LABEL-FREE, SINGLE-CELL OPTICAL MULTI-PARAMETER MONITORING OF APOLIPOPROTEIN E-NULL DIFFERENTIATED MACROPHAGES ON A CENTRIFUGAL MICROFLUIDI PLATFORM

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ABSTRACT

We report a novel, integrated opto-microfluidic technique for real-time measurements of the individual response of cells in a population to various stimuli. We use this method to study macrophages which are key cellular players in atherosclerosis. Naïve non-activated macrophages (M0) can differentiate into M1 or M2 phenotypic subtypes. In atherosclerosis, M2-macrophages activity is anti-inflammatory while M1-macrophages activity is pro-atherogenic. These cell-type specific changes are measured via their unique Photonic Fingerprints ("PINs") comprising of scattering, absorbance and auto-fluorescence signals, obtained in a label-free fashion.

KEYWORDS: atherosclerosis, apolipoprotein e-null differentiated macrophages, centrifugal microfluidics, label-free detection, living photonics.

INTRODUCTION

Atherosclerosis is an important risk factor for cardiovascular disease and its associated high mortality in developed countries [1]. The recruitment of immune cells to lipid depositions in artery walls is what triggers the inflammatory response responsible of atherosclerosis development. Among the immune cells, macrophages are critical players in atherosclerosis initiation and progression. Isolation, culture, and functional characterization of peritoneal & bone marrow-derived macrophages from mice are exceptionally powerful techniques used to examine aspects of macrophage biology in vitro [2].

We integrate centrifugal microfluidic and label-free optical technologies with highly efficient cell-to-light coupling to measure the real-time response of individual macrophages within a population. We demonstrate that the so-obtained "PIN" (Table 1) acts as a key reporter for the differential state of the macrophage and may thus inform on the early stage development of atherosclerosis [3].

Table 1. Single cell PIN parameters.

Optical Parameter	Single Cell Parameter
Light backscattered (polarized)	Size, shape and RI of nucleus Size, shape, RI and number of mitochondria
Light scattered at low angle (< 5º) (non-polarized)	Size, morphology, refractive index, viability
Light scattering at large angle (> 130º) (non-polarized)	Membrane roughness, refractive index
Absorbance bands	Specific indicators
Auto-fluorescence bands	Specific proteins

OPERATIONAL PRINCIPLE

To perform analysis at single-cell level, our centrifugal platform features scale-matched geometrical V-cup obstacles geared for capture of individual cells (Fig. 1) in stopped-flow mode at high capture efficiency. Once arrayed in the V-cups, the PINs of individual cells are acquired by optical modules mounted on the centrifugal test stand [4].

EXPERIMENTAL

The base part of the microfluidic chip exhibiting the inlets and V-cup array has been fabricated in PDMS (Sylgard 184, Dow Corning GmbH, Germany). Moulds for PDMS panel featuring the V-cup array and the reservoirs are surface micro-machined using SU8-3025 (Microchem, USA). For replication, PDMS was mixed at a ratio of 10:1 (base to curing agent by weight), poured on the mould and degassed under a vacuum for 1 hour. The PDMS was then cured in an oven at 70 ^{o}C for 3 hours and then removed from the mould using a standard scalpel. 1-mm diameter access holes were then punched at the inlet ports. The PDMS is then cleaned using nitrogen. The middle Poly(methyl methacrylate) (PMMA) layer (chip support holder) is structured by a laser cutter (Epilog Zing Laser, Epilog, USA). A pressure-sensitive adhesive (PSA) attaches the middle layer to the base plate of the chip; it consists of a standard borosilicate microscope slide. The so-obtained chip is then treated by air plasma (1000 mTorr for 5 minutes) and bonded together with the PDMS base to form the biochip. The selected materials and fabrication methods for the biochip all satisfy the key criteria of transparency, biocompatibility, absence of leaks and long term stability.

RESULTS AND DISCUSSION

We have used bone marrow-derived macrophages from apolipoprotein E-null (apoE-KO) mice, a common model for human atherosclerosis. Differentiation of M0 towards M1-macrophages was achieved by 24 hours of culture in the presence of the inflammatory stimuli LPS (10 ng ml^{-1}) and IFN-γ (20 ng ml^{-1}) [4]. M2-macrophages were obtained by culturing M0 for 24 hours in the presence of the interleukin IL-4 (20 ng ml^{-1}) [2]. M0 macrophages were retained on the V-cup array for analysis at the single-cell level. Cell morphology (Fig. 2a), scattering and absorbance bands (Fig. 3) as well as auto-fluorescence bands (Fig. 4) were recorded. Uniform cell morphology was observed and a baseline signal for the scattering and absorbance bands was measured. A primary peak appeared in the auto-fluorescent bands at 565 nm, with a slight signal increase also observed at 670 nm.

In comparison to the undifferentiated M0 type, M1 and M2 macrophages showed significant changes in cell morphology, displaying enhanced roughness of the cell membrane (Figs. 2b and 2c for M1 and M2, respectively). A notable increase in the scattering and absorbance bands was also observed (Fig. 3). Well-defined primary and secondary peaks were found in the auto-fluorescent bands at 530 nm and 670 nm, and at 565 nm and 630 nm for M1 and M2 phenotypes (Fig. 4).

CONCLUSION

Overall, the reported, label-free opto-microfluidic system provides a specific, label-free PIN for individual M1-proatherogenic and M2-antiatherogenic macrophages. The system thus bears great promise for early-stage detection of atherosclerosis on a compact and cost-efficient point-of-care platform.

ACKNOWLEDGEMENTS

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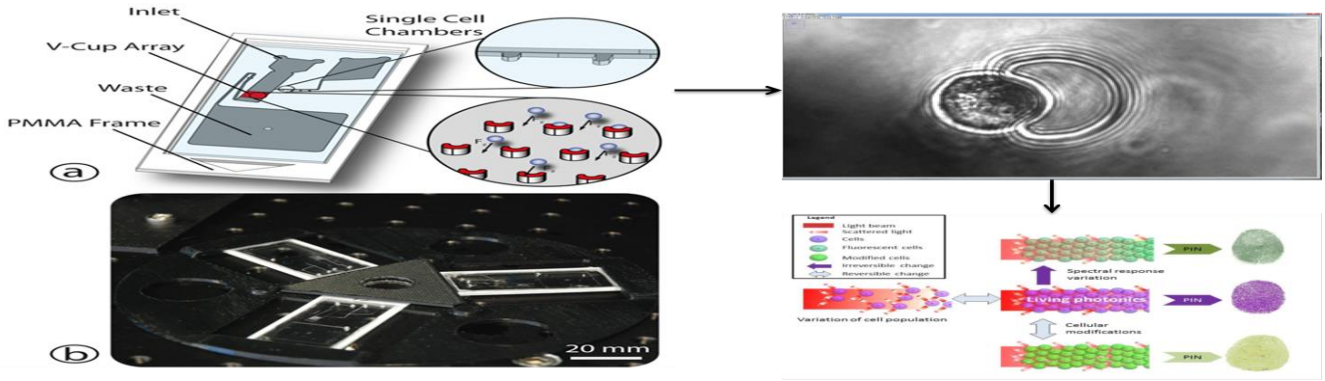

Figure 1. Single cell analysis by centrifugal cell capture with integrated PIN detection on an array of microscale V-cups.

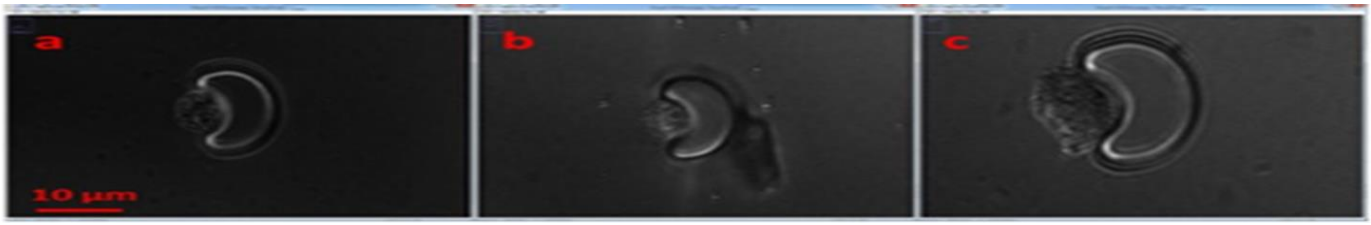

Figure 2. Macrophage morphology for bone marrow-derived macrophages with different phenotypes: a) M0, b) M1 (classical), and c) M2 (alternative) activation.

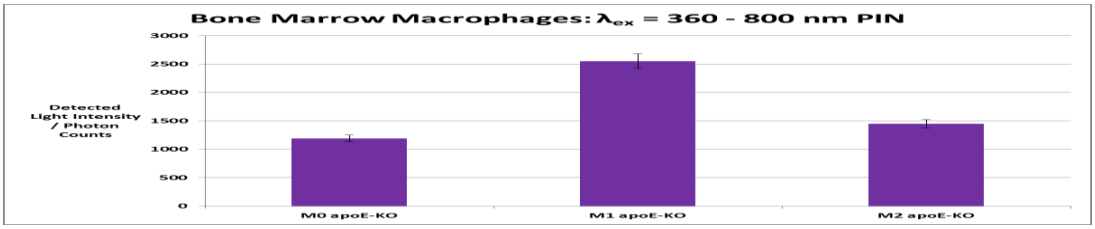

Figure 3. PIN for apoE-KO mouse bone marrow-derived macrophages based on broadband light interrogation.

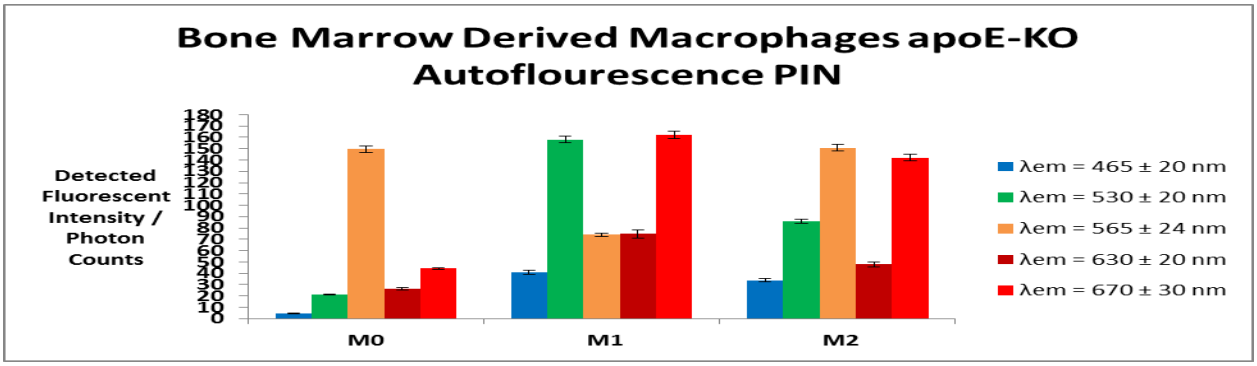

Figure 4. Auto-fluorescence properties of apoE-KO mouse bone marrow-derived macrophages have been examined for (a) M0, (b) M1 classical activation, and (c) M2 alternative activation conditions. A unique auto-fluorescence PIN is observed for each of the macrophage activation conditions.