Droplet-based Microfluidic Synthesis of Lipid Vesicles Containing Quantum Dots

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KAIST
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액적 방식의 미세유체기술을 이용한 양자점이 함유된 지질소포체의 합성

Droplet-based Microfluidic Synthesis of Lipid Vesicles Containing Quantum Dots
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by
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KAIST

A thesis submitted to the faculty of KAIST in partial fulfillment of the requirements for the degree of Master of Science and Engineering in the Department of Bio and Brain Engineering. The study was conducted in accordance with Code of Research Ethics.

Daejeon, Korea
2011. 12. 6
Approved by

[Signature]
Professor Je-Kyun Park

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박 윤 희

위 논문은 한국과학기술원 석사학위논문으로 학위논문심사위원회에서 심사 통과하였음.

2011 년 12 월 6 일

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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 containing quantum dots. To generate giant uni-lamellar vesicles (GUVs), we designed a simple microfluidic chip combining a parallel-flow channel with cross-flow channel. Previously, microfluidic-based uni-lamellar lipid vesicles have been synthesized by using T-junction and focusing channels. These approaches rely on partial surface treatment of channel, serially flowed fluids and off-chip process, thereby causing high complexity and contamination of lipid vesicles. In addition, dimension of uni-lamellar lipid vesicles is ranged from 10 to 120 μm. Due to the dimension of microfluidic device, the small sized lipid vesicles less than 10 μm cannot be easily synthesized. Here, we fabricated a simple microfluidic chip consisted of T-junction and cross flow region. Dimyristoylphosphatidylcholine (DMPC) was used for the construction of self-assembled membrane, in which the solvent of DMPC was octanol and octanol–chloroform solution. In the condition of octanol-chloroform solution, smaller droplets (diameters below 10 μm) were easily synthesized. We also found that the concentration of lipid was act as a primary role to determine mono dispersity as well as generate smaller droplets. Consequently mono dispersed droplets with a mean diameter of 10 μm were generated at the lipid concentration of 14 mg/ml. On the basis of these results, a synthesis of GUVs with a size below 10 μm was demonstrated by using microdroplet based device. To confirm encapsulation efficiency of nanoparticles, we synthesized GUVs containing quantum dots. This result will be useful to develop a new microfluidic platform for simple, rapid, continuous quantum dots containing GUVs synthesizer.

Keywords: Droplet, Giant uni-lamellar lipid vesicles, Quantum dots encapsulation
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### Nomenclature

#### Alphabetic Letters

<table>
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<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$Q_o$</td>
<td>Flow rate of oil</td>
</tr>
<tr>
<td>$Q_w$</td>
<td>Flow rate of water</td>
</tr>
<tr>
<td>$V$</td>
<td>Characteristic velocity</td>
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</table>

#### Greek Letters

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>$\gamma$</td>
<td>Interfacial tension</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Viscosity of liquid</td>
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#### Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>DMPC</td>
<td>Dimyristoylphosphatidylcholine</td>
</tr>
<tr>
<td>GUVs</td>
<td>Giant uni-lamellar vesicles</td>
</tr>
<tr>
<td>MLVs</td>
<td>Multi-lamellar lipid vesicles</td>
</tr>
<tr>
<td>PDMS</td>
<td>Poly(dimethylsiloxane)</td>
</tr>
<tr>
<td>Q-dots</td>
<td>Quantum dots</td>
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Chapter 1. Introduction

1.1 Backgrounds

To increase the efficiency of anti-angiogenic response, dual angiogenesis factors of tumor are sequentially targeted [1-3]. In other words, the controllable sequential release system for dual and multiple drug delivery is highly needed to reduce side-effects of therapy [4, 5]. For this reason, a fully quantitative drug package is required to target therapy like cancer treatment [6, 7]. For example, liposome is widely used to encapsulate a drug and deliver it to a desired location. To satisfy all of the requirements for controllable sequential drug release, liposome based drug delivery techniques have been actively explored [8-11]. In particular, nanoscale multi-compartmented liposomes can be used to control dose and rate of drug delivery [12]. However, in the nanoscale fabricating system, drug packages are randomly structured owing to their tiny scale. Therefore, quantitative dosage of nanoscale liposome drug compartment should be determined by statistical deduction. In addition conventional synthesis method of nanoscale liposome has limitation of controlling encapsulated drug concentration.

Lipid vesicles are verified by lipid vesicle diameter and lamellarity [13]. The multi-
lamellar lipid vesicles (MLVs), one of the variety lipid vesicles, are expected to be suitable for controllable sequential drug release. In MLVs, gaps between each uni-lamellar vesicle are used for containers which include differently controlled nanomedicines. To increase the drug contents, it is more favorable to use relatively larger sized uni-lamellar vesicle. In particular, the giant uni-lamellar lipid vesicles (GUVs, > 1 μm) are able to observe directly under optical microscope [14] and handle using microscale mechanical systems. Therefore, controlled fabrication of GUVs-based MLVs is highly desired for multipurpose drug delivery process.

Conventional synthesis method of GUVs is started with preparation of lipid solution and evaporation of organic solvent, rehydration, vortex and extrusion or sonication processes are followed. This method is too bulky to control uniform micro size and encapsulate homogeneous concentration of nanomaterial. After vortexing of lipid solutions, randomly generated lipid vesicles should be replaced in an extrusion equipment [13]. This procedure caused some contamination possibility in making liposome-based drugs.

To overcome these limitations for lipid vesicle formation, a droplet-based microfluidic encapsulation method has been applied to the GUV synthesis [15]. In addition, droplet-based
microfluidic chips have been used to the synthesis of various biocompatible or organic materials [16-19]. And the droplet-based microfluidic techniques can also be used to encapsulate of biomolecules and control multiple emulsions [21]. Microfluidic-based droplet techniques are suitable to synthesize GUVs which need to delicate control of size and encapsulation efficiency.
Table 1. Review of droplet-based uni-lamellar lipid vesicle synthesis technologies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Approach</th>
<th>Number of inlets</th>
<th>Liposome size</th>
<th>Encapsulation efficiency</th>
<th>Organic solvent</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Discrete (partially on-chip)</td>
<td>2</td>
<td>27–55 μm</td>
<td>High</td>
<td>Ethanol/H₂O</td>
<td>Symmetric bilayer</td>
</tr>
<tr>
<td>[16]</td>
<td>Discrete (partially on-chip)</td>
<td>2</td>
<td>0–120 μm</td>
<td>High</td>
<td>Decane</td>
<td>Asymmetric bilayer</td>
</tr>
<tr>
<td>[17]</td>
<td>Continuous (fully on-chip)</td>
<td>4</td>
<td>50–100 μm</td>
<td>High</td>
<td>Chloroform/Toluene</td>
<td>Spatially control the surface wettability (Hard to fabricate)</td>
</tr>
<tr>
<td>[18]</td>
<td>Continuous (fully on-chip)</td>
<td>6</td>
<td>15 μm</td>
<td>Low</td>
<td></td>
<td>Hard to collect (Laser-assisted)</td>
</tr>
<tr>
<td>[19]</td>
<td></td>
<td></td>
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1.2 Research objectives

This work aims to propose a new droplet-based microfluidic platform for simple, rapid, continuous GUVs generation, which leads to the controllable encapsulation of quantum dots. All of the syntheses of GUVs are fully realized in one droplet-based microfluidic chip which has three fluid inlets. The microfluidic chip is designed with two parts; the one is droplet generation part and the other is lipid bilayer construction part. In order to apply GUVs to core uni-lamellar lipid vesicle of MLVs, an optimized condition for small sized (< 10 μm) GUV synthesis is investigated by several parameters such as chip design, dynamic condition of fluids and material property.

1.3 Thesis outlines

This thesis consists of six chapters. Chapter 1 includes motivation of this thesis and research objectives. In Chapter 2, design strategy and microfluidic chip dimensions are mentioned. In Chapters 3 and 4, experimental details and results with discussion are described, respectively. Last two parts have conclusion of thesis and future work.
Chapter 2. Design Principle

2.1 Design strategy

![Diagram](image)

**Figure 1.** Schematic diagram of GUVs synthesis process in a microfluidic device.

Figure 1 shows overall schematic diagram of GUV synthesis in a microfluidic device.

The device includes three inlets and one outlet, which is divided into three microchannel regions for sequential reactions. Each channel region is designed to be carried out by three sequential steps for the reactions. In step 1, the lipid molecules in the continuous oil phase wrap water droplets to form lipid single layer structures due to the amphiphilic property of
the lipid molecules. Next, between lipid molecules dissolved in oil phase and another water phase, an interface for allowing lipid bilayer formed in step 2. Then the lipid bilayers are constructed on the interface. In step3, droplet, which means a single lipid layer wrapped the water droplet, penetrates into water phase, and uni-lamellar lipid vesicles are synthesized to form giant uni-lamellar lipid vesicles (GUVs). Finally, the GUVs formed are replaced with water media. Consequently, these three steps can be continuously performed on each module in a droplet-based micro fluidic device.

**Figure 2**. Two types of GUV generation method using a microfluidic device.

In this study, the droplet generation part in step 1 is consisted of T-junction and focusing channel, as previously developed droplet techniques [20-22]. For steps 2 and 3, two types of design are considered as shown in Figure 2. The main difference of between the two designs
is the direction of additional water flow. The case 1 exploits a parallel flow concept based on the uni-lamellar construction using low flow rate. This design should be easy to integrate with other microfluidic components because fluid flow has the same direction and compact size chip. And the case 2 has a cross flow to the droplet stream direction. This cross flow has advantage which causes a secondary droplet break-up process. This advantage becomes to overcome photolithographical limitation (~up to 10 μm) of droplet formation.

Two-step photolithography is generally used to fabricate two types of GUV synthesis channel. The droplet generation part of step 1 makes a plug shape of droplet because of the narrow width and low height channel. The plug shaped droplets are fully filled in four walls of channel. Therefore, lipid molecules are not attached to the droplet surface which adheres to channel wall. Partially wrapped droplets are not stable for constructing lipid bilayer. The generated droplet should be built in spherical shape for inducing a lipid fully wrapping. Through two step photolithography, a microchannel with a large height difference can be achieved. Therefore, a cross-sectional area of enlarged channel is designed to be relatively large compared to droplet diameter, consequently droplet surface is efficiently wrapped by lipid, and the reaction between PDMS channel wall and lipid is decreased.
Figure 3. Schematic diagram of microfluidic device fabricated by two step photolithography.

The cross section A–A’ depicts a lipid single layered droplet formation in the expansion channel.
2.2 Bilayer formation via parallel flow

As shown in Figure 4, the parallel flow design has a focusing-type droplet generation part (a) and second lipid layer interface (b). Three types of fluids flowed in parallel at different flow rates. As the fluid was closer to center of the channel, the flow rate decreases. Therefore, centered dispersed water phase containing quantum dots solution maintains the lowest flow rate. The flow rate difference should be induced to maintain the stable water and oil interfaces. Through two-step photolithography, the channel height between dispersed water phase channel and other two channels is different. This channel design with a height difference needs to make a spherical shaped droplet.
**Figure 4.** Design of real scale parallel flow device. (a) water droplet generation part, (b) for GUV synthesis interface part, and (c) spherical droplet generation part showing big height difference.
2.3 Bilayer formation via cross flow

The cross-flow type channel has two parts of droplet generation (Figure 5). In order to minimize microdroplets (less than 10 μm), plug shaped droplet generation part and spherical droplet formation part are separated. The droplet generation occurs at the T-junction. According to the y-axis direction of T-junction channel, dispersed water phase vertically flows into continuous oil phase. Here the size of droplets is determined according to the channel dimension and flow rate ratio of orthogonally flowed two fluids. In this experiment, generated droplet at the T-junction has plug shape and sufficiently long. The cross-flow fluid is a water phase dye solution for visualization and uni-lamellar media change. Flow rates of two flows at the T-junction are not high for stable droplet generation. However, the flow rate of cross-flow is relatively high enough to break droplet and the droplet should be penetrated into the water phase with cross flow. Before droplet meets the water phase with cross flow, channel height should be increased to generate fully wrapped spherical droplet with lipid molecules.
**Figure 5.** Design of real scale cross-flow device: (a) T-juction design for water droplet generation, (b) cross-flow droplet break-up and GUV synthesis part, having height difference for spherical droplet generation, (c) expansion channel for interface area.
Chapter 3. Experimental

3.1 Two-step microchannel fabrication

The microfluidic device was fabricated through soft lithography technique using poly(dimethylsiloxane) (PDMS) (Sylgard 184 Dow Corning, Midland, MI). The spin coated negative photoresist SU-8 2010 (MicroChem Corp., MA) was exposed to ultraviolet (UV) through the mask with partially opened channel. The UV exposed SU-8 cross-linked patterns did not react with SU-8 developer. Through washing process with isopropanol alcohol, the embossed patterns were fully boned on silicon wafer. After first step photolithography, second step patterning for partially different channel depth was conducted by using the same process as the first step. For opposite side channel design, one step photolithography progress was carried out using second patterning mask with mirror symmetry design. Liquid phase PDMS and curing agent solution in the ratio of 8:1 was filled with the mold and cured on 60 °C hot plate for 40 min. After uncovering the silicon wafer from solidified PDMS device, oxygen plasma treatment was used to change the surface property from hydrophobic to hydrophilic. Both of the top and bottom part of device were irreversibly adhered by hydrogen bonding.
Figure 6. Schematic processes for two-step microchannel fabrication.
3.2 Device fabrication

3.2.1 Parallel flow device

The parallel flow device has four key dimensions. Dispersed water phase of quantum dots solution was flowed in channel with 20 to 40 μm in width, and its height is 30 μm. For the sheath flow of continuous oil phase of lipid solution, channel was higher than water phase channel. The height of oil channel was 80 μm and the width was 30 μm. The width of wider chamber for oil phase was 140 μm. And media changing water phase channel was 300 μm in width and 80 μm in height, as the same with oil phase channel. The dimension of PDMS device was 1200 μm in height and 800 μm in length.
Figure 7. Configuration of a PDMS device with parallel flow design.
3.2.2 Cross flow device

![Cross flow device diagram]

**Figure 8.** Configuration of a PDMS device with cross-flow design.

The cross flow microfluidic device contains a number of critical design factors (Figure 8). The dimension of T-junction channel and cross-flow channel was particularly significant. The T-junction of this microfluidic channel was determined to consider droplet size and the dimension ratio of two orthogonal channels. In this T-junction channel design, dispersed water phase channel in y-axis had 20 μm width and 10 μm height. And continuous oil phase channel in x-axis had 30 μm width and 10 μm height. In the cross-flow part for smaller
droplet formation, three different dimension parts existed. The T-junction channel on $x$-axis became narrow toward the expansion channel. The narrowest width of the channel was 10 $\mu$m and 10 $\mu$m height. And enlarged channel had 900 $\mu$m width and 70 $\mu$m height. In the connecting point of T-junction part between pinched flow part, channel height was changed from 10 $\mu$m to 70 $\mu$m. And the dimension of PDMS chip was is 2000 $\mu$m high and 1500 $\mu$m length.

### 3.3 Materials

The continuous oil phase was solution of 1,2-dimyristoyl-snglycero-3-phosphocholine (DMPC, Sigma). Several solvents of DMPC, including chloroform (Sigma), 1-octanol (Sigma), and solution that mixed with chloroform and octanol were used. The dispersed water phase contained quantum dot and deionized water. For visualization of droplet under halogen lamp, water phase was mixed with yellow, green and red food dye.

For the control test, mineral oil (Sigma) was used. And quantum dots (Qdot®655 goat F(ab’)$_2$ anti-human IgG conjugate) was used in encapsulation feasibility test.
3.4 Experimental setup

The PDMS chip was loaded on an inverted microscope (Eclipse TS100, Nikon, Japan). Three types of solutions were injected into the device using glass syringes (Hamilton Company, NV) equipped on a syringe pump (Pump 11 Pico Plus; Harvard Apparatus). A real-time the charge-coupled (CCD) camera (DS-2M, Nikon instruments Inc., NT) was used to capture the experimental images in a microfluidic device (Figure 9).

The images of experimental results were analyzed using image analysis program (ImageJ).
Figure 9. Experimental setup for droplet-based microfluidic GUVs synthesis chip
3.5 Experimental method

In droplet-based microfluidic system, generated droplet diameter is predictable through evaluating capillary number. The capillary number, \( Ca \), representing viscous forces versus surface tension acting across an interface between immiscible liquid like water and oil is defined as below:

\[
Ca \overset{\text{def}}{=} \frac{\mu V}{\gamma}
\]

(1)

where \( \mu \) is the viscosity of the liquid, \( V \) is a characteristic velocity and \( \gamma \) is the surface or interfacial tension between the two fluid phase. For high capillary number, smaller droplet is generated easily. In addition, the viscosity of the liquid is controllable according to changing solvent type. In this thesis, two types of solvent were used. And the characteristic velocity corresponds to the velocity of continuous oil phase in channel. The surface tension is affected by lipid concentration. If the lipid concentration is high, surface tension is low. In this study, three evaluation factors of capillary number were exploited to find the smallest GUVs diameter.
Chapter 3. Results and Discussion

4.1 Limitation of parallel flow device

**Figure 10.** Droplet generation at the parallel flow device. Red is water droplet, green is water phase in parallel stream and colorless fluid is lipid solution. (a) The smallest droplet diameter was over 30 μm. (b) Droplet could not adopt interface between oil and green water.

In this study, the parallel-flow device adopted a focus type using a sheath flow for droplet generation. With this device, the droplets have been produced over a size of 30 μm (Figure 10(a)). In addition, three different fluids were immiscible with each other at low flow rates. Because of the flow directions were the same in the chip, droplets
were not adhering to the second interface (Figure. 10(b)). Furthermore, droplets penetration rate is very low.

Creation of small GUVs is needed to synthesize multi-lamellar lipid vesicle. However, in the parallel flow device, we could not make smaller droplets due to the above stated reasons. From the next experiments, we used a cross-flow device.

4.2 Characterization of the droplet diameter

For characterization of droplet diameter in cross-flow device, the droplet diameter was measured according to several flow rate ratios ($Q_o/Q_w$). As shown in Figure 11, droplet diameter ranged from 10 to 20 $\mu$m. On the basis of the capillary number (Equation 1), it is clear that higher $Q_o$ condition made smaller droplets.

In this experiment, continuous oil phase was mineral oil and dispersed water phase and cross-flow solution were distilled water (see Figure 5).

For the smaller droplet generation, we selected experimental condition at the flow rate ratio ($Q_o/Q_w =$) of over 5.
Figure 11. Effect of the flow rate ratio $Q_o/Q_w$ on the droplet generation, where $Q_o$ is the flow rate of oil phase and $Q_w$ is the flow rate of water phase in T-junction. (a) Snapshot of droplet generation according to $Q_o/Q_w$. Droplet diameters decrease as $Q_o/Q_w$ increases. (b) Change of droplet diameter according to $Q_o/Q_w$, ranging from 10 to 20 μm.
4.3 Effect of lipid solvent on the droplet diameter

The viscosity of lipid solvent acts as a significant role to determine droplet diameter. In general, the chloroform has been used to make the droplets. However, high vapor pressure and swelling effect on PDMS leads to the limitation of microfluidic experiments. For this reason, octanol was used for lipid solvent instead of chloroform.

In this study, octanol and octanol–chloroform solution were used to investigate small GUV formation. As a result, the small droplets were formed when octanol–chloroform solution was used (Figure 12).

For the smallest droplet generation, we used octanol–chloroform solution at the flow rate ratio ($Q_w/Q_o$) of 0.4.
Figure 12. Effect of solvent type on the droplet diameter. (a) Snapshot of droplet generation at the two ratios of $Q_w/Q_o$. (b) Change of droplet diameters according to $Q_w/Q_o$ decreases. As expected, octanol–chloroform solution made smaller droplets.
4.4 Effect of lipid concentration on the droplet diameter

In this experiment, we tested several lipid concentration conditions for droplet formation. At the flow rate ratio \((Q_w/Q_o)\) of 0.4, the lipid of oil phase causes decreasing surface tension of droplets. For this reason, as the lipid concentration increases, the diameters of generated droplets were decreased (see equation 1). As shown in Figure 13, the mono-dispersity showing small mean diameter of about 10 \(\mu\)m was obtained at the highest lipid concentration of 14 mg/ml. Meanwhile, at the lowest concentration condition of 2 mg/ml, mono-dispersed droplets with a mean diameter of 25 \(\mu\)m were produced. At the lipid concentration conditions of 6 or 10 mg/ml, a half of the droplets were smaller size than droplets produced from the lipid concentration of 2 mg/ml. Compared with 14 mg/ml condition, diameter of generated droplets was heterogeneous at 6 or 10 mg/ml. Due to the diameter of generated droplet in the T-junction was synchronized with cross-flow to the homogeneous droplet break-up. This result indicates that synchronized effect was very important for control of mono-dispersity.
Through these results, an optimum condition for smaller GUV formation was found to be the lipid concentration of 14 mg/ml.

**Figure 13.** Droplet size distribution depending on lipid concentrations varied from 2 to 14 mg/ml.
4.5 Giant uni-lamellar lipid vesicles for multi-lamellar lipid vesicles

Figure 14. Typical GUVs obtained from the center region of expansion channel.

We synthesized the smallest (~10 μm) GUVs for core of multi-lamellar vesicles at an optimized condition. To obtain these results, we optimized several experimental conditions such as, flow rate ratio, solvent type and lipid concentration. Flow rate ratio ($Q_w/Q_o$) of 0.4, octanol–chloroform (3:2) solvent, and the lipid concentration of 14 mg/ml were chosen to obtain the best results. As shown Figure 14, generated GUVs were included in yellow water phase showing large droplets. It seems that large droplet was generated by interfacial tension between cross-flow water and octanol–chloroform
solution. It was also shown that large droplet had inner vortex dynamics. This vortex
effect prevents GUVs from sticking to each other. The diameter of GUVs was found to
be $7.8 \pm 1.0 \mu m$.

4.6 Quantum dot encapsulated giant uni-lamellar lipid vesicles

For measuring nanoparticle encapsulation efficiency of droplet-based GUVs, we
demonstrated quantum dots encapsulation inside the lipid vesicle. The concentration of
encapsulated quantum dots was 10 nM. Figure 15 (a) shows quantum dot encapsulated
GUVs which flowed along the channel. In Figure 15 (b), GUVs were transferred to the
glass slide from EP tube after 30 min. The concentration of encapsulated quantum dots
was measured by intensity of GUVs on a glass slide. Synthesized GUVs, which had
similar diameter, had constant relative intensity. It means that concentration of GUVs
could be regulated with quantum dots solution concentration and the diameter of
GUVs.

These results suggested that droplet-based GUVs synthesis technique was
applicable to make higher drug encapsulation efficiency.
Figure 15. Quantum dots(Q-dots) encapsulated GUVs. (a) Q-dots encapsulated GUVs flowed along the channel. (b) Synthesized GUVs were collected at other tube for 30 min. (c) Relative intensity of Q-dots encapsulated GUVs. The distance indicates a–a’ in the inset.
Chapter 5. Conclusions

We have developed a new microfluidic platform for synthesis of small-sized uni-lamellar vesicles. The giant uni-lamellar vesicles were generated continuously using droplet based microfluidic technology. For the generated uni-lamellar diameter characterization, flow rate ratio at the T-junction, solvent type, and lipid concentration should be controlled. When the range of flow rate ratio ($Q_w/Q_o$) was below 0.5, small droplets were generated stably. It was found that octanol–chloroform solvent was suitable for microfluidic based liposome synthesis. The lipid concentration was important factor to synthesize monodispered small droplets. Because the lipid concentration can be used to control the interface tension of droplets, droplets diameter was decreased as lipid concentration increases. When droplet size controlling through the concentration of lipid was synchronized with flow rate of cross-flow, homogeneity of the droplet was increased. In this thesis, we demonstrated giant uni-lamellar vesicles below 10 μm under high lipid concentration, and low flow rate ratio conditions.

The water cross flow was applied to secondary droplet break-up and lipid bilayer construction. The droplets generated at the T-junction were controllable by changing flow rate...
ratio. However, the dimension of droplet generation part was major factor to determine the droplet diameter. Secondary break-up force was needed to make the smaller size droplet. In addition, the cross-flow causes to generate smaller spherical droplets. And the cross-flowed waster and continuous oil phase created an interface for lipid bilayer assembling and media changing.

Through the characterization of the factors for stable uni-lamellar formation, we developed a new microfluidic platform for simple, rapid and continuous synthesis of GUVs containing quantum dots.

This giant uni-lamellar generator will be contributed to develop an on-chip multi-lamellar generator. Since different kinds of drugs could be encapsulated sequentially, multi-lamellar vesicles can be used for site-specific controlled drug delivery.
References


액체 방식의 미세유체기술을 이용한 양자점을 함유한 지질소포체의 합성

지질소포체는 약물전달 연구분야에서 각광받고 있는 전달 매개체이다. 최근 약물전달 연구분야 중에서도 다중약물전달의 역할을 수행하기 위해서 다층지질소포체(multi-lamellar lipid vesicles)의 필요성에 대두되고 있다. 본 연구에서는 다층지질소포체의 각각 지질층의 사이 공간에 전달되어야 할 서로 다른 약물이 함유되어 약물의 전달에 있어서 순차적인 조절이 가능한 약물전달 시스템을 구상해 보았다. 이러한 다층지질소포체는 기존의 지질소포체 합성 방법을 통해서 무작위적인 형태로 합성되기 때문에 크기와 함유한 약물의 농도를 조절하기가 어렵다. 또 기존의 방식을 통해서는 다중약물을 함유시키는 것이 거의 불가능하다. 그렇기 때문에 순차적 조절이 가능한 다층지질소포체를 합성하기 위해서 액체 방식의 미세유체기술을 적용하였다. 특히 다층지질소포체의 합성에 앞서 근간이 될 수 있는 단일지질이중층막으로 둘러싸인 지질소포체(Uni-lamellar lipid vesicles)의 합성의 연구 목표로 정하였다. 특히 안정적인 상태의 다층지질소포체를 형성하기 위해서는 최소한의 크기를 가지는 지질소포체의 합성을 필요로 하므로 10 μm 이하의 지질소포체를 합성하기 위한 연구를 진행하였다.

이전의 액체 방식의 미세유체기술을 이용한 지질소포체의 합성 연구들은 미세유체 칩(microfluidic chip) 상에서 지질단일층으로 둘러싸인 액체를 형성하고 이를 다른 튜브로
올겨서 지질이중층막을 형성하도록 한다. 이러한 방법은 완전한 지질이중층막을 형성하기 전에 오염이 되거나 형태를 잃어버릴 가능성을 갖게 된다. 앞에서 언급된 단점을 보완한 것이 하나의 첩 상에서 지질이중층막으로 둘러싸인 지질소포체를 형성하는 방식인데 기존의 연구에서는 마이크로 사이즈의 첩을 부분적으로 표면처리를 하는 정교한 과정이 필요했다. 또는 지질이중층막 형성에 필요한 용액들을 순차적으로 흘려주어 지질소포체를 합성하는 방식도 연구되었다. 이 방식은 필요한 용액의 종류가 많고 널어준 용액의 양에 한정하여 지질소포체를 형성한다는 한계점을 갖는다.

본 연구에서는 위에서 언급된 기존 연구들의 한계점을 극복하기 위하여 단순한 구조의 미세유체 집에서 10 \( \mu \)m 이하의 지질소포체를 합성하고 양자점을 함유하여 약물의 정량적 함유량 조절이 가능함을 보였다. 액적 방식을 이용하기 때문에 얼마나 작은 크기의 액적을 만들 수 있는지에 따라 지질소포체의 크기가 결정된다. 일반적으로 미세 액적 기반의 연구에서는 액적의 크기를 유추하는데 유속의 비율, 지질용액의 점도, 지질용액과 수용액사이의 계면장력 값을 이용한다. 본 연구에서는 위 세가지 요인들을 조절하면서 10 \( \mu \)m 크기 이하의 액적을 생성하는데 필요한 조건들을 결정하였다.

지질용액의 농축이 수용액의 농축보다 상대적으로 높아질수록 작은 액적을 생성한다. 또 지질용액의 점도가 높아질수록 작은 액적을 생성하는데 실험에서는 지질용액에서 사용되는 용액의 종류를 바꾸어가며 더욱 높은 점도의 용액을 찾을 수 있었다. 사용된 용액은 octanol-chloroform(3:2) 용액이다. 계면장력은 크기가 작을수록 작은 액적을 생성할 수 있는데 이는 지질용액에 용해되어 있는 지질의 농도를 높임으로써 계면장력의 크기를 줄일 수 있었다.
실험들을 통해 얻은 용매의 종류, 지질의 농도, 유속 조건들을 이용해서 10 \(\mu m\) 이하의 지질소포체를 합성할 수 있었다. 또 수용액에 양자점을 함유하여 지질소포체를 만든 결과 일정한 농도의 양자점을 함유한 지질소포체를 합성할 수 있었다.

결론적으로 하나의 미세유체 칩 상에서 특별한 표면처리 기술 없이 연속적인 지질소포체의 합성을 가능하게 하였다. 이는 이전 연구들의 한계점을 극복하여 더 작은 크기의 지질소포체를 합성한 데에 의미가 있다.

본 연구에서 디자인한 미세유체 칩은 또 다른 칩과의 연결이 용이하여 앞에서 언급했던 다층지질소포체의 합성에 응용하기에 용이한 것으로 사료된다. 특히 다층지질소포체를 합성하는 과정에서 각각의 지질이중층막을 순차적으로 합성하기 때문에 지질이중층막간에 우리가 원하는 약물을 함유시킬 수 있다는 점에서 매우 큰 장점을 가진다. 합성된 다층지질소포체는 순차적인 약물 전달에 응용할 수 있으리라 기대된다.