Target-Specific Delivery of Small RNA Cargos Facilitated by BioDrone Platform Technology

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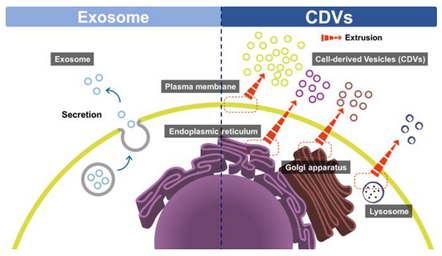
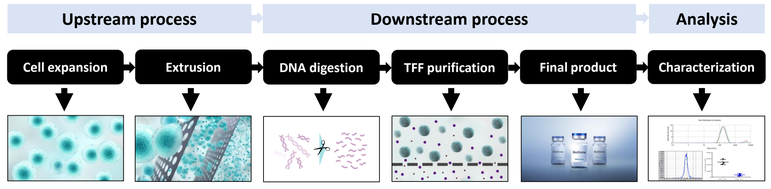
Cell-Derived Vesicles (CDVs) & BioDrone Drug Delivery Platform

At MDimune, we are developing an innovative drug delivery platform technology, BioDrone®, using cell-derived vesicles (CDVs) produced from diverse human cell sources using our proprietary extrusion process. We have previously demonstrated that CDVs, while sharing many physical and biochemical properties with exosomes, can serve as effective biomolecular carriers with highly efficient drug loading capabilities and excellent cellular uptake and tissue penetration properties.

(A)



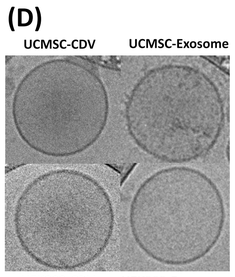
(B)



(C)

Cell source	CDV particle/cell	Fold Difference (CDV/Exosome)
UCMSC	~50,000	~20x
ADSC	~10,000	~10x
U937	~6,000	-
NK	~2,500	-
HEK293	~10,000	~20x-35x
Other cells	350~50,000	~10x~200x

(D)



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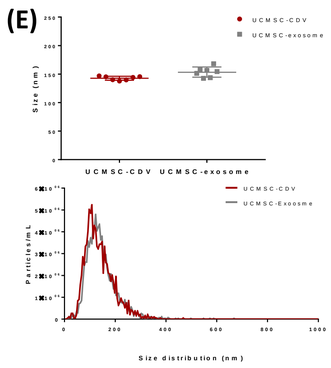

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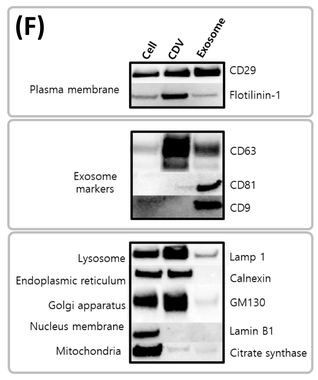
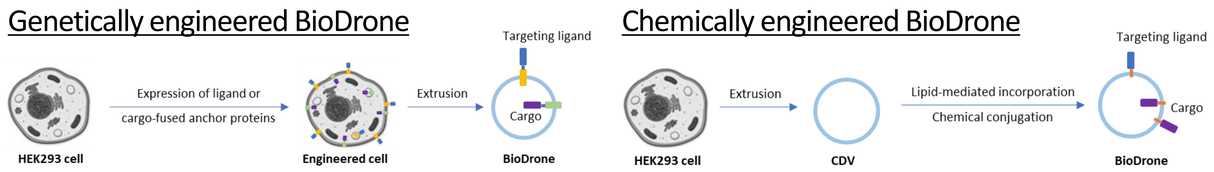


Figure 1. (A) A schematic diagram shows a possible mechanism for generating CDVs compared to exosomes. (B) The overall process of CDV manufacturing. (C) The yield of CDV production is compared to natural exosomes from diverse cell sources. Please note that the exosome yield couldn't be measured in U937 or NK due to the low level of exosome secretion. (D) Representative cryo-transmission electron microscopy (cryo-TEM) images of CDV and exosome. (E) CDVs produced from extrusion exhibit similar size and size distribution to exosomes from umbilical cord-derived mesenchymal stem cells (UCMSCs). (F) Western blot analyses show that some membrane components are well conserved between CDVs and exosomes, while other organelle markers or some classical exosome markers are over-represented in CDVs.

BioDrone Platform for CDV Therapeutics



- Anchor candidates were selected from proteome analysis.
- These are highly enriched in the CDV compared to cell and exosome.
- Overexpression of GFP-fused anchor candidates
- The distribution of the GFP signal and the quantified amount in the CDV have been analyzed.
- Membrane topology in the CDV has also been verified.
- CDV anchors are highly effective exclusively for MDimune BioDrone.
- Benchmarking the state-of-the-art technology of vesicle modification
- Targeting ligand: well-developed antibody therapeutics
- Cargo: currently siRNA, expanding to small chemicals
- Chemical conjugation: click chemistry (NHS-Azido + DBCO-Maleimide)
- Lipid-mediated incorporation: antibody and siRNA

Introduction of Antibody as a Targeting Ligand to CDV

For the active targeting of CDVs, we adapted the well-developed and clinically-proven monoclonal antibody as a targeting ligand. A monoclonal antibody, such as trastuzumab, was introduced to CDVs by click chemistry or lipid-mediated incorporation. Such antibody-CDV conjugates demonstrated increased specificity in the cell-binding assay.

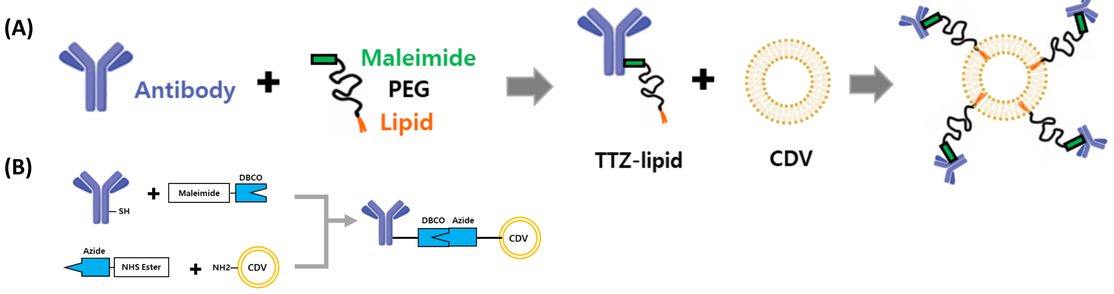
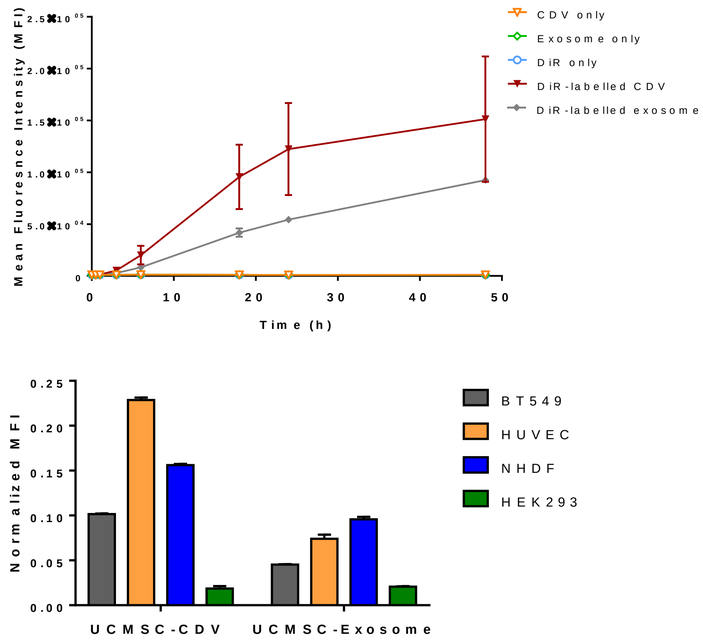


Figure 3. (A) A schematic diagram shows a lipid-mediated incorporation method of conjugating an antibody to the CDV. (B) A schematic diagram shows a click chemistry method of conjugating an antibody to the CDV.

Enhanced Cellular Uptake & Tissue Penetration

Next, we examined the cellular uptake of CDVs, in comparison with exosomes, in diverse recipient cells. We observed that cells intake more CDVs than exosomes. CDVs show equally effective or even better characteristics as cellular delivery vehicles compared to the same quantity of exosomes.

(A)



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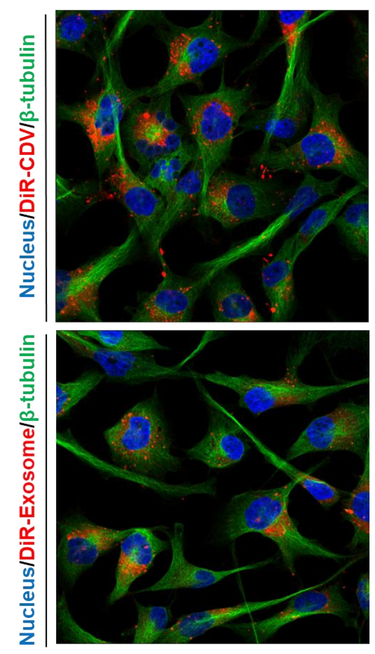
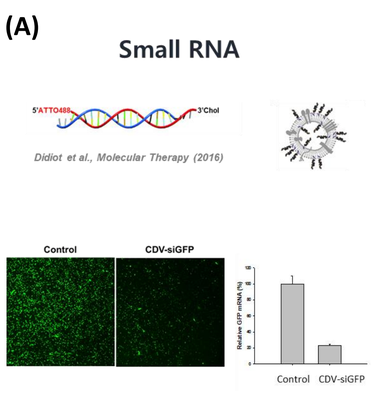


Figure 2. (A) Time-dependent cellular uptake of CDVs and exosomes was analyzed by flow cytometry. CDVs were favored by diverse recipient cells over exosomes (~2-fold enhanced uptake). (B) Cellular uptake of CDVs and exosomes were observed by confocal microscopy.

Cargo Loading to CDV – siRNA and mRNA

We demonstrated the therapeutic potential of CDVs by examining key characteristics as a drug carrier. We find that robust encapsulation of RNA and protein cargos leads to improved cellular responses and functional consequences in various target cells. (1) small RNAs are loaded onto CDVs highly efficiently using cholesterol conjugation. (2) mRNAs can be complexed with CDVs with help of PEI, which lead to enhanced target gene expression.

(A)



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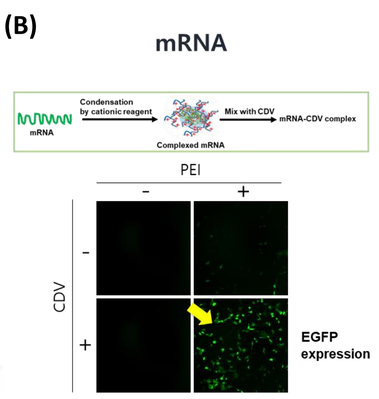
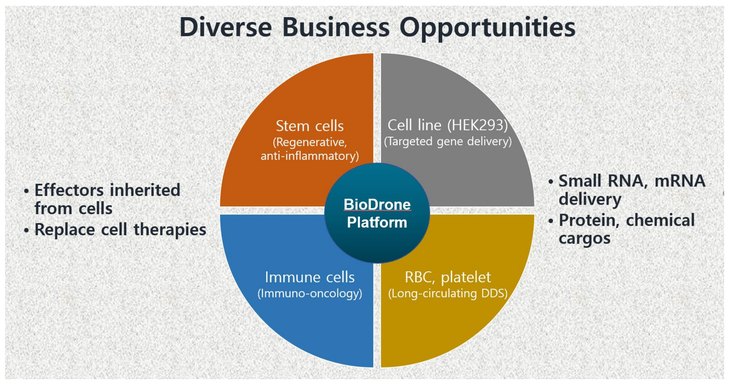
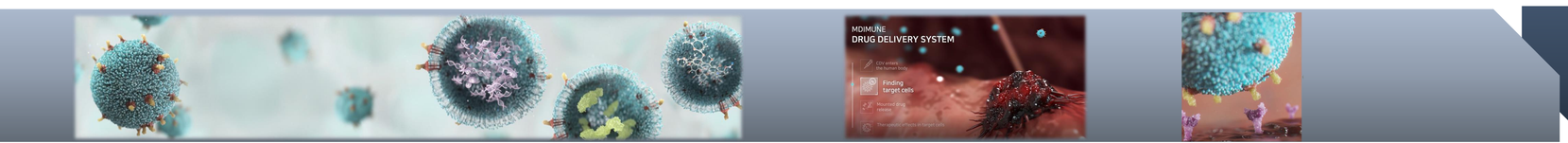


Figure 4. (A) A schematic diagram shows a siRNA encapsulation method using cholesterol conjugation. Typically, more than 2,000 copies of siRNAs are loaded per single CDV particle with >30 % loading efficiency. The siGFP-loaded CDVs were shown to suppress GFP expression effectively in the GFP-overexpressing cell line. (B) EGFP mRNAs were complexed with CDVs using PEI. When treated to cells, EGFP expression was drastically enhanced by triple (mRNA/PEI/CDV) complex compared to mRNA/PEI control with the same mRNA amount.

Summary and Business Opportunities

In summary, this study highlights the expandability and versatility of BioDrone platform technology for the drug delivery system. This novel platform can be utilized for diverse biotherapeutics and vaccine development targeting broad disease areas including cancer, CNS diseases, and rare genetic disorders.





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Thank you!