Development of microfluidic platform for efficient lipid formulation discovery of exosome-mimetic nanoparticles

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Introduction

❑Exosomes are nanovesicles of cellular origin, encapsulating bioactive molecules within lipid bilayers. They offer significant therapeutic advantages, including excellent biocompatibility and effective intercellular communication [1]. However, their widespread application has been constrained by intrinsic heterogeneity and scalability limitations [2]. To address these challenges, exosome-mimetic nanoparticles (ENPs), fabricated via reverse-Tesla microfluidic chips, present a promising alternative, as they allow for scalable production and stable CMC [3].

❑The necessity for disease-specific lipid formulations is underscored by the varying lipid composition ratios in exosomes, which depend on the parent cell type and functional activity [4]. A combinatorial library of ENPs, encompassing 2,978 possible discrete combinations, is essential for systematic exploration. Nevertheless, conventional manual pipetting methods are time-consuming and prone to error, necessitating an advancement in lipid solution fabrication.

❑Herein, we present convection-based “Christmas tree” concentration gradient generator (CGG) microfluidic chips designed to rapidly identify optimal lipid formulations for specific therapeutic applications. Initially, computational simulations were performed to validate the design's capacity to generate a concentration gradient. Subsequently, the overall CGG's efficacy was assessed by analyzing the fluorescence intensity of the solution collected from its outlet. This methodology significantly contributes to enabling more economical screening for disease-specific drug delivery.

Strategy of fabricating various ENPs using reverse-Tesla CGG (rT-CGG)

A

Composition	Figure	Ratio
DC-CHOL		?
CHOL		?
PC		?
SM		?
PS		?
PE		?

B

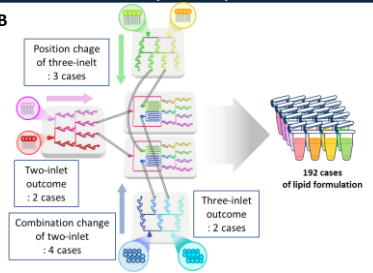

Fig 1. Schematic illustration of **(A)** lipid composition and its ratios utilized in ENPs, and **(B)** the procedure for producing lipid solutions employing rT-CGG, Each abbreviation is below: DC-CHOL: DC-Cholesterol, CHOL: Cholesterol, SM: Sphingomyelin, PC: Phosphatidylcholine, PS: Phosphatidylserine, PE: Phosphatidylethanolamine

Analysis of rT-CGGs using CFD simulation for diverse solutions of lipids

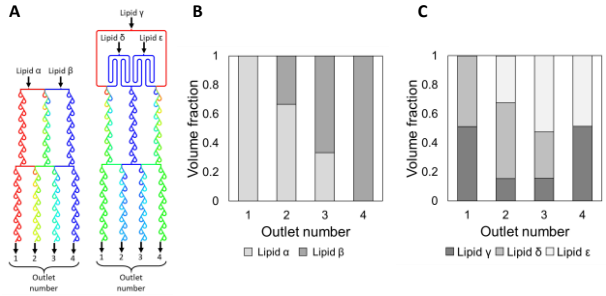

Fig 2. (A) Schemes of two-inlet rT-CGG and three-inlet rT-CGG with each outlet number. Volume fractions (determined by dividing one lipid volume by the total volume) for each of the four outlets in **(B)** the two-inlet rT-CGG and **(C)** the three-inlet rT-CGG.

Design enable full mixing for uniform lipid distribution

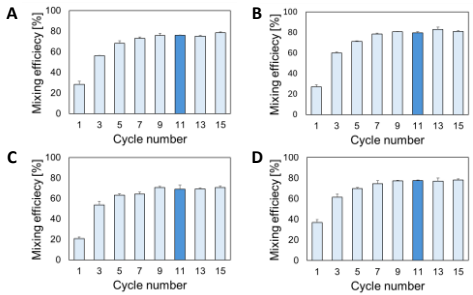

Fig 3. Assessments of fluorescence images demonstrating the reverse-Tesla chip's mixing efficiency per cycle at total flow rates of **(A)** 800, **(B)** 960, **(C)** 1,200, and **(D)** 1,600 μL/min. The blue and orange fluorescent beads were injected through inlets separately with same flow rate and pictured at specific cycles.

CGG performance evaluation via fluorescence intensity

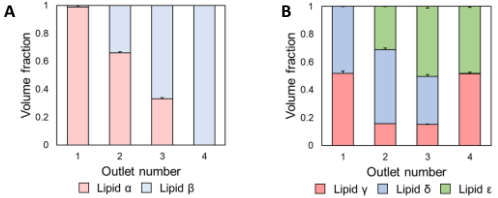

Fig 4. Fluorescence intensity quantification at each of the four outlets for both **(A)** the two-inlet rT-CGG and **(B)** the three-inlet rT-CGG. The volume fractions for each outlet were subsequently calculated by dividing the measured outlet fluorescence intensity by the initial fluorescence intensity.

Conclusions

❑Finding a lipid formulation of ENP that closely resembles the lipid composition of exosomes has become necessary for cell-specific targeting.

❑A two-inlet rT-CGG simulation showed a linear concentration gradient across channels at the exit (R^2 = 0.99). Furthermore, combining two-inlet and three-inlet rT-CGGs can yield up to 192 distinct lipid solutions, offering a more compact study compared to traditional mathematical methods.

❑For reliable and even lipid splitting, complete mixing is crucial before the splitter section, which is achievable using an 11-reverse-Tesla structure.

❑Fluorescence intensity comparison demonstrates the ability of producing lipid solutions using CGG, highlighting the possibility of improving precision targeting, speeding up the identification of lipid formulations for other illnesses and advancing nanomedicine in the future.

❑References

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