Elucidating the cellular uptake and tissue distribution mechanism of cell-derived vesicles, a novel therapeutic carrier

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INTRODUCTION

Cell-derived vesicles (CDVs) are emerging as a novel therapeutic carrier. One of the crucial factors in the development and therapeutic applications of CDVs is to understand the precise mechanism by which vesicles find and enter the target cells. In this study, we aimed to investigate the uptake mechanism of CDVs produced from a manufacturing process established at MDimune Inc. *In vitro* uptake assay of natural killer cell-derived CDVs (NK-CDVs) in human umbilical endothelial cells (HUVECs) or BT549 cells was performed to provide precise insights into that regard. We further explored if CDVs can target cells in the brain using adipose stem cell-derived CDV (ADSC-CDVs).

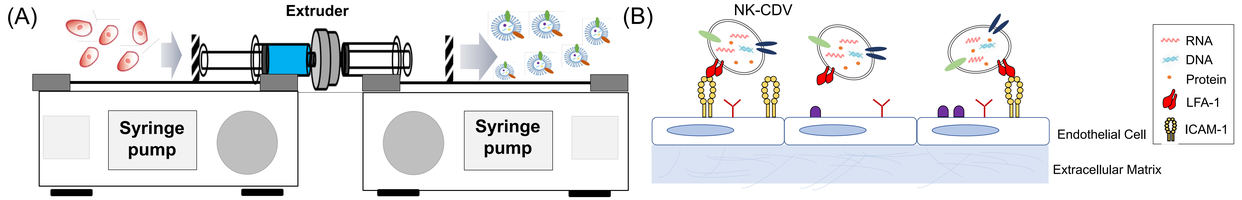
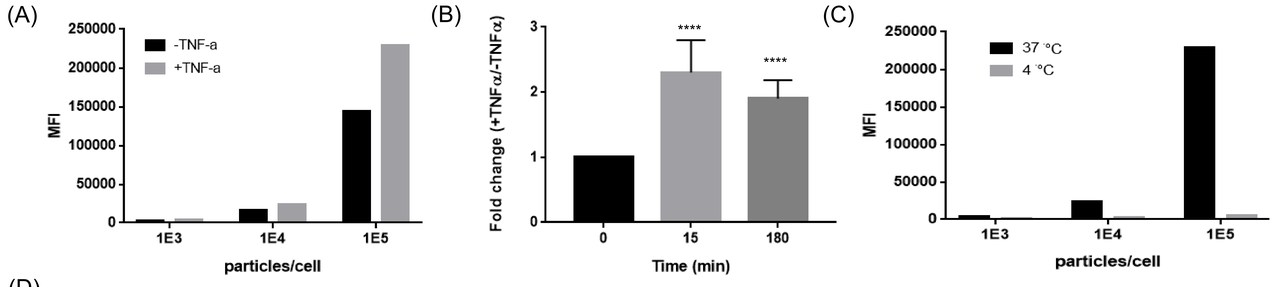


Figure 1: (A) Manufacturing-scale production of CDV using a syringe extruder. (B) Adhesion of lymphocyte function-associated antigen (LFA-1) expressed on NK-CDV to intercellular adhesion molecules-1 (ICAM-1) expressed on endothelial cells such as human umbilical endothelial cells (HUVECs) is the potential mechanism behind the applications of NK-CDVs as an anticancer drug delivery vehicle.

IN VITRO UPTAKE STUDY

HUVECs was treated with TNF-α to induce expression of ICAM-1. Then, CFSE-labeled NK-CDVs at different particle numbers were added to the culture media of the recipient cells and incubated for 180 mins at 37 °C. The degree of NK-CDV uptake into HUVECs was examined using the FACS and further visualized with a confocal microscope.



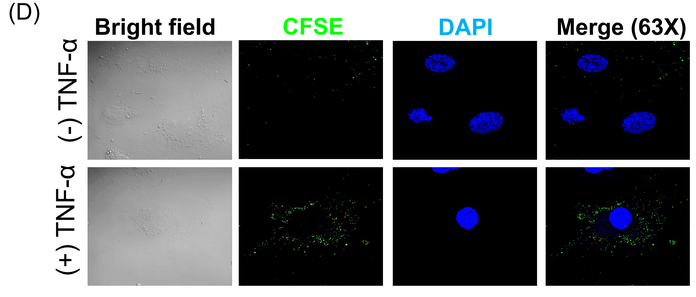


Figure 2. Cellular uptake of NK-CDVs into HUVECs. (A) As the particles number increases, the uptake of NK-CDVs was found to increase gradually. This suggests that uptake of NK-CDVs into HUVECs is dose-dependent. (B) Increased uptake was observed in TNF-α-induced HUVECs compared to non-treated HUVECs, indicating that ICAM-1 of HUVECs increased the binding of CDVs. (C) Incubation at 4°C attenuated the uptake significantly compared to 37°C, suggesting an energy-dependent process rather than a passive uptake or nonspecific binding. (D) Representative confocal microscopic images of CFSE-labeled NK-CDVs taken up by TNF-α-treated at 180 mins of treatment.

IN VITRO UPTAKE MECHANISM STUDY

Two different cells were used to examine the mechanism of NK-CDV uptake. Cells were preincubated with dynasore (dynamin-dependent endocytosis inhibitor), cytochalasin D (actin-dependent phagocytosis or micropinocytosis inhibitor), or heparin (heparan sulfate proteoglycan mediated endocytosis inhibitor) before the addition of NK-CDVs. The degree of uptake was examined using FACS.

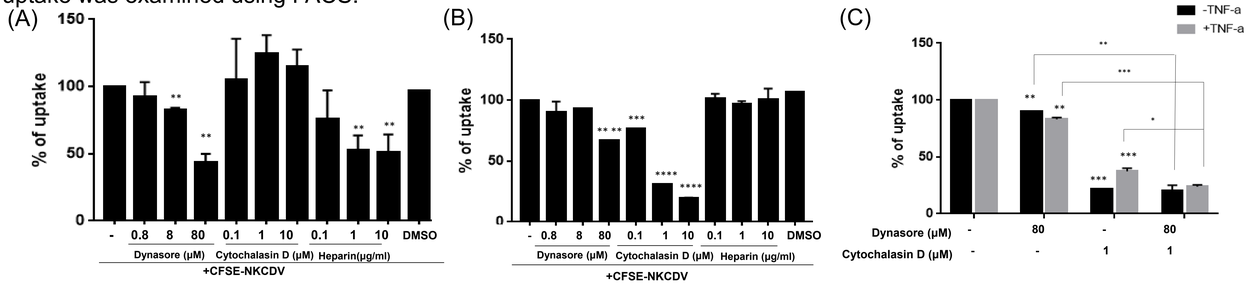


Figure 3: The uptake mechanism of NK-CDs was accessed in BT549 and HUVECs. (A) Heparin and dynasore inhibit the uptake of NK-CDV in BT549 cells. (B) Uptake of NK-CDVs in TNF-α-treated HUVECs occurs via dynamin-dependent endocytosis and actin-dependent phagocytosis, and micropinocytosis but not heparan sulfate proteoglycan mediated endocytosis. (C) A combination of dynasore and cytochalasin D was not sufficient to inhibit uptake completely, suggesting that a third uptake mechanism may also exist.

UPTAKE of CDVs in the BRAIN

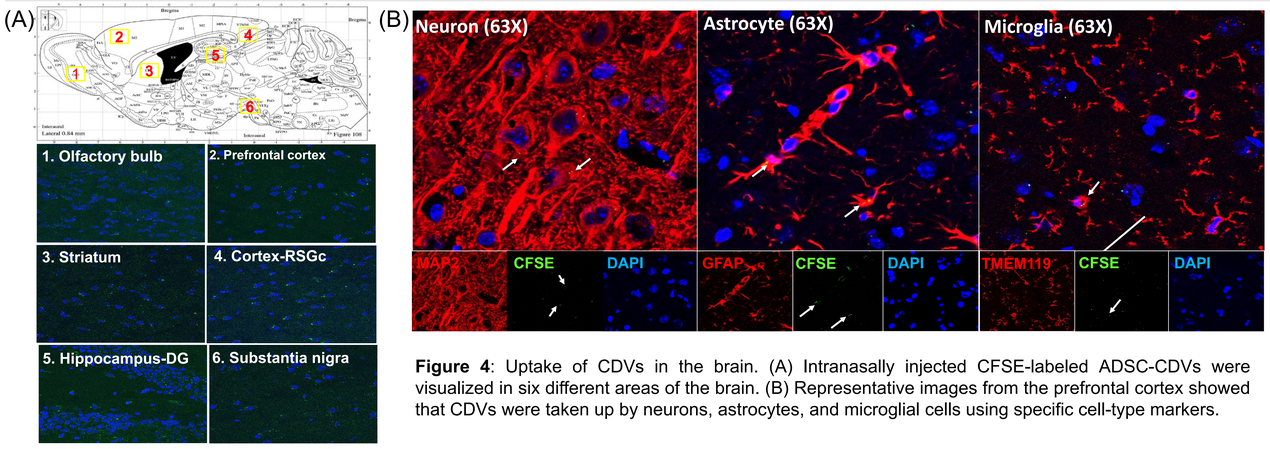


Figure 4: Uptake of CDVs in the brain. (A) Intranasally injected CFSE-labeled ADSC-CDVs were visualized in six different areas of the brain. (B) Representative images from the prefrontal cortex showed that CDVs were taken up by neurons, astrocytes, and microglial cells using specific cell-type markers.

CONCLUSIONS

- NK-CDVs uptake in HUVECs is LFA-1/ICAM-1 dependent.
- The entry route of CDVs is cell-dependent. The main entry route of NK-CDVs into HUVEC is via an actin-dependent phagocytosis and micropinocytosis pathway, whereas in BT549 cells, dynamin-dependent and heparan sulfate proteoglycan mediated pathways are the major routes of entry for NK-CDVs.
- ADSC-CDV reaches brain via intranasal injection, suggesting the potential application of CDVs as a drug delivery carrier for CNS diseases.