Enhanced Characterization of Fluorescently Labeled Extracellular Vesicles using ViewSizer 3000

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Introduction

Exosomes are small extracellular vesicles, 30-150 nm in diameter, which have been determined to play a crucial role in extracellular signaling. They have been observed in both prokaryotic and eukaryotic organisms, meaning they are incredibly widely spread in nature. Exosomes bud off from their parent cells in a sealed package, taking the properties of their parent cell walls with them and encasing many intracellular components. A wide variety of bioactive markers have been found encased in exosomes including but not limited to proteins, lipids, DNA, and RNA. Upon formation, exosomes are released into the extracellular space and have been found in many body fluids including: blood, urine, saliva, and breast milk.

The diversity of this cargo has led to exosomes having a litany of roles within the body including but not limited to: immune regulation, tissue regeneration, cancer progression, and neurodegenerative diseases. They can be released by healthy or diseased tissue, and their composition correlates with the health of their parent cell giving them significant potential for diagnosing diseases. In addition, since exosomes are already carrying cargo from cell to cell it has been shown that replacing that cargo with therapeutics could lead to more reliably targeted delivery of therapeutics. Since they take on the features of their parent cell they maintain their unique properties; like crossing biological barriers including the blood-brain barrier, or stem-cell derived exosomes showing regenerative or reparative properties.

Exosomes are important mediators in cell-to-cell communication, and are taken up into recipient cells, releasing their cargo in the process. The cargo is incredibly diverse including anything from growth factors, signaling molecules, enzymes, in addition to the ones mentioned previously.

Experimental Design

Exosomes were obtained from Hansa BioMed, and these studies were performed with HEK-293 cell derived material,

Characterize the size and concentration of EVs using the ViewSizer™ 3000.

these were stored at -80°C. The labeling was done by a kit produced by SBI, catalogue EXONTA110A-1. Instructions were followed as indicated. NTA and Fluorescent measurements were performed on the HORIBA ViewSizer 3000 Simultaneous Multi-laser Nanoparticle Tracking Analyzer (NTA). These experiments required two different sets of recording parameters one for NTA. Both sets of measurements use Brownian motion to determine particle size and count by tracking the movement of polydisperse particles in phosphate buffered saline (PBS).

For the multi-laser NTA only data (all particles), measurements were recorded with the following parameters: frame rate: 30 frames/sec; exposure: 15 msec; gain: 30; blue laser power: 210 mW; green laser power: 12 mW; and red laser power: 8 mW; temperature control: active, 22°C. 25 short videos were collected with 3 seconds of stirring between each video, and a 5 second wait to ensure completely independent sets of particles in each video.

For the fluorescent measurements, the parameters were similar but not identical and a 550 nm longpass filter was placed into the optics to eliminate any contribution from non-fluorescent particles and the red laser (635 nm) was turned off to ensure there was no contribution from scattering. For the Fluorescent only data (only labeled particles), measurements were recorded with the same parameters with the following exceptions: red laser power: 0 mW. 25 short videos were collected with 2 seconds of stirring, and a 3 second wait.

Results

Each measurement was repeated six times in NTA mode and Fluorescence mode, the results were plotted to emphasize NTA in Figure 1 and Fluorescence (FL) in Figure 2. Total count, labeled count and percentage can be seen below with their standard deviation.

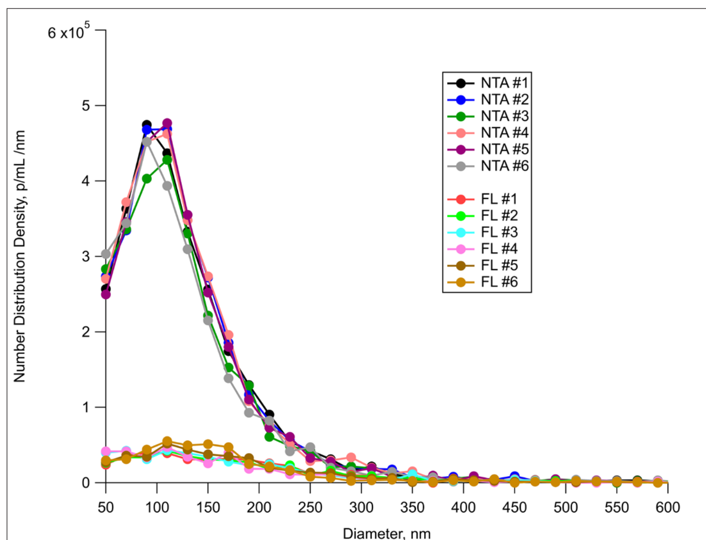

Figure 1: Unlabeled and Labeled HEK-293 Exosome Populations

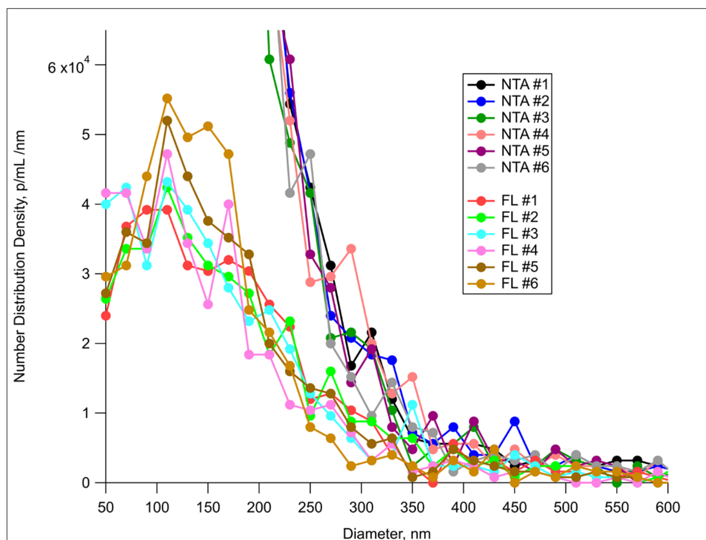

Figure 2: Fluorescently Labeled HEK-293 Exosome Population

Sample	Particle Count	% Labeled
FL 1	7.380E+06	14.76
FL 2	7.300E+06	14.46
FL 3	7.140E+06	15.66
FL 4	6.690E+06	13.22
FL 5	7.700E+06	15.62
FL 6	7.870E+06	17.61
NTA 1	5.000E+07	
NTA 2	5.050E+07	
NTA 3	4.560E+07	
NTA 4	5.060E+07	
NTA 5	4.930E+07	
NTA 6	4.470E+07	
Average:		**15.22**
Std dev.:		**1.47**

Table 1: Particle Counts Showing FL. Labeled and Unlabeled HEK Exosomes

The results show very consistent reproducibility in counts in both NTA and FL modes. NTA averaged concentration was 4.85e7 ± 2.61e06 (5.39%), while for FL it was 7.35e6 ± 4.19e5 (5.70%) (Figure 1). Labeling was quite consistent at 15.22 ± 1.47% (Figure 2). Labeling is crucial to exosome studies as they are isolated from complex biofluids, enabling the researcher to determine how many exosomes are present and to distinguish them from similarly sized particles.

Conclusion

By utilizing the ViewSizer in both NTA and Fluorescence mode we were able to detect labeled and unlabeled HEK-293 derived exosomes with strong reproducibility. Current literature suggests that some fluorescently labeled tags have very low efficiency (~5%)[1]. Here we observe ~15% labeling with a suitably small standard deviation. These results suggest that ViewSizer is an excellent tool for characterizing the size and concentration of EVs and that it can detect labeled populations of fluorescently tagged exosomes with high repeatability and accuracy. The size of the labeled population observed also suggests the labeling kit used from SBI to be very efficient and an excellent tool for investigating exosome populations when combined with ViewSizer.

References

1. Melling G, Conlon R, et al. *Nature Portfolio*, (2022) 12:262. https://doi.org/10.1038/s41598-021-04225-4

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