CONTROLLED ENCAPSULATION OF SINGLE-CELLS INTO MONODISPERSE PICOLITER DROPS

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ABSTRACT

Encapsulation of cells within picoliter-size monodisperse drops provides new means to perform quantitative biological studies on a single-cell basis for large cell populations. Variability in the number of cells per drop due to stochastic cell loading is a major barrier to these techniques. We overcome this limitation by evenly spacing cells as they travel within a high aspect-ratio microchannel; cells enter the drop generator with the frequency of drop formation.

KEYWORDS: cell biology, tissue engineering, inverse emulsions, emergence

INTRODUCTION

While drop-based microfluidics promises breakthrough applications such as directed evolution, tissue printing and bead-based PCR in emulsions, it also facilitates quantitative studies of biology at the most fundamental level, that of single cells. The power of such drop-based studies derives from their ability to study many cells in complete isolation from one another. Despite their great potential, studies of single cells in drops suffer from an intrinsic limitation; because the process of loading cells into drops is purely random, the distribution is dictated by Poisson statistics. Thus, the probability of a drop containing k cells is $\lambda^k \exp(-\lambda) / (k!)$, where λ is the average number of cells per drop. This means that to minimize the number of drops that contain more than a single cell requires very low average loading densities, meaning that most drops actually contain no cells whatsoever.

Recent work describes a method to passively sort drops containing single cells from smaller empty drops after each cell triggers its own encapsulation upon entering a narrow aqueous jet formed in a flow-focusing device [1]. This clever mechanism can also be used to sort cells based on their size since, for this system, drops are always slightly larger than the cell they contain; however, to control encapsulation of cells within uniformly-sized (monodisperse) drops, one (and only one) cell should be present whenever a drop is generated. This can be achieved manually for each drop [2], or passively, and with a much higher throughput, by organizing cells in the direction of flow so that they enter the microfluidic nozzle with the same frequency at which drops form. Here we report a method that causes cells to self-organize into two evenly-spaced streams whose longitudinal order is shifted by half the particle-particle spacing. This occurs when a high density suspension of cells or particles is forced to travel rapidly through a narrow high-aspect-ratio microchannel.

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EXPERIMENTAL

To demonstrate controlled single-cell microdrop generation, we used a flow-focusing geometry [3], fabricated with standard PDMS soft lithography, to emulsify concentrated suspensions (in PBS) of HL60 cells or 9.9-µm-diameter polystyrene beads immediately after they had traversed a 27-µm-wide × 52-µm-tall × 6-cm-long rectangular microchannel (Figure 1a). To ensure robust ordering, a syringe pump forced the aqueous (disperse) phase through the focusing channel at 10 µL/min for beads and 13 µL/min for cells. To generate drops slightly faster than the rate at which particles passed the nozzle, fluorocarbon oil (with dissolved surfactant) was injected at 50-60 µL/min for beads and 85 µL/min for cells, causing 21.7 pL drops to form at 7.7 kHz for beads and 14.6 pL drops to form at 14.9 kHz for cells. To confirm that the encapsulation process did not adversely affect the cells, we tested their survival rates after encapsulation with calcein AM and ethidium homodimer-2.

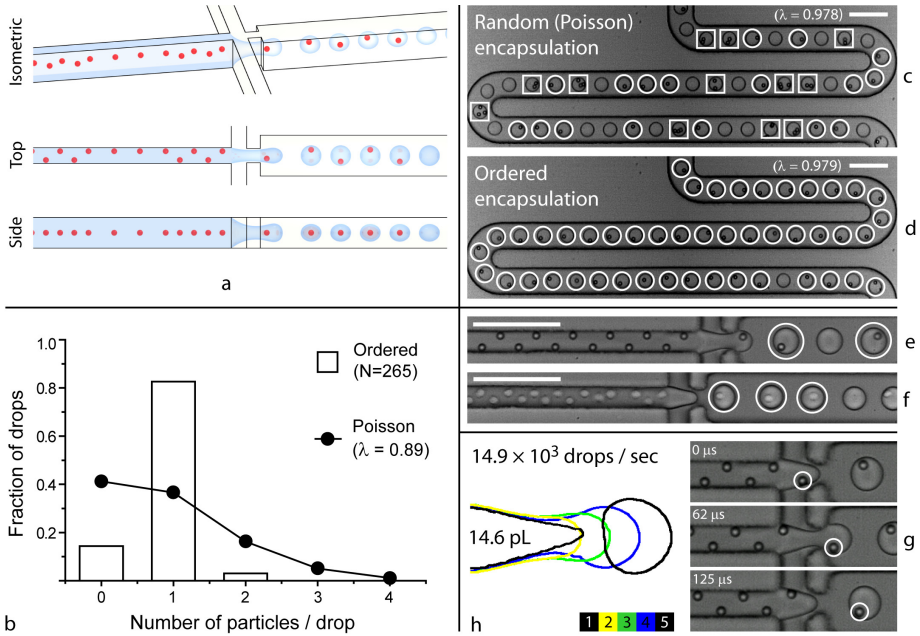

Figure 1. Ordered encapsulation. As depicted schematically in (a), hydrodynamic interactions cause particles to self-organize along one side of the microchannel or into a diagonal/alternating pattern. The uniform spacing in the flow direction (side view) leads to a formation of single-particle drops when two lateral flows of oil pull drops from the aqueous stream (isometric view) with the same (or higher) frequency that particles reach the microdrop generator (g). As the results for 0.89 beads per drop on average in (b) indicate, ordered encapsulation of beads (d-e) generates more single-particle drops (circles) and fewer empty (not marked) or multiple-particle drops (boxes) than would have been possible from (c) stochastic (Poisson) loading. With 92.9 % retaining membrane integrity compared to 96.2 % for controls, cells also self-organize (f), where drops formed as in (h). Scale bars: 100 µm.

RESULTS AND DISCUSSION

This phenomenon provides a passive method to controllably load single cells into drops, overcoming intrinsic limitations set by Poisson statistics and ensuring that virtually every drop contains exactly one cell (Figure 1b and compare stochastic encapsulation, Figure 1c, with ordered encapsulation, Figure 1d, where 46 out of 47 drops contain a single bead). Recent advances in inertial sorting of particles in microfluidic devices describe four independent streams of particles in square channels [4]. For the high-aspect ratio microchannel here, we superimposed a tightly controlled additional degree of particle ordering that generates two particle trains with precisely staggered longitudinal spacing; this is critical for controlled encapsulation.

CONCLUSION

The ability to apply drop-based microfluidics [5] for high-throughput single-cell biology, cell analysis, and tissue engineering is extremely promising but has yet to be realized. The passively-controlled cell and particle encapsulation that we report here is a critical missing component to attain these goals, and due to its simplicity, we anticipate that it will rapidly become common practice. See [6] for more details.

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