DROPLET-ENHANCED ON-CELL ENCODING OF SINGLE CELL SECRETORY FUNCTION

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ABSTRACT

Engineered T-cells have demonstrated profound promise towards the treatment of otherwise intractable cancers. However, the inherent heterogeneity of individual clones in cell products is often neglected, limiting reproducibility. We report a method of sorting single viable T-cells directly through secreted protein signatures using conventional flow cytometry. T-cells are rapidly encapsulated into 100 µm droplets with cell-linked anti-cytokine capture and detection antibodies, prompting the formation of fluorescent sandwich immunocomplexes on the surface of secreting clones. Partitioning samples enables continued signal accumulation without intercellular crosstalk, enhancing the potential to probe secreted proteins over a large dynamic range.

KEYWORDS: Droplet Microfluidics, Single Cell Analysis, Flow Cytometry, Cell Secretions

INTRODUCTION

Secreted proteins are critical for the coordination of immune defenses. Interestingly, novel insights from high-content functional screens have alluded to the presence of a small fraction of cells which dominate immune responses by secreting a multitude of cytokines simultaneously and in excess [1]. With increasing interest in harnessing the immune system to treat cancer, isolation and examination of these potent clones would greatly benefit clinical application. To date, approaches to sort cells from secreted protein signals are limited, and often reliant on co-encapsulation of single cells and particles into droplets simultaneously, limiting throughput [2,3]. In this work we develop an approach to rapidly screen and sort individual viable cells based on secreted factors that accumulate at high concentrations in nanoliter droplets and are encoded back onto the secreting cell's surface (Fig. 1); obviating the need for secondary capture phases, and allowing sorting using standard flow cytometry.

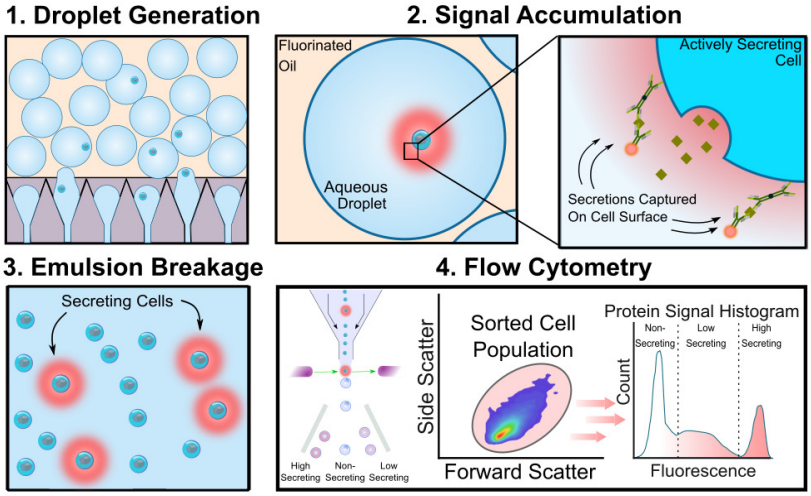

Figure 1: Overview of droplet-enhanced on cell secretion assay. Cells and anti-cytokine capture and detection antibodies are encapsulated into monodisperse droplets. The emulsion is incubated for several hours, allowing secretions to accumulate at high concentrations and associate directly with the cell surface in the form of fluorescent sandwich immunocomplexes. Once signals have accumulated the emulsion is disrupted and cells are sorted using standard flow cytometry.

EXPERIMENTAL

Primary immune cells were mitogenically activated for a period of 6 hours. After activation, cells were washed thoroughly and resuspended alongside anti-CD45/interleukin-2 (IL-2) bispecific capture probes and phycoerythrin-anti-IL2 detection probes (miltenyi biotec). Samples were injected into a microfluidic step-

emulsification device, partitioned into 100 μm droplets, and incubated at 37°C for several hours to allow accumulation of secreted product. Cellular IL-2 production was encoded back onto the surface of the secreting cell in the form of fluorescent immunocomplexes with the capture and detection probes. Once sufficient signal was accumulated, the emulsion was disrupted and the cell populations analyzed directly via flow cytometry.

RESULTS AND DISCUSSION

Droplet-enhanced on-cell secretion profiling enabled accurate detection of IL-2 production over several orders of magnitude from individual $CD3^+$ T-Cells. In a direct comparison between bulk and droplet based assays (Fig. 2a), non-stimulated immune cells yielded no fluorescent signal after three hours of incubation (Fig. 2b,d). In contrast, strong, mitogenic activation overwhelmed the conventional bulk assay, rendering, non-activated cells indistinguishable from actively secreting neighbors within one hour (Fig. 2c). Processing of identical cell mixtures following droplet encapsulation yielded no apparent crosstalk (Fig. 2e). Instead IL-2 production was observed from roughly one quarter of analyzed activated lymphocytes. Coupling droplet based partitioning with on-cell secretion capture enhances current approaches to measure single cell protein production by concentrating secreted products and integrating signal over time while eliminating crosstalk. Thus, this system can be used to probe the production of multiple proteins simultaneously, regardless of production rate or secretion kinetics, and subsequently enrich highly functional clones for immunotherapy applications.

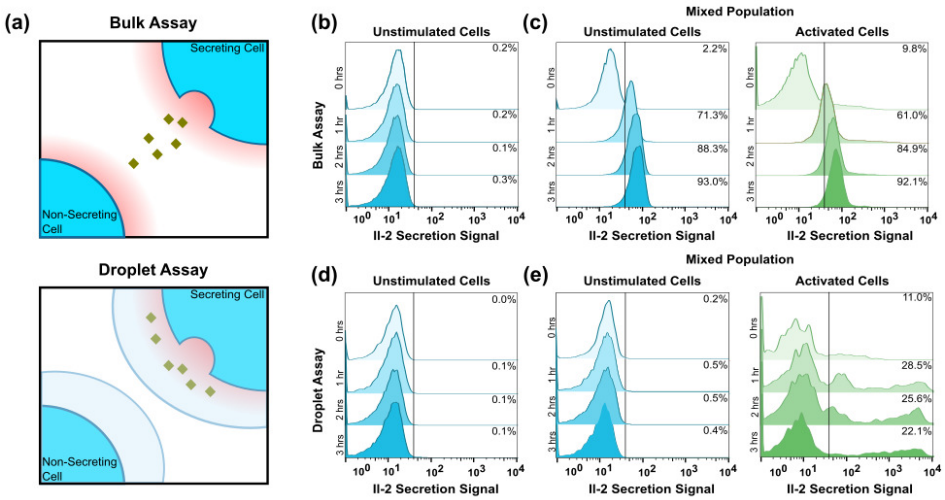

Figure 2: (A) Comparison between bulk and droplet based on-cell secretion assays. (B,D) Unstimulated controls yielded no detectable fluorescence signal after 3 hours in both conditions. (C) In contrast, when unstimulated (left, blue) and mitogen activated (right, green) cells were mixed in equal amount, fluorescent signal accumulated on all cells rapidly, rendering it impossible to distinguish between the two based on secretion. (E) In droplets, activated cells displayed a highly spread secretion signal dependent on the rate of cytokine production while unstimulated cells remained non-fluorescent.

ACKNOWLEDGEMENTS

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