SINGLE CELL IMPEDANCE CYTOMETRY FOR RAPID AND LABEL-FREE MONOCYTE PHENOTYPING

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Monocytes represent a highly heterogeneous leukocyte population with the ability to differentiate into macrophages, a major cell type involved in the pathogenesis of atherosclerotic plaque in cardiovascular diseases [1,2]. Label-free analysis of their native cellular phenotypes and functions not only reduces assay cost and time, but is also essential for understanding disease progression and development of novel therapeutic strategies. Impedance cytometry is an emerging technology for high throughput cell phenotyping based on intrinsic electrical properties without the use of antibodies [3,4]. While parallel electrodes offer higher detection sensitivity as compared to co-planar electrodes [3,4], it is limited by laborious microfabrication. Herein, we present the development of an efficient microfluidics impedance cytometer using coplanar electrodes for rapid monocyte identification and phenotyping based on differentiation status.

The microfluidics impedance cytometer consists of a two-inlet, two-outlet polydimethylsiloxane (PDMS) microchannel (30 μm (width) × 20 μm (height)) bonded on patterned coplanar electrodes (20 μm in width with 20 μm separation gap). Sample and sheath fluid are injected into the device to hydrodynamically focus the cells at the channel center prior electrical detection. As the cell moves through the detection region, it disrupts the electric field generated by coplanar electrodes, thereby causing a change in electrical impedance. Using our setup (Fig. 1), the change in electrical impedance was quantified based on current change, and various cellular information were extracted at different frequencies of the excitation signal. We measured two impedance parameters namely the 1) opacity (ratio of impedance magnitude at 0.3MHz ($|Z_{LF}|$) to impedance magnitude at 1.7MHz ($|Z_{HF}|$)) which reflects cell membrane capacitance, and 2) $|Z_{LF}|$ which characterize cell size.

For identification of different blood cell types, human monocytes and lymphocytes purified using Dean Flow Fractionation (DFF) [5], and diluted red blood cells (RBCs) samples were separately injected into the microdevice. As shown in figure 2, different cell types were clearly differentiated based on $|Z_{LF}|$ due to distinct cell size differences (monocytes: 10 – 12 μm, lymphocytes: 7 – 8 μm, and red blood cells: 6 – 8 μm). Characterization of primary monocytes and leukemic THP-1 monocytic cell line also showed distinct differences in size and opacity (Fig. 3). Lastly, we compared the impedance profile of THP-1 and differentiated macrophages (PMA stimulated), and found that macrophages were more heterogeneous in size with significantly lower opacity than THP-1, demonstrating the feasibility of real-time assessment of monocyte differentiation using impedance-based sensing (Fig. 4).

In conclusion, the developed microdevice enables continuous label-free monocyte phenotyping using coplanar electrodes configuration with sufficient sensitivity. With the design simplicity and low cost fabrication, we envision our method will greatly facilitate immunology research and point-of-care monocyte profiling in patients with cardiovascular diseases.

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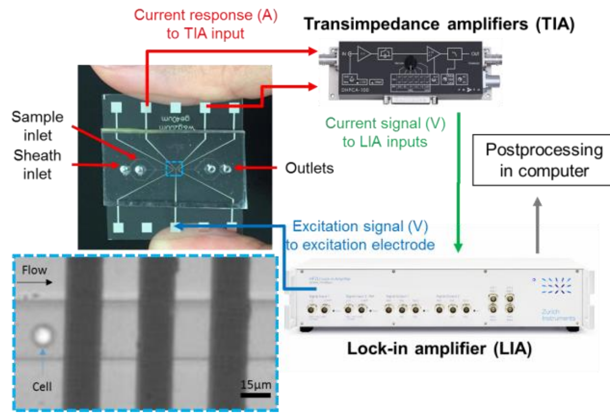

Fig. 1 Schematic of experimental setup: the microfluidic cytometer with microfabricated three electrodes. To establish the electrical measurement, the excitation signal is supplied by lock-in amplifier (LIA) and a change in current response corresponding to cell event can be recorded by transimpedance amplifiers (TIA) followed by signal extraction by LIA and postprocessing in computer. Optical image illustrating single cell flowing through the detection region (blue box).

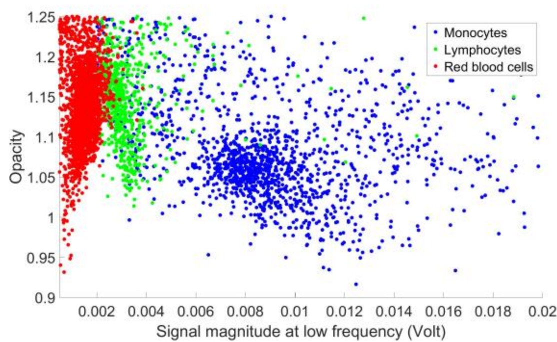

Fig. 2 Identification of different blood cell types: scatter plot of opacity versus impedance magnitude at 0.3MHz from monocytes (blue), lymphocytes (green) and red blood cells (red).

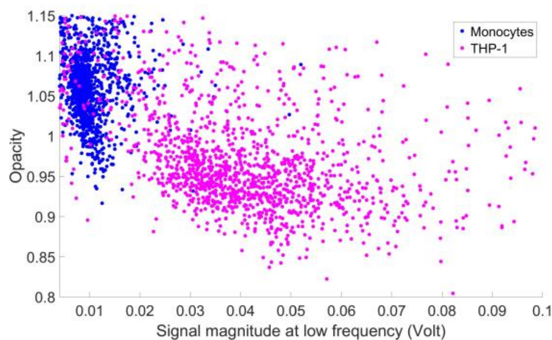

Fig. 3 Determination of primary monocytes and their leukemic counterpart (THP-1): scatter plot of opacity versus impedance magnitude at 0.3MHz from healthy monocytes (blue) and THP-1 (purple).

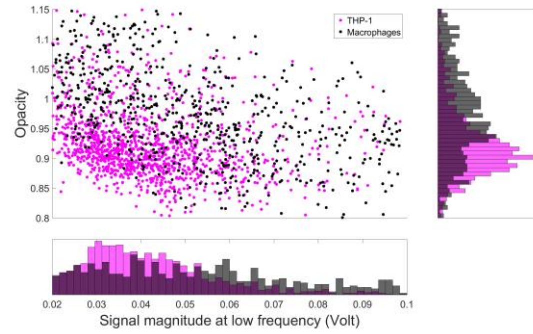

Fig. 4 Characterization of monocyte differentiation: scatter plot of opacity versus impedance magnitude at 0.3MHz from THP-1 (purple) and macrophages (black). The histograms of corresponding data were plotted on right side and below indicating distribution of opacity and distribution of signal magnitude at low frequency respectively.

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