BioDrone®, a novel drug delivery platform: From the basic science to potential therapeutic promises

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What is BioDrone®?

BioDrone® is an innovative drug delivery platform that relies on the proprietary extrusion method to obtain tiny vesicles from cells. Cells in suspension are passed through membrane filters with narrow pore sizes and revascularize into tiny nanovesicles, which are very similar to exosomes in size, shape, and many biochemical properties. These cell-derived vesicles (CDVs) can be produced in far greater numbers than exosomes, probably because CDVs are derived from multiple membrane sources, whereas exosomes are produced through a specific secretion pathway. In this study, we aimed to identify the key objectives to best assess its potential as a novel, nanosized drug delivery system, including comparisons between CDVs and exosomes.

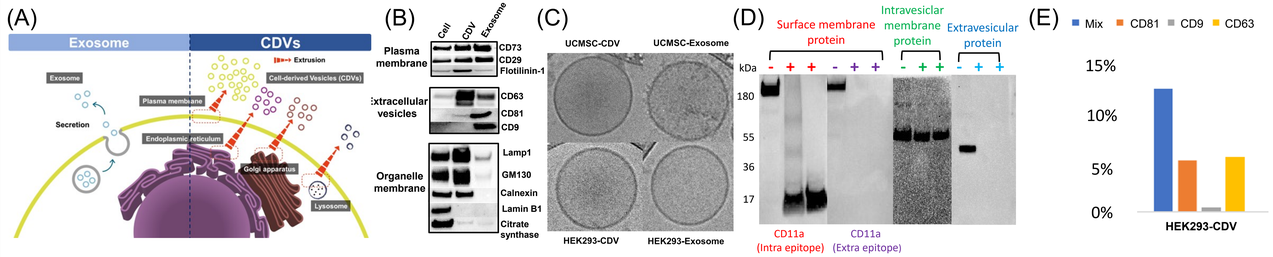


Figure 1. (A) Schematic diagram showed the possible mechanism of high productivity of CDVs compared to exosomes. (B) Western blot analyses showed that some membrane components are well conserved between CDVs and exosomes, while other organelle markers or even some classical exosome markers are over-represented in CDVs. (C) Representative cryo-transmission electron microscope (cryo-TEM) images demonstrated that it is nearly impossible to distinguish between the CDVs and exosomes. (D) Western blot analyses verified that vesicles maintain the original membrane topology of cells. (E) CDVs can be classified with their surface markers at single-particle level using nano-flow cytometry. Initial results showed that approximately 12% of CDV particles contain three classical exosome marker sets. A more unique marker panel for CDVs will further identify the entire subpopulations that constitute CDVs.

Cellular uptake of CDVs

Cellular uptake of CDVs and exosomes labeled with a fluorescent dye were examined in diverse recipient cells. Recipient cells intake more CDVs than exosomes, as shown by flow cytometry. A similar intracellular trafficking pattern between the CDVs and exosomes was observed by confocal imaging over time. Furthermore, the reconstituted CDVs from powder maintain the cellular uptake capability as efficiently as CDVs, suggesting lyophilization can be one of the CDV formulations.

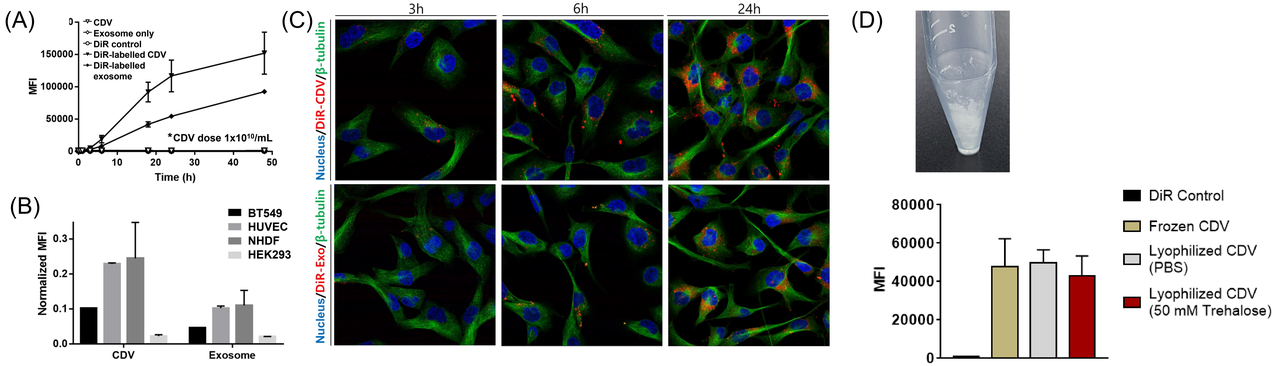
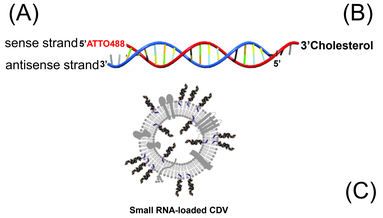


Figure 2. (A) Time-dependent cellular uptake of CDVs and exosomes was analyzed by flow cytometry. (B) Flow cytometry analyses showed that CDVs and exosomes were taken up by diverse recipient cells. (C) Cellular uptake and intracellular trafficking of CDVs and exosomes were observed by a confocal microscope. (D) Cellular uptake of lyophilized CDVs was compared to frozen CDVs after 24 h of incubation.

Encapsulation of small RNA cargos into CDVs

Small RNAs were loaded into the CDVs highly efficiently, more than 2000 copies per single CDV particle by incubation method using RNA containing cholesterol moieties. CDVs from two different cell sources (HEK293, UCMSC) showed similar or higher loading results than exosomes obtained from the same cells. RNA loaded CDVs effectively knocked down the target genes. Additionally, RNA cargos loaded onto CDVs can enter cells efficiently, demonstrating that CDVs can serve as effective drug delivery system.



Number of CDV particles	7.55E+10
Associated RNA	309 pmol
RNA molecules/CDV particle	2460
RNA loading efficiency	30.9%

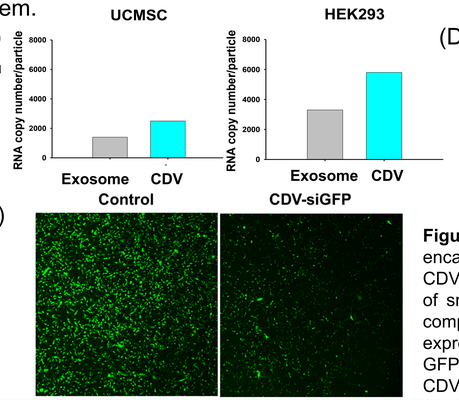


Figure 3. (A) Schematic diagram showed small RNA encapsulation method and the RNA loading efficiency of CDV is shown in table below. (B) Encapsulation efficiency of small RNA into CDVs from different cell sources was compared to exosomes. (C) Suppression of target gene expression by small RNA loaded CDVs was verified in the GFP-cell line. (D) Uptake of small RNA cargo loaded onto CDV was observed by confocal microscopy.

Tissue penetration of CDVs

In vivo penetration of CDV was demonstrated using a retinal pigment epithelium model. The results showed that CDVs can penetrate retinal tissues quite effectively and reach the epithelial barrier that has been a target of many debilitating eye diseases. In addition, CDVs showed similar or better tissue penetration results compared to the same number of exosomes.

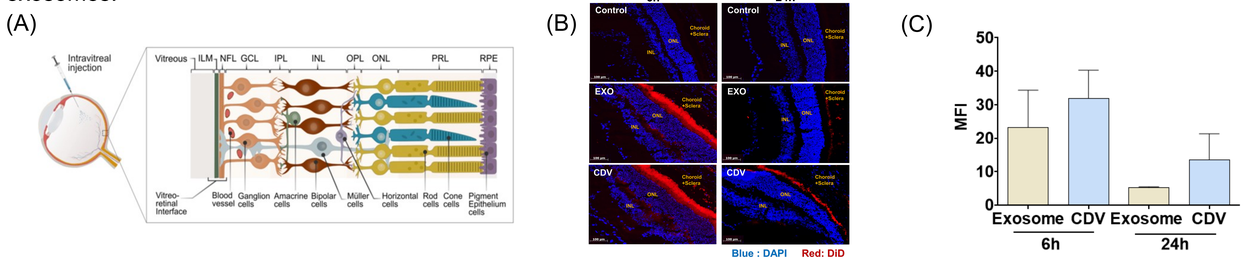


Figure 4. (A) Schematic diagram showed tissue penetration of CDV in eye via intravitreal injection. (B) CDVs and exosomes were injected into the rodent eye through the intravitreal space and tissue penetration of CDVs and exosomes was demonstrated by immunostaining. (C) Fluorescence intensity of CDVs and exosomes in retinal pigment epithelium was quantified at two different time points using ImageJ software.

Summary and future direction

- We have demonstrated the potential of BioDrone® as a drug delivery vehicle in comparison with exosomes.
- We further aim to
  - Understand surface markers of CDVs comprehensively at a single particle level.
  - Combine drug loading and surface engineering and demonstrate functional consequences of BioDrone® in various disease models.