LABEL-FREE IMPEDANCE MAPPING OF NEUTROPHIL DYNAMIC IMMUNE RESPONSES FOR RAPID MULTI-PARAMETRIC INFLAMMATORY PROFILING

Chayakorn Petchakup[1], Sheng Yuan Leong[1], Hui Min Tay[1], Rinkoo Dalan[2], King Ho Holden Li[1] and Han Wei Hou[1,3*]

[1]School of Mechanical and Aerospace Engineering, Nanyang Technological University, Singapore
[2]Endocrinology Department, Tan Tock Seng Hospital, Singapore
[3]Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

ABSTRACT

In this work, we report an integrated inertial-impedance cytometer for label-free neutrophil sorting and "impedance-based multifunctional phenotyping" from whole blood directly within minutes (<5 mins).

KEYWORDS: Single cell analysis, Functional phenotyping, Leukocyte sorting, Impedance cytometry

INTRODUCTION

Neutrophils are the most abundant and key effector cells of the innate immunity [1]. Conventional neutrophil studies require labeling for specific markers to assess inflammation status which are laborious and expensive. Impedance cytometry is a label-free cell characterization method but measurement of cellular native state cannot reflect the pathological phenotypes such as metabolic activities and responses to inflammatory stimulus which are more clinically-relevant. Herein, we develop a novel integrated platform for label-free neutrophil sorting and impedance phenotyping. Two key features include the 1) measurement of leukocyte impedance from whole blood, and 2) single-stream 3D cell focusing to enhance detection. As proof-of-concept for diabetes testing, we demonstrated for the first time, time-dependent functional phenotyping of healthy and inflamed neutrophils (tumor necrosis factor-alpha (TNFα)) treated with calcium ionophore (CaI), Phorbol myristate acetate (PMA) and N-Formylmethionine-leucyl-phenylalanine (fMLP) and electrically mapped out their dynamic responses.

EXPERIMENTAL

The polydimethylsiloxane (PDMS)-based device (Figure 1) consists of a Dean Flow Fractionation neutrophil sorter, inertial particle focuser in serpentine channels, and an impedance detector using coplanar electrodes (30µm×20µm (W×H)). The detector provides 1) opacity (ratio of impedance response at 1.7MHz ($|Z_{HF}|$) to impedance response at 0.3MHz ($|Z_{LF}|$)) reflecting cell membrane properties, and 2) $|Z_{LF}|$ which reflects cell size [3]. For enhanced impedance detection (Figure 1B), we use a stepped sample inlet to hydrodynamically confine the cells to the lower half of the channel, resulting in single-stream particle focusing closer to the electrodes.

RESULTS AND DISCUSSION

Flow rate study was performed and the optimal flow rate ratio was 1:20 (sample: sheath) to achieve single particle position (Figure 2A, B). For separation efficacy, we found that 100× blood dilution was optimal with high neutrophil recovery and purity (>80%) (Figure 2C). Next, we characterized neutrophil impedance characteristics in untreated (control) and "inflamed" blood treated with TNFα for 4 h, and observed that inflamed neutrophils had lower opacity and larger size in their native state (Figure 3A). Under the influence of inflammatory stimulus, both

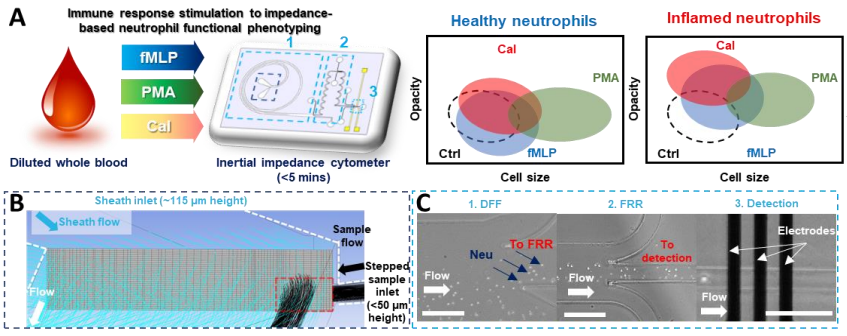

*Figure 1: Impedance mapping of neutrophil immune response using inertial impedance cytometer. (**A**) Experimental workflow (**B**) Simulation showing 3D focusing at the inlet region. Red box indicates confined sample stream. (**C**) High speed images showing neutrophil sorting and focusing at different stages of the platform. Scale bar = 100 µm.*

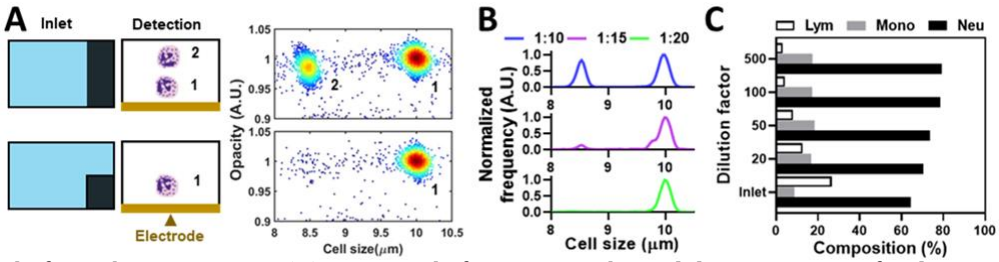

*Figure 2: Platform characterization (**A**) 3D particle focusing at inlet and detection region for devices with and without stepped inlet. Black and blue indicate sample stream and sheath stream respectively. (left) Impedance profiling of 10 μm beads without and with stepped inlet (right). (**B**) Quantification of particle equilibrium positions based on cell size at different flow rate ratios. (below) Relation between blood dilution and (**C**) sorted leukocyte compositions*

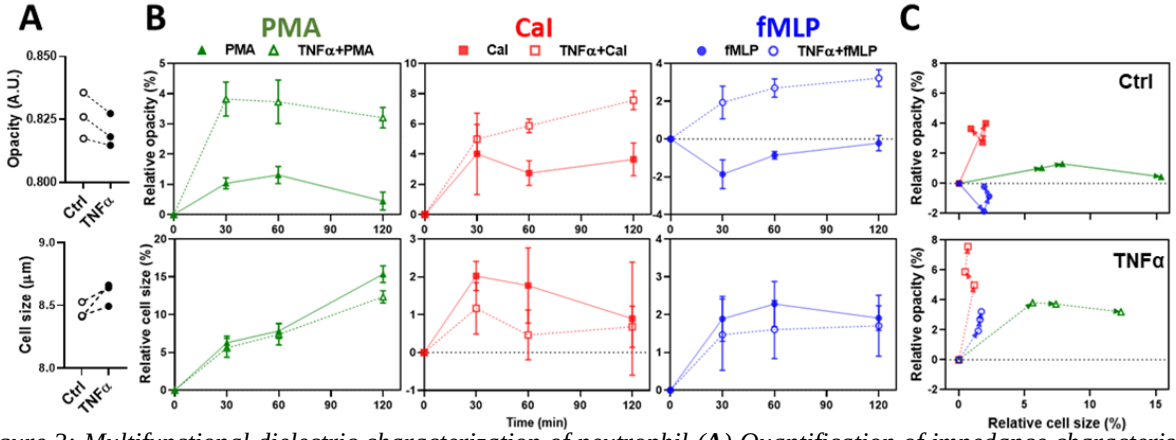

*Figure 3: Multifunctional dielectric characterization of neutrophil (**A**) Quantification of impedance characteristics of neutrophils from untreated blood (control, ctrl) and from inflamed blood with TNFα for 4hr (**B**) Average relative impedance characteristics (opacity and cell size) of neutrophils treated with different stimulus with respect to their unstimulated neutrophils (0 min) for control and TNFα treated conditions. (**C**) Functional dielectric mapping of control (top) and TNFα treated neutrophils (bottom). Arrows indicate time progression.*

NETosis stimulus induced significant changes in cell size and opacity at different timepoints, with larger size and opacity changes observed for PMA and CaI, respectively (Figure 3B). On the contrary, fMLP-treated neutrophils had a decrease in opacity and increase in size. By comparing the dielectric mapping of inflammatory-induced neutrophil responses between untreated and inflamed neutrophils (Figure 3C), it is clear that inflamed neutrophils exhibited distinct differences in dynamic responses which warrant further investigation.

CONCLUSION

Overall, these results highlighted the importance of dynamic dielectric responses in neutrophils which can lead to a paradigm shift in developing next-generation impedance cytometry. Current studies include testing in diabetes and elucidating mechanistic insights between neutrophil dysfunction and dielectric properties. We envision the platform can be readily translated into a point-of-care testing for metabolic and inflammatory diseases.

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CONTACT

* H.W. Hou; phone: +6567904950; hwhou@ntu.edu.sg