

**Objective:** This study aims to fabricate a three-dimensional (3D) micromixer with high mixing efficiency using a 3D printing technique that offers high resolution as well as ease and rapidity of fabrication. The mixing performance of the fabricated micromixer is evaluated, and lipid nanoparticles are synthesized within the micromixer.

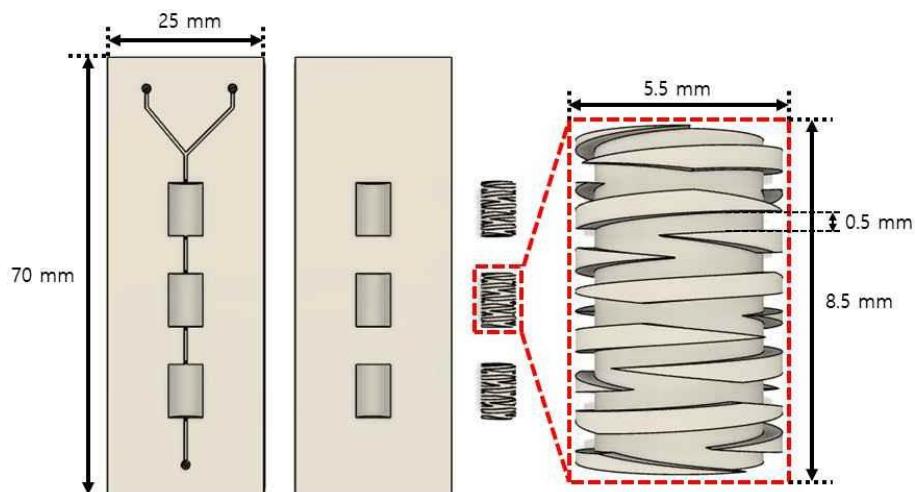
## Experimental Methods

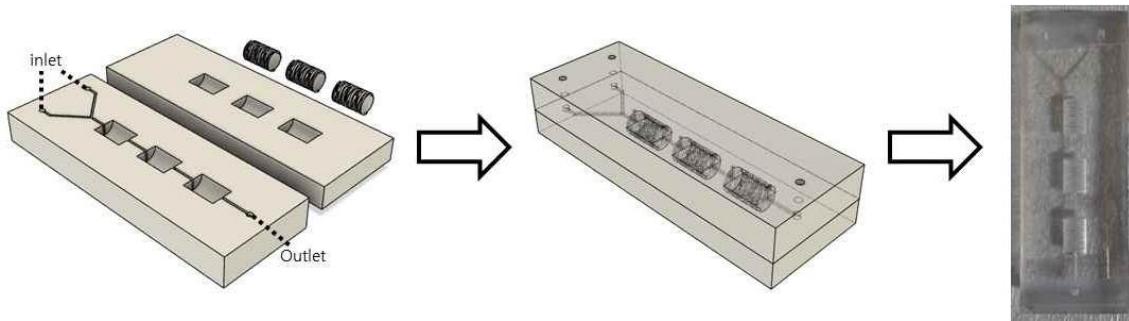
### 1) Fabrication of the Micromixer

The micromixer was designed using Fusion 360 software (Autodesk Inc., San Rafael, CA, USA). In this study, a three-dimensional coil-shaped pattern was designed to induce chaotic flow of two fluids. To evaluate the mixing efficiency of the coil-shaped pattern, straight channels were intermittently incorporated at regular intervals to allow visualization of fluid mixing using fluorescence microscopy.

Due to the characteristics of DLP printing, it is challenging to fabricate a single chip containing the coil-shaped pattern without supports; therefore, the coil pattern was printed separately. Subsequently, a main bottom chip to enclose the coil pattern, a top chip, and the coil pattern were fabricated independently. The components were assembled by pressing them together on a slide glass and bonded through thermal curing at 70 °C for 24 hours using residual resin after printing.

The channel diameter and depth of both the main bottom chip and the coil pattern were 0.5 mm. Each coil pattern was fabricated with a diameter of 5.5 mm and a height of 8.5 mm. A DLP printer (Phrozen Sonic Mini 8K, Phrozen 3D, Hsinchu City, Taiwan) and a clear resin (G217 Clear Non-yellowing Tough ABS-like 3D Printer Resin, Resione, Dongguan City, China) were used for fabrication. During printing, the printed structures adhered to the rough surface of the printer plate and were suspended upside-down. As curing occurred on the rough plate surface, even transparent resin resulted in uneven surfaces, reducing transparency and making internal fluid observation difficult. Therefore, a secondary resin coating was applied, and the chip was pressed using a slide glass and subjected to UV irradiation (INNO-CURE 850, Lichtzen, Korea) for secondary curing, producing a transparent chip suitable for internal observation.





## 2) Evaluation of Mixing Efficiency

To evaluate the mixing efficiency within the micromixer, two different fluorescent dyes were used: green fluorescent dye (Bright Dyes® FLT Yellow/Green Liquid, Kingscote Chemicals, Ohio, USA) and red fluorescent dye (Bright Dyes® FWT Red 25 Liquid, Kingscote Chemicals, Ohio, USA), each mixed with distilled water. The two fluids were introduced into the micromixer inlets at flow rates of 3, 5, 10, 30, and 50 mL/h. Syringe pumps (KDS 100, KD Scientific, Boston, MA, USA) and Tygon® tubing (1/32 inch I.D. × 1/16 inch O.D.) were used for fluid delivery.

The flow within the micromixer was imaged using a fluorescence microscope (IX71, Olympus Co. Ltd., Tokyo, Japan) at multiple locations along the channel. Fluorescence intensity profiles were obtained from the images using ImageJ® software. The mixing efficiency at each location was then calculated according to the following equation:

$$\eta = 1 - \sqrt{\frac{1}{N} \sum_{i=1}^N \left( \frac{I_i - I_i^{CM}}{I_i^{CM}} \right)^2}$$

$\eta$  = the mixing efficiency,  $N$  = the number of pixels in a given region,  $i$  = the pixel index,  $I_i$  = the intensity of pixel  $i$ ,  $I_i^{CM}$  = intensity at pixel  $i$  for a fully mixed state

## 3) Synthesis of LNPs in the Micromixer

In microfluidic environments, lipid nanoparticles (LNPs) are typically formed by introducing a discontinuous phase containing lipids dissolved in ethanol and a continuous phase containing mRNA dissolved in a buffer solution, such as PBS, into the microfluidic chip. The optimal flow rate ratio between the continuous and discontinuous phases is reported to be 3:1.

In this study, the discontinuous phase was prepared by dissolving 0.25 wt% lecithin (Lecithin from Egg, TCI) in ethanol (ethyl alcohol 99.9%, Duksan Chemical). To evaluate the effects of mixing efficiency on LNP size and dispersity, DPBS (Dulbecco's Phosphate Buffered Saline, Welgene) without nucleic acids was used as the continuous phase.

## Results

### 1) Mixing Efficiency in the Micromixer

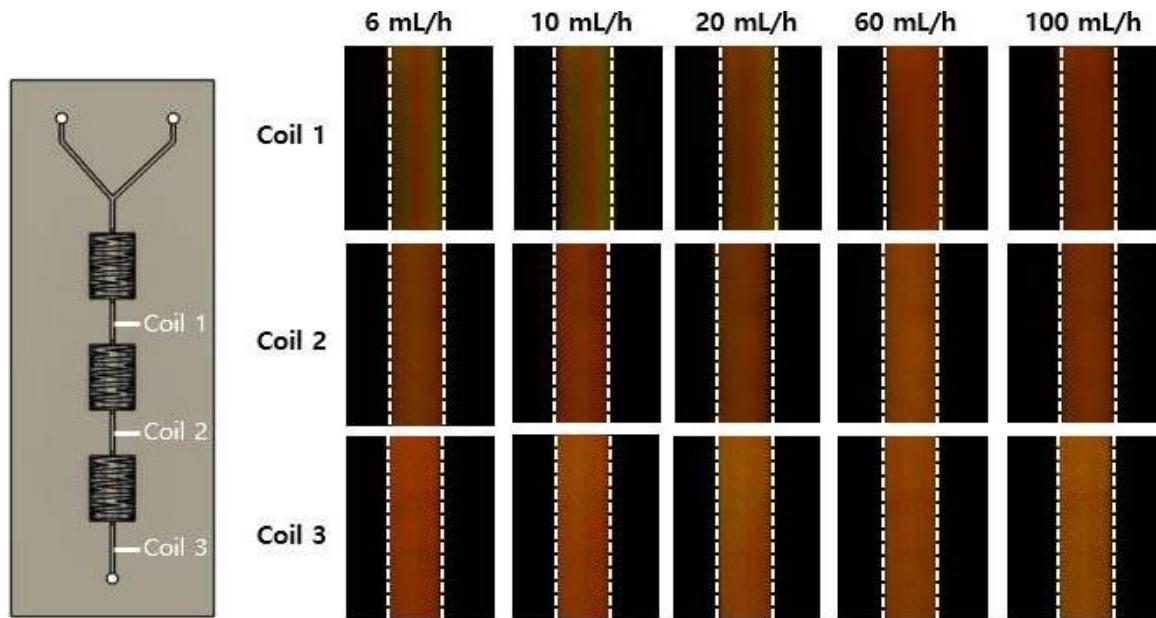


Figure 1. Fluorescence images of green and red dyes at Coil 1, Coil 2, and Coil 3 under different flow rates

As shown in Fig. 1, the green and red fluorescent dyes became progressively more mixed from Coil 1 to Coil 3 along the coil-shaped pattern. In addition, higher flow rates resulted in improved mixing efficiency.

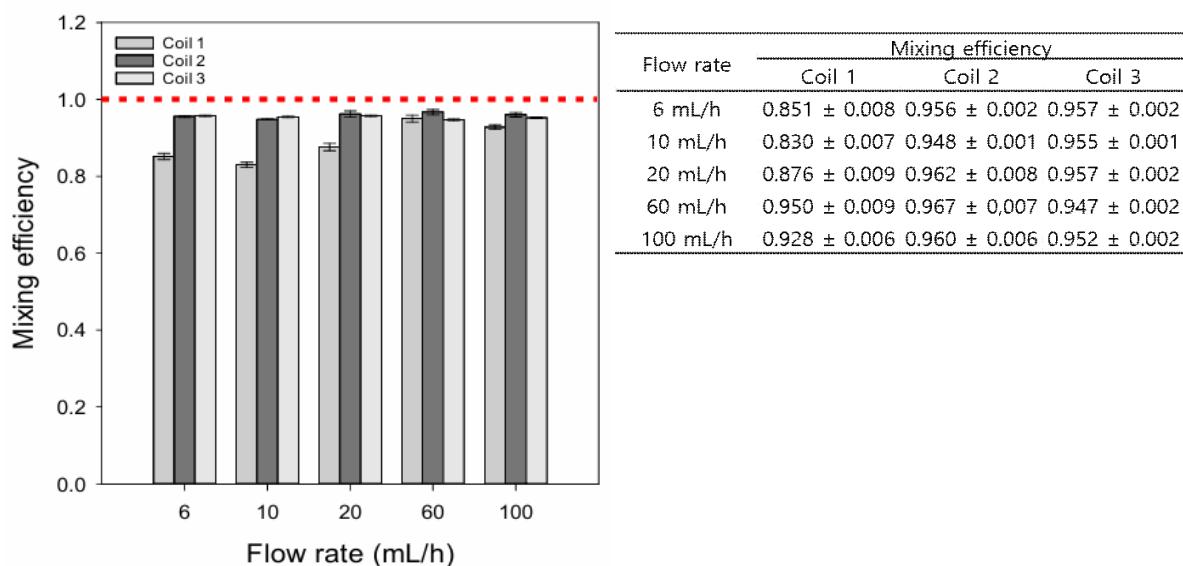


Figure 2. Comparison of mixing efficiencies at Coil 1, Coil 2, and Coil 3 under different flow rates

Mixing efficiencies at Coil 1 were  $0.851 \pm 0.008$ ,  $0.830 \pm 0.007$ , and  $0.876 \pm 0.009$  for flow rates of 6, 10, and 20 mL/h, respectively (Fig. 2). At higher flow rates of 60 and 100 mL/h, Coil 1 alone achieved efficiencies of  $0.950 \pm 0.009$  and  $0.928 \pm 0.006$ . Following Coil 2, the micromixer exhibited consistently high mixing efficiencies above approximately 0.95, independent of the flow rate.

## 2) Size and Polydispersity of LNPs Synthesized in the Micromixer

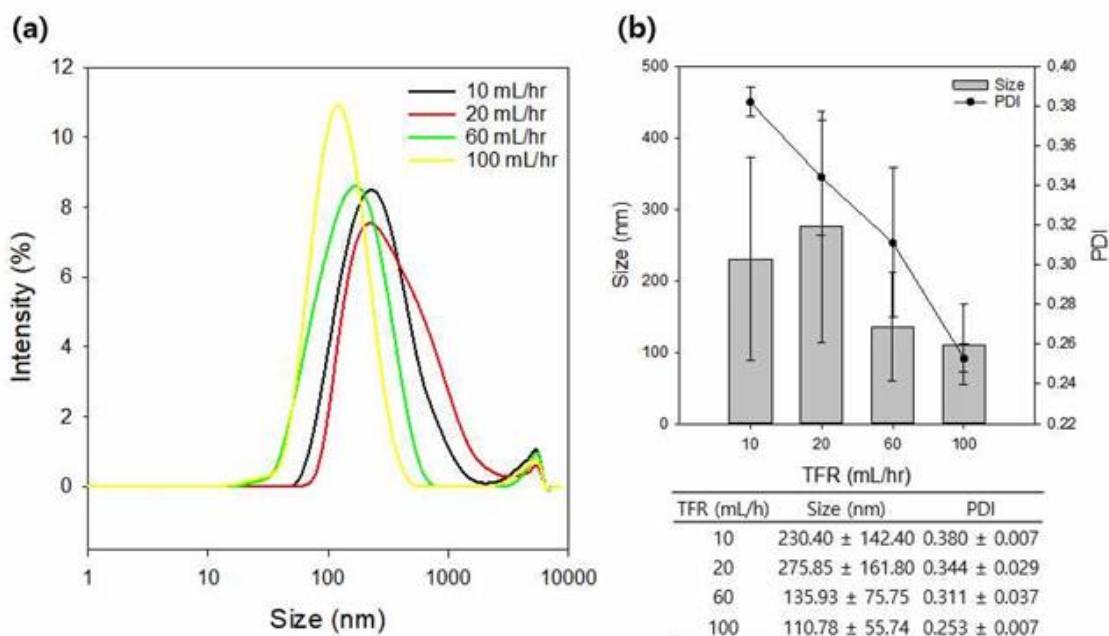


Figure 3. Size and polydispersity index of LNPs at different flow rates

The size and polydispersity index (PDI) of the LNPs decreased with increasing flow rate (Fig. 3). The PDI was slightly higher compared to previous studies, which is likely due to the chip being designed to maximize mixing efficiency rather than providing an optimal stabilization region for LNP synthesis, as well as non-ideal lipid conditions for nanoparticle formation.

## Conclusion

Among 3D printing techniques, the DLP method enabled precise fabrication of internal channels with a diameter of 0.5 mm, with an average printing time of approximately 40 minutes, allowing rapid chip production. This combination of high precision and fast printing facilitates the rapid and straightforward realization of complex microchannel structures. Furthermore, the use of transparent photopolymerizable resin allows real-time observation of fluid flow within the chip, which is effective for validating fluid simulations and collecting data, enabling applications in fluid dynamics research, biomedical experiments, and other fields.

The fabricated 3D coil-structured micromixer achieved a maximum mixing efficiency of 0.967. At low flow rates, a single pass through the short 8.5 mm coil segment already resulted

in high mixing efficiencies above 0.85, while at higher flow rates, efficiencies exceeded 0.93. This high performance is primarily attributed to the design of the coil structure, which induces vortices and chaotic fluid motion. As the fluid follows a curved path in the coil-shaped channel rather than flowing straight, centrifugal and Coriolis forces generate small vortices. These vortices increase the contact area between fluids and disrupt laminar flow, thereby enhancing mixing. The irregular coil pattern further promotes chaotic advection, increasing the frequency of fluid contact and remaining effective even under low Reynolds number conditions, which is more efficient than mixing in conventional straight channels.

Additionally, inserting straight channel segments within the coil structure temporarily stabilizes the flow before generating vortices again, and this repeated variation optimizes mixing efficiency. High mixing efficiency enables rapid and uniform mixing of lipids and water-soluble drugs, resulting in LNPs with small and uniform particle sizes. These structural features significantly enhance the reproducibility and stability of drug delivery systems. Moreover, high mixing efficiency promotes effective encapsulation of drugs within the lipid core. LNPs formed through uniform mixing exhibit excellent physical and chemical stability, as well as improved stability in biological environments.

At a total flow rate (TFR) of 100 mL/h, LNPs with an average size of 110 nm were produced, approaching the previously reported optimal size of 100 nm. Further optimization, such as higher flow rates or narrower channels, could allow more precise control over particle size.