OCEAN CARBON CYCLE STUDIED BY SINGLE-CELL IMPEDANCE CYTOMETRY ON CALCIFYING ALGAE

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ABSTRACT

Algae play an important role in the global carbon cycle. We explore the use of microfluidic single-cell impedance spectroscopy in the field of calcifying algae. The ratio of the impedance at high frequency vs. low frequency, known as opacity, allows us to discriminate between calcified coccolithophores and coccolithophores with a calcite exoskeleton dissolved by acidification (decalcified). In the experimental work we demonstrate that we can discriminate between calcified and decalcified coccolithophores with an accuracy of 94.1% using opacity.

KEYWORDS: Flow Cytometry, Electrical Impedance Spectroscopy, Single-cell Characterization, Algae, Calcification, Equivalent Circuit Model

INTRODUCTION

Since the industrial revolution 30% of the anthropogenic CO2 is absorbed by oceans [1], which results in ocean acidification being a threat to calcifying algae [2]. The species studied, coccolithophores Emiliania huxleyi, is considered to be globally the single most dominant calcifying algae [3]. Coccolithophores create a unique exoskeleton from inorganic calcium carbonate platelets. The PIC (particulate inorganic carbon):POC (particulate organic carbon) ratio describes the relative amount of inorganic carbon and is a critical parameter in the ocean carbon cycle.

In this research [4], the use of microfluidic single-cell impedance spectroscopy is explored in the field of calcifying algae. Microfluidic impedance spectroscopy enables characterization of single-cell electrical properties in a non-invasive and label-free way. The ratio of the impedance at high frequency vs. low frequency, known as opacity, is used to discriminate between calcified coccolithophores and coccolithophores with a calcite exoskeleton dissolved by acidification (decalcified). In other words, the high resistivity of calcium carbonate is used to assess changes in the PIC:POC ratio.

THEORY

The impedance of cells in suspension can be expressed by an equivalent circuit model (ECM), as developed by Foster and Schwan [5]. This model is extended to account for the electrical response of the inorganic exoskeleton of coccolithophores as shown in Figure 1a. The calcification factor α is introduced to account for the resistive behavior of calcite. At low frequencies the cell membrane is blocking the signal, giving information about the cell volume, whereas the cell interior is probed at high frequencies. As the model shows in Figure 1b, calcified cells have a higher opacity compared to cells without any calcite, because calcite still behaves resistive at 20 MHz. The model predicts that calcified cells have a higher opacity than decalcified cells.

EXPERIMENTAL

A glass-PDMS chip was used as described by de Wagenaar et al. [6] The two-layered chip consists of a PDMS chip with channels and a glass chip with platinum coplanar electrodes as displayed in Figure 1c. The constriction channel was designed to increase the volume fraction of the cell with respect to the medium between the electrodes and thereby increase the sensitivity.

Simultaneously, the impedance was measured at 0.5 and 20 MHz using a Zurich Instruments HF2LI lock-in amplifier. The absolute impedance difference $\Delta|Z|$ of a passing cell was found by post processing the recorded data in MATLAB (R2017b). The 'find peak' function was used after baseline removal with a 6th order polynomial fit.

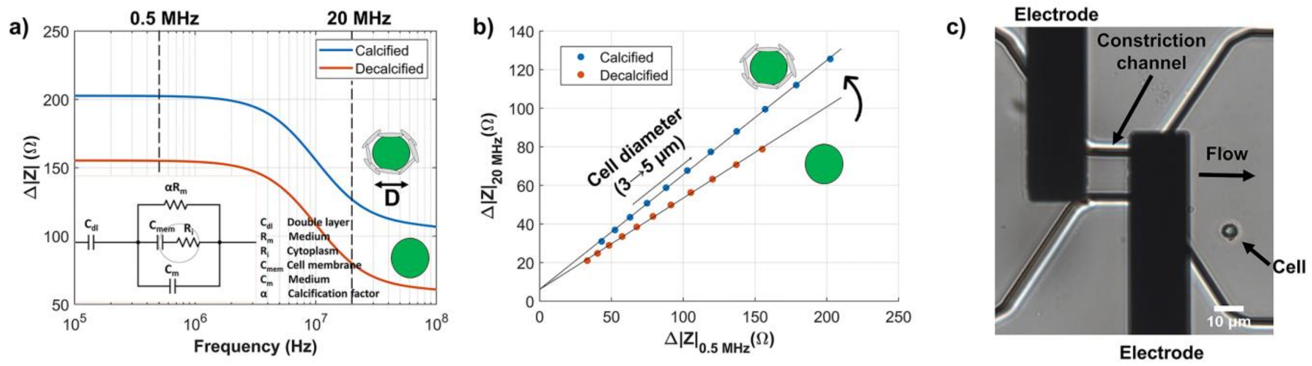

Figure 1: (a) Modelling results for a simple system with planar electrodes in a volume of 20x20x20 μm^3. Impedance spectra for a 5 μm cell with exoskeleton (calcified) and a bare cell (decalcified). The inset shows the ECM. (b) High versus low frequency response for calcified and decalcified cells ranging from 3 to 5 μm in diameter. (c) Image of microfluidic channel with coplanar electrodes with a constriction channel of 10x30x20 μm^3. The electrodes are 20 μm in width and separated by a 15 μm gap.

RESULTS AND DISCUSSION

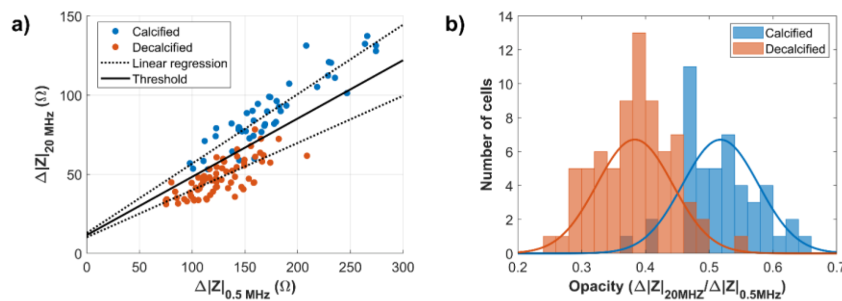

Figure 2: (a) Experimental result showing Δ|Z| at 0.5 and 20 MHz for calcified and decalcified cells. Linear regression of both populations was performed and the optimal threshold was determined, separating the two populations with an accuracy of 94.1%. (b) Opacity of calcified and decalcified cells with the normal distribution of each population.

The resulting impedance difference and opacity for calcified and decalcified cells is plotted in Figure 2a and b. As expected from the model, the calcified cells showed a significantly higher opacity compared to the decalcified cells (t-test; $P<0.05$; $N=119$). Linear regression analysis was used to improve the separation between calcified and decalcified cells to an accuracy of 94.1%.

CONCLUSION

A microfluidic impedance cytometer has been used to investigate calcification of the coccolithophore Emiliania huxleyi. The experimental work has shown that we can discriminate between calcified and decalcified coccolithophores with an accuracy of 94.1% using the impedance magnitude at high and low frequency.

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