Droplet encapsulation of particles in different regimes and sorting of particle-encapsulating-droplets from empty droplets

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K. S. Jayaprakash and A. K. Sen^{a)}

AFFILIATIONS

Department of Mechanical Engineering, Indian Institute of Technology Madras, Chennai 600036, India

^{a)}Author to whom correspondence should be addressed: ashis@iitm.ac.in

ABSTRACT

Encapsulation of microparticles in droplets has profound applications in biochemical assays. We investigate encapsulation of rigid particles (polystyrene beads) and deformable particles (biological cells) inside aqueous droplets in various droplet generation regimes, namely, squeezing, dripping, and jetting. Our study reveals that the size of the positive (particle-encapsulating) droplets is larger or smaller compared to that of the negative (empty) droplets in the dripping and jetting regimes but no size contrast is observed in the squeezing regime. The size contrast of the positive and negative droplets in the different regimes is characterized in terms of capillary number Ca and stream width ratio ω (i.e., ratio of stream width at the throat to particle diameter $\omega = w/d_p$). While for deformable particles, the positive droplets are always larger compared to the negative droplets, for rigid particles, the positive droplets are larger in the dripping and jetting regimes for $0.50 \leq \omega \leq 0.80$ but smaller in the jetting regime for $\omega < 0.50$. We exploit the size contrast of positive and negative droplets for sorting across the fluid–fluid interface based on noninertial lift force (at $Re \ll 1$), which is a strong function of droplet size. We demonstrate sorting of the positive droplets encapsulating polystyrene beads and biological cells from the negative droplets with an efficiency of $\sim 95\%$ and purity of $\sim 65\%$. The proposed study will find relevance in single-cell studies, where positive droplets need to be isolated from the empty droplets prior to downstream processing.

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I. INTRODUCTION

One of the unique capabilities of microfluidics technology is to perform single-cell studies on an unprecedented scale.^{1,2} Analysis of single cells could pave the way in the profound understanding of cancer cell heterogeneity, pluripotent stem cells, and variability in neurons.³ Droplet microfluidics enables this by compartmentalizing single cells and confining them from the bulk population.^{2,3} Conventional analysis of the bulk cell population could reveal nothing more than an average behavior of the population.^{3,4} Moreover, at times, the enzymes or proteins secreted by one or a few odd cell(s) in a population can be lost in the cell medium.⁴ Confining individual cells in droplets and analyzing those separately, by optical, electrical, or mechanical techniques, can precisely pick the cells of interest and will be helpful in studying their influence on the population.

Encapsulation of cells or particles in droplets has been studied in detail and it was found that the process of encapsulation follows

Poisson distribution in the case of a diluted cell sample.⁴ One of the main disadvantages in cell or particle encapsulation is the stochastic nature, which limits high throughput processing of single cells to a great extent. Following statistics, the probability of a given number of particles (n) to be encapsulated in a droplet is given by $p(n) = e^{-\lambda} \lambda^n / n!$, where λ is the average number of particles in a droplet, which can be defined as the ratio of the initial volume fraction of the particles in the sample to the volume fraction of one particle to one droplet.⁴ An increase in the initial concentration of particles in the sample will result in an increase in the chances of encapsulation of multiple particles in a droplet. Further increase in the initial particle concentration will lead to a shift from Poisson distribution to binomial distribution.⁴ Therefore, for high throughput processing of single cells by encapsulation, either the encapsulation process should be deterministic (by focusing and/or ordering the particles prior to encapsulation or by using active encapsulation techniques) or the empty droplets has to be eliminated by sorting

(after encapsulation). These were the two broad approaches that had been used to minimize/eliminate the number of empty droplets and multiple particle encapsulation.

One of the simplest techniques, yet difficult to achieve particularly with beads and cells, is to order the particles closely to the size of one particle so that the particles arrive one after another at the droplet junction. This type of close-packed ordering was demonstrated in the case of deformable gels, which do not stick to the walls of microchannels.⁵ Inertial focusing of rigid particles and cells using a high aspect ratio rectangular channels has shown a tremendous increase in the single particle encapsulation rate with a lesser number of empty droplets and multiple particle encapsulation.⁶ Similarly, curved microchannels, exploiting Dean forces, were used to order particles prior to encapsulation.⁷ There are several applications wherein two different particles have to be coencapsulated in a droplet. Successful coencapsulation resulting from the Poisson type of encapsulation would be difficult to achieve.⁸ Hence to enhance successful coencapsulation, two streams of particles were ordered in separate rectangular microchannels using inertial forces and then coencapsulated in a droplet. It was also found that for coencapsulation, low aspect ratio channels would result in a single line focusing and thereby increase the encapsulation rate. Recently, hydrodynamic trapping of cells and rigid particles and subsequent release, one particle at a time, in droplets was demonstrated.⁹ The particles were trapped in the micro vortices at the expanded flow-focusing droplet junction unlike the normal junctions. The single particle encapsulation efficiency was found to be ~50%.⁹ Optical forces have been used to trap and drive particles to the droplet junction.¹⁰ Once the particles reached the interface between the discrete phase and the continuous phase, droplets were generated. Electrical methods have been employed for generating cell-encapsulating droplets. Impedance of a cell crossing a pair of electrodes was detected and fed back to a piezo actuator to direct the cell to the droplet generating junction to achieve on-demand droplet generation.¹¹

Another approach to eliminate empty droplets is to sort the particle-encapsulated droplet (positive droplet) from the empty droplets (negative droplets) that can be achieved using either passive^{12–15} or active^{16–21} techniques. In one of the works, Chabert and Viovy used asymmetric flow at a flow-focusing junction and produced droplets in the jetting regime closer to one of the walls to sort positive droplets.¹² In the jetting regime, the presence of a cell produced larger positive droplets than the negative droplets. The larger positive droplets moved to the channel center due to the shear gradient and were sorted from the smaller negative droplets.¹² Deterministic lateral displacement (DLD) technique was used to sort positive droplets from negative droplets after encapsulation.^{13,14} The size difference between positive and negative droplets to realize different DLD was achieved by generating droplets in the jetting regime.¹⁴ Pinched flow fractionation (PPF) technique was investigated to sort positive droplets from the negative droplets, wherein plugs containing cells were broken down into smaller droplets before sorting.¹⁵ Active techniques such as standing surface acoustic wave (SAW),²¹ fluorescence activated sorting of droplets by detecting the positive droplets with the help of fluorescence tagging and subsequent sorting using dielectrophoresis,¹⁶ and SAW¹⁷ have been demonstrated.

Here, we first study encapsulation of polystyrene beads and biological cells inside aqueous droplets in different droplet generation regimes, namely, squeezing, dripping, and jetting. The size difference between positive and negative droplets is characterized in terms of capillary number Ca and stream width ratio ω , in various regimes. The positive droplets are found to be larger or smaller compared to the negative droplets in the dripping and jetting regimes but no size difference is observed in the squeezing regime. For cells, the positive droplets are always larger compared to the negative droplets, whereas for polystyrene beads, the size contrast of the positive droplets is decided by the stream width ratio ω . Finally, the size contrast between the positive and negative droplets is exploited to demonstrate size-based sorting.²²

II. MATERIALS AND METHODS

A. Materials

Silicone oil of viscosity 1000 mPa s (Sigma Aldrich, Bangalore) was used as the continuous phase 1 (CP₁) to generate droplets. Particles, namely, polystyrene microbeads, (Sigma Aldrich, Bangalore) of sizes 10 μ m and 15 μ m, cultured cancerous cell lines, namely, HeLa and MDA-MB-231 (NCCS, Pune, India), and Peripheral Blood Mononuclear Cell (PBMC) extracted from human blood were suspended in the aqueous phase and used as the discrete phase (DP). The polystyrene microbeads were suspended in a 23% w/w glycerol solution with 0.5% w/w Tween 80 to make the beads neutrally buoyant. In the sorting experiments, mineral oil (Sigma Aldrich, Bangalore) of viscosity 28.7 mPa s was used as the second continuous phase (CP₂).

B. Preparation of cell suspension

Cancerous cell lines, HeLa and MDA-MB-231 cells, were cultured and extracted at 90% confluence. The extraction was done by adding 3 ml of the dissociation reagent, 0.25% trypsin +0.53 mM EDTA solution (HiMedia, Mumbai), at 37 °C. The mixture of cells and solution was incubated for 5 min at 37 °C. After incubation, the cells were removed and resuspended in fresh Dulbecco's Modified Eagle Medium (DMEM) (HiMedia, Mumbai) with FBS (Fetal Bovine Serum, HiMedia, Mumbai) in the ratio 10:1. The cells are then washed twice in PBS before use. The PBMCs were extracted from 2 ml blood from healthy volunteers by density gradient centrifugation using Histopaque-1077 for 30 min at 1500 rpm (Sigma Aldrich, Bangalore). The PBMCs were pipetted out from the buffy coat and washed twice with PBS (phosphate buffered saline). To nullify the gravitational force, the washed cells were suspended in 20% dextran (Sigma Aldrich, Bangalore, 450 000–650 000 MW) solution in PBS with 10% FBS, resulting in a density of ~1069 kg/m³.

C. Experimental

The CP₁ and DP were taken in glass syringes and pumped into the microchannels by syringe pumps (Cetoni GmbH, Germany) to generate droplets. The syringes are connected to the chip by polytetrafluoroethylene tubing of outer diameter 1.6 mm and inner diameter 0.25 mm. The CP₁ flow rate was in the range 0.2 μ l/min–1.5 μ l/min, and the DP flow rate was in the range 0.1 μ l/min to 0.5 μ l/min. The maximum droplet generation rate was

~350 Hz. In sorting experiments, CP_2 flow rate was in the range $0.6 \mu\text{l}/\text{min}$ – $3.0 \mu\text{l}/\text{min}$. The microchannels were fabricated in polydimethyl siloxane (PDMS) by the soft lithography technique,³² using an SU-8 master mold on a silicon wafer by the standard photolithography. The width of the throat was $25 \mu\text{m}$ and the width of the sorting region was $75 \mu\text{m}$ and the height of the channel was $50 \mu\text{m}$. The microchannel flows were observed under an inverted microscope (IX73, Olympus Corporation, Japan) that was attached with a high-speed camera (FASTCAM SA5, Photron Inc., UK) at 5000 fps and 1008×256 resolution. The videos of the flow were recorded and analyzed using Photron Fastcam Viewer (PFV) software, Photron Inc., UK. Sorting of positive droplets from empty droplets is quantified in terms of sorting efficiency and purity. The sorting efficiency is defined as the percentage of number of desired droplets (positive droplets) collected at the target outlet to the sum of the desired droplets collected together at the target outlet and waste outlet. Purity is defined as the percentage of the number of desired droplets (positive droplet) at the target outlet to the sum of the desired droplets and the empty droplets at the target outlet.

III. RESULTS AND DISCUSSION

First, encapsulation of rigid particles (i.e., polystyrene microbeads) is studied over a range of capillary numbers in different droplet generation regimes (i.e., squeezing, dripping, and jetting). The capillary number of the flow is defined as $Ca = \mu_{CP_1} v_{CP_1} / \sigma_d$, where v_{CP_1} is the velocity of the CP_1 in the droplet generation region and σ_d is the interfacial tension between the droplet and silicone oil (CP_1). The Ca was varied in the range 0.001–2.30 by changing the flow rate ratio r (i.e., ratio of flow rate of DP to CP_1) to observe all three different regimes of droplet generation. The size contrast between the positive and negative droplets with respect to the size ratio of the negative droplets is studied

that is then utilized for size-based sorting of positive and negative droplets. Next, similar studies are carried out for biological cells. Finally, experiments are performed to sort positive and negative droplets based on the size contrast, and the sorting process is characterized in terms of sorting efficiency and purity.

A. Droplet encapsulation of polystyrene beads

In general, droplets can be generated in three different regimes, namely, squeezing, dripping, and jetting, using a flow-focusing junction. The occurrence of the different regimes depends on the flow capillary number thus different regimes can be obtained by simply varying the Ca . In the present study, polystyrene microbeads of sizes $10 \mu\text{m}$ and $15 \mu\text{m}$ are encapsulated in droplets generated in different regimes, as depicted in Fig. 1.

Squeezing regime was observed at a very low $Ca < 0.02$. Here, no considerable change between the diameters of the positive and negative droplets was observed [see Figs. 1(a) and 1(e)]. This may be attributed to the process of droplet formation in the squeezing regime, wherein the DP completely obstructs the flow-focusing junction at the throat and pinches off owing to the upstream pressure in the CP_1 . The device used for the study had a throat of $25 \mu\text{m}$ and the sizes of the microbeads (i.e., $15 \mu\text{m}$ and $10 \mu\text{m}$) are less than the throat size. As the throat size is adequate to accommodate the microbeads, the microbeads do not influence the droplet generation, i.e., the microbeads do not deform the interface (between DP and CP_1) at the throat, and hence there is no contrast between the sizes of the positive and negative droplets.

When the Ca is increased to a value in the range $0.02 < Ca < 0.1$, droplet generation shifts from the squeezing regime to the dripping regime. Here, the stream width of the DP at the throat ($\sim 14 \mu\text{m}$) becomes comparable to the size of the microbead $d_p = 15 \mu\text{m}$ [see Figs. 1(b) and 1(f)]. Here, the

FIG. 1. Schematic representation and the corresponding experimental images of different regimes of droplet generation with $10 \mu\text{m}$ bead encapsulation. (a)–(d) show the schematic representation and (e)–(h) show the corresponding experimental images. (a) and (e) Droplets are generated in the squeezing regime at $Ca < 0.001$ and the stream width ratio at the throat $\omega \sim 1.66$ to 2.50 . (b) and (f) Droplets are generated in the dripping regime at $0.02 < Ca < 0.1$ and the stream width ratio at the throat $\omega \sim 0.85$ to 1.55 . (c) and (g) Droplets are generated in the jetting regime at high $Ca > 0.1$ and the stream width ratio at the throat $\omega \sim 0.80$ to 0.50 . (d) and (h) Droplets are generated in the jetting regime at high $Ca > 0.1$ and the stream width ratio at the throat $\omega \sim 0.50$ to 0.35 . Scale bar represents $50 \mu\text{m}$. Ca is the capillary number of CP_1 .

nondimensional stream width at the throat, defined as $\omega = (\text{width of the DP stream at throat, } w / \text{diameter of the particle, } d_p) \sim 1.0$ and the positive droplets are found to be larger than the negative droplets. In the dripping regime, when $\omega < 1.4$, the interface of the DP at the throat is deformed outward to accommodate the microbead, resulting in a larger positive droplet. The size of the positive droplet D_{+ve} depends on the size of the particle d_p encapsulated as well as the size of the negative droplet D_{-ve} , which is further detailed in the following sections.

If we further increase $Ca > 0.1$, droplets are generated in the jetting regime. Here, the stream width ratio at the throat ω is lesser than the stream width in the dripping regime $\omega_j < \omega_d$. This results in a further increase in the size contrast Δ between the positive and the negative droplets, which is evident from Figs. 1(c) and 1(g).

Sustaining the droplet generation in the jetting regime and reducing the flow rate of discrete phase reduce the stream width to $\omega \leq 0.5$ for the same Ca [see Figs. 1(d) and 1(h)]. Interestingly, such very small stream width ratios result in the generation of positive droplets that are smaller than the negative droplets, which is explained later in the section.

The process of encapsulation of rigid particles in droplets in different regimes of droplet generation is studied with respect to the Ca of CP_1 and the nondimensional stream width ratio $\omega = w/d_p$, and the encapsulation regime plot is shown in Fig. 2(a). In the squeezing regime, the stream width of the DP at the throat is equal to the throat size of $25 \mu\text{m}$ and we get the nondimensional stream width ratio ω as 1.67 and 2.50 for the $15 \mu\text{m}$ and $10 \mu\text{m}$ microbeads, respectively. In the dripping regime, the stream width of the DP at throat is smaller than or comparable to the throat size of $25 \mu\text{m}$, with $0.85 \leq \omega \leq 1.55$, and the size of the microbeads is

adequate to deform (bulge) the DP- CP_1 interface at the throat. This deformation due to the presence of particles at the throat results in a larger positive droplet size than the negative droplets.

In the jetting regime, when $0.50 \leq \omega \leq 0.80$, Δ is positive (i.e., positive droplets are larger than the negative droplets) which we refer to as jetting regime 1 and when $0.35 < \omega \leq 0.50$, Δ becomes negative (i.e., positive droplets are smaller than the negative droplets) which we refer to as jetting regime 2. The two contrasting observations in the jetting regime in terms of the size difference Δ between the positive and the negative droplets are shown in two rectangles, marked as 1 and 2 in Fig. 2(a). This phenomenon is unique for rigid microbeads at smaller jet width ratios ω that was not observed for deformable objects such as cells (presented in Sec. III B, encapsulation of cells in the jetting regime always resulted in positive Δ).

When the jet width w is less than the half of the diameter of the microbead ($\omega < 0.50$), the resulting positive droplets are smaller than the negative droplets. This is very much evident in Figs. 2(b)–2(d) wherein for the same Ca of CP_1 and flow rate ratio r , resulting in a fixed stream width of $\sim 6.5 \mu\text{m}$, the positive droplets encapsulating $15 \mu\text{m}$ beads are smaller, whereas the positive droplets encapsulating $10 \mu\text{m}$ beads are larger compared to the negative droplets. This is because; for the $10 \mu\text{m}$ microbeads [indicated by blue arrow in Fig. 2(b)], the nondimensional stream width $\omega \sim 0.6$ that is greater than 0.5 and hence these microbeads exhibit jetting regime 1 [Fig. 2(c)]. On the other hand, for the $15 \mu\text{m}$ microbeads [indicated by the red arrow in Fig. 2(b)], the nondimensional jet width $\omega \sim 0.4$, that is less than 0.5 and these microbeads exhibit jetting regime 2 [Fig. 2(d)]. Hence, for a fixed Ca and flow rate ratio r , it is possible to achieve positive and

FIG. 2. (a) Different regimes of rigid particle encapsulation defined by the capillary number Ca of CP_1 and the nondimensional stream width at the throat $\omega = w/d_p$. The insets in the plot show the experimental images at the respective regimes of droplet generation. The rectangle marked as 1 demarcates jetting regime 1, and the bottom rectangle with a dotted line shows jetting regime 2 of encapsulation wherein Δ is negative. (b)–(d) For a fixed $Ca = 0.9$ and jet width w , jetting regime 2 was observed for the $15 \mu\text{m}$ particles and jetting regime 1 was observed for the $10 \mu\text{m}$ microbeads. (b) Thin arrows indicate $10 \mu\text{m}$ bead and thick arrows indicate $15 \mu\text{m}$ bead, jet width $\sim 6 \mu\text{m}$ prior to particle encapsulation, (c) $10 \mu\text{m}$ bead exhibits jetting regime 1 as $\omega \sim 0.6$, (d) $15 \mu\text{m}$ bead exhibits jetting regime 2 as $\omega \sim 0.4$ for the same flow rate ratio $r = 0.15$ and $Ca = 0.9$. Scale bar represents $50 \mu\text{m}$. Experimental images of $10 \mu\text{m}$ bead exhibiting jetting regime 2 are shown in Fig. S2 in the [supplementary material](#).

negative Δ depending on the size of rigid particles (see video 1 in the [supplementary material](#)).

In the jetting regime, when a microbead enters into the jet, having width less than the diameter of the microbead, it blocks the flow and thus instantaneously breaks the continuous flow of jet. For relatively higher stream width ratio of $0.50 < \omega \leq 0.80$, the jet breakage is partial and regenerates itself into an intact jet [see Fig. 2(c)] due to relatively smaller size of the microbeads resulting in the positive droplets that are larger than the negative droplets. On the other hand, for relatively smaller stream width ratio of $0.35 < \omega \leq 0.50$, the jet gets broken completely, owing to the smaller ω (or larger size of microbeads), resulting in positive droplets that are smaller than the negative droplets [see Fig. 2(d)].

The sizes of the positive droplets resulting from encapsulation of two different sizes of microbeads, $10\mu\text{m}$ and $15\mu\text{m}$, in all three regimes of encapsulation, namely, dripping regime, jetting regime 1 and jetting regime 2, are characterized as shown in Fig. 3. From experiments, we observed that the percentage change in the size of the positive droplet D_{+ve} depends on the size of the negative droplet D_{-ve} , the size of the particle encapsulated d_p , and the regime of encapsulation.

We have nondimensionalized the size of the negative droplets D_{-ve} with the particle diameter d_p as $\bar{D}_{-ve} = D_{-ve}/d_p$. In the case of dripping regime, as the ratio $\bar{D}_{-ve}$ increases, irrespective of the encapsulated particle size, the percentage increase in the positive droplet size decreases. As the negative droplet size D_{-ve} approaches the size of the particle d_p , the percentage size difference increases. It was also found that the percentage increase depends on the ratio $\bar{D}_{-ve}$ and not on the individual sizes of the negative droplets D_{-ve} or particles d_p . The maximum percentage increase in the size difference was found to be $\sim 29\%$ for $\bar{D}_{-ve} \approx 1.30$ for a negative droplet size of $12.8\mu\text{m}$, which is the minimum droplet size produced in the dripping regime.

FIG. 3. Plot showing the variation of percentage in size difference with the ratio of negative droplet diameter to the particle diameter $\bar{D}_{-ve} = D_{-ve}/d_p$ in three different regimes of encapsulation.

In jetting regime 1, when the ratio $\bar{D}_{-ve}$ is increased further from an initial value of $\bar{D}_{-ve} = 2.30$, the percentage size difference increases up to a maximum value of 38% at $\bar{D}_{-ve} = 3.0$ beyond which it decreases. Similar to the dripping regime, in this regime, the percentage increase in the size difference depends on the ratio $\bar{D}_{-ve}$. Jetting regime 1 occurs for a minimum droplet size of $25.5\mu\text{m}$, below which the transition to jetting regime 2 occurs. In jetting regime 2, the size difference Δ is negative and hence the percentage change is always negative. Similar to the dripping regime, we found that the percentage decrease in the size difference depends only on the ratio $\bar{D}_{-ve}$. When the ratio $\bar{D}_{-ve}$ increases, the percentage decrease in the size difference also increases but attains a steady value of 31% for $\bar{D}_{-ve} \approx 2.60$. In jetting regime 2, the minimum size of the droplet produced was $12.0\mu\text{m}$. Among the three different regimes, we found that in jetting regime 1, the percentage change was maximum, i.e., 38% for $\bar{D}_{-ve} \approx 3.0$.

B. Droplet encapsulation of biological cells

Encapsulation of cells (HeLa and MDA-MB-231 cell lines and PBMCs) of different sizes was studied in three different regimes of droplet generation, namely, squeezing, dripping and jetting regimes (see video 3 in the [supplementary material](#)). Cells of sizes d_c in the range of $10.1\mu\text{m}$ – $19.5\mu\text{m}$ were encapsulated, and the size contrast Δ between the positive and negative droplets was studied. Figures 4(a)–4(c), respectively, show the encapsulation of cells in the squeezing, dripping, and jetting regimes. Similar to the encapsulation of rigid particles, encapsulation of cells in the squeezing regime did not produce any change in the size of positive droplets as compared to the negative droplets. In the dripping regime, positive droplets were found to be larger than the negative droplets and as the size of the negative droplets $\bar{D}_{-ve}$ approaches the size of cells, i.e., $\bar{D}_{-ve} \rightarrow 1$, the size difference between the positive and the negative droplets Δ increases, which is shown in Fig. 4(d). On the other hand, when the size of the negative droplets $\bar{D}_{-ve}$ is much larger than the cell size, the size contrast approaches zero, $\Delta \rightarrow 0$. For instance, for a negative droplet size of $27.4\mu\text{m}$ and cell size of $10.1\mu\text{m}$, size contrast, $\Delta \sim 0$.

Encapsulation of cells in the jetting regime always produced larger positive droplets, i.e., Δ is always positive. Unlike the encapsulation of rigid particles, wherein a negative size contrast Δ was observed in jetting regime 2 for $\omega < 0.5$, in the case of encapsulation of cells, the positive droplets were found to be positive always (even when $\omega < 0.5$), as shown in Fig. 4(c). When a cell passes through a jet of width smaller than the cell diameter, both the interfaces of the jet and the cell deform so that there is always a layer of DP surrounding the cell (also, cells tend to stay in the aqueous phase³³). In the case of rigid particles, when $\omega < 0.5$, the particles while passing through the jet completely block the continuous jet due to which the jet breaks into smaller positive droplets. On the other hand, cells which are deformable in nature, do not stop the continuous flow of jet rather they deform themselves and the DP interface to maintain the flow resulting in an increase in size of the positive droplets. For example, a cell of diameter $21\mu\text{m}$ is squeezed to the width of the stream ($\sim 16\mu\text{m}$) as it enters the throat of the droplet generator (refer to Fig. S4 in the [supplementary material](#)). The percentage increase in size of the positive droplets again

FIG. 4. Cell encapsulation in different regimes of droplet generation. (a) Squeezing regime for which $\Delta = 0$, (b) dripping regime, and (c) jetting regime in which Δ is always positive. (d) Percentage increase in the size of the positive droplets with $\bar{D}_{-ve} = D_{-ve}/d_c$. Scale bar represents $50 \mu\text{m}$.

depends on the ratio of the size of the negative droplets to the size of the cells $\bar{D}_{-ve}$, as shown in Fig. 4(d). We observe that, in addition to $\bar{D}_{-ve}$, the percentage increase in size of the positive droplets depends on the droplet generation regime. For a fixed $\bar{D}_{-ve}$, the percentage increase in size in the jetting regime was found to be higher than that in the dripping regime, which is due to the fact that the jet/stream width in the jetting regime ω_j is much smaller than that in the dripping regime ω_d , i.e., $\omega_j < \omega_d$. As the droplets produced in the jetting regime are smaller than that in the dripping regime, we observed an increase in positive droplet size as high as 73% for $\bar{D}_{-ve} = 0.97$.

C. Sorting of positive and negative droplets encapsulating particles and cells based on size contrast

The device used for the present study consists of two regions, namely, a flow-focusing droplet generator and a droplet sorter as shown in Fig. 5. The device design with experimental images of droplet generator, sorting region, and separation is shown in the supplementary material, Fig. S1. Particles (rigid microparticles and cells) are suspended in an aqueous solution that acts as the discrete phase (DP). Droplets are generated at the flow-focusing junction in continuous phase 1 (CP₁) that get transported into the sorting region, where another immiscible continuous phase (CP₂) coflows along CP₁. The viscosity of CP₁ (μ_{CP_1}) is much greater than the viscosity of CP₂ (μ_{CP_2}); thus, irrespective of the flow rates, the maximum velocity (i.e., region of zero shear) of the coflow always lies in the CP₂. Using a coflowing system of two immiscible phases, extraction of rigid polystyrene particles (from aqueous phase to oil phase)²³ and soft hydrogel microparticles (from oil phase to aqueous phase)²⁴ has been demonstrated, at low Re , in which the working principle was based on the interfacial properties of the liquid phases and the particle. In contrast, here, we have employed hydrodynamic (noninertial lift) force that acts on the deformable objects at low Re to selectively sort droplets of interest. Deformable objects (such as cells, vesicles, droplets), at low Reynolds number

(i.e., $Re \ll 1$), are subjected to a lateral force toward the region of zero shear (or maximum velocity) called the noninertial lift force^{25–29}. In the present case, since the region of zero shear lies in CP₂, the droplets tend to move from CP₁ to CP₂ due to the noninertial lift force^{28,30} given by $F_l \sim \mu_{CP_1} \dot{\gamma} R^3 / \gamma$, where $\dot{\gamma}$ is the local shear rate, R is the radius of the droplet, and γ is the position of the droplet from the wall. However, in order to cross the interface between the CP₁ and CP₂, the droplets need to overcome the interfacial tension force that scales as $F_i \sim \sigma R$, where σ is the interfacial tension between CP₁ and CP₂. The interfacial migration of the droplets would depend on the ratio of the above two forces $k \sim (\mu_{CP_1} \dot{\gamma} R^2) / \sigma \gamma$, which can give rise to selective migration depending on the size and viscosity ratio of the droplets as described in our previous works.^{22,31} The controlling parameters which govern the interfacial migration from CP₁ to CP₂ are the strain rate $\dot{\gamma}$ and the radius of the droplet R . The strain rates can be varied by varying the flow rates of the continuous phases. From the expression of the force ratio, we see that the noninertial lift force is a strong function of droplet radius. Experimentally, we observe that there is a size contrast between the droplets that encapsulate particles (rigid microbeads or cells) and the empty droplets thus the positive droplets can be sorted from the negative droplets. The rigidity of the particles affects only the droplet generation mechanism and does not play any role in the migration process (as demonstrated in the supplementary material, Fig. S6).

From the above studies on the size contrast between the positive and negative droplets in different regimes, one could think of two different methods to sort the positive and negative droplets. First, we could operate in the dripping regime or jetting regime 1, wherein the positive droplets are always larger than the negative droplets. Thus, the positive droplets could migrate across the CP₁–CP₂ interface due to higher noninertial lift force acting on these positive droplets and get separated from smaller droplets [Fig. 5(b)]. Second, we could operate in jetting regime 2 wherein the positive droplets are always smaller than the negative droplets. Thus, the negative droplets could undergo interfacial migration and get separated from the positive droplets [Fig. 5(a)].

FIG. 5. Schematic of particle encapsulation and sorting. (a) Encapsulation of rigid particles in the jetting regime and subsequent sorting of positive droplets. (b) Encapsulation of cells in the jetting regime and subsequent sorting of positive droplets.

Here, we utilized the second method as shown in Fig. 5(a) in the case of rigid particles for the following reasons. In jetting regime 1, the minimum size of the negative droplets D_{-ve} (generated using our design) was $25.5\mu\text{m}$, and the size contrast Δ was found to be in the range $6\text{--}12\mu\text{m}$. For this size contrast, even for a very low stream width of the CP_2 , (for example, at a flow rate of $1\mu\text{l}/\text{min}$) giving rise to a very low strain rate of 140 s^{-1} , both the positive and the negative droplets migrate across the interface thus size-based sorting is not possible (refer to the [supplementary material](#) Fig. S3). In the dripping regime, although the minimum negative droplet size was only $12\mu\text{m}$ and a good size contrast Δ in the range $0\text{--}6.5\mu\text{m}$ was obtained, the interdistance between the droplets was found to be very small ($\sim 5\mu\text{m}$), which is less than the droplet size (refer to the [supplementary material](#) Fig. S3). This small interdistance between the droplets causes perturbations of the CP_1 – CP_2 interface leading to a poor sorting efficiency and purity.

Sorting of positive droplets containing a $10\mu\text{m}$ bead from the negative droplets by employing jetting regime 2 is shown in Fig. 6. As the droplets move along the sorting channel [Fig. 6(a)], due to the higher noninertial lift force owing to the larger size, the negative droplets are pulled toward the CP_1 – CP_2 interface faster than the positive droplets [Fig. 6(b)]. Here, the positive droplets are of size $18.2\mu\text{m}$ and the negative droplets are of size $\sim 26.0\mu\text{m}$.

Since the negative droplets are ~ 1.43 times larger than the positive droplets, the noninertial lift force, which scales as the square of droplet size, acting on the negative droplets is approximately twice that acting on the positive droplets. Thus, the negative droplets approach the interface [Fig. 6(c)], undergo interfacial migration and get separated from the positive droplets (Fig. 6(d), see video 2 in the [supplementary material](#)).

Figure 7 shows the variation of sorting efficiency and purity with respect to the ratio $(D_{+ve}/D_{-ve})^2$. For a given ratio of the negative droplet size D_{-ve} to particle size d_p , i.e., $\bar{D}_{-ve}$, we get a fixed positive droplet of size D_{+ve} ; thus, the ratio $(D_{+ve}/D_{-ve})^2$ would decide the efficiency of interfacial migration (since the strain rate for a given experiment is fixed). For sorting of the positive droplets using the second method [Fig. 5(a)], the ratio $(D_{+ve}/D_{-ve})^2$ needs to be smaller. From the experiments, it was observed that when the ratio $(D_{+ve}/D_{-ve})^2 < 0.5$, most of the negative droplets migrate across the CP_1 – CP_2 interface (i.e., when the size contrast is more than 30%) which gives rise to a sorting efficiency $> 80\%$. Similarly, it was found that the sorting purity increases as the ratio $(D_{+ve}/D_{-ve})^2$ decreases. A maximum purity $\sim 35\%$ was obtained for an efficiency of $\sim 95\%$ for $(D_{+ve}/D_{-ve})^2 = 0.45$. The maximum efficiency and purity is observed at the ratio, $(D_{+ve}/D_{-ve})^2 \approx 0.45$, and further decrease in the ratio, $(D_{+ve}/D_{-ve})^2$ did not improve the purity. It is observed that the change (increase) in efficiency and purity of sorting

FIG. 6. Sorting of positive droplets from negative droplets. (a) Positive droplets of size $18.2\mu\text{m}$ encapsulating $10\mu\text{m}$ microbeads and negative droplets of size $26.0\mu\text{m}$ entering the sorting region, (b) the larger negative droplets move closer to the CP_1 – CP_2 interface owing to higher noninertial lift force, (c) the negative droplets migrate laterally across the interface, and (d) the positive droplets are separated from the negative droplets.

decreases when $(D_{+ve}/D_{-ve})^2 < 0.5$ and becomes negligible when $(D_{+ve}/D_{-ve})^2 \approx 0.4$. This is due to the fact that the purity is affected because of the disturbance of the interface between the two continuous phases (CP_1 – CP_2), at the bifurcation junction of the sorting channel. When the flow of migrated larger

FIG. 7. Variation of sorting efficiency and purity with the ratio $(D_{+ve}/D_{-ve})^2$.

(negative) droplets is separated at the bifurcation, the interface is disturbed, occasionally, for a small interval of time resulting in the escape of the negative droplets to the outlet of the positive droplets.

The effect of the presence and absence of particles inside droplets on the cross stream migration of droplets was also investigated. It was found that the presence of particles inside droplets did not affect the migration pattern, i.e., positive and negative droplets of the same size exhibited similar behavior, until the volume fraction of particle to the droplet is smaller than 0.33 (refer to Figs. S5 and S6 in the [supplementary material](#)).

As discussed, positive droplets containing cells are always larger than the negative droplets, in both dripping and jetting regimes, and hence scheme 2 shown in Fig. 5(b) was followed for sorting. Similar to the rigid microparticle case, operating in the dripping regime produced droplets with small interdistance between them which adversely affect the sorting process. So, droplets were generated in the jetting regime and two types of cancerous cells, HeLa and MDA-MB-231 and PBMCs, were encapsulated. Experiments were performed to study the migration and sorting of positive droplets from empty droplets for each type of the cancerous cells and PBMCs. Unlike rigid microparticles, cells are heterogeneous and have a size distribution. Figure 8 shows the sorting of three different sizes of positive droplets, $D_{+ve} = 33.0\mu\text{m}$, $28.0\mu\text{m}$, and $19.0\mu\text{m}$ containing HeLa [Figs. 8(a) and 8(b)], MDA-MB-231 [Figs. 8(d) and 8(e)], and PBMCs [Figs. 8(g) and 8(h)], respectively, from the corresponding negative droplets of sizes $D_{-ve} = 23.4\mu\text{m}$, $17.2\mu\text{m}$, and $11.0\mu\text{m}$, respectively (see video 4 in the [supplementary material](#)). Irrespective of the type of cell, we study the effect of diameter of the positive droplets and the ratio of diameter of the positive droplets to that of the negative droplets on the sorting efficiency and purity at different strain rates or flow rates (for different cell type).

For a fixed strain rate (used for a given cell type), as the diameter of the positive droplets increases, the sorting efficiency also increases from 0% to 100%, as shown in Fig. 9(a). For every positive droplet size in the range of $15\mu\text{m}$ to $35\mu\text{m}$, there exists a critical strain rate at which 100% efficiency is attainable, compromising the purity. The three curve in Fig. 9(a) corresponds to the sorting of three sets of droplet sizes shown in Fig. 8. We also observed that for a fixed strain rate (cell type), the sorting efficiency depends on the square of the ratio of diameter of the positive droplets to that of the negative droplets, i.e., $(D_{+ve}/D_{-ve})^2$, which is representative of the ratio of the noninertial lift force acting on the droplets. Figure 9(b) shows the variation of sorting efficiency and purity with the ratio $(D_{+ve}/D_{-ve})^2$. We observe that an increase in the ratio $(D_{+ve}/D_{-ve})^2$ gives rise to a higher sorting efficiency and purity. For a maximum sorting efficiency of $\sim 95\%$, the purity was observed to be $\sim 65\%$. Similar to rigid particles, the effect of the presence and absence of cells in the droplets on cross stream migration is studied. The migration of droplets containing single HeLa and MDA-MB-231 cells in droplets is compared with negative droplets of same diameter and was found that the presence of cells and the rigidity of the cells did not affect the migration pattern (refer to Fig. S5 in the [supplementary material](#)). Finally, for the different types of cells used in the experiments, cell viability was

FIG. 8. Sorting of positive droplets encapsulating cells of different types and corresponding sizes. (a) Positive droplet of size $D_{+ve} = 33.0 \mu\text{m}$ containing HeLa cells enters the sorting channel, the negative droplet size $D_{-ve} = 23.4 \mu\text{m}$, (b) the positive droplet is separated after interfacial migration and (c) shows the droplets collected at the negative droplet outlet. (d) Positive droplet of size $D_{+ve} = 28.0 \mu\text{m}$ containing MDA-MB-231 cells enters the sorting channel, the negative droplet size $D_{-ve} = 17.2 \mu\text{m}$, (e) positive droplets are sorted from the negative droplets after interfacial migration and (f) shows the droplets collected at the negative droplet outlet. (g) Positive droplet of size $D_{+ve} = 19.0 \mu\text{m}$ containing PBMCs enters the sorting channel where the negative droplet size $D_{-ve} = 11.0 \mu\text{m}$, (h) and is sorted from the negative droplets, and (i) shows the droplets collected at the negative droplet outlet.

checked using trypan blue test by encapsulating the cells in both the oils used in the experiments (refer to the [supplementary material](#)). It was found that all three types of cells were viable in both the oils for at least 2 h with negligible or no compromise in viability compared with the control (Fig. S7 in the [supplementary material](#)).

All the experiments were conducted at a cell concentration such that the total volume of the cells is less than 5% of the total

sample volume of the dispersed phase, so as to minimize the number of droplets containing more than one cell. Within this limit, there is no effect of the particle concentration on the sorting efficiency and purity. However, the efficiency and purity of sorting could be affected if the initial concentration is increased, mainly due to the interfacial perturbation of droplets moving from one phase to the other phase.

FIG. 9. (a) Variation of sorting efficiency from 0% to 100% for three strain rates 140 s^{-1} (HeLa cells), 211 s^{-1} (MDA-MB-231 cells), and 352 s^{-1} (PBMCs) as D_{+ve} is varied from $29 \mu\text{m}$ to $33 \mu\text{m}$, $21.5 \mu\text{m}$ to $28.0 \mu\text{m}$, and $15.5 \mu\text{m}$ to $19.0 \mu\text{m}$, respectively. (b) Variation of sorting efficiency and purity with the ratio $(D_{+ve}/D_{-ve})^2$.

IV. CONCLUSION

We investigated the particle encapsulation regimes and demonstrated a passive technique based on interfacial migration phenomena to sort particle-encapsulating droplets (positive droplets) from empty droplets (negative droplets). Encapsulation of both rigid particles (polystyrene microbeads) and biological cells (HeLa, MDA-MB-231 and PBMCs) inside aqueous droplets in a continuous phase (CP_1) is investigated in three different droplet generation regimes, namely, squeezing, dripping, and jetting. We observed that the size of the positive droplets was larger or smaller compared to the negative droplets in the dripping and jetting regimes but no size contrast was observed in the squeezing regime. The size contrast of the positive and negative droplets in different regimes was illustrated in terms of capillary number Ca of CP_1 and stream width ratio (with respect to particle diameter) at the throat $\omega = w/d_p$. We observed that, for cells, the positive droplets were always larger compared to the negative droplets. On the other hand, for rigid particles, the positive droplets were found to be larger in the dripping and jetting regimes (for $0.50 \leq \omega \leq 0.80$) and smaller in the jetting regime (for $\omega < 0.50$). Rigid particles and cells behaved differently in the jetting regime of droplet generation, which could be due to the deformability of the cells. In the case of rigid microbeads, jetting regime 1 occurs for a minimum droplet size of $25.5 \mu\text{m}$, below which the transition to jetting regime 2 occurs. A maximum of $\sim 38\%$ increase in positive droplet size was observed in jetting regime 1 and a maximum of $\sim 32\%$ decrease in positive droplet size was observed in jetting regime 2. Operating in jetting regime 2, sorting of positive droplets (with rigid particles) from negative droplets was studied and the maximum sorting efficiency of $\sim 95\%$ and purity of $\sim 35\%$ was attained when $\left(\frac{D_{\text{posve}}}{D_{\text{negve}}}\right)^2$ is less than 0.5. In the case of cells, dripping and jetting regimes showed only an increase in the size of positive droplets and a maximum percentage change of $\sim 75\%$ was obtained in the jetting regime (when $D_{\text{ve}} \sim d_c$, i.e., $\bar{D}_{\text{ve}} \sim 1.0$). In the case of both rigid particles and cells, the percentage change in positive droplet size depends on the ratio D_{ve}/d_p . Finally, the sorting of positive droplets encapsulating cells from negative droplets was studied and a maximum efficiency of $\sim 95\%$ and purity of $\sim 65\%$ was attained. In summary, we have provided an improved understanding of droplet encapsulation and demonstrated sorting of positive droplets by the noninertial lift induced interfacial migration. The proposed sorting technique can also be used with fluorinated oils that are biocompatible and thus preferred for biological cells. The proposed understanding and technique may find applications in encapsulation and sorting of particles/cells for single-cell studies.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for the device design with experimental images of droplet generator, sorting region and separation, experimental images of $10 \mu\text{m}$ bead exhibiting jetting regime 2, migration of both positive and negative droplets across interface, deformation of cells at the throat of the flow-focusing junction during encapsulation, effect of particles/cells encapsulated in droplets on migration, and cell viability results. Video 1 shows the different regimes of encapsulation of rigid microbeads. Video 2

shows the sorting of positive droplets encapsulating $10 \mu\text{m}$ beads from negative droplets. Video 3 shows the encapsulation of cells in different modes of droplet generation. Video 4 shows the sorting of positive droplets encapsulating cells of different sizes from negative droplets.

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