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Development of microfluidic impedance cytometry enabling the quantification of specific membrane capacitance and cytoplasm conductivity from 100,000 single cells

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ABSTRACT

This paper presents a new microfluidic impedance cytometry with crossing constriction microchannels, enabling the characterization of cellular electrical markers (e.g., specific membrane capacitance (C_{sm}) and cytoplasm conductivity (σ_{cy})) in large cell populations (~ 100,000 cells) at a rate greater than 100 cells/s. Single cells were aspirated continuously through the major constriction channel with a proper sealing of the side constriction channel. An equivalent circuit model was developed and the measured impedance values were translated to C_{sm} and σ_{cy}. Neural network was used to classify different cell populations where classification success rates were calculated. To evaluate the developed technique, different tumour cell lines, and the effects of epithelial-mesenchymal transitions on tumour cells were examined. Significant differences in both C_{sm} and σ_{cy} were found for H1299 and HeLa cell lines with a classification success rate of 90.9% in combination of the two parameters. Meanwhile, tumour cells A549 showed significant decreases in both C_{sm} and σ_{cy} after epithelial-mesenchymal transitions with a classification success rate of 76.5%. As a high-throughput microfluidic impedance cytometry, this technique can add a new marker-free dimension to flow cytometry in single-cell analysis.

1. Introduction

As label-free bioelectrical markers, membrane capacitance and cytoplasm resistance have emerged as promising electrical indicators for cell type classification and cell status evaluation (Morgan et al., 2007; Valero et al., 2010; Xu et al., 2016). Variations in cellular electrical properties are closely related to physiological and pathological processes involving blood cells (Prieto et al., 2016; Su et al., 2013), tumour cells (Memmel et al., 2014; Taruvai Kalyana Kumar et al., 2016) and stem cells (Xavier et al., 2017; Zhou et al., 2016b).

Conventional techniques for characterizing single-cell electrical properties mainly include dielectrophoresis (DEP), electrorotation (ROT), and micro electrical impedance spectroscopy (μEIS) (Xu et al., 2016; Zheng et al., 2013). In DEP, the numbers of cells attached to dielectrophoretic electrodes as a function of frequencies are quantified and translated to cellular intrinsic electrical properties. However, DEP can only provide electrical properties at a population base rather than at the single-cell level (Henslee et al., 2016; Taruvai Kalyana Kumar et al., 2016; Zhou et al., 2016a). In ROT, a rotating electric field is applied to rotate single cells as a result of the Maxwell-Wanger polarization. But in ROT, the cell manipulation and positioning are very time-consuming, which makes this technique as a low-throughput approach (e.g., tens of (Lannin et al., 2016) or hundreds of (Memmel et al., 2014) tumour cells characterized by ROT). In μEIS, single cells are trapped by microfabricated electrodes, where a frequency-dependent signal is applied and the electrical responses of the cells are recorded. This approach again suffers from limited throughput (e.g., tens of tumour (Hong et al., 2012; Malleo et al., 2010), or stem cells (Zhou et al., 2016b) characterized by μEIS).

By combining μEIS with flow cytometry, a new technique,

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microfluidic impedance cytometry is developed, in which cells rapidly pass through microchannels and result in equivalent impedance variations. This technique provides a high-throughput characterization of single-cell electrical properties (Cheung et al., 2010). However, the majority of previously reported microfluidic impedance cytometry can only collect raw electrical signals rather than membrane capacitance and cytoplasm resistance due to the problem of current leakage and the lack of equivalent circuit models (Bürgel et al., 2015; Haandbaek et al., 2014; McGrath et al., 2017; Myers et al., 2013; Sabounchi et al., 2008; Spencer et al., 2016; Xavier et al., 2017). Recently, a modified microfluidic impedance cytometry was reported to quantify intrinsic cellular electrical parameters of specific membrane capacitance and cytoplasm conductivity, which, however, still suffered from limited throughput and can only report data from hundreds of single cells due to limitations in geometrical structures of the microfluidic chips (Chiu et al., 2017; Zhao et al., 2013a).

This paper presents a new microfluidic impedance cytometry, enabling the characterization of intrinsic cellular electrical markers (e.g., specific membrane capacitance (C_{sm}) and cytoplasm conductivity (σ_{cy})) in large cell populations (~ 100,000 cells) at a rate larger than 100 cells/s. The developed microfluidic system contains a crossing constriction channel as the flow channel with a cross-sectional area smaller than biological cells (see Fig. 1(a)). In operations, single cells are aspirated continuously through the major constriction channel, effectively sealing the side constriction channel and generating changes in impedance values, which are further translated to C_{sm} and σ_{cy}, based on an equivalent circuit model.

Due to the concerns of 1) cellular aggregations at the inlet of the constriction channel and 2) multiple cells travelling in the constriction channel simultaneously, previously reported designs of constriction channels were incompatible with high aspiration pressures and could usually report C_{sm} and σ_{cy} of hundreds of single cells in an experiment (Chiu et al., 2017; Zhao et al., 2013a). In this approach, a much higher pressure can be used to aspirate cells through the crossing constriction channel, leading to significant improvements in throughput. Furthermore, in this approach, image processing was no longer required, which significantly decreased the workloads of data processing. Owing these advantages, the new approach can collect C_{sm} and σ_{cy} from ~ 100,000 single cells within half an hour.

2. Materials and methods

2.1. Materials

Unless otherwise indicated, all cell-culture reagents were purchased from Life Technologies Corp. (USA). Materials required for device fabrication included SU-8 photoresist (MicroChem Corp., USA), AZ serial photoresist and developer (AZ Electronic Materials Corp., USA) and 184 silicone elastomer (Dow Corning Corp., USA).

More specifically, relevant materials used in cell cultures and treatments include RPMI-1640 Media (GIBICO, Life Technologies Corp., USA, Cat: 11875), DMEM Media (GIBICO, Life Technologies Corp., USA, Cat: 11995), Fetal Bovine Serum (GIBICO, Life Technologies Corp., USA, Cat: 10099), Penicillin-Streptomycin (GIBICO, Life Technologies Corp., USA, Cat: 15140), 0.25% Trypsin (GIBICO, Life Technologies Corp., USA, Cat: 15050), Phosphate Buffer Saline (GIBICO, Life Technologies Corp., USA, Cat: C10010500BT), and Transforming Growth Factor-Beta (GIBICO, Life Technologies Corp., USA, Cat: PHG9211).

2.2. Cell culture and treatment

All cell lines were purchased from China Infrastructure of Cell Line Resources and cultured in a cell incubator (3111, Thermo Scientific, USA) at 37 °C in 5% CO_2. More specifically, the lung cancer cell lines of H1299 and A549 were cultured with RPMI-1640 media supplemented with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin. The cervical cell line of HeLa was cultured with DMEM media supplemented with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin.

In experiments of inducing epithelial-mesenchymal transitions of A549 cells, after 24 h of cell seeding (~ 60% surface coverage), RPMI-1640 media supplemented with 0.25% Fetal Bovine Serum and 1%

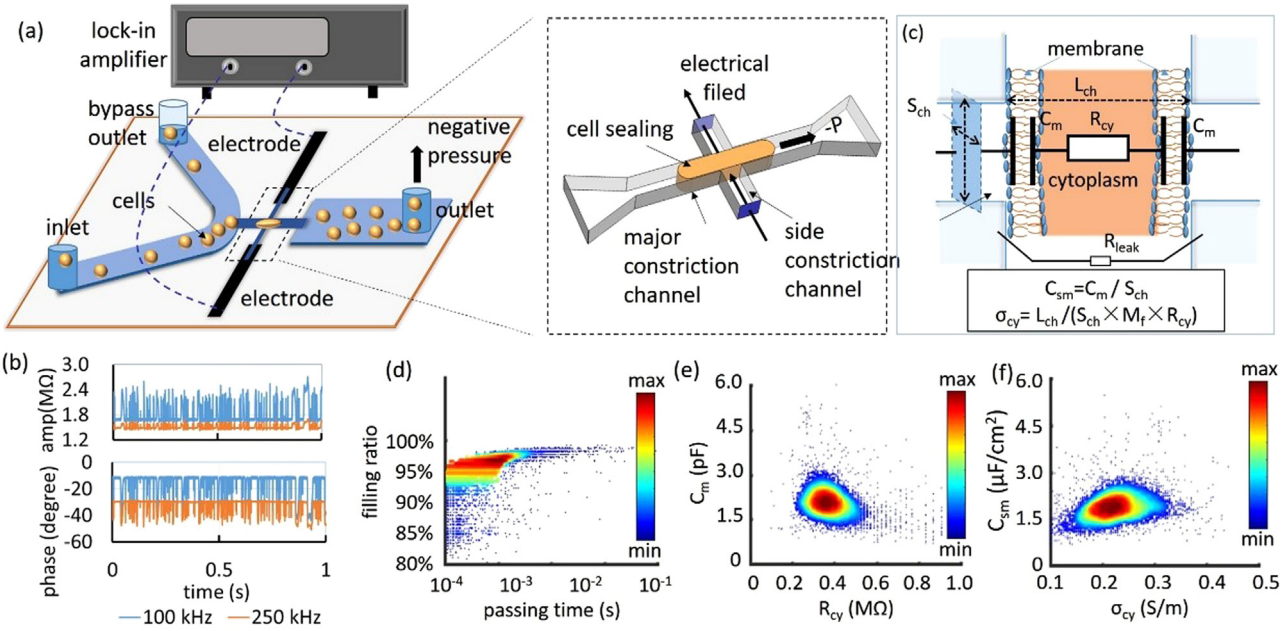

Fig. 1. High-throughput microfluidic impedance cytometry: schematic of the microfluidic system with a crossing constriction channel as the following channel, an impedance analyser, and a pneumatic controller (a). In operation, a negative pressure generated from the pneumatic controller aspirates single cells continually through the major channel of the crossing constriction channel, with impedance data sampled through the side channel (b). With an analytical equivalent circuit model (c), recorded raw impedance changes can be processed to passing time vs. filling ratio (d), membrane capacitance vs. cytoplasm resistance (e), which are further translated to intrinsic electrical markers of single cells, including specific membrane capacitance (C_{sm}) vs. cytoplasm conductivity (σ_{cy}) (f).