HIGH-THROUGHPUT SYNTHESIS OF NANOSCALE LIPID VESICLES FOR CONTROLLING SIZE AND SIZE DISTRIBUTION IN A CONTRACTION-EXPANSION ARRAY MICROCHANNEL

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ABSTRACT

We report a new synthesis method of liposomes with tunable size as well as enhanced monodispersity of the size distribution and high throughput in a contraction–expansion array (CEA) microchannel. Lipid vesicles were generated in the CEA microchannel by injecting lipids in isopropyl alcohol as a sample flow and phosphate buffered saline as a buffer flow, leading to spontaneous formation of liposomes. Generated lipid vesicles from the CEA microchannel showed narrower size distribution than ones from the linear microchannel. The throughput of the lipid generation in the CEA microchannel was ten times higher than previous works.

KEYWORDS: Contraction–expansion array microchannel, Liposome, High-throughput

INTRODUCTION

Manipulation of the liposome size and size distribution plays a pivotal role in implementation of drug delivery for *in vivo* applications. In order to control the size distribution of liposomes, the formation of lipid vesicles in a microchannel has been reported [1]. Even though the uniform size distribution of liposomes has been achieved in the microchannel, there are still demands to enhance the performance such as throughput and monodispersity. Recently, a contraction–expansion array (CEA) microchannel gives strong attention due to the simplicity of fabrication, no external forces and high throughput [2]. In such a CEA microchannel, Dean vortices lead to three-dimensional (3D) lamination by continuously splitting and redirecting fluid streams, resulting in enhancement of fluid mixing. In this paper, we report a new synthesis method of liposomes with tunable size as well as enhanced monodispersity of the size distribution and high throughput in a CEA microchannel.

PRINCIPLE

Figure 1a shows a schematic of the formulating liposomes in the CEA microchannel. The injected sample and buffer fluid experience transverse flows, Dean flows characterized with two counter-rotating vortices in the upper and lower plane of symmetry of the channel. When the fluid streams curve, they receive centrifugal force, resulting in movement of fluid to lateral space. Then other fluid streams move toward the empty space by mass conservation. The CEA microchannel generates centrifugal forces that enable to act as vortex flows along curved channels. The centrifugal forces above the critical Dean number (typically De ≈10) induce a secondary flow field where two vortex flows exist rotation in opposite directions each other. The induced Dean flow flows split and redirect the different fluid streams, leading to 3D lamination effect, which results in increase of interfacial area of the sample and buffer fluids. In addition, the high shear stress is induced in the CEA microchannel, especially in the contraction regions, due to the high pressure driven flow. The homogeneity of the lipid vesicles are related to these two kinds of effects. From the interplay between high shear stress and 3D laminating effect, the lipid vesicles are generated with small and narrower size distribution.

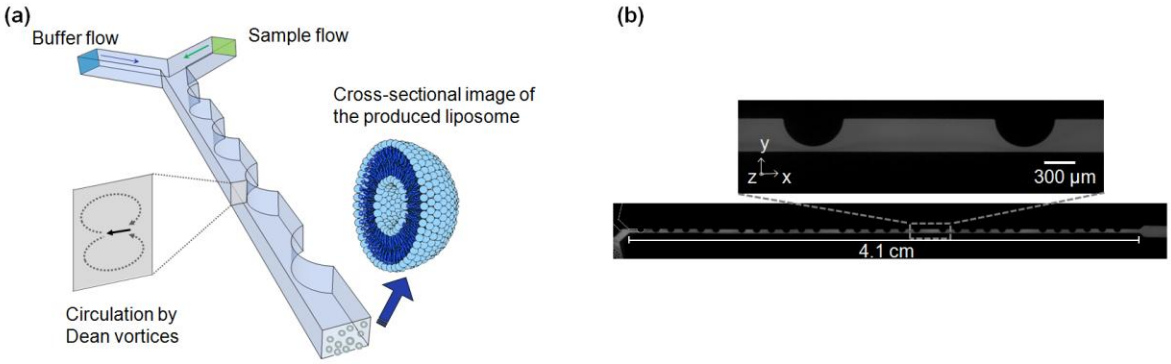

Figure 1. (a) A schematic of a microfluidic device to formulate liposomes. Sample flow was injected to upper inlet channel and buffer flow was introduced to lower inlet channel. Dean vortices occurred at the tip of the contraction region, thereby improved mixing between sample and buffer flow.(b) The micrographs of the fabricated CEA microchannel.

EXPERIMENTAL

As shown in Figure 1b, we fabricated a CEA microchannel in the shape of semicircular groove arrays by using poly(dimethylsiloxane) (PDMS) molding technique. The device has the 50 μm deep microchannel containing a uniform array of contraction–expansion microchannel. The channel has a width of 350 μm and 50 μm on the expansion and contraction region, respectively. The interval between each region is 600 μm. The rounded array microchannel is composed of 30 contraction–expansion regions. Lipid vesicles were generated in the CEA microchannel by injecting lipids—dimyristoylphosphatidylcholine (DMPC) and cholesterol in a molar ratio of 1:1—in isopropyl alcohol (IPA) as a sample flow and phosphate buffered saline (PBS) as a buffer flow, leading to spontaneous formation of liposomes.

RESULTS AND DISCUSSION

The size of lipid vesicles can be controlled by adjustment of the total flow rate. Figure 2a shows that increasing the total flow rate results in smaller vesicle radii. In addition, lipid vesicles formulated in a CEA microchannel produced more homogeneous than those obtained in a linear microchannel (Figure 2b). Increasing the total flow rate has a limiting effect, however, as more than 18 mL/h, which requires a longer microchannel to ensure complete mixing. The total flow rate can therefore be used to fine-tune the vesicle size distribution and increase liposome synthesis throughput.

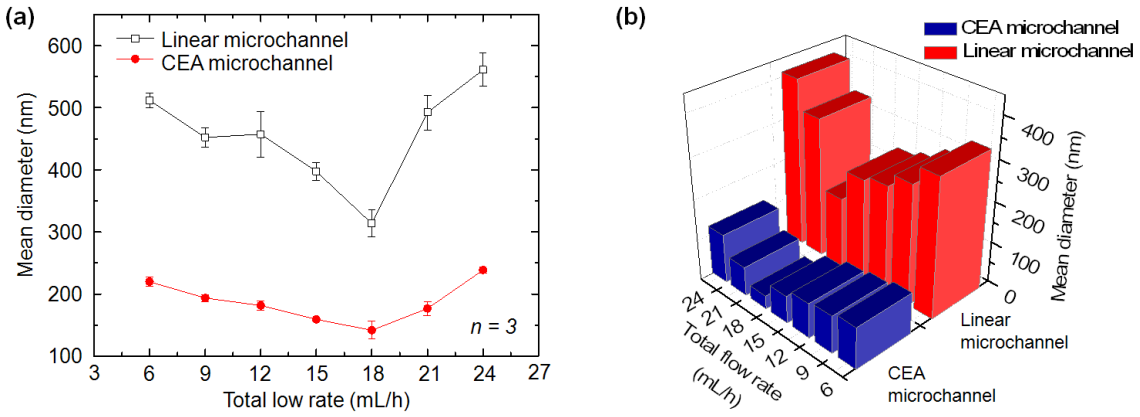

Figure 2. Effect of liposome size and size distribution on the total volumetric flow rate. For a constant ratio of sample to buffer flow as 1:9, the average liposome size decreases with increasing the total volumetric flow rate. (a) Liposome size distributions produced in the linear and CEA microchannel are shown. (b) Liposome size distribution as a function of total flow rate in both linear (red) and CEA (blue) microchannels.

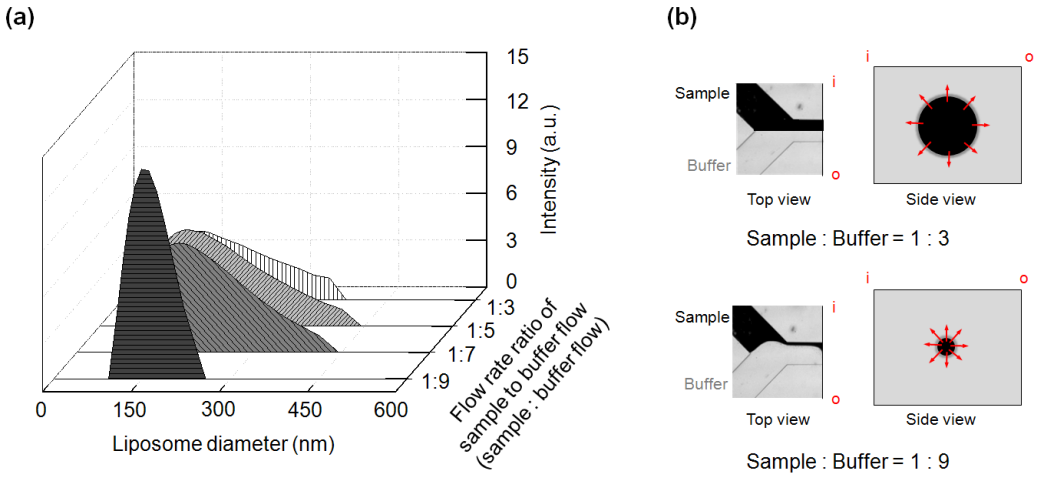

Figure 3. Controlled liposome size and size distribution as a function of the sample and buffer flow ratios at a constant total flow rate of 18 mL/h in the CEA microchannel. A decrease in sheath flow increases the vesicle size and size distribution. (b) Higher ratio of sample flow gives less IPA/PBS interface, making it difficult for large amount of sample flow to diffuse into buffer flow.

We investigated the influence of various flow ratio conditions on the liposome size and size distribution. As shown in Figure 3a, the liposome size and size distribution decrease when increasing the ratio of buffer flow. This is due to the fact that higher ratio of sample flow gives less IPA/PBS interface, making it difficult for large amount of sample flow to diffuse into buffer flow, thereby making it hard to synthesize into smaller liposomes by self-assembly as shown in Figure 3b. From this experiment, we can control liposome size and size distribution by changing the ratio of sheath flow.

Compared with the linear microchannel, liposome synthesis in the CEA microchannel yields smaller and monodisperse lipid vesicles as shown in Figure 4. In order to confirm the size distribution of liposomes, we obtained transmission electron microscopy (TEM) images at a flow rate of 18 mL/h in Figure 5. As we expected, generated liposomes in the CEA microchannel show more homogeneous than ones produced in the linear microchannel.

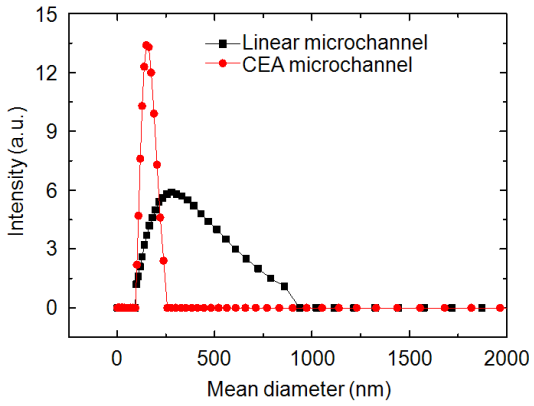

Figure 4. For a constant ratio of sample to buffer flow as 1:9, liposome size distributions in both of the linear and CEA microchannel at 18 mL/h. Liposomes generated in a CEA microchannel show narrower size distribution than ones obtained in a linear microchannel.

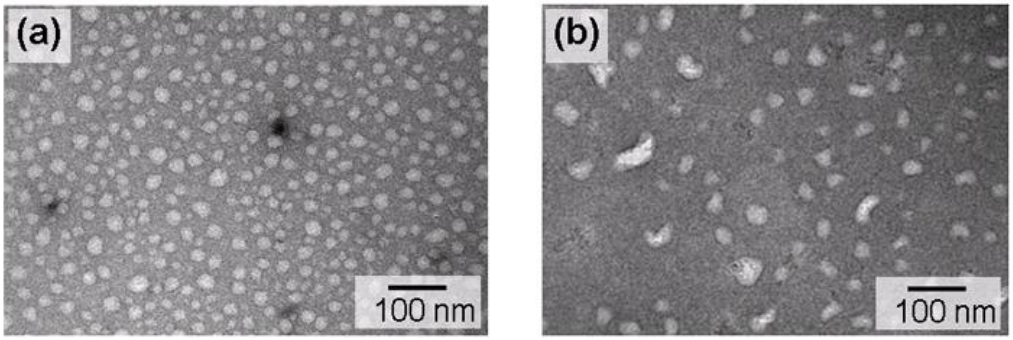

Figure 5. TEM images of generated lipid vesicles at a total flow rate of 18 mL/h in (a) the CEA microchannel and (b)the linear microchannel. For simple size comparison, dry TEM images were shown.

CONCLUSION

We have obtained the rapid generation of lipid vesicles with monodispersity in the CEA microchannel. The throughput of the lipid generation in the CEA microchannel was ten times higher than previous works [1]. We expect that this technology can provide practical application with mass production in the field of nanomedicine such as precise dosage delivery.

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