Size and Charge Fingerprints of Extracellular Vesicles by Nanoparticle Tracking Analysis (NTA/PTA)

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Particle Tracking Analysis – size and more

Particle Tracking Analysis, also referred to as Nanoparticle Tracking Analysis (NTA/PTA) is a technique suitable for the characterization of particles typically between 20 nm and 3 µm (depending on particle properties). According to our experience for extracellular vesicles (EVs) the limit of detection is ~40 nm.

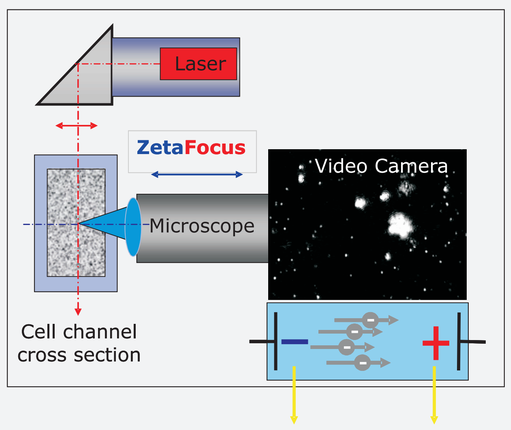

Figure 1: Principle of measurement setup. Particles are suspended in a cuvette and illuminated by a laser. The 90° scattering light is detected by a camera.

In **multiparameter** PTA, size concentration and zeta potential can be measured on the same sample.

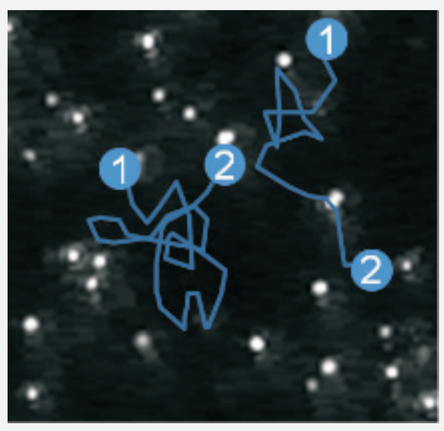

Figure 2: Tracking of EVs.

Size Fingerprints

The optimization of EV isolation is a crucial step. This abstract focuses on the question how the incubation time affects the particle size distribution of EVs.
After blood collection (venous), serum was incubated at 37°C. Alliquots were taken after 0, 3, 6, 9 and 24h of incubation. Isolation of EVs were performed by precipitation (10% PEG 8000 in 0.9% NaCl).

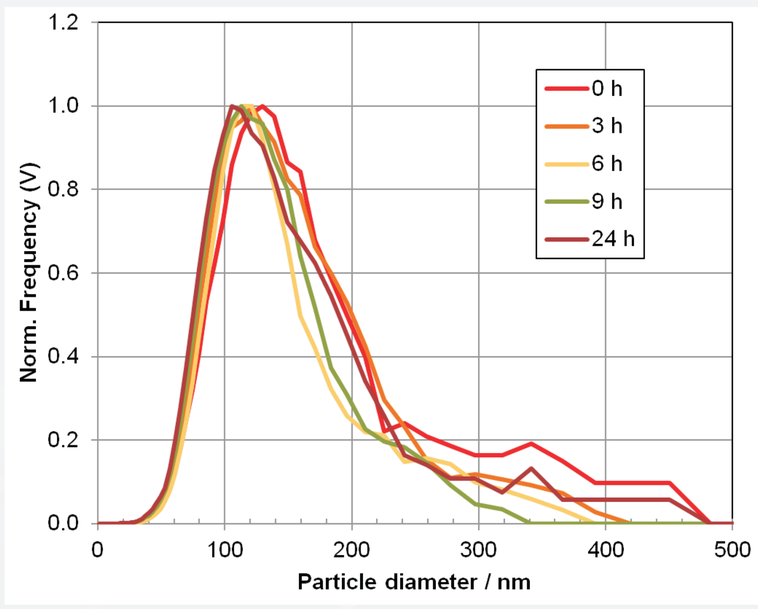

Figure 3: Particle size distributions of EVs. The particle size distributions show variations over incubation time.

- Particle size distributions of 0, 3 and 24 h show comparable particle size distributions.
- At incubation times 6 and 9 h the PSDs reveal a somewhat lower content for particles >200 nm .
- The increase of larger particle content (24 h) it is not fully understood and part of ongoing research.

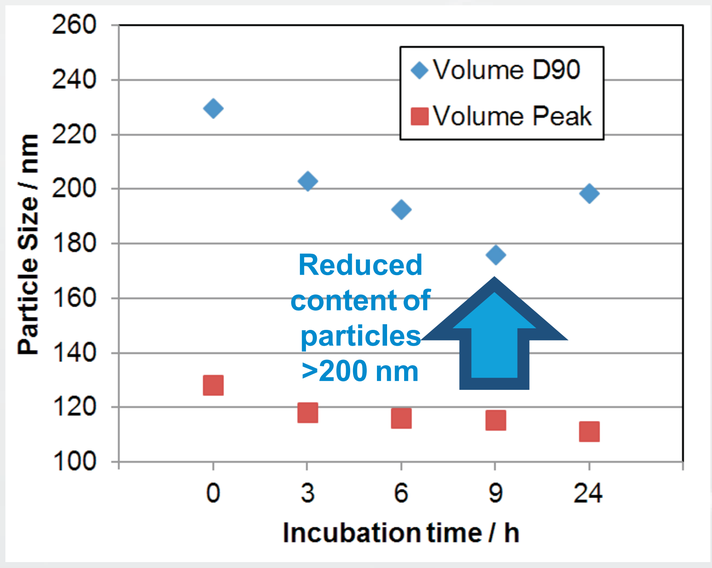

Figure 4: Monitoring of peak diameter and D90.

Zeta Potential - It's all about the surface

Nanoparticles are thermodynamically instable and suffer agglomeration over time. In the same way, it would be expected that EVs undergo fusion. Stability mechanisms such as steric or **electrostatic stabilization** reduce the rate of agglomeration.

Stability is important as **the properties of aggregates may be completely different from single particles.**

Stability can be monitored via the zeta potential.The stability of particles depends on e.g. temperature, ionic strength and pH. In buffer (e.g. saline buffer) the salt content is rather high, resulting in a short range of electrostatic forces enhancing EV interaction.

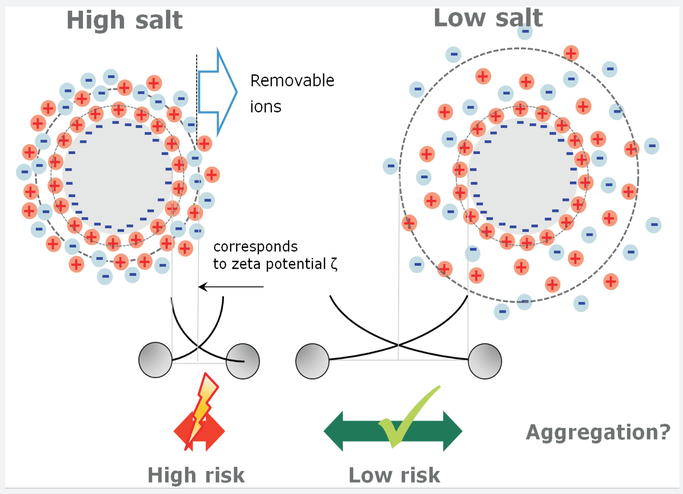

Figure 5: Explanation of the electrostatic stability mechanism.

Zeta Potential : Observing EV fusion?

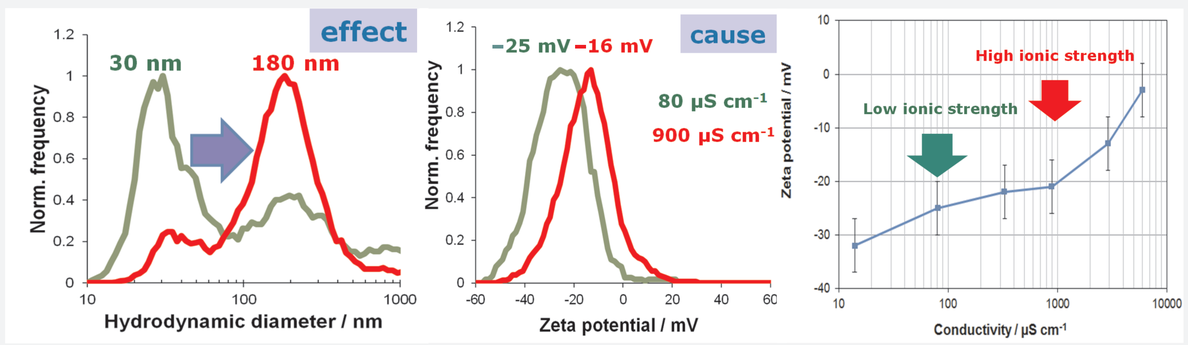

Figure 6a: Particle size distribution of HEK cell line EVs isolated by ultracentrifugation (UC) resuspended in Soerensen buffer pH7. At high ionic strength, the particle size distribution shifts towards larger size.

Figure 6b: Zeta potential distribution shift to higher zeta potential for high ionic strength

Figure 6c: Zeta potential fingerprint of EVs over conductivity

At low ionic strength, a population of particles with 30 nm were present. The 30 nm signal almost vanished at high ionic strength and a population around 180 formed. In correlation with the zeta potential distributions, the lower zeta potential of the larger population indicates agglomeration.

Zeta Potential Fingerprints

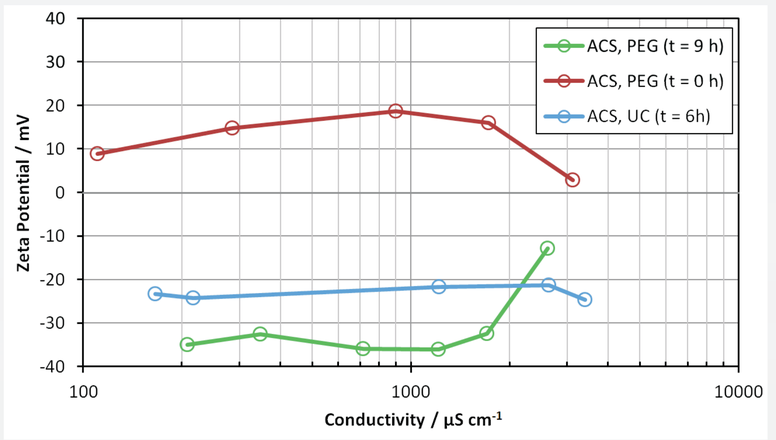

Figure 7: Zeta potential fingerprints of EVs isolated from blood serum, incubated at 37°C. The isolation methods ultracentrifugation (UC, resuspension in PBS) and precipitation (10% PEG 8000 in NaCl) are compared.

The zeta potential is influenced by ionic strength (buffer concentration) and the individual sample preparation.

In general, zeta potentials (absolute numbers) decrease for high conductivity, indicating instability at high ionic strength (high possibility of particle-particle interaction). Before incubation PEG isolated EVs show cationic nature and change sign of charge after 9 h incubation.

Conclusion

- **Incubation time** has an effect on particle size distributions.
- **Zeta potential fingerprints allow quantification of stability.** This would allow to quantify the influence of sample preparation on EVs such as composition of buffer, multiple thawing-freezing cycles and pH.

Acknowledgement

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