MICROFLUIDIC IMMUNOPHENOTYPING ASSAY PLATFORM FOR IMMUNOMONITORING OF SUBPOPULATIONS OF IMMUNE CELLS

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ABSTRACT

Functional immunophenotyping of immune cells is a promising method for diagnosis of immune dysfunctions. In this study, an integrated microfluidic immunophenotyping device was developed that could achieve rapid and efficient isolation, enrichment, stimulation and functional immunophenotyping of subpopulations of immune cells directly from blood specimens. This new microfluidic immunophenotyping technology can provide an unprecedented level of information depth on the distribution of immune cell functionalities. Such an innovative tool will allow comprehensive and systems-level immunomonitoring to define and characterize the "immune status" of healthy individuals and patients, critical for clinical interventions and managements of patients with immune system disorders.

KEYWORDS: Immunophenotyping, Immune Cells, Cytokines, Microfluidics

INTRODUCTION

Immune cells are important blood constituents that play a major role in immune responses against pathogenic infections, allergic conditions, and malignancies [1, 2]. The conventional clinical methods to determine functional status of immune cells are based on whole blood stimulation assays to measure cytokine secretion from peripheral blood mononuclear cells (PBMCs). However, measurements of the overall capacity of the whole population of immune cells to produce cytokines may not be informative enough to accurately reveal the immune status of patients, as in these 'bulk' assays it is difficult to pinpoint the phenotype or real identity of the reactive cells involved. Therefore, there is a significant need for new immunomonitoring platform for rapid, accurate and functional cellular immunophenotyping of subpopulations of immune cells from patients' blood, hence provide a promising new approach for rapid and accurate diagnose of immune status for infectious and inflammatory diseases across a broad patient spectrum.

RESULTS AND DISCUSSION

Here, we demonstrated an integrated microfluidic immunophenotyping assay (MIPA) platform that can perform efficient isolation, enrichment, and enumeration of peripheral PBMCs as well as subpopulations of immune cells from human blood, and simultaneously perform quantitative measurements of multiple inflammatory cytokines secreted from these isolated immune cells using a no-wash, homogeneous chemiluminescence ("AlphaLISA") assay (Fig. 1). We generated a novel PDMS microfiltration membrane (PMM) of a large surface area and high porosity (>30%) for microfluidic isolation and immunophenotyping of subpopulations of immune cells using our newly developed surface micromachining technique for soft materials (Fig. 1&2A-D) [3]. Integrated in a microfluidics-absed MIPA device (Fig. 2A), the PMM served as a highly efficient microfiltration material for bioparticle separation during a sequence of processes entailing cell isolation, enrichment, enumeration, stimulation, incubation, and cytokine secretion detection on a single chip.

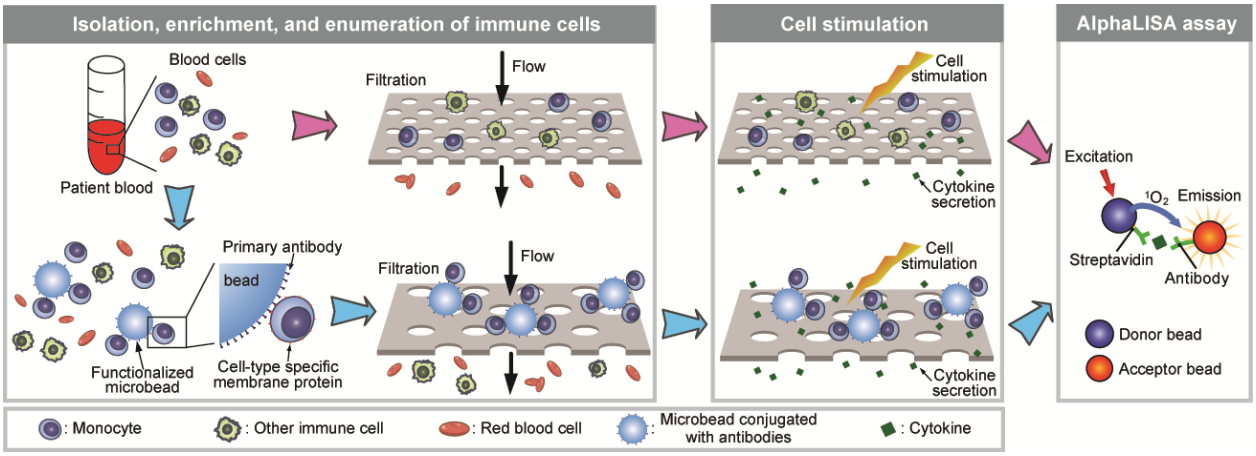

Figure 1: *Schematic of isolation and immunophenotyping of PBMCs (top) and subpopulations of immune cells (bottom) from blood specimens.*

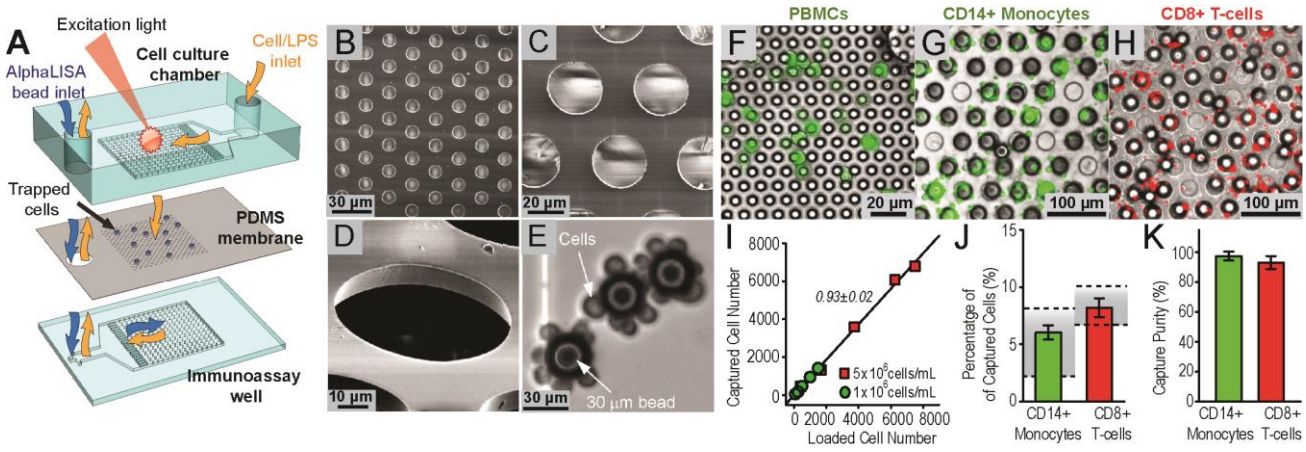

Figure 2: *Isolation of PBMCs, CD14+ monocytes and CD8+ T-cells from human blood. (A) Schematic of the MIPA device. (B-D) SEM images showing free-standing PMMs with the hole diameter of either 6 µm (B), 30 µm (C), or 60 µm (D). (E) Representative brightfield image showing CD14+ monocytes captured on microbeads. (F-H) Representative merged brightfield and fluorescence images showing flurescence-tagged leukocytes (F), mcieobead-labled CD14+ monocytes (G) and CD8+ T-cells (H) isolated from lysed blood on the PMM. (I) Number of leukocytes captured on the PMM using undiluted (5 × 10^6 mL^{-1}) as well as diluted (1 × 10^6 mL^{-1}) lysed blood samples as indicated. (J) Percentages and (K) purities of CD14+ monocytes and CD8+ T-cells captured using functionalized microbeads on the PMM from lysed blood. The shaded areas in J indicate the normal range of percentage of CD14+ monocytes and CD8+ T-cells in blood from healthy adults.*

For isolation and purification of subpopulations of immune cells from blood specimens, we employed a combined use of a PDMS microfiltration membrane (PMM) in the MIPA device and antibody-conjugated polystyrene microbeads. Using the biotin-streptavidin conjugation chemistry, polystyrene microbeads can be coated with antibodies against cell surface antigens that are specific to the desired subpopulation of immune cells (such as monocytes, T-cells). When mixed with a blood specimen for 30-60 min, the microbeads could selectively capture desired subpopulation of immune cells by recognizing and binding covalently to the specific surface proteins of the cells (Fig. 2E). Owing to their significant physical size increase after captured by microbeads, when the blood sample is passing through the PMM, the desired subpopulation of immune cells could be easily isolated and retained on the PMM, while the other blood cells including undesired immune cells and erythrocytes (red blood cells) would be eluded through the PMM (Fig. 2G-H).

We have demonstrated isolation and enrichment of monocytes and T-cells from lysed and unprocessed human blood specimen using microbeads (diameter of 32 µm) conjugated with antibodies directed against CD14 or CD8, unique surface protein markers of monocytes and T-cells. This new microfluidic platform permitted high capture yield (> 70%) and excellent capture purity (> 97%) for PBMCs, CD14+ monocytes and CD8+ T-cells from both spiked samples and blood samples. Using our MIPA device, about 6.0% and 8.2% of leukocytes were captured from lysed whole blood samples as CD14+ monocytes and CD8+ T-cells, respectively (Fig. 2J). Our results here were consistent with the fact that the normal range of percentage of CD14+ monocytes in blood from healthy adults is from 2-11% [4]. Owing to its large surface area and high porosity, the PMM permitted a heightened volume throughput (up to 20 mL min^{-1}) for processing blood specimens without causing clogging of the PMM. Thus, the cell isolation process can be achieved extremely fast (within several seconds) and it allows a sample loading directly using a pipette, which is very convenient for applications in clinical site.

Our MIPA device further allowed *in situ* quantitative immunosensing of cell-secreted cytokines by detecting optical signals resulted from the AlphaLISA assay (Fig. 3) [5]. Our MIPA device using the AlphaLISA assay provides an efficient way for quantitative detection of cytokine production, without involving complex reagent loading and washing steps as in the conventional ELISA. Our MIPA device required much less assay time (< 4 h, about 7 times faster) and sample volume (< 5 µL; 20-fold less) than that required for conventional ELISA [6]. The sensitivity of our MIPA device (75 pg/mL) is very compatible with that of ELISA (10 pg/mL) method [7]. Using our MIPA platform, we systematically quantified the concentration levels of TNF-α, IFN-γ, and IL-6 secreted by THP-1 monocytes as a function of the cell number (n = 1,000, 5,000, and 20,000 cells) and the lipopolysaccharide (LPS) concentration (10, 50, 100 ng/mL) (Fig. 3A). Our result demonstrated that as expected, secreted cytokines would increase with both the cell number and the LPS concentration. Further, as a proof of concept, we performed measurements for different cytokines from PBMCs isolated from immunoparalyzed pediatric patients following cardiac surgery with cardiopulmonary bypass (CPB) and compared the results with healthy donor samples (Fig. 3B). Our results demonstrated an acquired immunosuppressed state - also termed immunoparalysis [8], in these patients following CPB, suggesting these immunoparalyzed patients at a heightened risk for developments of post-operative infections [8] and may require an accurate immunomonitoring and immediate immunomodulatory therapy.

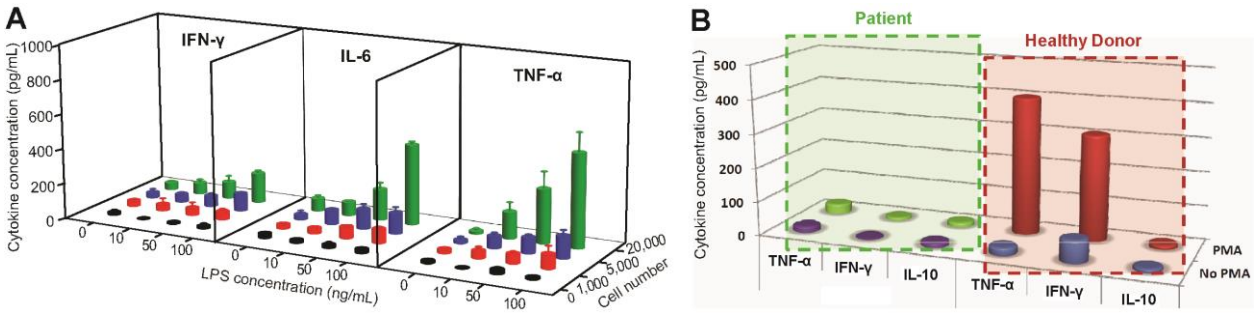

Figure 3: *Multiparametric detection of cytokines secreted from monocytes and PBMCs from healthy donor and patient samples using the MIPA device. (A) Plot of concentrations of TNF-α, IFN-γ, and IL-6 secreted by LPS-stimulated monocytes as a function of cell number and LPS concentration. (c) Plot of cytokine concentration secreted by PBMCs (n = 20,000) from healthy donor and patient samples with or without PMA stimulation.*

CONCLUSION

Together, our MIPA platform permits efficient isolation and accurate functional immunophenotyping of subpopulations of immune cells within one device, providing an unprecedented level of information depth on the distribution of immune cell functionalities. Our microfluidics-based innovative technology can potentially serve as a comprehensive and standardized immune monitoring platform to define and characterize the "immunotype" of healthy individuals and patients with inflammatory diseases before, during, and after targeted immunomodulation, thus leading to interventions that can significantly reduce or eliminate symptoms, side effects, disease progression, recurrence, and ultimately inflammatory diseases associated mortality.

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