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Optimizing Exosome Size Measurement Accuracy: Impact of Medium Conductivity

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Abstract **-** Exosomes are nano-sized vesicles produced by cells and containing identifying cellular content, which play important and emerging roles in intercellular communication Exosomes have emerged as potentially vital players in both diagnostics and therapeutic applications, since their unique molecular composition mirrors that of their parent cells, making them exceptional biomarkers and promising tools for disease detection and targeted treatment. Accurate characterization of exosomes is therefore essential for unlocking their full potential in clinical and research settings. One method for exosome characterisation is the measurement of their size. However, since exosomes are non-metabolising and are therefore unable to drive ion channels to maintain cell size, they may be susceptible to swelling or other sources of size instability in low ionic strength media due to the formation of Donnan potentials from interaction with ions and membrane-impermeable charges such as proteins. In this paper we examine the effect of extra-exosomal conductivity of MCF-7-derived exosomes which suggests that exosomes suspended in media of greater than ca. 15% of physiological strength are similar to those at physiological strength, but that below this, they appear to be both highly variable and subject to swelling.

Keywords Exosomes, Diagnosis, Biomarkers, Characterization, Zeta sizer, and Conductivity.

1. Introduction

Extracellular vesicles (EVs) are membrane-enclosed particles released by nearly all cell types; however, in contrast to cells, they lack the capacity to replicate. They vary significantly in size, ranging from 20 nm to 10 μm, though most are smaller than 200 nm [1]. The variation in size and the mechanisms behind their formation contribute to the diversity of EVs, which include exosomes, microvesicles, and apoptotic bodies. EVs typically carry proteins, genetic material, lipids, and other cellular components from their source cells. This makes them promising candidates as biomarkers for various diseases and cancers, as they can transfer genetic material like RNA between cells. Studies have shown that EVs significantly influence the behavior of recipient cells [1]; for example, EVs secreted by colorectal cancer cells play an important role in stimulating fibroblast proliferation by enhancing cell-to-cell communication[1].

Exosomes, a subtype of EVs, are defined by their double-layered membrane and are secreted by a wide range of eukaryotic cells. They are found in numerous tissues and bodily fluids, such as blood, urine, and saliva, and typically measure between 30 and 150 nm in diameter[2]. Exosomes are known for bearing specific molecular signatures derived from their originating cells and participate in a range of biological functions. As shown in **Figure 1,** they encapsulate lipids, proteins, DNA, and various forms of RNA, such as mRNA and miRNA, which differ from their intracellular counterparts. Through the transfer of these biomolecules between cells, exosomes serve as critical mediators of intercellular communication, impacting processes such as immune response modulation and protein production[3].

Exosomes are characterized by several attributes. In addition to standard molecular assays, exosomes are commonly categorized according to physical parameters such as particle size and surface charge. Given the size of exosomes, determination of size cannot be performed microscopically, relying instead on the inference of size through some other

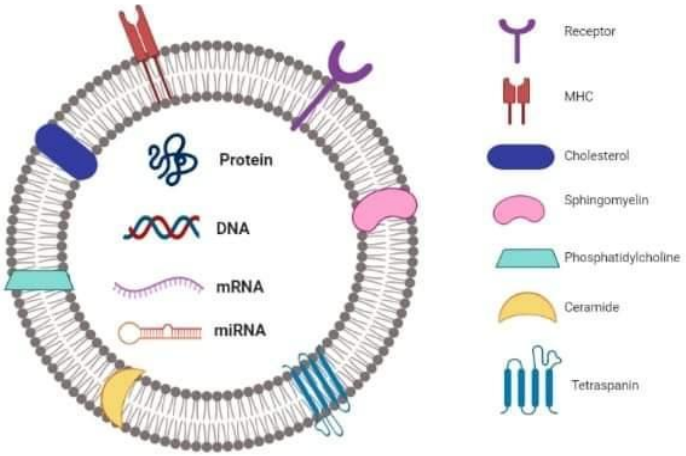


Figure 1: The main components of exosomes. Created with BioRender.

physical manifestation such as measurement of Brownian motion that allows determination of radius through the Einstein-Stokes relationship[4]. Several techniques have been developed to measure this, including nanoparticle tracking analysis (NTA) that uses video analysis of nanoparticles to determine the distribution of nanoparticle size; another common approach is the use of dynamic light scattering (DLS) to estimate the mean particle size, though not the size distribution.

An important question about the behavior of exosomes is the way in which they behave in media of different ionic strengths. Since they have no active pumping mechanism and have relatively large surfaces compared to their interiors (due to the so-called square-cube law), we would anticipate rapid equilibration of ions across the membrane. However, the presence of large molecules (which cannot pass through the membrane) will attract water due to osmotic pressures, creating osmotic, chemical and electrostatic gradients across the membrane. These typically resolve to a so-called Donnan equilibrium, with increased ionic and water content inside the exosome. This will in turn have an impact on size. This is of particular importance when exosomes are analyzed in non-physiological ionic strength, using methods such as dielectrophoresis (DEP) [5] which typically occurs in low-conductivity media.

In this paper, we have used DLS to measure the size of exosomes derived from the human breast cancer cell line MCF-7 across a range of different medium ionic strengths and compare these to gold-standard NTA measurements at physiological strength. The results suggest that exosomes are most stable at values of medium conductivity of 200 mS/m or greater, corresponding to approximately 15% of physiological ionic strength.

2. Materials and Methods

2.1. Cell culture

MCF-7 breast adenocarcinoma cells (ATCC® HTB-22™) were obtained from Biomss (Al Ain, UAE). Dulbecco's Modified Eagle Medium (Gibco™ DMEM High Glucose, 41965-039), Fetal Bovine Serum (Gibco™ FBS, 10270-106), a Penicillin-Streptomycin mixture (Pen/Strep, 09-757F), and Phosphate Buffered Saline (PBS, 10X, 17-516Q. All reagents were sourced from Lonza-Bioscience (Basel, Switzerland).

MCF-7 cells were initially seeded into 75 cm^2 culture flasks (Corning) and passaged at a 1:3 ratio upon reaching 80%–90% confluence. All culturing steps were conducted in accordance with the manufacturer's instructions. For both two-dimensional (2D) and spheroid cultures, phenol red-free DMEM medium was used, supplemented with 10% fetal bovine serum (FBS), 0.01 mg/mL bovine insulin, and a standard penicillin-streptomycin mixture.

2.2. Isolation of Exosomes

The appropriate volume of cell-free culture media was transferred, followed by the addition of 0.5 volumes of the Total Exosome Isolation Reagent. The mixture was then thoroughly blended using either vortexing or pipetting. The samples were then stored overnight at 2°C to 8°C. Following incubation, they were centrifuged at 10,000 × g for 1 hour at the same temperature. The resulting supernatant was carefully removed and discarded, leaving the exosomes as a pellet at the bottom of the tube. The pellet was then resuspended in an appropriate volume of 1X PBS.

2.3. Media

To make 35 mL of DEP (dielectrophoresis) media with adjustable conductivity, begin by mixing 2.975 grams of sucrose with 0.105 grams of dextrose. Next, incorporate 35 µL of calcium chloride ($CaCl_2$) and 87.5 µL of magnesium chloride ($MgCl_2$). Finally, add distilled water to bring the total volume to 35 mL. After preparing the base solution, gradually adjust the conductivity by slowly adding small quantities of 10x DPBS (Dulbecco's Phosphate-Buffered Saline). Because this solution is highly concentrated, it's important to add it incrementally at first to prevent exceeding the target conductivity.

2.4. Particle Size measurement

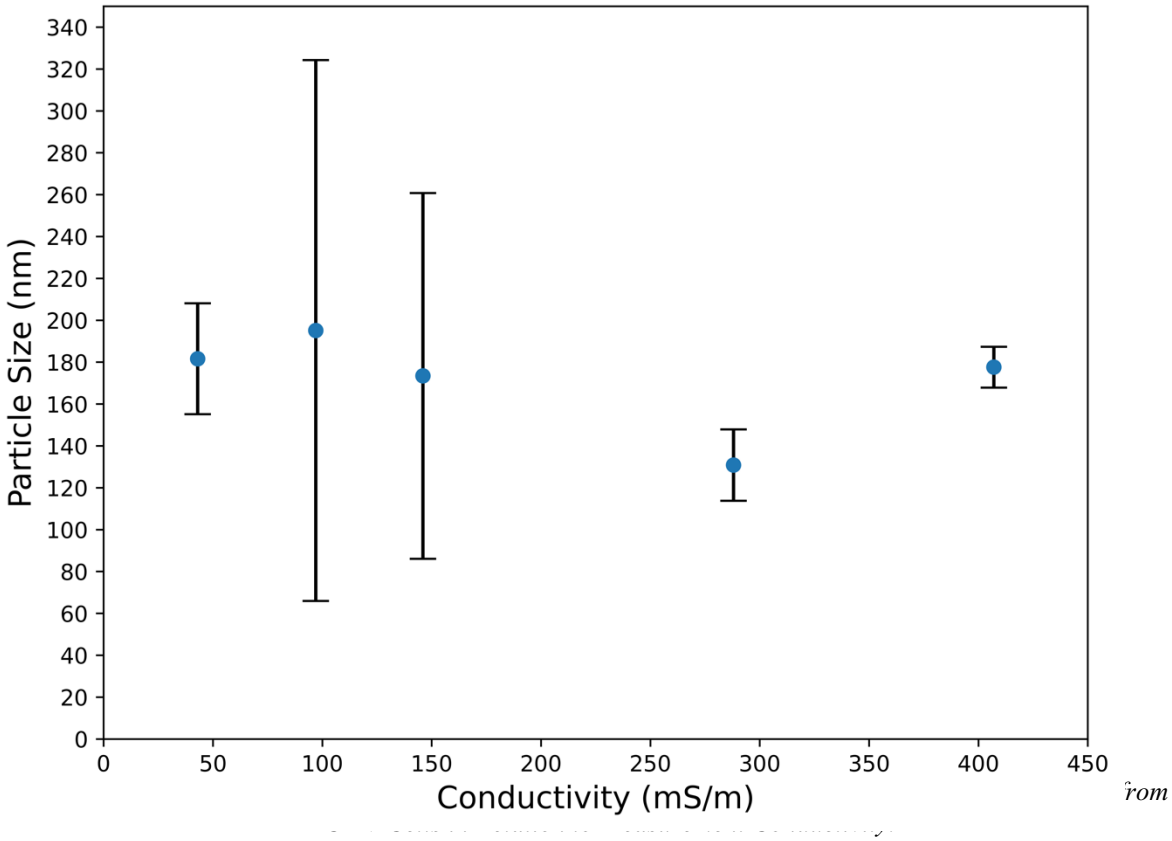
Nano Tracking Analysis (NTA) was used to determine the size and concentration of exosomes isolated from the MCF7 cell line. Various exosome samples derived from MCF-7 cells were analyzed to determine their mean particle size using Dynamic Light Scattering (DLS). Each sample was measured three times.

3. Results and Discussion

The measured particle sizes of exosomes derived from MCF-7 cells are shown in Figure 2, including their associated standard deviations. In exosomes at lower conductivities (below around 200 mS/m) substantial variation was observed in exosome size, suggesting that exosomes may respond differently to the extracellular ionic medium according to their macromolecular content. The lowest conductivity showed somewhat reduced variation, though this may be due to osmotic stress having damaged or destroyed some of the larger exosomes.

Conversely, measurements above 200 mS/m showed little variation, suggesting a highly homogeneous size profile. The particle size diameter observed at conductivity 407 mS/m was 177.6 nm. When this was compared to NTA measurements in physiological medium, we found them to be highly comparable (average size of 138 nm at a concentration of $8.3x10^9$ particles ml^{-1}). Similarly, the value observed at 288 mS/m was 130.9, again similar to that observed in NTA, suggesting that there is a minimal effect on exosome size under these conditions.

Understanding size distributions is important, as they affect exosome function and interactions. This study emphasizes the need for standardized protocols to reduce variability and improve exosome characterization. These results suggest that for these MCF-7-derived exosomes, the diameters measured by DLS and NTA are comparable, but that exosome stability is adversely affected by low ionic strength media, leading to wide variations in size. This needs to be considered when using methods such as dielectrophoresis to measure exosomal properties.



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4. Conclusion

In this research, exosomes derived from MCF-7 cells were efficiently isolated and examined using both NTA and DLS, with the latter used to measure exosomes at low ionic strength. Exosomes showed considerable variability in particle size within a single sample at lower conductivities, suggesting instability in low ionic strength media. This may arise from the lack of active ion transport leading to osmotic swelling as part of the formation of Donnan equilibria. Optimizing medium conductivity within a defined optimal range significantly improves the stability and accuracy of exosomal measurements, offering valuable advancements for characterization methods in exosome research.

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