# DROPLET-BASED MICROFLUIDIC SYNTHESIS OF GIANT UNI-LAMELLAR LIPID VESICLES CONTAINING QUANTUM DOTS

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## ABSTRACT

This paper describes a new droplet-based microfluidic platform for synthesis of small sized (~10 μm) giant uni-lamellar vesicles (GUVs) containing quantum dots (QDs). To generate GUVs, we introduced an additional cross-flow to break vesicles into small size. Dimyristoylphosphatidylcholine (DMPC) was used in construction of self-assembled membrane, in which the solvent of DMPC were octanol and octanol–chloroform mixture. Consequently, we have demonstrated that the monodispersed GUVs with 7−12 μm diameter containing nano-sized QDs were successfully fabricated. The proposed synthesis method of cell-sized GUVs would be highly desired for the multipurpose drug delivery and the study of artificial cells.

## KEYWORDS

Giant uni-lamellar lipid vesicles, Microdroplet, Quantum dots encapsulation

## INTRODUCTION

Monodispersed lipid vesicles have been utilized as a drug delivery vehicle and a biochemical reactor. To generate monodispersed lipid vesicles in nano- to micrometer size range, an extrusion step must be included in conventional hand-shaking method [1]. Although droplet-based microfluidics is suitable to synthesize giant uni-lamellar vesicles (GUVs), previous method depends on sophisticated control of vesicle size and shows low encapsulation efficiency [2]. In addition, lipid vesicles as a drug carrier still need to be improved for effective encapsulation of the concentrated biomolecules such as cells, proteins and target drugs [1]. To overcome these limitations, this paper reports a new microfluidic platform for continuous synthesis of cell-sized (~10 μm) GUVs containing quantum dots (QDs).

## EXPERIMENTAL

Figure 1 shows an overall schematic diagram of GUVs synthesis in a microfluidic device. Droplets of an aqueous solution containing QDs were generated at a T-junction, in which the oil phase consisted of octanol and octanol:chloroform 3:2 (v/v) containing dimyristoylphosphatidylcholine (DMPC) as a lipid solution. Next, the control water flow from the additional inlet promoted secondary droplet breakup and formation of a lipid monolayer at the interface between oil and water phase of droplets. Furthermore, the generated lipid vesicles were allowed to stabilize at the expansion channel with an enlarged channel height (Figure 1b). The microfluidic device was fabricated through a two-step soft lithography technique using poly(dimethylsiloxane) (PDMS). The dimension of fabricated device was shown in Figure 2. The widths of the water and oil channel were 20 and 30 μm, respectively. The channel height of the T-junction and the expansion channel were 10 and 70 μm, respectively.

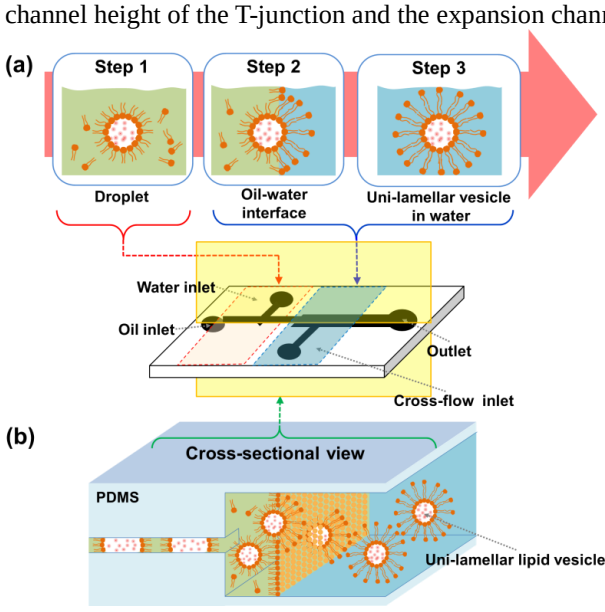


*Figure 1. Diagram of giant uni-lamellar vesicles (GUVs) synthesis on a microfluidic chip. (a) Essential three-steps of GUVs synthesis process. (b) The generated lipid vesicles were allowed to stabilize at the expansion channel, which was fabricated by two-step photolithography.*

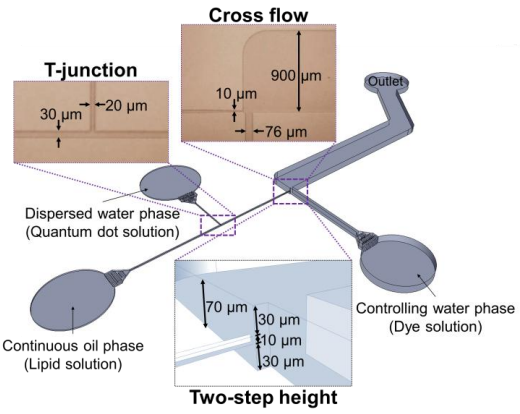


*Figure 2. Channel design and an enlarged view of the fabricated microfluidic device. The microfluidic chip is designed with two parts; the one is droplet generation part and the other is lipid bilayer construction part.*



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## RESULTS AND DISCUSSION

As capillary number (Ca = μv/γ) increases, the droplet size becomes smaller. In addition, the viscosity (μ) of the liquid is controllable according to the type of solvent used as oil phase. If the lipid concentration is high, surface tension (γ) is low. In this study, three factors of capillary number were exploited to find the smallest diameter of GUVs. Figure 3 shows the plot of the droplet diameter versus the flow rate ratio ($Q_o/Q_w$) on the droplet generation, where $Q_o$ and $Q_w$ are the oil and water flow rates, respectively. We confirmed the range of droplet diameter according to $Q_o/Q_w$. When the $Q_o/Q_w$ was adjusted at 5, 4, and 2, the droplet size was changed to 16.4 ± 1.4, 17.6 ± 2.2 and 20.3 ± 3.2 μm, respectively. On the basis of the Ca, it is clear that higher $Q_o/Q_w$ could make smaller droplets. To maintain a smaller and more stable droplet generation status, we selected an experimental condition of $Q_o/Q_w$ to be greater than 5.

We also characterized the effect of two solvents of octanol and octanol:chloroform 3:2 (v/v) to optimize the droplet size. The viscosity of lipid solvent acts as a significant role to determine droplet diameter. When the chloroform was utilized to generate the droplets, its high vapor pressure and swelling effect on PDMS would hinder the long-term production of vesicles within a microchannel. On the other hand, the swelling effect on PDMS microchannel was not accompanied by octanol. Moreover, in order to improve the lipid solubility and increase lamellarity of lipid molecules, we used octanol and octanol–chloroform mixture for lipid solvent instead of chloroform (Figure 4a). As a result, the monodispersed droplets which were around 10 μm in diameter were successfully fabricated when the octanol–chloroform solution was used as a solvent (Figure 4b).

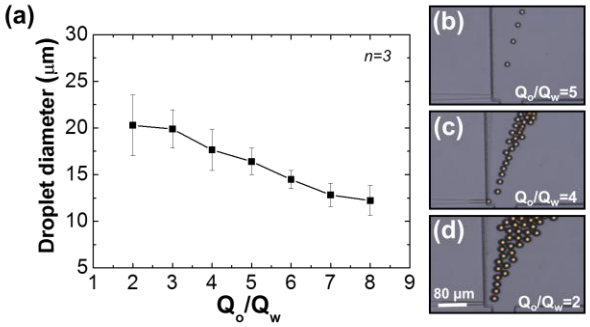


*Figure 3. (a) Plot of the droplet diameter versus the flow rate ratio $Q_o/Q_w$ on the droplet generation, where $Q_o$ and $Q_w$ stand for the oil and water flow rates, respectively. The microscopic images of the droplet break-up by the additional cross-flow when the ratio of $Q_o/Q_w$ was (b) 5, (c) 4 and (d) 2.*

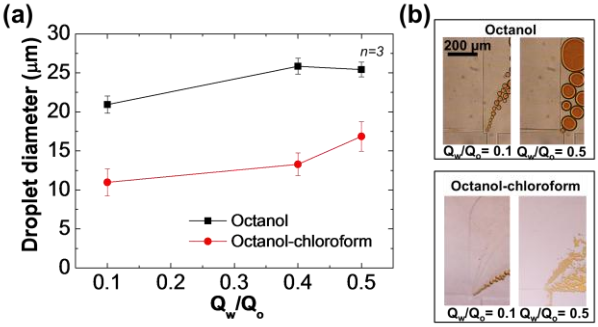


*Figure 4. Effect of solvent types on the droplet diameter. (a) Plot of the droplet diameter versus the flow rate ratio $Q_w/Q_o$. (b) Microscopic images of droplets in different solvents and flow rate conditions.*

Figure 5 shows the histogram of droplet diameter with respect to the lipid concentration from 2 to 14 mg/ml at the $Q_w/Q_o$ of 0.4. Because the lipid molecule reduces the surface tension of droplets, the size of the generated droplets was decreased as the lipid concentration increases. At the highest lipid concentration of 14 mg/ml, the size of monodispersed droplet was approximately 10 μm. However, at the lipid concentration of 6 and 10 mg/ml, the droplets were produced from a few micrometers to several tens of micrometers.

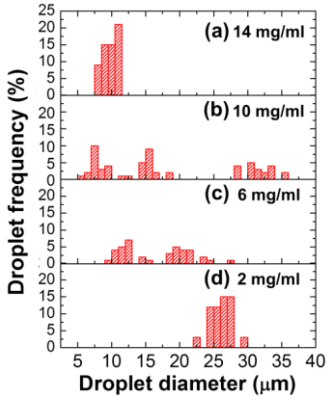


*Figure 5. Plot of the droplet size distribution depending on the lipid concentration of (a) 14, (b) 10, (c) 6, and (d) 2 mg/ml. Lipid concentration was related to droplet diameter and uniformity.*

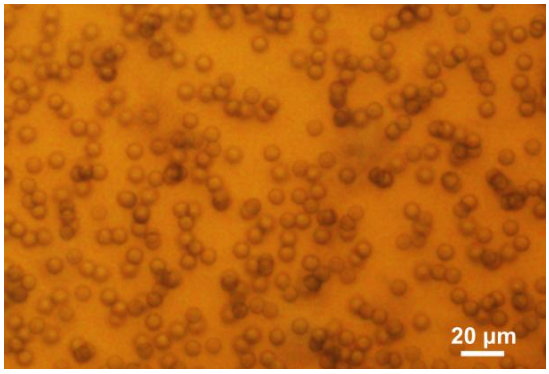


*Figure 6. Microscopic images of the fabricated GUVs containing quantum dots (QDs). Highly uniform-sized GUVs were synthesized with a diameter of 7.8 ± 1.0 μm.*

Under the fixed flow rate condition, the lipid concentration should be significantly considered to determine the droplet diameter and monodispersity.

As seen in Figure 6, a bright-field image of the GUVs which were stably stored in the microfluidic outlet. We successfully synthesized the small-sized GUVs (smaller than 10 μm) using the octanol–chloroform solution when the $Q_w/Q_o$ and lipid concentration were 0.4 and 14 mg/ml, respectively. The diameter of vesicles after breakup was found to be 7.8 ± 1.0 μm.

In order to confirm the efficient encapsulation to GUVs, we verified the encapsulation of QDs inside the lipid vesicle (Figure 7). The concentration of CdSe QD (with fluorescence emission peaks of 655 nm) solution was 10 nM. In Figure 7a, the QD-encapsulated GUVs transferred from the microfluidic outlet were stored on the glass slide at room temperature for 30 min. The fluorescence intensity profile on the line A–A' in Figure 7a was measured as shown in Figure 7b. Synthesized GUVs, which had uniform diameter, had constant relative intensity.

These methods would be practicable to control the concentration of QDs in lipid vesicle according to the concentration of QDs in injected solution. This possibility will overcome limitation of hand-shaking method about low encapsulation efficiency.

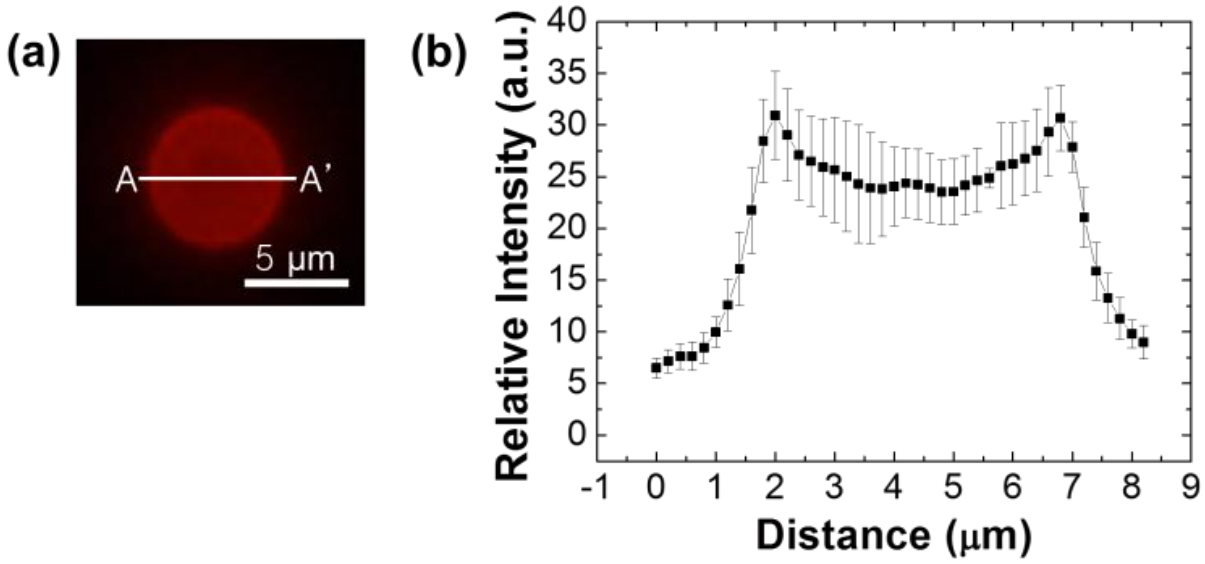


*Figure 7. (a) A microscopic image of a fabricated vesicle containing QDs. (b) The fluorescence intensity profiles of a vesicle across the line A–A' of panel a.*

## CONCLUSION

We have presented a method to synthesize GUVs containing QDs based on the droplet-based microfluidics. Uni-lamellar vesicles were continuously generated and split in the range of particular dimensions by an additional cross-flow. The fabrication method could be applied to collect highly monodispersed GUVs with high encapsulation efficiency, in the various experimental conditions such as the ratio of flow rates at T-junction, solvent type and lipid concentration. The fabricated GUVs containing QDs were easily collected from the outlet and stably stored. This paper suggested that droplet-based GUV synthesis technique would be applicable to make high efficiency of drug encapsulation and pseudo-cell study.

## ACKNOWLEDGEMENT

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