Summer Scholar Report

Encapsulation of Motor Particles in Vesicles using Microfluidic Devices

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The interior of a cell is filled with organelles, macromolecules, proteins, sugars, microtubules of the cytoskeleton, and a host of other biochemicals; it is complex, dynamic, and crowded. For a living cell, an issue of scale arises when considering the transport of molecules and macromolecules. In eukaryotic cells, where translational diffusion rates of molecules across the cell are on the order of tens or hundreds of seconds,[1] the cell employs transport proteins to enable molecule transport on faster time scales. Furthermore, the aggregate motion of motor activity in cells directly affects the motion of molecules, macromolecules, and supramolecular structures.[2] We aim to examine the underlying physics of these interesting dynamics by building a model system.

The most basic requirement for a cell is an artificial membrane to define the boundary of the cell. As a result, we looked at vesicles, since vesicles are simply fluid enclosed by a lipid-membrane. In order to build a foundation for more complexity and a domain to encapsulate cellular machinery, we created vesicles for characterization. There are a few methods of creating vesicles[3-4] , but we desired a method to produce stable, monodisperse, and relatively pristine inverted emulsion precursors and vesicles. In particular, we looked to microfluidics to produce vesicles of these desired qualities. Reliability of vesicle production allows more definite characterization of the vesicles and offers a foundation to build more complexity when vesicles are used to encapsulate proteins and small organelles. From there, we introduced swimming or faster-than-diffusion Janus particles[5] in vesicles to simulate aggregate effects of motor activity in living cells. In this way, we hope to understand the role of random motion in cellular transport. We have been able to successfully encapsulate silica Janus particles using a PDMS (polydimethylsiloxane) microfluidics technique and non-swimming, polystyrene particles via glass capillary microfluidics

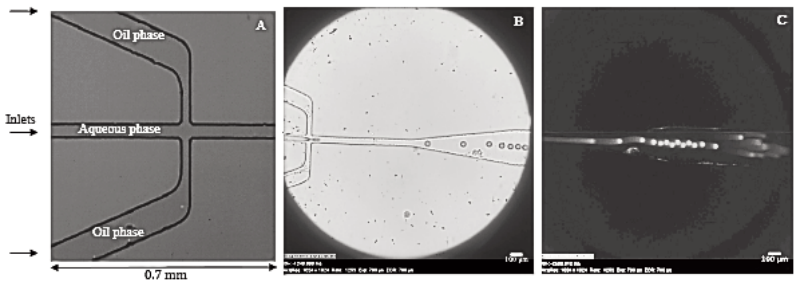

Figure 1. *(A) Labels for the direction of flow of the oil and aqueous phase from the inlets. (B) Brightfield image of the formation of inverted emulsion droplets containing 200mM sucrose, 100mM Bis-Tris, 0.7mg/mL sulforhodamine B, and silica Janus particles. (C) Laser-induced fluorescence image of the formed inverted emulsion. Figures 1B and 1C are viewed from a 10x objective.*

Both the PDMS microfluidic technique and as the glass capillary technique are employed to make mono disperse inverted emulsion and double emulsion, respectively. In addition, they provide a relatively easy way to introduce desired materials into the inner solution of the inverted emulsion or double emulsion. The difference between a double emulsion and a vesicle is a layer of oil between the two layers of phospholipid. In our experiments, we let this layer of oil evaporate so that vesicles form. Since the PDMS microfluidics techniques form inverted emulsion, a pull through technique is used to add on the second layer of phospholipids to form a vesicle. This all physically happens in small chips that have either PDMS channels or glass capillaries that run an aqueous solution and oil to a juncture where the flow and shear forces produce either inverted emulsion or double emulsion, respectively (Figure 1).

As the oil and aqueous phase is pumped into the PDMS device, the two laminar flows of liquid meet at the junction of the device (Figure 1A). After fine adjustments to the flow rates of the syringe pumps for the aqueous and oil phase, the oil phase, flows at a rate in which the oil flow shears and pinches off droplets of aqueous phase forming stabilized inverted emulsions. The droplets are surrounded by a single layer of phospholipid, which stabilizes these droplets. The high-speed Phantom camera allows us to capture the formation of inverted emulsions, and fluorescence allows us to verify the production of inverted emulsions. We see that the produced inverted emulsions are monodisperse, with a diameter of 50μm (Figures 1B and 1C). Although the majority of these inverted emulsions appear stable over the length of the device through which they travel, we have observed multiple instances where the droplets will merge with each other and form larger inverted emulsion droplets. The frequency in which merging occurs is rather unpredictable, but roughly 1 in 5 to 1 in 20 droplets merge by the time droplets exit the PDMS device.

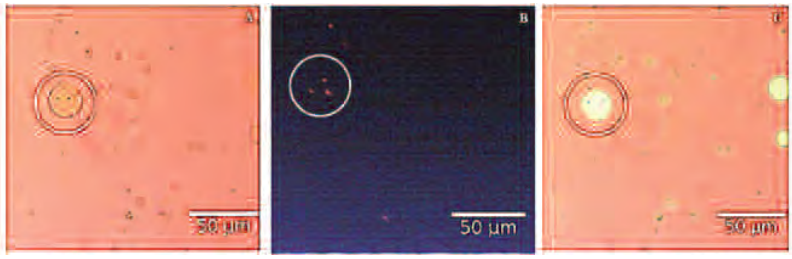

Figure 2. *(A) DIC (differential interference capture) image of a vesicle encapsulating three polystyrene particles. (B) Fluorescence image of the vesicles with the polystyrene particles tagged with Nile Red dye excited with 485nm light. (C) DIC image with fluorescence to excite the HPTS (8-hydroxypyrene-1,3,6-trisulfonate) with a 407nm light. A white circle is used to indicate the vesicle of interest. The vesicles was formed from an aqueous phase of 200mM sucrose, 100mM Bis-Tris, 0.7mg/mL HPTS, and 10uL of polystyrene particles tagged with Nile Red dye to simulate Janus particles. Figure 2A, 2B, and 2C are viewed with a 100x objective.*

After the inverted emulsion is collected and centrifuged to produce vesicles, thin samples of vesicles are studied via brightfield, DIC, fluorescence, and digital holography imaging. To ensure that we are observing vesicles, we encapsulate an aqueous fluorescent dye inside the vesicles and

image fluorescently. A higher intensity of emitted light indicates a higher concentration of dye. It is clear from the shape and intensity of the emitted turquoise light that we are observing vesicles (Figure 2C). The phospholipid membrane clearly contains the fluorescing aqueous phase of the vesicle. We also tagged the polystyrene particles with Nile Red dye so we could easily determine if the particles were encapsulated or not. We see three distinct red-spots indicative of material tagged with Nile Red (Figure 2B). In addition, these particles are localized in the position of the vesicle visible as pictured in Figures 2A and 2C. Since standard microscopy views distinct planes along the z-axis, particles may appear to be moving inside the vesicles whereas after careful inspection the particles are moving close to the surface of the vesicle. Therefore, we adjusted the focus of the microscope to ensure that the particles move inside the vesicle for all planes along the z-axis. We used digital holography to produce a light-scattered image of the vesicle (Supplemental information 1). Using an optical trap, we held the particle at a certain position in three-dimensional space while moving the sample stand. When we try to move the particle outside of the bilayer membrane, the membrane blocks the movement of the vesicle past the membrane. We moved the particle along all three axes to show that the polystyrene particle is indeed encapsulated.

The reason we substituted HPTS with sulforhodamine B is because we desired to obtain better 2-channel fluorescence measurements. In fact, we could see the background fluorescing lower intensity, light turquoise upon excitation with the solid state illuminator. Although there appears to be some fluorescing sulforhodamine B in background images, the intensity appears to be lower and thus, making sulforhodamine B a better fluorescent dye. Background fluorescence of the medium may indicate that vesicles have burst or that droplets did not form vesicles upon crossing the interface during the pull-down process. The observed vesicles in sample slides are often polydisperse, filled-with substructures, and often smaller than 50μm which were the observed diameter of the inverted emulsion. Thus, the observations seem to suggest bursting or dividing vesicles. The main source of this may very well be the centrifugation to pull through vesicles in which high forces may strain and cause loss of vesicle stability, or it may be a lack of osmotic matching between the inner and outer aqueous phases.

We observe multiple vesicles containing Janus particles and other substructures (Supplemental information 2). Upon addition of hydrogen peroxide the Janus swimmers exhibit rapid, random translational motion. This distinguishes them from surrounding substructures, which do not follow similar movements upon addition of hydrogen peroxide. In addition, we see in brightfield images that Janus particles seem to alternate between dark and light, which is the optical result of particles half-coated in platinum. The vesicles are polydisperse and the internal environment of the vesicles is dynamic with substructures of oil or even smaller vesicles enclosing high concentrations of fluorescent dye.

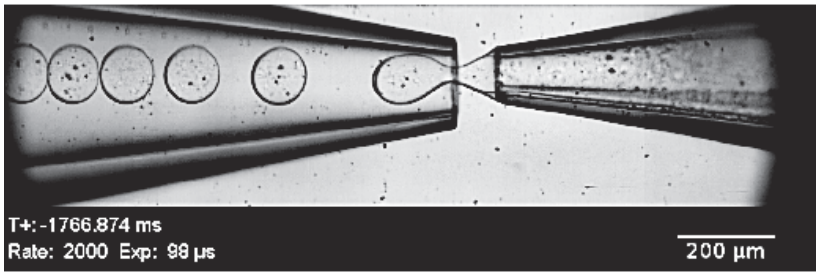

Figure 3. *Brightfield image of tapered ends of the glass capillaries forming double emulsios. The dark spots inside the double emulsions are polystyrene particles.*
The double emulsions are vesicles with a layer of oil (chloroform and hexane) between the phospholipid layers.

We see inverted emulsion moving from the right, injection capillary across the juncture and into the left, collection capillary (Figure 3). There is flow from an outer aqueous phase that shears and pinches off double emulsion droplets in a similar manner to that of the oil phase in the PDMS microfluidic device. The dark spots in the images are polystyrene particles that are encapsulated in the double emulsions. These double emulsions become vesicles when the chloroform-hexane layer evaporates. However, it is precisely this process that leaves a permanent oil scar on the phospholipid membrane, which makes accurate digital holography difficult; it interferes with successful tracking of encapsulated particles. Despite this, we have shown that particle encapsulation is possible with multiple techniques.

We have developed a method of creating relatively clean and stable vesicles via PDMS microfluidics, subsequently encapsulated Janus particles, and observed swimming or faster-than-diffusion motion of these particles. With the glass capillary microfluidics, we have shown that particle encapsulation is possible with other methods, which facilitates future endeavors to actively employ a variety of techniques to encapsulate motor particles. In the vein of studying cellular transport rates, further study of the physics of the effect of aggregate motion of motor particles will allow us to answer questions about how such mechanics affects rates of many chemical reactions such as protein transcription, DNA synthesis, and more. It seeks to answer how a cell is able to function given such a crowded internal environment in which crowding would seem to hinder, not facilitate, efficient transport of cellular material on reasonable time scales. Understanding the underlying physics will enable scientists to exercise greater control and mastery over different elements in the construction of an artificial cell.

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Resources

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