Quality assessment of cell-derived vesicles using compartment labeling and detergent susceptibility assay

Hui-Chong Lau, Jihye Lee, Yerim Kwon, Seung Wook Oh, and Sung-Soo Park*

BioDrone Research Institute, MDimune Inc., #602 KOLON Digital Tower-III, 49 Achasan-ro, Seongdong-gu, Seoul 04790, KOREA

Abstract

The development of membranous vesicles as therapeutics has been one of the active research areas since extracellular vesicles were recognized as natural intercellular messengers. However, the production and purification of lipid vesicles need to be further standardized due to the lack of quality assessment methods. For example, nanoparticle tracking analysis provides particle size and number but has a drawback in counting non-vesicle particles. This study developed analytical methods for the quality assessment of vesicles, using cell-derived vesicle (CDV) and nanoflow cytometry (nFCM). We aimed to identify and quantify genuine lipid vesicles. First, the degree of fluorescent labeling of the vesicle compartment was examined with lipophilic dyes to stain the lipid membrane and intracellular dyes to stain the vesicle lumen. The nFCM analyses showed that both dyes fluorescently labeled 80 to 100 % of particles in most CDV products. Next, double staining of membrane and lumen was applied to further identify intact lipid vesicles. Nanoflow cytometry revealed that 80 to 92 % of CDVs were double-stained population, which were regarded as intact vesicles. To further confirm the lipid vesicle, stained CDVs were subject to a detergent, Triton X-100, and the detergent-susceptible lipid vesicles were assessed. We observed that nearly all the double-stained population disappeared, implying that these were genuine lipid vesicles. Altogether, we developed analytical methods using nFCM to assess the vesicle quality of CDV products. Currently, these methods are being tested and optimized for CDVs in various manufacturing processes.

Vesicle labeling and lysis methods

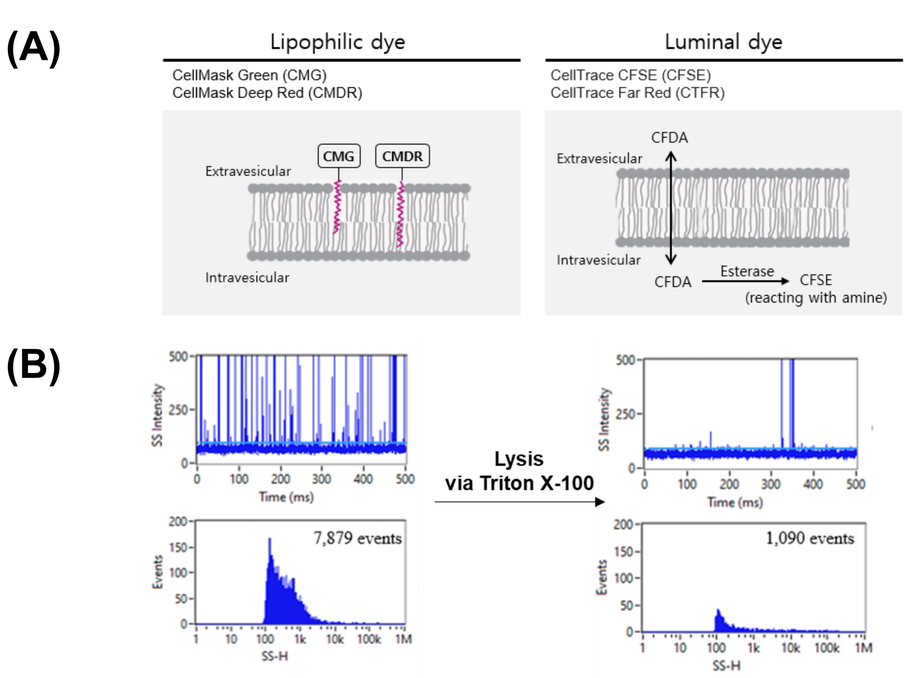

Figure 1. (A) Graphical presentation of fluorescence labeling methods of CDV. Two types of staining dyes (lipophilic and luminal dye) with different excitation and emission wavelengths were used to determine the labeling efficiency. Commonly used membrane dyes, such as CMG and CMDR, were used for this study. At the same time, CFSE and CTFR were selected for luminal staining. (B) Vesicle lysis via Triton X-100. Triton X-100 disrupted the membrane vesicle in CDV, resulting in the particle decrease.

Double staining of CDV using CFSE and CMDR

Nanoflow cytometry analyses of double staining displayed distinct populations of particles. For example, double-stained particles might be intact lipid vesicles in the CDVs. Other populations might be impurities, including lipophilic aggregates, protein aggregates, damaged vesicles, or cell debris.

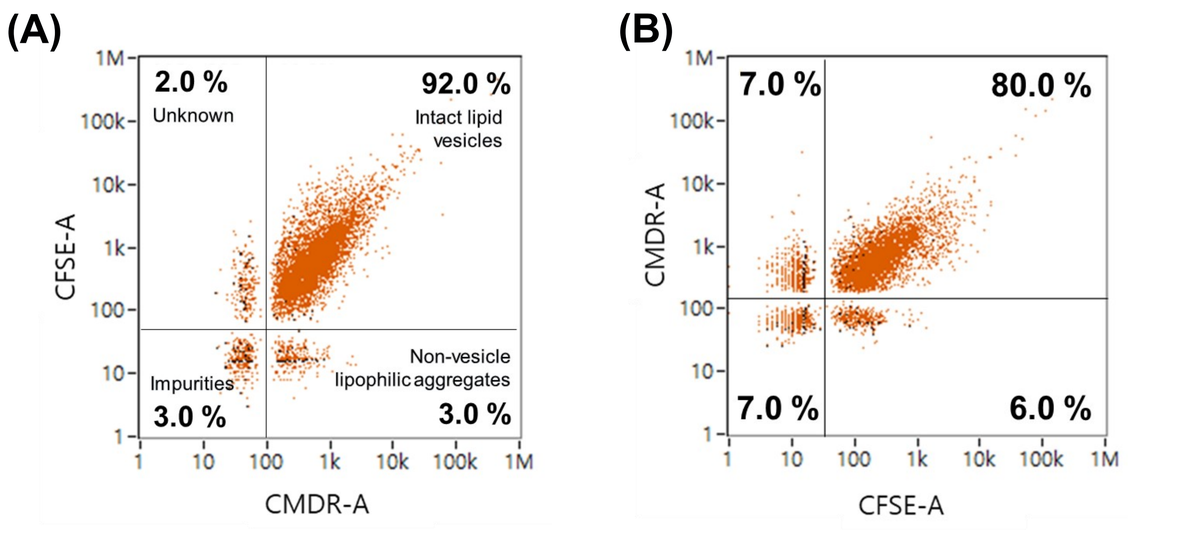

Figure 3. Double staining of CDV using CFSE and CMDR combination. CDVs were double stained, and the nFCM analyses were performed to obtain fluorescence dot plots. (A) Dot plot of CDVs treated in a CFSE and CMDR order. (B) Dot plot of CDVs treated in a CMDR and CFSE order.

Luminal and membrane staining of CDV

Staining conditions were optimized using different particle numbers and dye concentrations. Both dyes showed efficient labeling for CDVs.

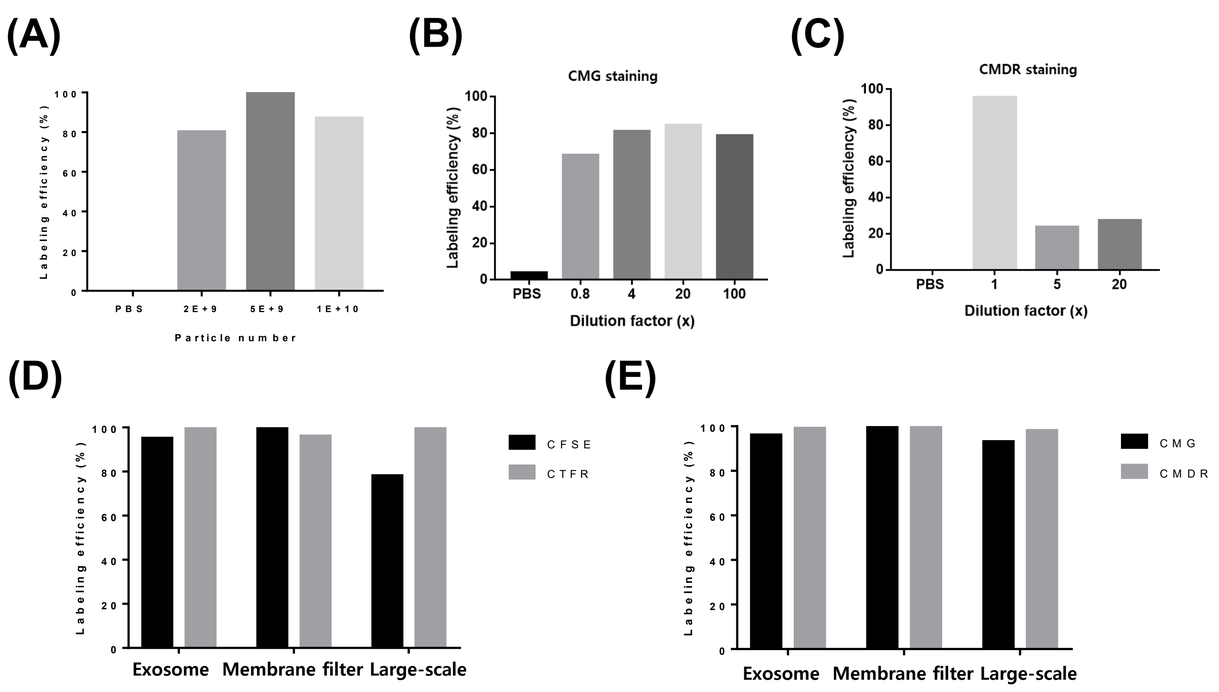

Figure 2. (A) Three different particle numbers were labeled with 10 μM CFSE dye CDVs. CDVs were labeled with different amounts of CMG (B) and CMDR (C). Three different vesicles were labeled with luminal staining dye (D) and membrane staining dye (E).

Vesicle purity assessment via Triton X-100

Most of the labeled CDVs were sensitive to the detergent. But their detergent lysis might result in the formation of nonspecific aggregates and the increase of background signal. However, the remaining particles of potential impurities after detergent lysis were not labeled with fluorescent dyes.

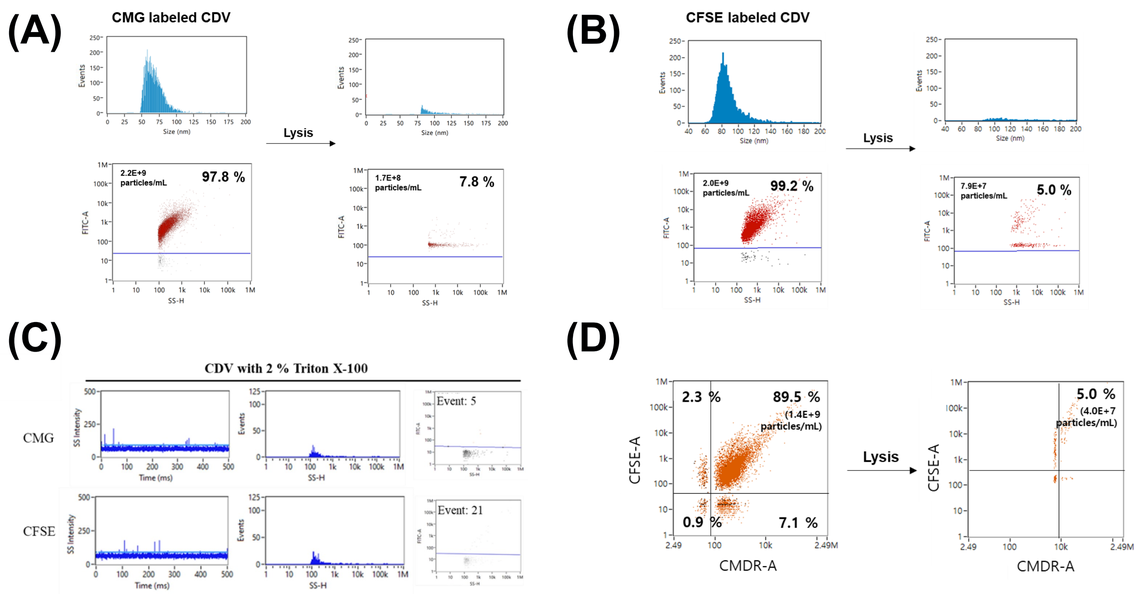

Figure 4. Triton X-100 lysis of labeled CDVs. The Triton X-100 lysis determined whether the labeled CDVs were sensitive to detergent. (A) Triton X-100 lysis of CMG-labeled CDVs. (B) Triton X-100 lysis of CFSE-labeled CDVs. (C) CMG or CFSE labeling after Triton X-100 lysis. (D) Double stained CDVs were subject to Triton X-100 lysis.

Conclusion & Future Prospects

- CDV analysis in a single-particle resolution provided a valuable window to understand the CDV characteristics.
- CDV staining using luminal or lipophilic dyes demonstrated analytical methods to assess CDV as lipid vesicles.
- The detergent lysis was an easy and effective assessment tool to evaluate CDV purity.
- A single method might lead to a biased result due to the nonspecific staining and aggregate formation.
- We suggest applying two orthogonal analytical methods to assess CDV characteristics, such as luminal staining and detergent lysis.