Microfluidic Synthesis of Liposomes for Antigen Presentation in Cell Culture

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Introduction
Liposomes are vesicles composed of phospholipids forming a spherical bilayer. They are useful in medical applications, for they can be synthesized in an efficient and reproducible manner. Among alternative methods, synthesizing liposome using microfluidics provides the most reliable production of liposomes due to its superior control over mixing various components in a continuous manner (Figure 1). A series of experiments by changing operational parameters, namely, total flow rate (TFR) and flow rate ratio (FRR), was conducted to understand their effects on the size, polydispersity, and stability of resulting liposomes.

Methods
- Lipids in the following molar composition were combined with ethanol for a total concentration of 4 mg lipids/1 mL ethanol: 62.9% DOPC, 35.8% Cholesterol, 1.3% DSPE-PEG Biotin
- The lipids in ethanol were pumped in a central stream with two outer streams of phosphate-buffered saline (PBS) at their corresponding flow rates, causing hydrodynamic focusing to occur (Figure 2).
- Two microfluidic chips with vastly different chip geometries were compared for differing TFRs.
- Post processing of the liposomes included dialysis and filtration to ensure all ethanol and other possible contaminants are removed before placing in cell culture.
- Liposomes were characterized on a Malvern Zetasizer for size reported as Z-average and polydispersity.
- Total Flow Rate (TFR) = Sum of all flow rates
- Flow Rate Ratio (FRR) = (PBS flow rate) / (Ethanol lipid solution flow rate)

Results & Discussion
- Regardless of TFRs, higher FRRs yielded smaller Z-averages at low FRRs (below 15), which agrees with the previous report (Figure 3).2
- Contrary to the general expectation, higher FRRs yielded bigger Z-averages at higher FRRs (Figure 3).
- At such higher FRRs, the higher TFR yielded significantly bigger liposomes (Figure 3).
- A higher FRR for the same TFR yielded a higher PDI, which agrees with the previous report (Figure 4).
- The device with an extra layer for enhancement of mixing enabled creation of liposome with lower PDIs for similar FRRs (Figure 4).

Conclusions & Future Plans
Z-averages decreased as FRR increased only up to a certain FRR and the trend reversed above that threshold FRRs. In contrast, PDI increased as FRR increased for the entire range of FRRs tested. The extra layer in the microfluidic device for enhancement of mixing enabled creation of liposomes with lower PDIs. Next, the stability in size and PDI of the liposomes will be monitored. The liposomes of the selected sizes will be created for conjugation of antigens on their surfaces; and will be used in culture of immune cells to mimic the physiological antigen presentation.

References
1. Simulated graphics created with Biorender.

Acknowledgements
Immense gratitude to Dr. Kyung-Ho Roh, as well as the rest of the lab for their mentoring throughout this project. Thanks to Dr. Vogler and the Chemistry department for the use of the Zetasizer for characterization, and special thanks to Adelkunle Akinmole for Zetasizer training.

Table 1: Experimental Conditions (Flow Rates) and corresponding Z-averages and PDIs.