
Nishantika Mitra
nishantika.mitra@auckland.ac.nz
The Liggins Institute, The University of Auckland

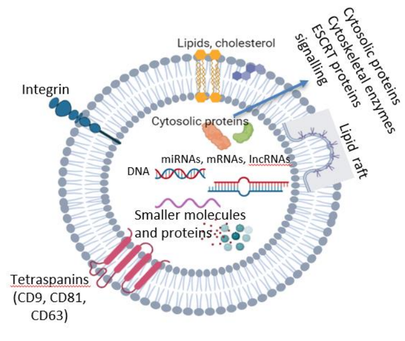
Optimising a protocol for the isolation and characterisation of plasma extracellular vesicles (EVs)

Nishantika Mitra [1], Hui Hui Phua [1], Cherie Blenkiron [2], Anastasiia Artuyants [2], Chris Pook [1], Fiona Lithander [1], Mark Vickers [1], Richard Mithen [1] , Farha Ramzan [1]

[1]Liggins Institute, The University of Auckland, Auckland, New Zealand, [2]Molecular Medicine and Pathology, The University of Auckland, Auckland, New Zealand

Background

- Extracellular vesicles (EVs) are lipid bound particles (30-5000 nm) secreted by the cells and carry a cargo of important bioactive molecules (**Figure**).
- EVs are involved in mediating intercellular communication through transfer of their cargo and have potential utility as disease biomarkers and therapeutic agents(1).
- Diet is shown to impact the composition of EVs. However, methods to accurately profile EVs require robust and standardised isolation and analytical approaches(2).



Abbreviation
ESCRT: Endosomal sorting complex required for transport

Structure and composition of EVs

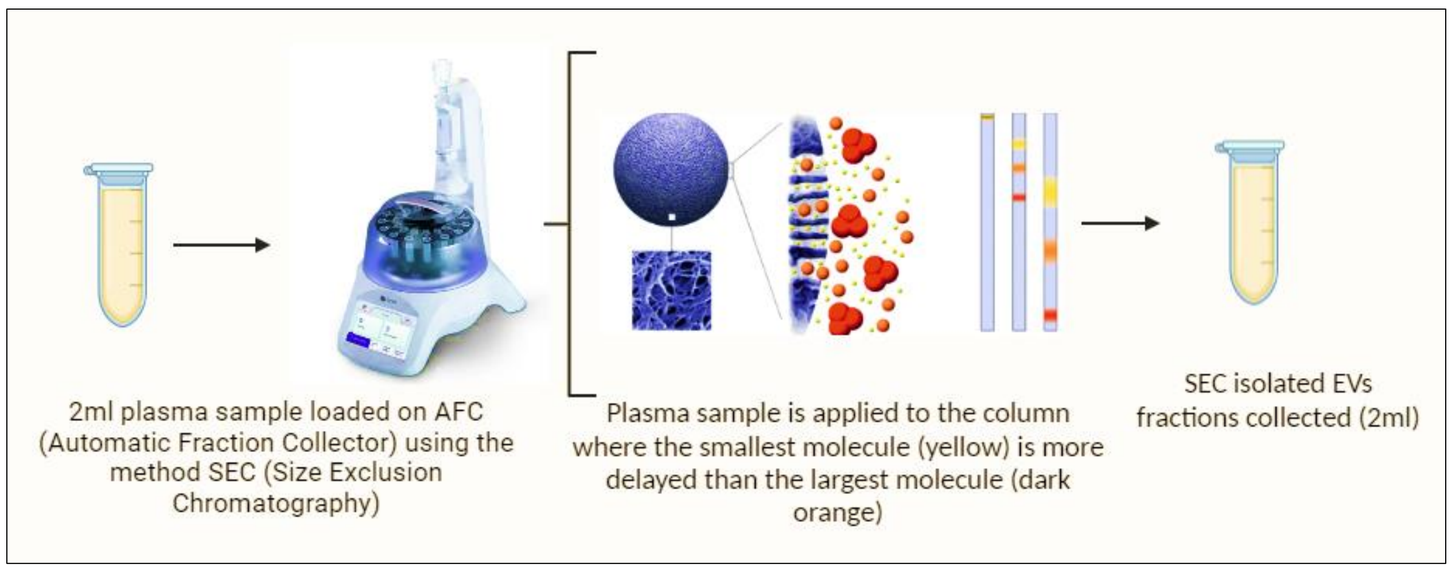
Hypothesis

- Size exclusion chromatography (SEC) will effectively isolate EVs from plasma, resulting in isolation of EVs that are enriched and free of lipoprotein contaminants, thereby preserving the integrity and functionality of the EVs for accurate downstream molecular and functional analyses.

Aim

- Optimise and standardise a method for EVs isolation and characterisation using SEC method.
- Characterise the obtained EV enriched samples using MISEV 2023 guidelines.

Method



Characterisation

1. Bicinchoninic acid (BCA Assay)
2. Nanoparticle tracking analysis (NTA)
3. SYPRO ruby gel stain
4. Western blot
5. Transmission electron microscopy (TEM)

Results

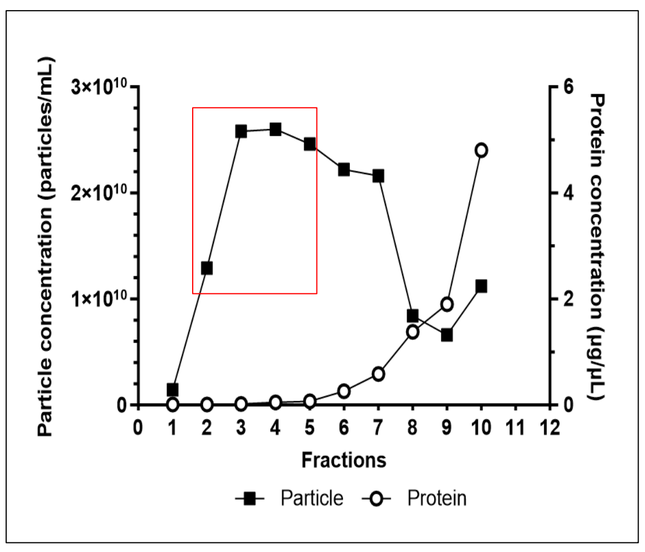


Figure 2: BCA Assay and NTA analysis

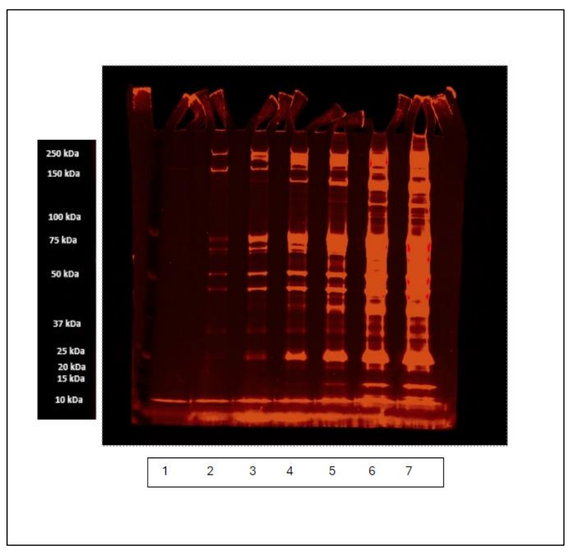


Figure 3 : SYPRO Ruby gel stain

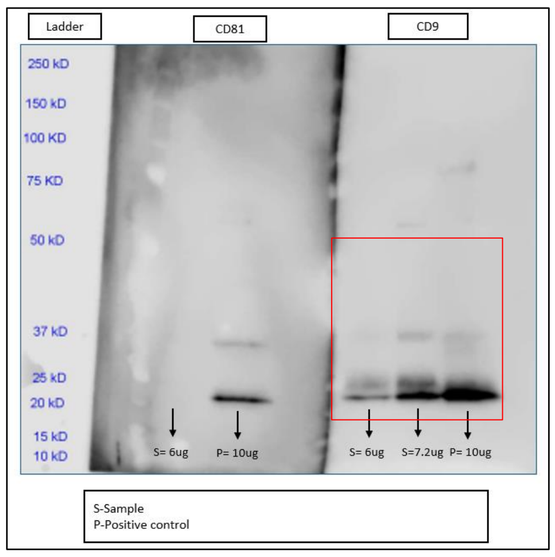


Figure 4 : Western blot

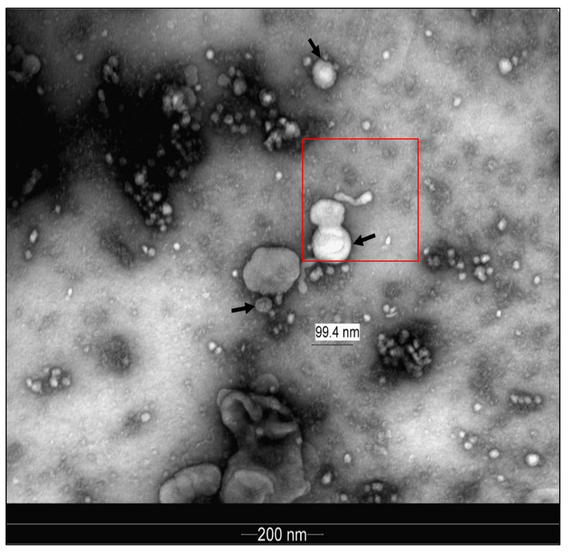


Figure 5: TEM

Discussion

- SEC is a highly robust and effective method for isolating EVs from small volumes of plasma, with SEC fractions 2-5 showing a high purity of EVs with minimal contamination from lipoproteins.
- Further confirmation of EV-specific markers, confirmed their biological integrity, which is crucial for downstream functional and molecular analyses.
- Given these results, SEC is our preferred method for downstream analysis, offering a reliable approach to obtaining intact and uncontaminated EVs for further research.

References

1. Santín-Márquez R, Alarcón-Aguilar A, López-Diazguerrero NE, Chondrogianni N, Königsberg M. ***Sulforaphane - role in aging and neurodegeneration***. GeroScience. 2019;41(5):655–70.
2. Urabe F, Kosaka N, Ito K, Kimura T, Egawa S, Ochiya T. ***Extracellular vesicles as biomarkers and therapeutic targets for cancer***. Am J Physiol - Cell Physiol. 2020;318(1):C29–39.