MICROFLUIDIC APPROACH FOR PRODUCTION OF LIPID NANOPARTICLES-BASED NANO MEDICINE

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ABSTRACT

This paper described a simple preparation method for small-size and monodispersed lipid nanoparticles (LNPs) by using microfluidic devices. The fundamental role and importance of chaotic micromixer in the microfluidic device was demonstrated. The suitable cycle number of chaotic micromixer was confirmed for precise controlling LNPs size with narrow distribution under the any flow rate conditions. In addition, LNPs containing siRNA was synthesized for evaluation of penetration efficiency via in vivo experiment. The PEGylated LNPs containing siRNA with a diameter of 30 nm could penetrate to the mouse parenchymal liver cells rather than the LNPs with a diameter of 50 nm.

KEYWORDS: Chaotic mixer, Lipid Nanoparticles, Nano Medicine

INTRODUCTION

Lipid nanoparticles (LNPs) are of the great interest as a nanometer-sized drug carriers. LNPs have remarkable features for drug delivery system such as a prolonged circulation time in the blood, low cytotoxicity, high accumulation efficiency into the tumor cells. In particular, LNPs in the size range of 30-100 nm are suitable for the cancer therapy. Therefore, precise control of the small-size LNPs with narrow distribution is a big challenge for the development of LNPs-based nanomedicine, because accumulation efficiency into the tumor cells strongly depends on the LNPs size [1]. Microfluidic device has been reported as a tool to produce monodispersed small-size LNPs[2]. However, the effect of fluid dynamics in the microfluidic device on the LNPs size has not been elucidated in detail. In this study, we investigated the role and importance of micromixer for the small size LNPs synthesis using the microfluidic device. Moreover, the LNPs containing siRNA was synthesized by using the microfluidic-based approach and was evaluated the penetration efficiency via in vivo experiment.

EXPERIMENTAL

Microfluidic devices were fabricated by double layer lithographic technique. The height of the first SU-8 layer was 79 μm and the second SU-8 layer was 31 μm. Figure 1 shows the schematic illustration of experimental system for producing the LNPs. To confirm the effect of the chaotic micromixer on LNPs formation behavior, the cycle number of chaotic micromixer was changed from 0, 6, 10, and 20 cycles. 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was dissolved in ethanol to obtain a concentration of 10 mg/mL lipid solution. Saline was used for the aqueous solution. These solutions were fed to the microfluidic device by using syringe pumps. The flow rate of the lipid solution was 0.1 mL/min and that of the aqueous solution was varied from 0.3 to 0.9 mL/min. The size of the LNPs was measured by dynamic light scattering. For in vivo experiment, we synthesized a multifunctional envelope-type nano device called MEND, which composed a pH-sensitive cationic lipid (YSK05), cholesterol, PEG-DMG by using microfluidic device. The PEGylated YSK-05 MEND was administered into the mouse liver cells to evaluate the effect of the LNPs size on the penetration and the gene knockdown efficiency. FITC-isoelectin B4, Hoechst 33342, and Cy5-siRNA were used for staining the parenchymal liver cells, liver sinusoidal endothelial cells, and YSK-05 MEND, respectively.
RESULTS AND DISCUSSION

LNPs was continuously formed by mixing of aqueous and lipid solutions in the microfluidic device. We found that the LNPs size was decreased with increasing the flow rate ratio (FRR; aqueous/lipid). Figure 2 shows the size distribution of LNPs formed in the microfluidic device at FRR of 3. The size distribution of LNPs was gradually shifted to the larger LNPs size with wider distribution by reducing the number of chaotic micromixers. On the other hand, the LNPs with a diameter of 30 nm formed at the FRR of 9 regardless of the cycle number of chaotic micromixer. 10-cycle number of chaotic micromixers was enough to form the small-size LNPs with narrow distribution for all FRR conditions, although the solutions were not completely mixed in the microfluidic device (data not shown). From these results, we propose the LNPs formation process as shown in below: (1) aggregation of lipid molecules, (2) formation of intermediate structures called bilayered phospholipid fragments (BPFs), and (3) transformation of BPFs to LNPs[3]. The transformation kinetics of the intermediate structures is essential to produce the small-size LNPs. Thus, the small-size LNPs can synthesize by the rapid dilution of ethanol concentration via the microfluidic device.

For in vivo experiment, the PEGylated YSK-05 MEND was synthesized by using the microfluidic device. The LNPs with a diameter of 30 and 50 nm were prepared to confirm the penetration efficiency into...
the mouse parenchymal liver cells. The size of LNPs was able to control precisely by the microfluidic device and the concentration of PEGylated lipid compared with the conventional method. Figure 4 shows the confocal microscopic images of the liver cells administered the LNPs. The YSK-05 MEND (red) with a diameter of 30 nm could penetrate to the parenchymal liver cells (blue) passing through the liver sinusoidal endothelial cells (LSEC, green) and showed a high gene knockdown efficiency. In contrast, the LNPs with a diameter of 50 nm remained into the LSEC (Figure 4 (b)). For these reasons, we consider that the microfluidic-based approach can easily produce the small-size LNPs and be expected to become a fundamental process for production of LNPs-based nanomedicines.

**CONCLUSION**

We demonstrated the role and importance of chaotic micromixer in the microfluidic device for producing the small-size of LNPs. The slight difference in the size of LNPs influenced the penetration efficiency into the mouse liver cells.

**ACKNOWLEDGEMENTS**

Part of this work was supported by the Hosokawa Powder Technology Foundation. M. Maeki acknowledges support from the Japan Society for the Promotion of Science (JSPS).

**REFERENCES**


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