SIZE-CONTROLLED FABRICATION OF POLYDIACETYLENE SENSOR LIPOSOMES USING A MICROFLUIDIC CHIP

Gang-June Kim,[1] Sumi Lee,[2] Jong-Man Kim,[2] and Simon Song[1]

[1]Department of Mechanical Engineering, Hanyang University, Seoul, KOREA

[2]Department of Chemical Engineering, Hanyang University, Seoul, KOREA

ABSTRACT

Polydiacetylene (PDA) is a conjugated polymer sensor with unique optical properties that it exhibits color (blue-to-red) and fluorescence changes under the application of thermal or chemical stress. When we produce PDA liposomes, it is important to make the size distribution of the liposomes uniform because they significantly affect the phase transition and the fluorescent emission. So far, PDA liposomes are prepared by mixing of bulk phases. As a result, they are polydisperse in size and require tedious 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.

KEYWORDS: Liposome, Size Control, Polydiacetylene, Sensor, Microfluidic Chip

INTRODUCTION

Polydiacetylene is a conjugated polymer sensor that changes its visible color and fluorescence upon stimulus[1]. By exposing them to 254 nm UV light, translucent diacetylene (DA) liposomes become non-fluorescent blue, and the blue PDA liposomes change their color to the "fluorescent" red when thermal or chemical stresses are applied, as shown in Figure 1. DA liposomes are commonly produced by mixing of bulk phases, resulting in polydisperse distribution in size[2]. The polydispersity probably deteriorates the quality of PDA sensor so that the individual liposome emits different intensity in visible red and fluorescence in spite of applying the same stress to them. Therefore, additional post-processes such as micro-filtering and sonication are required to improve the size uniformity though they often cause the fracture of the liposome configuration.

Recently, the size control of micro or nano-particles has been actively studied using a microfluidic chip. Zourob et al. reported a micro-reactor using droplets to produce monodisperse polymer beads instead of using the suspension[3]. In addition, Jahn et al. used a hydrodynamic focusing technique for improved size distribution, and controlled the self-assembly process of liposomes by altering a flow rate and a flow rate ratio in a microfluidic chip[4].

We present a novel microfluidic method to generate uniform PDA liposomes using hydrodynamic focusing on a microfluidic chip. By varying the flow rate ratio of injected solutions for sample and sheath flows, we control the self-assembly process

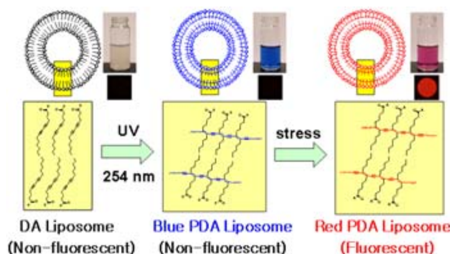

Figure 1. Phase change of PDA liposomes.

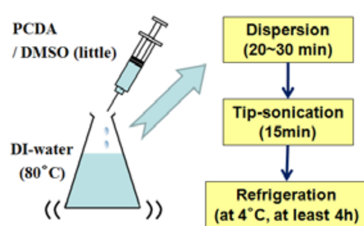

Figure 2. Preparation of bulk DA liposomes.

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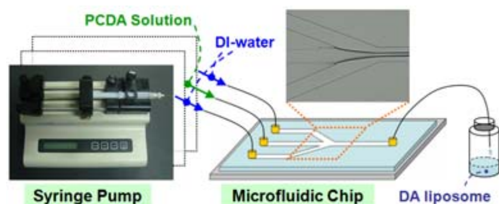

Figure 3. Schematic of experimental setup

Figure 4. PCDA Preparation.

of PDA liposomes, and examine the liposome size using SEM and DLS.

EXPERIMENTAL SETUP

Bulk Preparation of PDA Liposomes

Figure 2 shows a common procedure for the preparation of DA liposome in aqueous solution. First, 10, 12-pentacosadiynoic acid (PCDA) powder is dissolved in a little amount of dimethyl sulfoxide (DMSO). 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 carried out at a room temperature by exposing the solution to 254 nm UV light.

On-Chip Preparation of PDA Liposomes

Figure 3 shows a schematic of a microfluidic chip system to produce PDA liposomes. We used standard soft lithography and molding technique to fabricate a PDMS microfluidic chip with one main channel and three inlet channels. The cross section of a main channel is 100 μm in height and 50 μm in width.

To prepare a PCDA solution, PCDA are dissolved in chloroform, and the solution is dried with nitrogen gas to form a dry film of PCDA as shown in Figure 4. The dried lipid film is then redissolved in DMSO at 1 mM concentration of total lipid. Then, a PCDA solution and 80℃ deionized (DI) water are injected into a sample channel and two sheath channels, respectively. DA liposomes are generated by self-assembly process at the interface of the three flows, and are collected in a vial. They are refrigerated at least for 4 h and are polymerized at room temperature by exposing them to 254 nm UV light. DA and PDA liposomes are characterized using SEM and DLS.

RESULTS AND DISCUSSION

We examined PDA liposomes produced by a bulk method and on-chip method using SEM. Figure 5 evidences that the liposomes obtained on a microfluidic chip are smaller and more uniform than those formed in bulk. The diameter range of PDA liposomes is approximately 30~50 nm in diameter for the microfluidic method. Note that the liposomes were exposed to 254 nm UV light to keep them in shape before taking the SEM images. Figure 6 presents DLS results of PDA 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, respectively for the microfluidic method. The mean and standard deviation of PDA 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.

We investigated the effects of the flow rate ratio of inlet flows on PDA liposome size distributions. Figure 7 shows preliminary DLS results of PDA liposomes for various flow rate ratios. Both the mean and standard deviation of liposome diameters dramatically decrease until the ratio of sheath to sample flow rates increases to 7, and they show little changes for the further increase of the ratio. The increased rate produces the narrower sample stream so that the mixing of the sample and sheath solutions occurs quicker because of a short diffusion length. This indicates that the liposome size can be

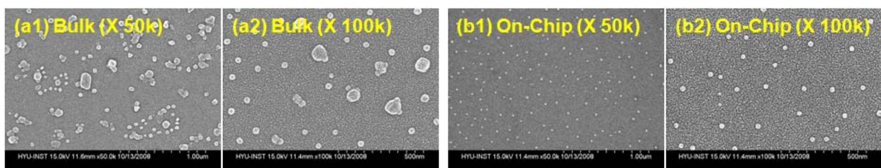

Figure 5. SEM images of PDA liposomes generated on chip (a) and in bulk (b)

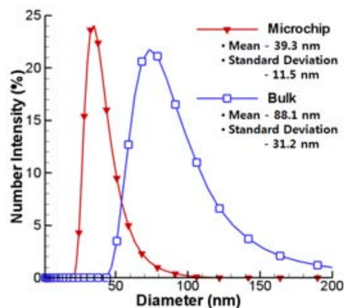

Figure 6. Size distribution of PDA liposomes generated on chip (gradient) and in bulk (square).

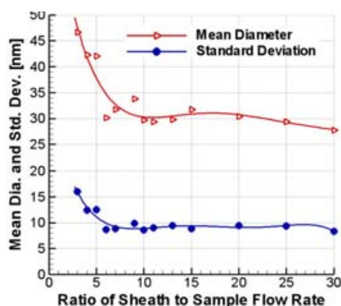

Figure 7. DLS results of PDA liposomes generated on chip varied with the flow rate ratio.

controlled by precisely varying the flow rate ratio or mixing time. However, it requires further experiments to reach a firm conclusion.

CONCLUSIONS

We studied a novel microfluidic method to fabricate PDA liposomes and control their size. The DLS and SEM results reveal that the size and its distribution of PDA liposomes are smaller when produced by the microfluidic method. In addition, we were able to control the PDA liposome size by varying the flow rate ratio of the inlet flows on the microfluidic chip. Increasing 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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REFERENCES

[1] E. K. Ji, D. J. Ahn, and J. M. Kim, The Fluorescent Polydiacetylene Liposome, Bulletin of the Korean Chemical Society, Vol. 24, pp. 667-670, (2003).
[2] J. M. Kim, Y. B. Lee, D. H. Yang, J. K Lee, G. S. Lee, and D. J Ahn, A Polydiacetylene-Based Fluorescent Sensor Chip, Journal of the American Chemical Society, Vol. 127, pp. 17580-17581, (2005).
[3] M. Zourob, S. Mohr, A. G. Mayes, A. Macaskill, N. Pe´rez, P. R. Fielden, and N. J. Goddard, A micro-reactor for preparing uniform molecularly imprinted polymer beads, Lab on a Chip, Vol. 6, pp. 296-301, (2006).
[4] A. Jahn, W. N. Vreeland, D. L. DeVoe, L. E. Locascio, and M. Gaitan, Microfluidic Directed Formation of Liposomes of Controlled Size, Langmuir, Vol. 23, pp. 6289-6293, (2007).