USING MULTI-FREQUENCY ELECTRICAL IMPEDANCE SPECTROSCOPY TO MONITOR SINGLE BUDDING YEAST CELLS

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ABSTRACT

This work presents a microfluidic device that enables reliable immobilization and multi-frequency electrical impedance spectroscopy (EIS, 10 kHz – 10 MHz) of single budding-yeast cells, S. cerevisiae. Immobilized cells with different shapes in 4 different orientations can be discerned by using multi-frequency EIS data. The budding process can be continuously monitored in real-time through EIS and distinguished from potential movements of immobilized cells by extracting representative vectors from the multi-frequency EIS data.

KEYWORDS: Single-cell analysis, Electrical impedance spectroscopy, Cell trapping, Cell culturing

INTRODUCTION

Electrical impedance spectroscopy (EIS) enables the frequency-dependent multi-parametric readout of cellular information. By using microfabrication techniques, EIS can be integrated in microfluidic devices to detect single cells. Most of those devices, called electrical impedance cytometers, were used to characterize suspended biological samples in a flow-through setup [1, 2]. However, EIS has not yet been used for real-time monitoring of cellular dynamics, e.g., cell growth, at single-cell resolution.

The concept of single-cell immobilization and EIS has been demonstrated previously [3–5]. In this work, the device features capture and cultivation of single cells while performing real-time EIS of immobilized budding yeast cells. Different orientations and different shapes of immobilized cells could be discerned by using multi-frequency EIS data. Moreover, we have been able to continuously monitor the budding process, and to detect potential movements of immobilized single yeast cells during measurements by extracting representative vectors from the multi-frequency EIS data.

EXPERIMENTAL

The device, as schematically shown in Figure 1, included a glass substrate with patterned electrodes to perform localized impedance measurements, a SiNx insulation layer to reduce electric crosstalk between adjacent electrodes, and microchannels structured in SU-8 (30 µm high) and sealed with a PDMS cover for optical access. Application of a slightly lower pressure to the suction channel enabled single-cell capturing at the cell traps (4 µm wide) along the cell-culturing channel. EIS was performed by applying an AC signal to the common stimulus electrode and by recording the resulting signal on the individual recording electrodes at the traps. The orifices of the traps prevented cells from passing into the suction channel and constrained the electric current to flow through the narrow opening, thereby improving the sensitivity of EIS. After each measurement, cells were released, and EIS was performed for the empty trap, which was then used to calculate relative magnitude $A_r$ and relative phase $\theta_r$.

Figure 1: Schematic 3D view of the microfluidic device showing the cell traps and immobilized single cells. The PDMS cover is not shown in the schematic for better illustration.
RESULTS AND DISCUSSION

As a consequence of the specific configuration of cell traps and of the cell morphologies (with buds and without buds), single yeast cells were immobilized at the traps in 4 different orientations as shown in the micrographs in Figure 2a: unbudded cells (UB); horizontally-immobilized cells with the bud inside the trap (HBI); horizontally-immobilized cells with the bud outside the trap (HBO); and vertically-immobilized cells with the bud and mother cell stacked (VB). VB cells were directly discriminated from other orientations by using the relative magnitude at 1 MHz and the relative phase at 4 MHz (Figure 2b). By analyzing the multi-frequency EIS data with principal component analysis, HBO, HBI and UB cells were classified by means of linear discriminant analysis on the full multi-frequency EIS data set (Figure 2c).

![Image](image_url)

Figure 2: Discrimination of immobilization orientations of budding yeast cells by using multi-frequency EIS. (a) Micrographs of 4 orientations. Buds are marked with arrow heads. Scale bar is 5 μm. (b) Separation of VB cells. (c) Classification of UB, HBI and HBO cells (projections on first two principal components shown).

Among the 4 orientations, VB cells were chosen to perform the bud-growth monitoring through EIS, as EIS was most sensitive to growth in this orientation. Figure 3a shows the real-time EIS recording of the budding process of immobilized VB cells. The size increment of the bud, which is difficult to determine optically within short time periods (inserts in Figure 3a), could be clearly observed through EIS signals. Figure 3b shows the real-time EIS recording of cell motion of a VB cell. The impedance measurements also reflected changes in position or motion of single yeast cells in the trap.

![Image](image_url)

Figure 3: Real-time EIS recording of the budding process and cell motion of immobilized single yeast cells. (a) Budding process of 5 immobilized cells. Inserts show images of the monitored cell at the beginning and the end of Rec 2. (b) Cell motion of a cell with inserted images showing its mother cell moving towards the outside of the trap. Buds are marked with arrow heads. Scale bar is 5 μm.

By analyzing the multi-frequency EIS data of bud-growth and cell-motion recordings with principal component analysis, two principal vectors were extracted that represent bud growth in Figure 3a and cell motion in Figure 3b. The multi-frequency EIS data were then projected to the two representative vectors, as shown in Figure 4. Cell movement (trajectory along vertical axis) during the real-time recordings could then be qualitatively discerned from bud growth (trajectories horizontal axis). Therefore, this type of projection enables the discrimination of cell activities during the overall recording duration.
CONCLUSION

A microfluidic device that combines immobilization and localized multi-frequency electrical impedance measurements of single cells has been presented in this work. The experiments using budding yeast cells have validated the functionality and sensitivity of the EIS-integrated microfluidic device. The results demonstrate that single-cell multi-frequency EIS can be used to monitor cell growth, while also detecting potential cell motion in real-time and label-free, and that EIS constitutes a sensitive tool for single-cell analysis.

ACKNOWLEDGEMENTS

The yeast cells used in this study were kindly supplied by Diana Ottoz and Dr. Fabian Rudolf, ETH Zurich, D-BSSE, CSB Group. This work was financially supported through the Swiss SystemX.ch program within the RTD project “CINA” and the FP7 ERC Advanced Grant “NeuroCMOS”.

REFERENCES


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