Microfluidic Resistive Pulse Sensing (MRPS) Measurements of EVs and EV Standards

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Introduction

As science based on EVs advances, comparison of EV measurements across different manufacturing sites and manufacturing methods is important. To isolate differences or drifts in EV formulations, it is necessary to have stable metrology so that sample differences can be properly attributed to changes in the formulation and not the metrology. Establishing stable metrology in turn relies on the development of standards measured by multiple orthogonal methods.

With this goal in mind, this poster reports measurements of EVs and EV standards using Microfluidic Resistive Pulse Sensing (MRPS) and other measurement techniques.

The results validate Spectradyne's MRPS technology for quantifying vesicles and vesicle standards, and highlight the unique insights MRPS delivers that optical technologies cannot.

The MRPS Technique

MRPS is rapidly being adopted as a powerful technique for measuring the size and concentration of extracellular vesicles.

MRPS:

- Uses electrical sensing, not optical
- Counts and sizes EVs one-by-one in solution
- Measures size directly, without inferring from diffusion
- Measures concentration directly, by measuring sampled volume

MRPS is therefore independent of the material properties of the particles, and measures samples accurately no matter their polydispersity.

Practical EV Quantification

Spectradyne's nCS1 delivers significant practical benefits that make it ideally suited for routine and quantitative EV analysis:

- Only 3 microliters sample required
- Results in minutes
- Pre-calibrated, disposable cartridges
- No cleaning required between runs
- No user-adjustable parameters

0.5 in (13 mm)

Methods

Liposomes (used as vesicle standards), red blood cell-derived EVs (RBC EVs), and platelet-derived EVs (PLT EVs) were produced by Cellarcus Biosciences. Nanoparticle Tracking Analysis (NTA) and Vesicle Flow Cytometry (VFC) measurements were made on these particles by Cellarcus, and MRPS measurements were done by Spectradyne. In a final lability experiment, the effect on EVs of exposure to Triton X-100 detergent was measured.

MRPS measurements were performed using a Spectradyne nCS1 instrument. Sample (3 microliters, typically at 1:10 dilution in PBS with 0.05% BSA) was analyzed using TS-300 cartridges (sizing range 50 nm – 300 nm) and/or TS-400 cartridges (sizing range 65 nm – 400 nm), and the data processed using Spectradyne software (v2.4).

NTA measurements were done using a Nanosight LM-10 equipped with a 532 nm laser, sCMOS camera and a syringe pump. Ten videos of 10 seconds each were acquired for each sample, with the syringe pump introducing a new sample into the field of view between acquisitions. Data were analyzed using NanoSight software (version 3.2). Standard acquisition settings were: camera level 16, detection threshold 6, with maximum jump length, blur, and min track length all set to Auto. Sample volume was 1 ml, at 1:100 or 1:1000 dilution.

VFC measurements volume was typically 100 microliters, after a 1:10,000 dilution. Samples were stained with a membrane stain (vFluorRed, Cellarcus Biosciences) plus fluorescence-labeled antibodies for one hour at ambient temperature, before being diluted and analyzed using fluorescence triggering (excitation: 488 nm; emission: 690/50 nm) on a Beckman Coulter Cytoflex or CytoFLexS flow cytometer. Membrane fluorescence was calibrated in terms of vesicle size (surface area) using a synthetic vesicle size standard (Lipo100, Cellarcus).

Results 1: MRPS Liposome Measurements

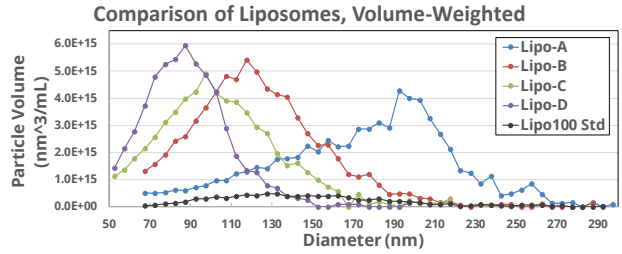

Figure 1. MRPS size distributions of five liposome samples (which serve as EV standards) show detailed size and concentration information. The volume weighting provides an assessment of total material in any size interval.

Results 2: EV Measurement Comparison

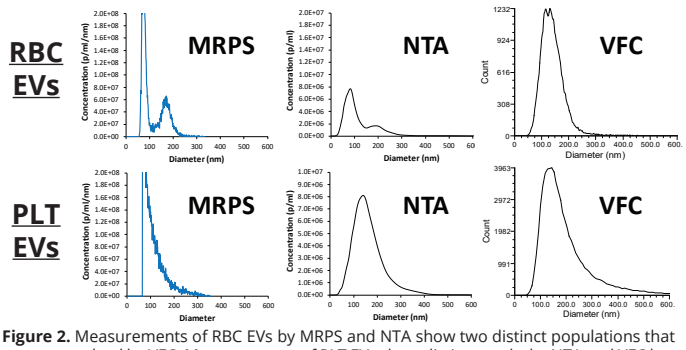

Figure 2. Measurements of RBC EVs by MRPS and NTA show two distinct populations that are not resolved by VFC. Measurements of PLT EVs show distinct peaks by NTA and VFC but not by MRPS. Other experiments support the interpretation that a large population of small weakly scattering particles is present in the PLT EV samples that are accurately quantified by MRPS but go undetected by the optical methods. This finding underlines the importance of using orthogonal metrologies when assessing formulation purity.

Results 3: Effect of Detergent on EVs

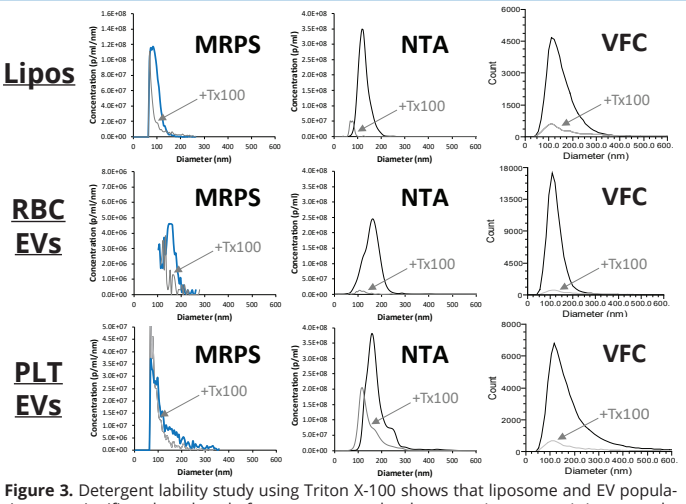

Figure 3. Detergent lability study using Triton X-100 shows that liposome and EV populations are significantly reduced after exposure to the detergent (grey traces). Importantly, MRPS shows a significant concentration of non-vesicle "background" particles that remain intact despite the addition of the detergent and that go undetected by the optical methods.

Conclusion

The experiments demonstrate that MRPS measurements of EVs and EV standards are sensitive to both EV and non-EV populations, giving critical information about the purity of EV samples. Researchers relying only on optical methods likely underestimate contamination and overestimate the quality of EV isolation, thereby hindering the development of high-quality EV-based diagnostics and therapeutics.