Design and Production of Lipid Vesicles and Nanoparticles with Exosome-Mimetic Composition Using a Crossflow Membrane Mixing Device

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Background

Although lipid-based drug nanocarriers are indispensable for modern drug delivery, their production method still requires significant improvement. The crossflow membrane mixing device is one of the promising methods for producing lipid vesicles and lipid nanoparticles (LNPs) in a large scale. In this study, impacts of various process parameters on the size and the internal structure of the resulting particles were studied.

Methods

Dipalmitoylphosphatidylcholine (DPPC), cholesterol (CH), and sphingomyelin (SM), were used for the preparation of the vesicles and LNPs. Its physicochemical characterization was conducted using nanoparticle tracking analysis, zeta potential measurements, differential scanning calorimetry, circular dichroism spectra, fluorescent lifetime analysis, and so on. In the preparation process using the crossflow membrane mixing device, lipids were dissolved in ethanol to be injected into flow of aqueous buffer solution. Effects of the operation parameters on resultant particles were investigated using dynamic light scattering, nanoparticle tracking analysis, and transmission electron microscopy.

Results

An optimum molar ratio of lipids to accommodate helical peptide was determined to be DPPC/CH/SM = 45/15/40, for which CH and SM played important roles to increase softness of the membrane and to provide highly-mobile phase, respectively1). These properties were essential for the transmembrane-domain peptide of integrin family to form and maintain α-helical structure; and thus, this composition was identified as exosome-mimetic. In the production of such exosome-mimetic vesicles (EMVs) using the crossflow membrane mixing device, we examined the effects of various process parameters on the morphology of resulting particles. While the product was generally in the form of vesicles, LNP-like dense particles were formed when a highly concentrated lipid solution was injected at relatively low flow rate at room temperature, which is likely the result of lipid aggregation caused by rapid dilution. Also, when vesicles were formed, their lamellarity varied depending on the process parameters including the lipid concentration, the flow rates, and temperature. The lipid density of the vesicles was strongly affected by the temperature, the lipid concentration in ethanol, and its flow rate, that is, both vesicles and LNPs were manufactured by changing the operation conditions using the crossflow membrane mixing device.

Conclusion

Factors influencing the morphology of exosome-mimetic vesicles/LNPs manufactured by the crossflow membrane mixing device were clarified.

Keywords:
Crossflow membrane mixing, exosome-mimetic vesicles, lipid nanoparticle

References:

[1] Shiho Tsutsumi, Yuki Takechi-Haraya, Yasuhiro Abe, Kohsaku Kawakami. Japan. 2025.