (b) a site with an immobilized particle and (c) a site with an immobilized cell.

In order to illustrate the expected differences among impedance measurements of the empty site, the site with an immobilized particle, and the site with an immobilized cell, simple equivalent-circuit models are shown in figure 2. For an empty site, the measured impedance includes the electrical double-layer capacitance (C_{dl1}, C_{dl2}) of both electrodes, the resistance of the bulk medium around the stimulus electrode (R_{e2}) and the recording electrode (R_{e1}), and the resistance of the bulk medium across the trapping site (R_{ts}). Thus the impedance across the stimulus electrode and recording electrode is:

$$Z_{empty} = R_{e1} + R_{e2} + \frac{1}{j\omega C_{dl1}} + \frac{1}{j\omega C_{dl2}} + R_{ts} \quad (1)$$

For the case of an immobilized monodisperse particle, an additional element, the resistance of the particle (R_{ptc}) is added in parallel to R_{ts} and is responsible for the measured impedance change, which can be expressed as:

$$Z_{particle} = R_{e1} + R_{e2} + \frac{1}{j\omega C_{dl1}} + \frac{1}{j\omega C_{dl2}} + R_{ts} /\!/ R_{ptc} \quad (2)$$

The dielectric properties of cells are more complex than those of particles and become decisive contributions to the total impedance. When a yeast cell is immobilized at the cell-trapping site, the cell membrane capacitance (C_m) is also detectable by EIS at higher frequencies besides the resistance of the cell cytoplasm (R_{cyto}). The measured impedance in this case is:

$$Z_{cell} = R_{e1} + R_{e2} + \frac{1}{j\omega C_{dl1}} + \frac{1}{j\omega C_{dl2}} + R_{ts} /\!/ \left(R_{cyto} + \frac{2}{j\omega C_m}\right) \quad (3)$$

According to the 3 equations above, a polystyrene particle adds a contribution to the resistive component of the impedance, which is expressed in the amplitude of the EIS signal. Yeast cells do not only have a resistive but also a capacitive contribution to the impedance, so they should yield variations in both, the amplitude and phase of the EIS signal.

RESULTS AND DISCUSSION

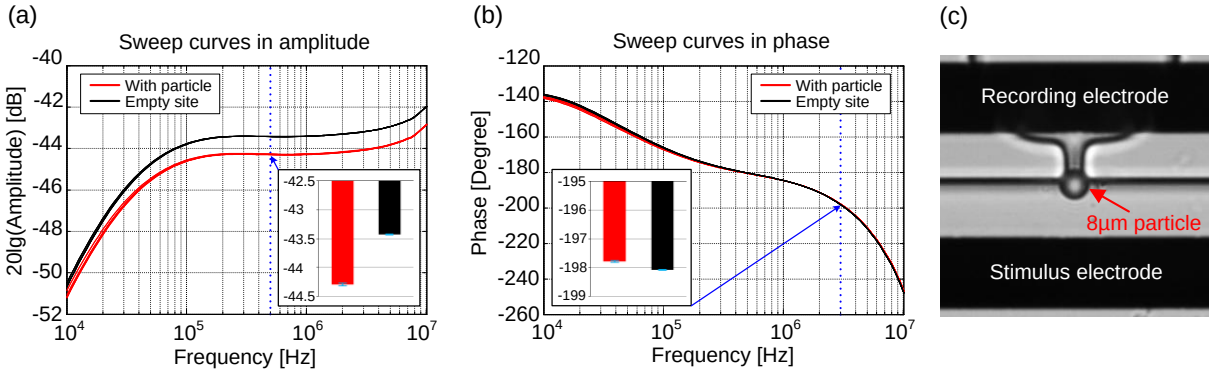

Figure 3: Multi-frequency EIS of 8-μm particles (n=10): (a) amplitude and (b) phase of recorded signals, and (c) a microscope photo of an immobilized particle. Inset histograms show the mean values of amplitude at 500 kHz and phase at 3 MHz with corresponding standard deviations.

8-μm particles are first used to characterize the EIS system, as illustrated in figure 3. After a single particle has been immobilized at the cell-trapping site, the amplitude curves in figure 3a show a significant difference over the frequency range from 100 kHz to 10 MHz, while the phase curves in figure 3b exhibit no visible difference over the whole frequency range. Because a monodisperse particle consists of uniform material without any membrane, the resistance of the particle is the only detectable parameter in the impedance measurement, which shows a good agreement with the experimental result. Moreover, the small standard deviations in the histograms prove the high resolution and sensitivity of this EIS system.

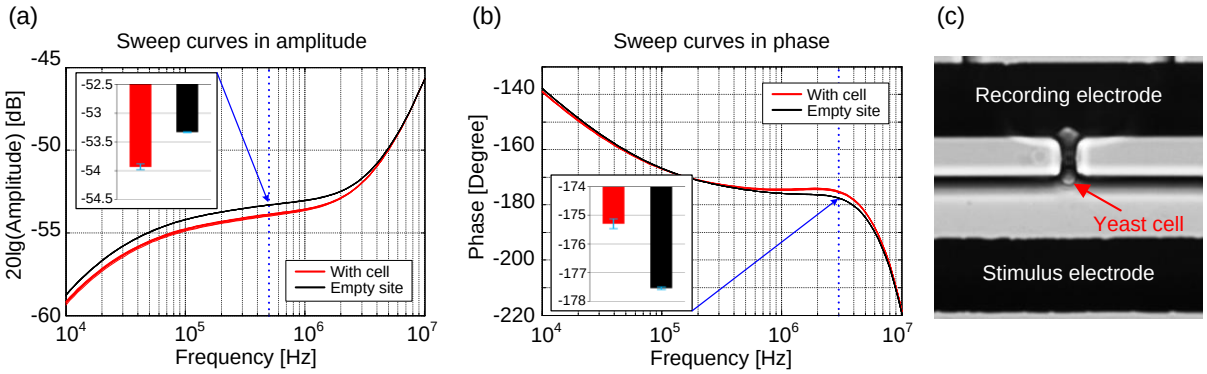

Figure 4: Multi-frequency EIS of yeast cells (n=7): (a) amplitude and (b) phase of recorded signals, and (c) a microscope photo of an immobilized cell. Inset histograms display the mean values of amplitude at 500 kHz and phase at 3 MHz with corresponding standard deviations.

Yeast cells, serving as biological model cells in this work, are loaded in cell suspension, and only cells without buds have been selectively trapped, monitored by microscope imaging (figure 4c), and measured by EIS (figure 4a, 4b). The amplitude curves show that the signal from a site with an immobilized yeast cell exhibits a significant difference from the empty site, especially between 100 kHz and 1 MHz, which is due to the detectable resistance of the cytoplasm at lower frequency. Furthermore, the phase curves display a visible difference as well, specifically around 3 MHz, which proves that the membrane capacitance is a detectable parameter at higher frequency. In contrast to measurements of particles, the slightly bigger standard deviations in the histograms here are due to the size variation of cells. However, these results confirm the high reliability of this EIS system for detection of single cells.

CONCLUSION

We presented a microfluidic chip that integrates the functions of immobilization and EIS of single particles and/or yeast cells. The experimental results demonstrate that this EIS system can be used to differentiate single particles and cells according to recorded phase signals. These are due to the detectable membrane capacitance of cells at higher frequency, which is consistent with theoretical considerations. The small standard deviations demonstrate the high sensitivity and resolution of this EIS system. Combined with fluorescent microscopy, this system constitutes a promising approach to single-cell analysis in systems biology.

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CONTACT

*Zhen Zhu 41-61-387-3296 or zhen.zhu@bsse.ethz.ch