Effective Delivery of RNA and Protein Cargos into Target Cells Using BioDrone® Platform Technology

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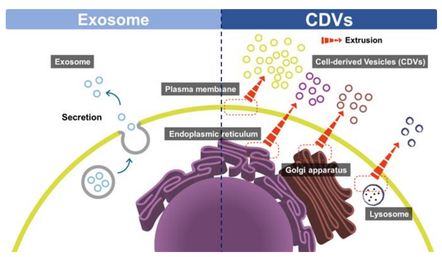
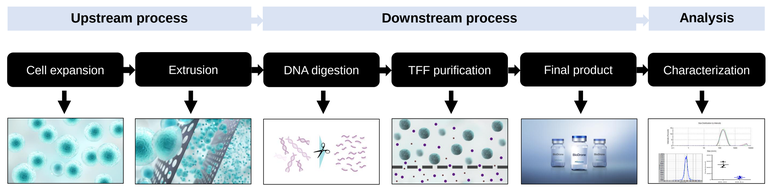
Cell-derived Vesicles (CDVs) & BioDrone® Drug Delivery Platform

At MDimune, we are developing an innovative **drug delivery platform technology, BioDrone®**, using **cell-derived vesicles (CDVs)** produced from diverse human cell sources by using our proprietary extrusion process. We have previously shown the establishment of a manufacturing-scale extrusion process to allow the production of a large number of nanovesicles. Here, we further confirm the advantage in productivity and compatibility of extrusion technology in multiple cell types.

(A)



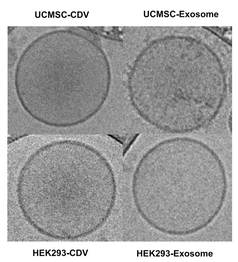
(B)



(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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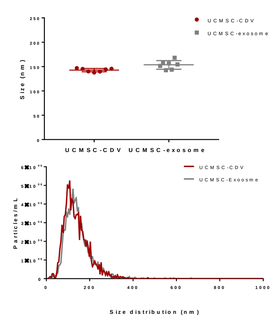

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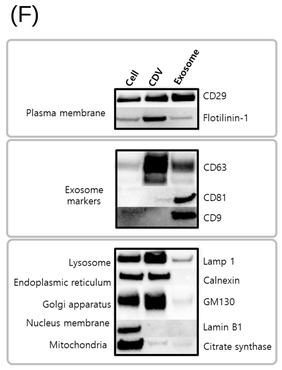
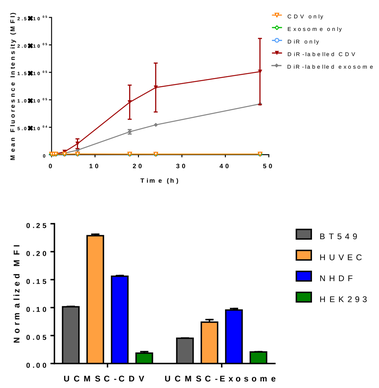


Figure 1. (A) A schematic diagram shows the possible mechanism of high productivity of CDVs compared to exosomes. (B) The overall manufacturing process to produce CDVs. (C) The yield of CDVs is compared to natural exosomes from diverse cell sources. Please note that the exosome yield couldn't be measured in U937 or NK due to the low level of exosome secretion. (D) Representative cryo-transmission electron microscopy (cryo-TEM) images (E) CDVs produced from extrusion exhibit similar size and size distribution as exosomes as illustrated 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 even some classical exosome markers are over-represented in CDVs.

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, and such enhanced uptake appears to be mediated by selected surface proteins that are enriched in CDVs. In addition, *in vivo* penetration results in the retina show that CDVs can penetrate retinal tissues effectively and reach the epithelial barrier that has been a target of many debilitating eye diseases. Taken together, CDVs show equally effective or even better characteristics as cellular delivery vehicles compared to the same quantity of exosomes.

(A)



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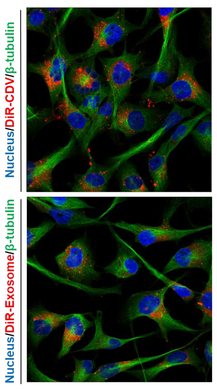

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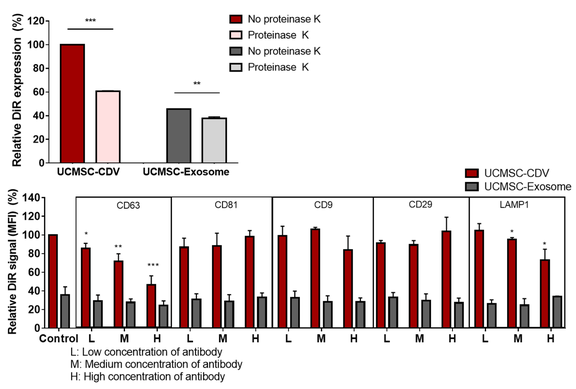

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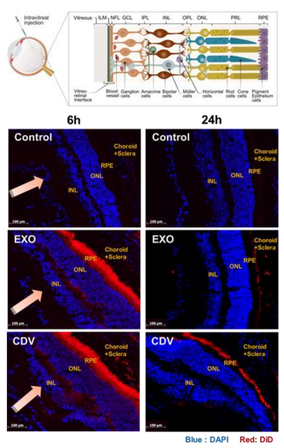


Figure 2. (A) Time-dependent cellular uptake of CDVs and exosomes was analyzed by flow cytometry. CDVs were favored by diverse recipient cells over exosomes (~2 fold enhanced uptake). (B) Cellular uptake of CDVs and exosomes were observed by confocal microscopy. (C) Digestion (proteinase K) or blockade (specific antibodies) of membrane proteins resulted in a target-specific reduction in cellular uptake. (D) A schematic diagram shows tissue penetration of CDVs in eyes via intravitreal injection. When CDVs and exosomes were injected into the eye through the intravitreal space, CDVs were shown to have equally effective or better tissue penetration compared to exosomes.

Encapsulation of RNA & Protein Cargos

We demonstrated the therapeutic potential of CDVs by examining key characteristics as a drug carrier, such as the drug loading capability of RNA or protein cargos. 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. (3) CDVs harboring protein cargos can be generated by engineering source cells and were shown to restore lost cellular functions in target cells. Further progress is being made to optimize the RNA and protein cargo loading onto CDVs.

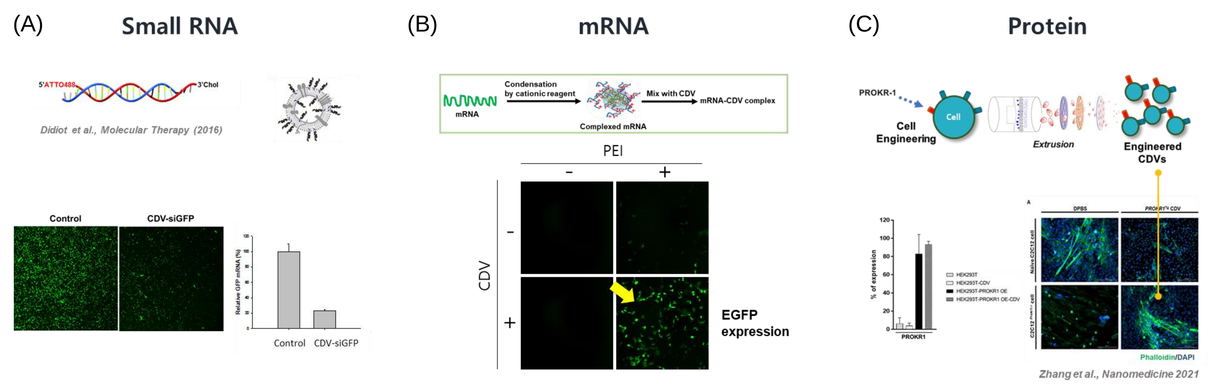


Figure 3. (A) A schematic diagram shows a small RNA encapsulation method using cholesterol conjugation. Typically, more than 2,000 copies of small RNAs are loaded per single CDV particle with >30% loading efficiency using this method. The siRNA(GFP)-loaded CDVs were shown to suppress GFP expression effectively 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. (C) Prokineticin receptor 1 (PROKR1) was overexpressed in HEK293 cells. Upon extrusion of PROKR1+ cells, CDVs were obtained with a high level of PROKR1 expression on the surface as assessed by flow cytometry. Treating PROKR1-CDVs rescued the functional phenotype (myogenesis) in PROKR1-deficient cells as visualized by actin filaments staining (Phalloidin, green).

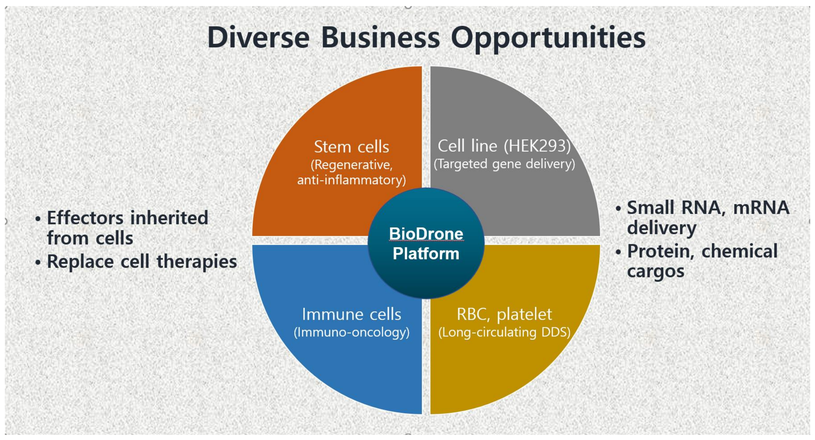
Strategy Toward Targeted Biotherapeutics Delivery & Vaccine Development

Surface modification of CDVs can be accomplished by (1) obtaining CDVs from engineered source cells or (2) conjugating extra ligands (peptide, antibody, aptamer, etc.) to CDVs directly. Both methods offer flexible means to engineer CDVs further to achieve targeted drug delivery or even effective antigen presentation to the host immune system.

Figure 4. The surface of CDVs can be engineered at either cell ("endogenous") or vesicle ("exogenous") level. The resulting CDVs decorated with diverse ligands can be used to direct CDVs toward tumor targets, facilitate CDVs to cross the blood-brain barrier for CNS targeting, or present antigens contained in CDVs for potent immune response.

Summary and Business Opportunities

In summary, this study highlights the expandability and versatility of BioDrone® platform technology in RNA and protein drug delivery. 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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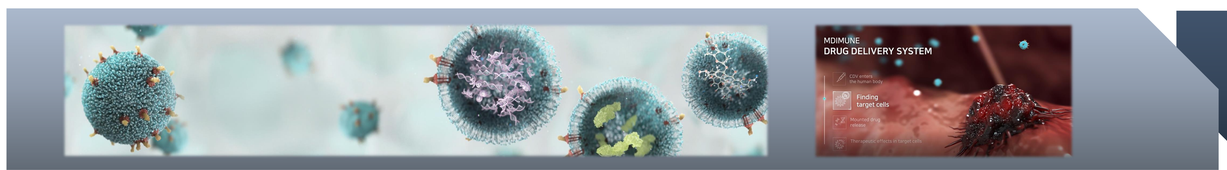

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