5.02 Single-Cell Analysis
1.02 Droplet Microfluidics
Oral

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DEMOCRATIZED HIGH-THROUGHPUT SINGLE-CELL SECRETION SCREENING USING DROPLETS FORMED BY STRUCTURED MICROPARTICLES

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We introduce a new approach to collect and quantify single-cell secretions without crosstalk in monodisperse droplets formed by precisely structured microparticles, enabling high-throughput screening based on this critical cell function. The ability to analyze and sort cells based on secretions (antibodies, cytokines, proteases, or other enzymes) has implications in understanding cellular heterogeneity fundamental to biology and creating new biotechnology products, such as biologics and cell therapies. Recently, droplet microfluidics has emerged as a powerful approach to perform single-cell secretion screening in high-throughput, using compartmentalization in a small volume to accumulate secreted factors to high levels for accurate detection. Despite this utility, *the necessity of specialized equipment and expertise on the end user hinders its widespread adoption*. A platform that is fully compatible with standard lab equipment (e.g. pipettes, flow cytometers) has the potential to dramatically extend the reach of single-cell screening technology.

Our particle-templated droplet, i.e. "Particle-drop", approach is unique in that pre-fabricated particles are used to form monodisperse emulsions that encapsulate single cells, requiring only standard lab equipment for the end user. Cells are loaded into cavity-containing microparticles and adhere via integrin binding sites (Fig.1a). Oil and surfactant are added and the suspension is agitated by pipetting to create incrementally smaller water-in-oil droplets (Fig.1b) [1,2]. These resulting particle-drops retain a fluid volume which is uniform and defined by the particle geometry, while excess fluid is partitioned into surrounding smaller satellite droplets. Secretions from encapsulated cells are captured on the associated particles (Fig.1c), and transferred back to an aqueous phase by breaking the emulsions enabling downstream analysis that is compatible with standard flow cytometers (Fig.1d).

Structured microparticles with a cavity to hold cells are fabricated utilizing an aqueous two-phase system combined with droplet microfluidics (Fig.2) [3]. This approach ensures precise fabrication ensuring both uniform particle and resulting particle-drop size (Fig.3). Since all fabrication steps are independent of the cell assay work flow, particles can be fabricated in bulk and stored for later use. To encapsulate cells, particles are first seeded into well plates and due to their morphology settle with their exposed cavity upright [4]. Cells are seeded into the well plate where they settle into the cavities of the particles according to single-poisson statistics (in contrast to typical double-poisson statistics for single-cell, single-particle pairs in drops) (Fig.4c). Particle-drops are formed with cells, demonstrating high viability over 24 hours (Fig.4a,b). Initial tests with anti IL-8 producing chinese hamster ovary (CHO) cells demonstrate the ability to capture and label secretions on particles containing cells without crosstalk to neighboring particles (Fig.5).

Using this particle-drop platform researchers can perform droplet based assays using standard lab equipment without sacrificing the precision of droplet microfluidics. Since droplets are formed simultaneously assays are performed homogeneously and sample throughput can be scaled to an even higher level than serial microfluidic droplet processes. The associated particle enables additional functionality such as physicochemical cues or cell specific capture antibodies to select out specific cell subpopulations. This platform promises to create a foundation for expanding the capabilities of *lab-on-a-particle* technologies.

Word Count: 500

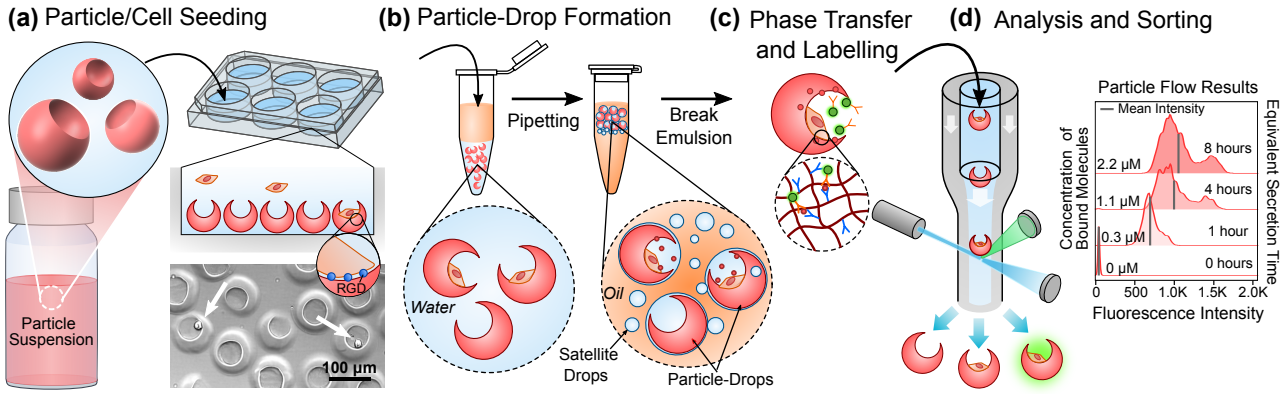

Figure 1. *Overview of particle-drop based single cell secretion screening platform. (a) Prefabricated cavity containing microparticles are seeded into a well plate and settle upright. Cells are seeded and adhere to the particle matrix via integrin binding sites (RGD). (b) Particles and associated cells are transferred and agitated by pipetting with oil and surfactant to generate monodisperse particle-drops. (c) Particles and cells are incubated to accumulate secretions and transferred back to buffer for labelling. (d) Particle/cells can be sorted using high-throughput commercial flow cytometers.*

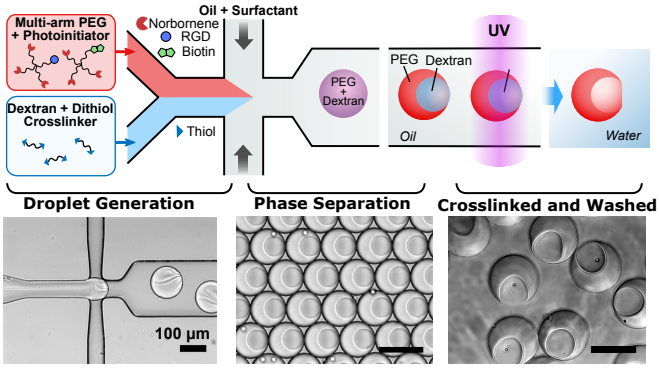

Figure 2. *Fabrication of cavity-containing particles using an aqueous two-phase based approach. UV reactive PEG and photoinitiator is coflowed with dextran in a flow focusing device. PEG and dextran undergo phase separation and are crosslinked via UV light downstream. Particles are collected and washed for later use.*

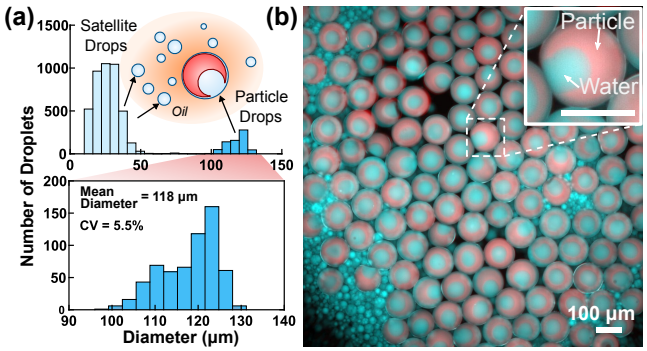

Figure 3. *(a) Droplet size analysis after emulsification shows two distinct subpopulations: smaller heterogeneous satellite drops and monodisperse partice-drops. (b) Fluorescent image of particle-drops with dye added to water phase to aid in visualization.*

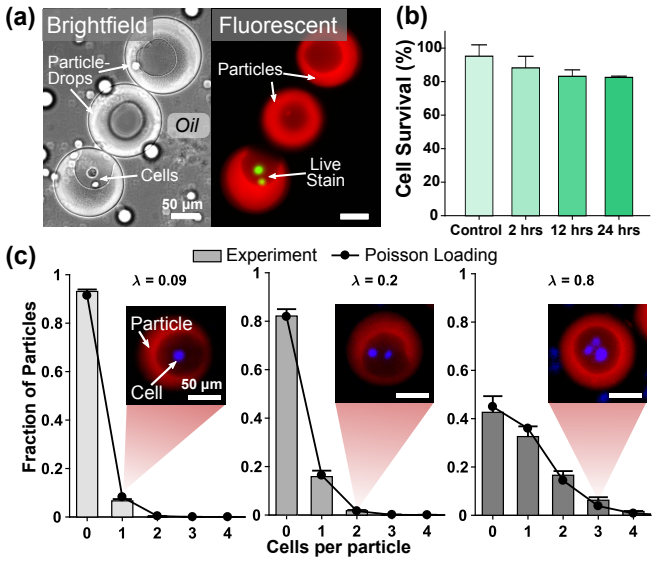

Figure 4. *Cell loading characterization in the particle-drop system. (a-b) Chinese hamster ovary (CHO) cells remain associated with particles after particle-drop formation and maintain high viability over 24 hours. (c) Cell loading into particles follows single-poisson statistics where λ is the average number of cells per particle.*

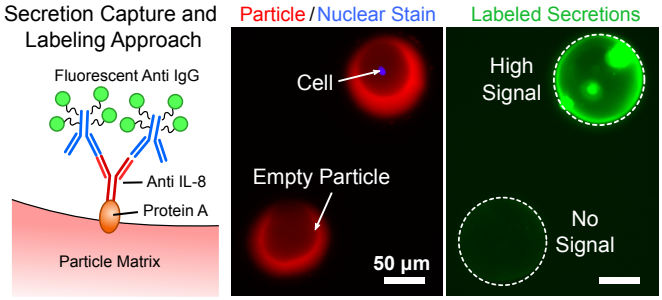

Figure 5. *Secretion capture assay using particle-drop system. Anti IL-8 producing CHO cells are encapsulated and secreted proteins are attached to the particle matrix using protein A and labelled with FITC Anti IgG. Fluorescent microscopy shows clear signal associated with cell loaded particles and no cross-talk.*

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