

MICROFLUIDIC LIPOSOME GENERATION FROM MONODISPERSE DROPLET EMULSION—TOWARDS THE REALIZATION OF ARTIFICIAL CELLS

Yung-Chieh Tan (1), Kenneth Longmuir (2), and Abraham P. Lee (1,3)

(1) Department of Biomedical Engineering
University of California at Irvine
Irvine, CA.

(2) Department of Physiology and Biophysics
University of California at Irvine
Irvine, CA.

(3) Department of Mechanical and Aerospace
Engineering
University of California at Irvine
Irvine, CA.

We report here for the first time that a hydrodynamically actuated microfluidic device, capable of precisely controlling the liposome self assembly process, has successfully synthesized liposome of defined size with potentially perfect encapsulation efficiency. This microfluidic platform will enable the control of concentrations of solvents, lipids, co-block polymers to alter size, shape, ionic strengths and composition of vesicle membrane to create a artificial cells with desired functional properties.

The emulsion generation microfluidic device consists of micro channels with inlet flow rates controlled by the Harvard 2000 pumps. The micro channels are fabricated by molding PDMS in SU-8 mold[1] and is designed to control the local shear forces between the co-flow of organic and aqueous boundary interface to generate monodispersed droplet emulsions.

To generate liposomes, naphthol Green dissolved in water and dioleoylphosphatidylcholine with fluorescently labeled dioleoylphosphatidylethanolamine dissolved in ether were infused into the channel (Fig. 1A). Water in lipid emulsions were generated as droplets at the ladder end of the channel (Fig. 1B).

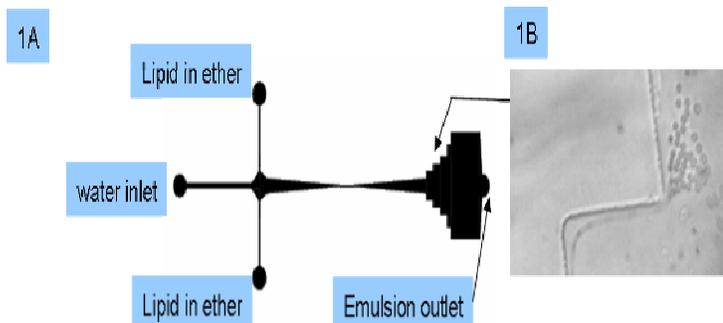


Figure 1A. Schematic drawing of the ladder channel
Figure 1B. Emulsion generated in the ladder design.

The resulting droplets when examined under the fluorescent microscopy indicated the abundance of lipids present on the water in ether emulsion (Fig. 2).

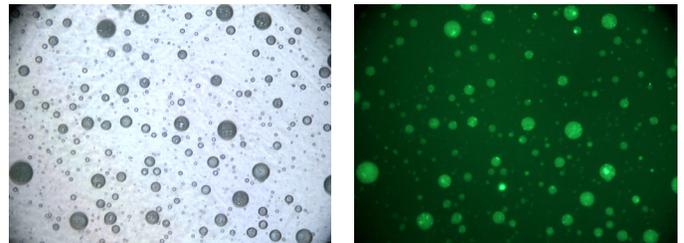


Figure 2. (left) Light microscopy of emulsion suspended in azure colored olive oil. The green color indicates water encapsulation. (right) Fluorescent microscopy of the same emulsion. The green fluorescent indicates the presence of lipids.

In order to verify the stability, the droplet emulsions were extracted, centrifuged and later suspended into olive oil and water. Unlike water in olive oil emulsions, which fuses easily with the surrounding droplet (Fig. 3), these lipid coated emulsion did not fuse with any others nor did it mix with DI water as indicated by the deep green color present inside the droplet. This verifies the formation of a liposome.

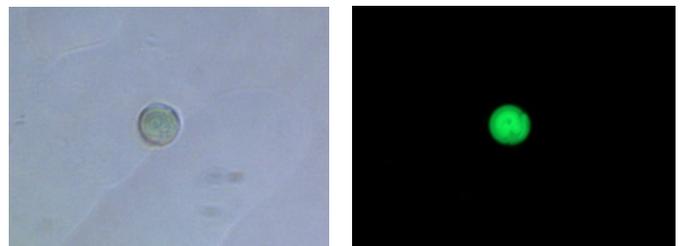


Figure 3. (left) Light microscopy of emulsion suspended in DI water. The deep green color indicates good dye encapsulation. (right) Fluorescent microscopy of the same emulsion. The green fluorescence indicates the presence of lipids.

Variations of channel designs resulted in uniform monodispersed droplet emulsions when the inlets are infused with olive oils and water. Through this bowtie channel (Fig. 4A), the emulsions were generated at the cross junction allowing the size of the droplet emulsion to be manipulated by the flow rate of the oil phase as indicated by (Fig. 5) that the radius varies inversely with the flow rate of the oil phase. Under this design emulsions as small as 0.5 μm in radius have been generated (Fig. 6).

The results presented here verified the certainty to create artificial cells through a microfluidic system capable of controlling and rectifying the size of emulsions. This system holds promise to create a user defined liposome with controllable parameters including colloidal size, shape, surface properties, membrane properties, high encapsulation efficiency, programmed cell to cell recognition properties and biocompatibilities. In medical field the applications includes gene and protein therapy, cancer chemotherapy, and other clinical treatments that involves targeted drug delivery mechanisms[4]. In computation science, individual liposome may be designed to have specific surface and integral property to form a unit that express live computational algorithms. In electrical sciences, cell batteries, conductors, and switches may be created from liposome with specific membrane permeability and conductance that respond to signal cues.

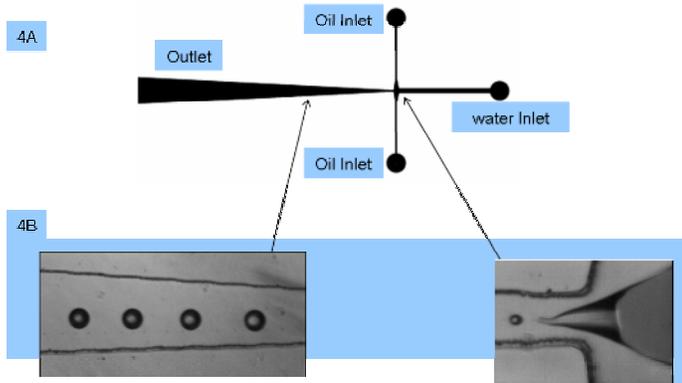


Figure 4A. Schematic drawing of the bowtie channel
Figure 4B. (right) Emulsion generated at the junction (left) Monodisperse droplet emulsion

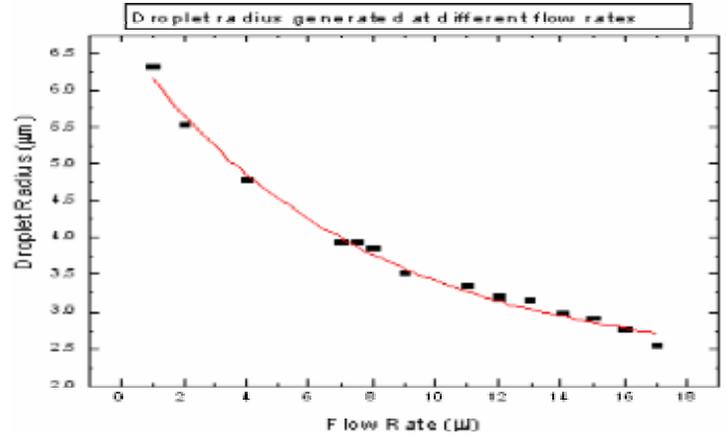


Figure 5. Droplet size decreases as oil flow rate increases.

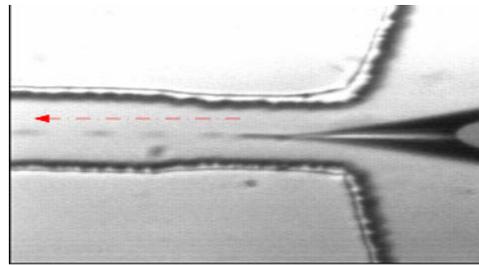


Figure 6. Emulsion droplets with size of <math><0.5 \mu\text{m}</math> in radius were captured. The arrow indicates the direction of droplet generation. The image is captured at 500 frames/s. The droplets are blurred due to flow speed exceeding range of capture rate

REFERENCES

1. McDonald, J. C., et al. (2000). "Fabrication of microfluidic systems in poly(dimethylsiloxane)," *Electrophoresis* **21**(1):27-40.
2. Tan, Collins and Lee (2002). "Monodisperse Droplet Emulsions in Co-flow Microfluidic Channels," (Submitted to Transducer conference 2003)
3. Tan, Collins and Lee (2002). "Controlled fission of droplet emulsions in bifurcating microfluidic channels," (Submitted to Transducer conference 2003)
4. Zhang, H. and Lu (1997). "Preparation of liposomes with a controlled assembly procedure," *Journal of Colloid and Interface Science* **190**:76-90