Quantification of Recombinant Outer Membrane Vesicles for Vaccine

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Principal Investigator(s): David Putnam

User(s): Mariela Rivera-De Jesús

Affiliation(s): Biomedical Engineering, Cornell University

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Contact: dap43@cornell.edu, mar524@cornell.edu

Primary CNF Tools Used: Malvern NS300 NanoSight

Abstract:

Bacteria-produced outer membrane vesicles (OMVs) are of great interest in the development of subunit vaccines. One challenge in their use as a vaccine platform is the difficulty of precisely quantifying OMVs. By using nanoparticle tracking analysis system, we can directly quantify the amount of recombinant OMVs produced and better adapt them for further applications. Recombinant ClearColi OMVs were indirectly quantified using bicinchoninic acid (BCA) assay and directly quantified using the Malvern NS300 NanoSight system, at different concentrations determined through the BCA assay. While no correlation was established between BCA-determined protein concentration and NTA-determined particle concentration, we have established a protocol for a direct quantification method of OMVs.

Summary of Research:

Outer membrane vesicles (OMVs) are 20 to 200 nm lipid nanoparticles produced by Gram negative bacteria. These vesicles contain the same surface proteins and sugars that are on the bacterial surface and are strong immunomodulators, an ability that has highlighted OMVs as a potential vaccine platform [1]. Bacteria can be recombinantly modified to produce OMVs with desired characteristics such as reduced toxicity and displaying of surface proteins. Our team is using recombinant bacteria to design a versatile OMV vaccine and investigate the co-presentation of antigens on OMVs. Indirect methods are the most employed, such as quantifying the total protein concentration or the dry mass of the vesicles, but it is important to have a precise measurement of the components of vaccines to produce reproducible results. One method for directly quantifying OMVs is through nanoparticle tracking analysis (NTA), using systems such as NanoSight [2].

Our goal is to develop a more precise method for measuring OMV concentration through NTA. In addition, if possible, we would like to establish a correlation between indirect quantification methods and NTA quantification methods.

Our group had previously modified ClearColi® (CC) BL21(DE3) cells to hypervesiculate through gene knockouts [3]. Three batches of CC OMVs were produced. In brief, liquid CC cultures were centrifuged (4°C, 15 min, 5,000 rcf) and the supernatant passed through a 0.2-mm filter. The filtrate was ultracentrifuged (4°C, 3 hr, 26,000 rpm) and decanted, and the pellets resuspended in PBS. The protein concentration of each batch was determined using the Pierce™ Bicinchoninic Acid (BCA) Protein Assay Kit. Batches were then diluted to 1.50, 1.00, 0.75, 0.50, 0.25, and 0.10 ng/μL according to the BCA assay. Each dilution of each batch was finally measured using the Malvern NS300 NanoSight (five replicates, 1 min each) to determine particle concentration.

Conclusions and Future Steps:

Figure 1 compares the protein concentration measured using the BCA assay and the particle concentration using the NanoSight. Each slope was significantly different from each other, as further highlighted by Figure 2.

Further study is needed to establish a potential correlation between BCA and NTA quantification. Nonetheless, a successful method for measuring OMV concentration through NTA was established. While indirect quantification is enough for in-lab samples, NTA quantification can be applied to sample where a more precise quantification is required, such as determining vaccine dosing.

References:

[1] Jan, AT. Front Microbiol. 2017; 8: 1-11.

[2] Gerritzen, MJH, et al. J. Extracell. 2017; 19(8): 271.

[3] Valentine, JL, et al. Cell Chem Biol. 2016; 23(6): 655-665.

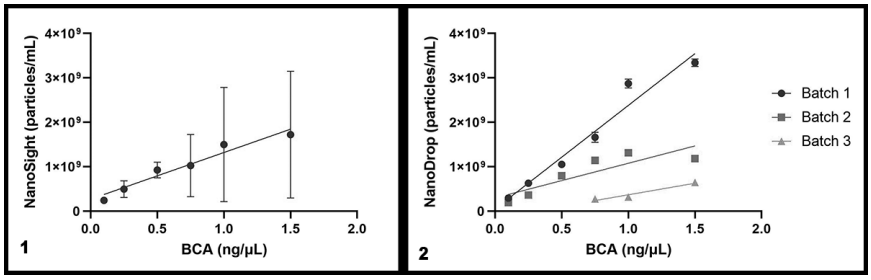

Figure 1. left: Comparison between BCA concentration and NanoSight concentration of CC OMVs, in individual batches. STD calculated by NanoSight. In Batch 3, the concentration of OMVs was too low to be measured below 0.75 ng/µL.

Figure 2, right: Comparison between BCA concentration and NanoSight concentration of CC OMVs, between all batches.