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SHEAR-FLOW DIRECTED SELF-ASSEMBLY OF DIACETYLENE LIPOSOMES OF CONTROLLED SIZE

Gang-June Kim, Simon Song*
Department of Mechanical Engineering,
Hanyang University
17 Haengdang-dong, Seongdong-gu, Seoul, 133-791, Korea
*simonsong@hanyang.ac.kr

Bora Yoon, Jong-Man Kim**
Department of chemical Engineering,
Hanyang University
17 Haengdang-dong, Seongdong-gu, Seoul, 133-791, Korea
*jmk@hanyang.ac.kr

ABSTRACT

This study addresses a novel microfluidic method to fabricate diacetylene (DA) liposomes and control their size. Polydiacetylene (PDA) is a conjugated polymer sensor with unique optical properties which are color (blue-to-red) and fluorescence change under the application of thermal or chemical stress. The liposome size distribution is important because they significantly affect the phase transition and the fluorescent emission. So far, DA liposomes are prepared by mixing of bulk phases. As a result, they are polydisperse in size and require post-processes to improve the size uniformity. Here, we report a novel strategy to generate uniform PDA sensor liposomes and control their size using a microfluidic chip and hydrodynamic focusing technique. PDA liposomes fabricated on a chip are analyzed using scanning electron microscope (SEM) and dynamic light scattering (DLS). The results present that the microfluidic strategy generates more monodispersed liposomes than a bulk method. It is also observed that the PDA liposomes size becomes smaller and their size distribution becomes more homogeneous as the flow rate ratio of inlet flows increases

INTRODUCTION

Liposomes, a class of vesicles that consist of spherical lipid bilayers, have attracted great interest for a wide range of chemical, biological, pharmaceutical and industrial applications due to their abilities to encapsulate and keep apart the aqueous component in its core and the lipophilic substance between two lipid layers. Among various liposomes, polydiacetylene (PDA), a conjugated polymer sensor, has unique optical characteristics that it changes its visible color and fluorescence upon the stimulus (Ji et al., 2003). Translucent diacetylene (DA) liposomes become non-fluorescent blue-phase PDA liposomes by exposing them to 254 nm UV light. Then, the blue PDA liposomes change their color to the “fluorescent” red when thermal or chemical stresses are applied, as shown in Figure 1. A PDA sensor uses these unique properties of the blue-to-red color and fluorescence change.

PDA liposomes are commonly fabricated through mixing of bulk phases, but it results in heterogeneous and polydisperse distribution in size (Kim et al., 2005). The polydispersity, however, deteriorates the quality of PDA sensor. For example, individual liposome emits different intensity in visible red and fluorescence in spite of applying the same stress to them. Therefore, additional post-processes required such as micro-filtering and sonication to improve the size uniformity even though they often cause the fracture of the liposome configuration. Thus, it is required to discover a novel strategy to form uniform PDA liposomes to improve the PDA sensor quality.

Recently, the size control of micro- or nano-particles has been actively studied using a microfluidic chip. Zourob et al. (2006) reported a micro-reactor using droplets to produce monodisperse polymer beads instead of using the suspension. In addition, Jahn et al. (2004 and 2007a) used a hydrodynamic focusing technique for better size distribution, and controlled the self-assembly process of liposomes by altering a flow rate and a flow rate ratio in a microfluidic chip.

We presented a microfluidic method to generate uniform PDA liposomes and control their size. They are generated by self-assembly in a microfluidic channel with hydrodynamic focusing. The flow rate ratio of injected solutions for sample and sheath flows appears to control the self-assembly of PDA liposomes. We show the detailed analysis on the liposome size using SEM and DLS.

EXPRIMENTAL SECTION

Preparation of Bulk PDA Liposomes

A common procedure for the preparation of DA liposome in aqueous solution is shown in Figure 2. In the first step, 10, 12-pentacosadiynoic acid (PCDA) powder is dissolved in a little amount of dimethyl sulfoxide (DMSO) in a test tube. This solution is dropped by a syringe into 80°C deionized (DI) water in a flask, and is thoroughly mixed for 20~30 min to yield a total lipid concentration of 1 mM. The resulting solution is sonicated for 30 min and is cooled at 4°C at least for 4 hrs. Then, polymerization is

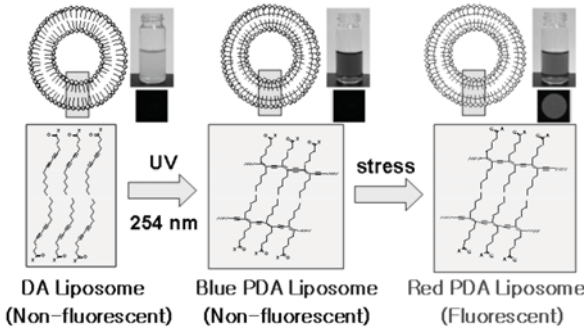

Figure 1. Phase change of PDA liposomes.

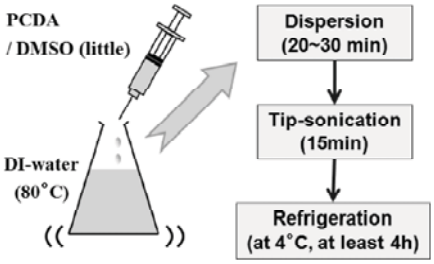

Figure 2. Preparation of bulk DA liposomes.

carried out at room temperature by exposing the solution with 254 nm UV light.

Preparation of Microfluidic Chip

Chip Fabrication. Figure 3 shows the schematic of experimental setup. We used standard soft lithography and molding technique (Xia and Whitesides, 1998) to fabricate a polydimethylsiloxane (PDMS) microfluidic chip. A standard lithography to make a mold for a microchannel pattern includes wafer cleaning, PR coating, soft-backing, UV exposure, hard backing, developing of PR, and washing and drying. PDMS (DC-184A, Dow corning) is thoroughly mixed with a curing agent (DC-184B, Dow corning) in a 10:1 ratio by volume, and the mixture is degassed in a vacuum chamber. After pouring the PDMS mixture on to the mold of the wafer, we cure it in an oven at 65℃ for 2 hours. Then, the microchannel-patterned PDMS substrate is obtained after it is peeled from the wafer. A chip fabrication is completed by bonding the PDMS susbtate with a glass slide after treating the PDMS with UV ozone. A chip has one main channel and three inlet channels: one for sample flow and the others for sheath flows. The cross section of a main channel is 100 μm in height and 50 μm in width. Typical flow rates are 0.1 ml/h in the sample channel and 0.3 ml/h in each sheath channel.

PCDA Solution. As presented in Figure 4, PCDA were dissolved in chloroform, and the solution is dried with nitrogen gas to form a dry film of PCDA. The dry lipid film was then redissolved in DMSO at 1 mM concentration of total lipid.

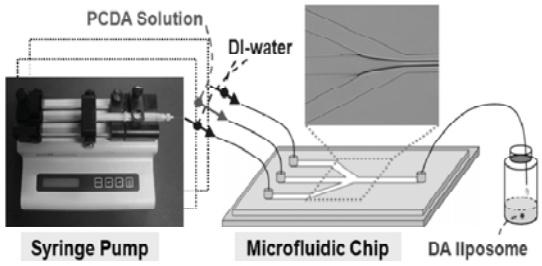

Figure 3. Schematic of experimental setup.

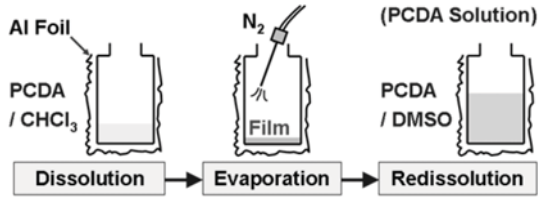

Figure 4. Preparation of PCDA solution.

On-Chip Liposome Fabrication. Using syringe pumps, a PCDA solution and 80℃ deionized (DI) water are injected into a sample channel and two sheath channels, respectively. After the three flows intersect at the junction of microchannels, DA liposomes are fabricated by self-assembly process at the interface between the PCDA solution and DI water, and are collected in a vial. Then, they are refrigerated at least for 4 h and are polymerized at room temperature by exposing them with 254 nm UV light. DA and PDA liposomes are characterized using SEM and DLS.

RESULTS AND DISCUSSION

Comparison of Microfluidic and Bulk Methods on Liposome Formation

Figure 5 presents DLS results of DA liposomes generated by both microfluidic and bulk methods. The flow rates were 0.1 and 0.3 mL/h in the sample and sheath channels for the former. The mean and standard deviation of DA liposome diameters are about 39 nm and 12 nm for the microfluidic method while they are about 88 nm and 31 nm for the bulk method. In addition, the diameter range of DA liposomes is approximately 30~50 nm in diameter for the microfluidic method, it is smaller than that of the bulk method. SEM images, as shown in Figure 6, evidence that the liposomes obtained on a microfluidic chip are smaller and more uniform than those formed in bulk. Note that the liposomes were exposed to 254 nm UV light to keep them in shape before taking the SEM images.

Effect of Flow Rate Ratio on Liposome Size

We investigated the effects of the flow rate ratio of inlet flows on a microfluidic chip. Figure 7 shows preliminary DLS results of DA liposomes for various flow rate ratios. As the ratio of sheath to sample flow rates increases, both the mean and standard deviation of liposome diameters

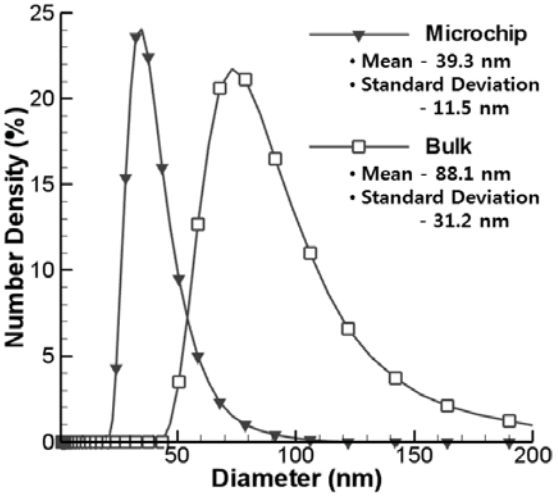

Figure 5. DLS results of DA liposomes generated in a microfludic chip (gradient) and in bulk (square).

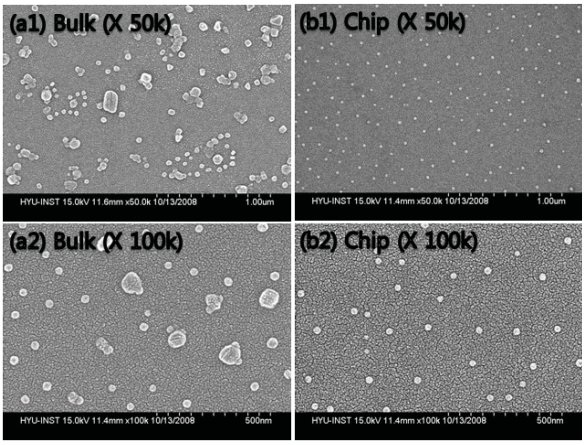

Figure 6. SEM images of PDA liposomes generated in a microfludic chip (a) and in bulk (b)

decrease, indicating that the liposome size becomes more homogeneous and can be controlled by varying the flow rate ratio. However, it requires further experiments to reach a firm conclusion.

CONCLUSION

We studied a novel microfluidic method to fabricate PDA liposomes and control their size. The DLS and SEM results reveal that the average size of PDA liposomes produced on a microfluidic chip is about 39 nm whereas a conventional bulk method results in PDA liposomes of 88 nm in diameter. Their size distribution is also smaller in the microfluidic method. In addition, we were able to control the PDA liposome size by varying the flow rate ratio of the

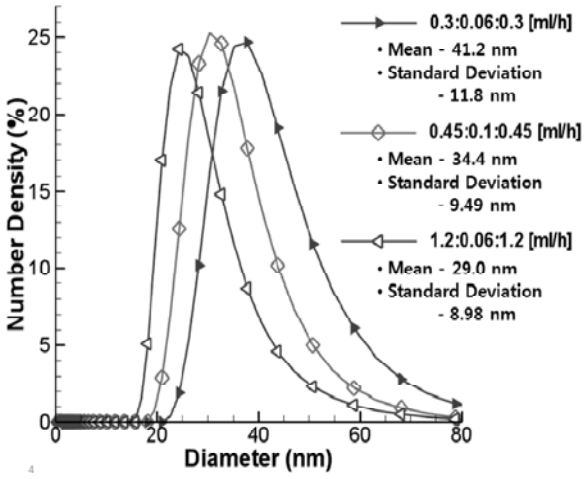

Figure 7. DLS results of DA liposomes generated in a microfluidic chip varied with flow rate ratio.

inlet flows on the microfluidic chip. Decreasing the ratio of sheath to sample flow rates allows to produce smaller and more monodisperse PDA liposomes in size. We expect that PDA liposomes produced by the microfluidic method would exhibit better sensor quality. This is under investigation, and will be included in the presentation.

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