Synthesis of exosome-mimicking nanoparticles using a microfluidic chip

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Introduction

❑Exosomes, which are released from cells, are replicating natural biological mechanisms and have lower immunogenicity compared to synthetic nanoparticles, play a vital role in intercellular communication and the transport of therapeutic agents [1]. However, obstacles like heterogeneity and problems with large-scale production continue to prevent their widespread use [2].

❑Therefore, We developed biocompatible exosome-mimetic nanoparticles (ENPs) composed of lipids and encapsulated anti-fibrotic microRNA (miRNA) using microfluidic chip. The fabricated ENPs were evaluated for their particle size distribution, morphological similarity, miRNA encapsulation efficiency, and cell viability and uptake efficiency in comparison with natural exosomes.

Schematic illustration of generating ENPs using microfluidic-driven self-assembly approach

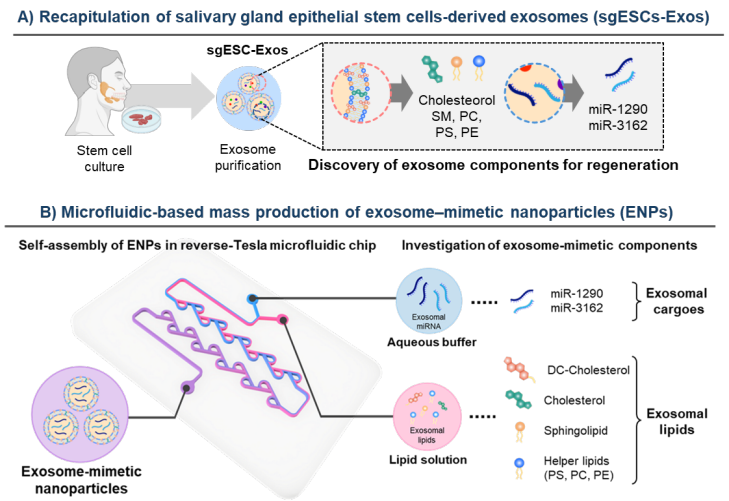

Fig 1. Strategy for fabrication of exosome-mimetic nanoparticles (ENPs) including A) the investigation of components used to replicate sgESC-Exos, B) the fabrication method of ENPs using a reTesla microfluidic chip.

Design of microfluidic chip for enhanced solution mixing

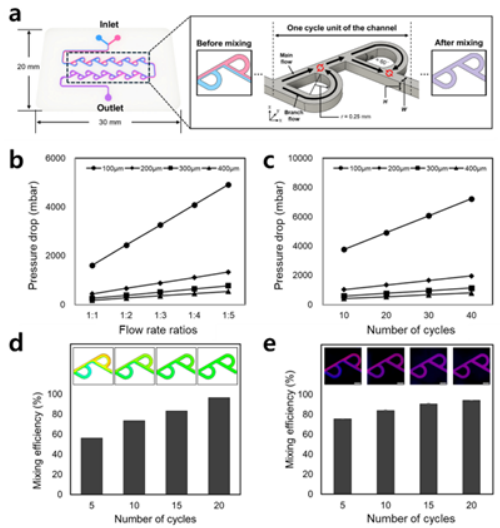

Fig 2. Design and mixing efficiency of microfluidic chip for ENP production. (a) Schematic illustration of mixing channel dimensions within chip. Pressure drop within microchannel by (b) flow rate ratio and (c) number of cycles with various channel widths. (d) Computational analysis of solution mixing in the channel with height of 200 μm. (e) Mixing efficiency of two fluorescent microbeads with number of mixing cycles.

Encapsulation of exosomal miRNA in ENPs

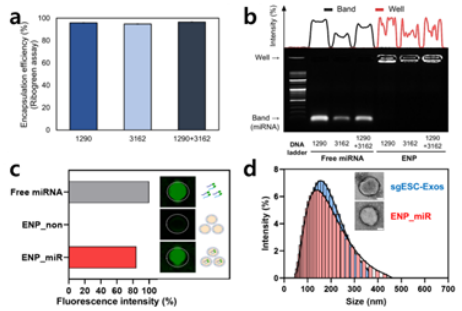

Fig 3. Investigation of encapsulation effects of nucleic acids using (a) Ribogreen assay, (b) gel retardation assay, and (c) fluorescence intensity (d) Comparison of size distribution profiles and morphological images of natural exosomes and ENP_miR.

Evaluation of therapeutic effects of ENPs

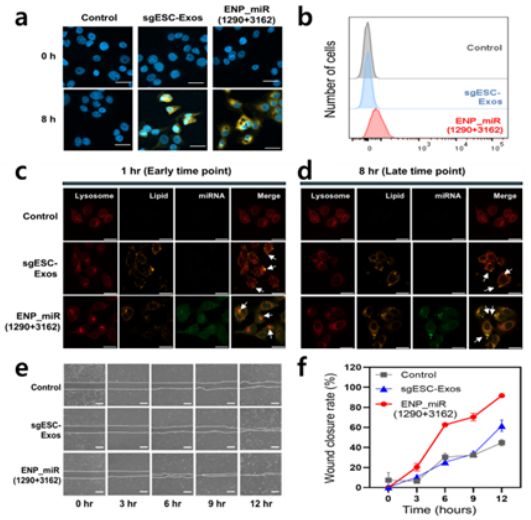

Fig 4. Evaluation of *in vitro* therapeutic effects of ENPs. (a) Confocal images and (b) flow cytometry analysis of cellular uptake (Scale bars = 40 μm). Intracellular localization visualized by confocal microscopy at (c) 1 h and (d) at 8 h after co-incubation (Scale bars = 20 μm). (e) Scratch wound healing assay of HaCaT cells for 12 h (Scale bars = 100 μm). (f) Statistics on HaCaT cell migration rates in each group.

Conclusions

❑In this study, we developed ENPs with microfluidic chip induced vortex-driven mixing of lipid solution and aqueous buffer, achieving a mixing efficiency greater than 95%.

❑The fabricated ENPs exhibited a uniform size of 140 nm, over 95% miRNA encapsulation efficiency, excellent biocompatibility (>99% cell viability), and 56-fold higher cellular uptake than the control.

❑ References

[1] S. Park *et al.*, *Bioactive Materials*, 44, 229-247 (2025).
[2] J. Kim *et al.*, *Sensors and Actuators B: Chemical*, 394, 134396 (2023).

❑ Acknowledgement

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