Recent Technical Developments in the Standardized Separation and Measurement of Extracellular Vesicles

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The Need for Standardised Analysis In Extracellular Vesicle Research

The concentration, size, and charge of vesicles reflect the health of the organism or tissue they are extracted from[1]. Accurate measurement of these parameters, therefore, has critical implications in the clinic. Furthermore, sample preparation can influence the vesicle characteristics[2].

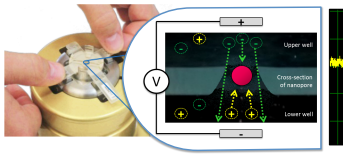
A standardised method of vesicle preparation and analysis is needed to allow comparison between research groups.

Current purification methods have problems with aggregation, or long, laborious processes. Commonly used characterization techniques lack sensitivity, and resolution[3].

We have developed a method of isolation and characterization, which together provide a sensitive method for determining vesicle size and concentration.

TRPS: Detailed Size, Charge and Concentration of EVs

Izon TRPS use a tunable elastic pore to measure particle properties. The size, charge and concentration of vesicles are determined with high resolution and sensitivity from the ionic current pulse signal they generate as they pass through the elastic pore[4].



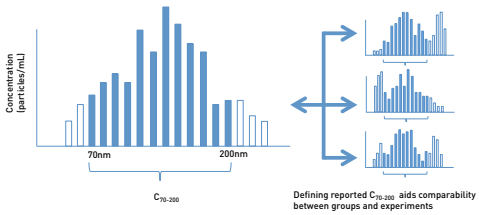
Tuning the pore size, applied pressure and voltage improves the measurement sensitivity and enables a wider range of particles to be analysed.

Particle Size:	50nm – 20µm+
Conc. Range:	10^5 – 10^{12} particles/mL
Sample Size:	30µL
Sample Media:	Aqueous Buffer

Size-Based Concentration

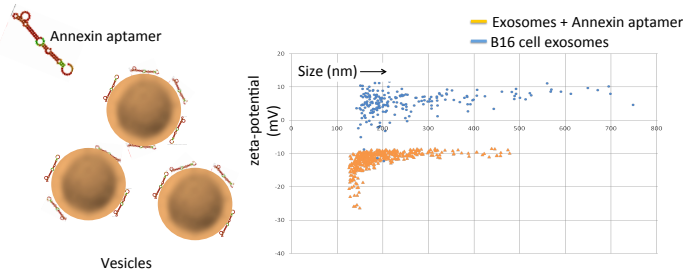
A new definition of particle concentration based on the size range being examined has been proposed, e.g. $C_{70\text{-}200}$

This is more meaningful than the standard definition in that it specifies the range under consideration and of interest to the researchers (e.g. 70 – 200nm), and will aid comparability between measurements.



EV Identification

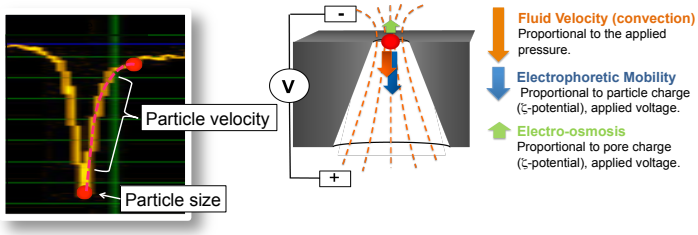
Aptamer based tool kit in development, to be used in conjunction with qNano to provide biological information.



The interaction of aptamers with biological targets on the vesicle surface, e.g. annexin, can provide discriminatory information based on the size and charge profiles of the different populations.

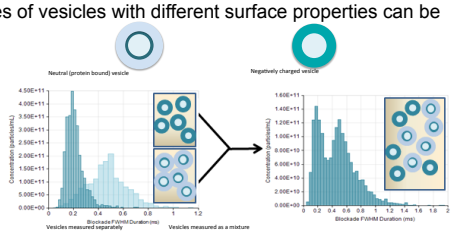
Particle Surface

Analysis of ζ-potential & surface modification



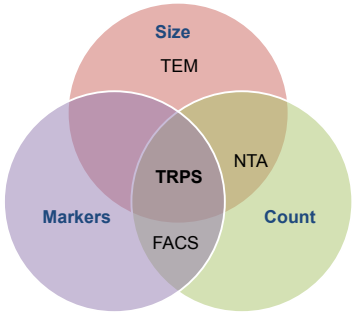
ζ-potential is calculated from the velocity of the particle as it moves through the nanopore. Changes to the particle surface through, for example, protein or aptamer binding can cause changes in ζ-potential. Due to the particle-by-particle measurement, mixtures of vesicles with different surface properties can be measured.

Surface charge of vesicles decreases vesicles protein binding. Ratios of differently charge vesicles can be detected.



Conclusions

- TRPS is the only high resolution technique which measures Size and Count, as well as surface Biomarkers (Figure)
- Size-Based Concentration is essential for meaningful comparison of measurements
- qEV columns provide a simplified approach to EV separation and isolation, and are expected to become the standard method.



Comparison of the capabilty of techniques for analysing vesicles.

References

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