A validated technique for exosome isolation from cerebrospinal fluid

Julia Milliex*, Ana Gabriela Henriques, Aled Clayton and Michael Jones

Cell Guidance Systems, Cambridge, UK (JM and MJ), Institute of Biomedicine, Aveiro, Portugal (AH) and Cardiff University, Cardiff, UK (AC).

**julia.milliex@cellgs.com*

Abstract

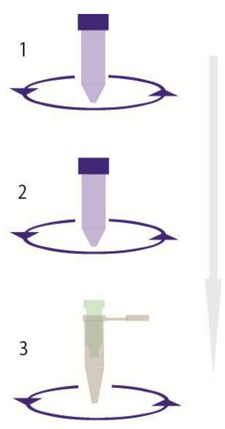
Exosomes (a subcategory of Extracellular Vesicles (EVs)) are lipid bilayer structures, released by most cell types. They range in size from 30–150 nm and are present in almost all body fluids. They play a central role in intercellular communication by carrying proteins, small molecules and genetic material (mRNA and miRNA) to other cells, which make exosomes particularly promising as reservoirs of diagnostic and prognostic biomarkers.

Exosome isolation from cerebrospinal fluid (CSF) is commonly investigated to discover potential protein candidates for diagnostics in Central Nervous System (CNS) diseases. However, the isolation step is still challenging and an efficient method for isolating exosomes from small volumes of CSF needs to be identified.

In this poster, we highlight the Exo-spin™ Exosome isolation kit as a novel tool to help exosome isolation in neurodegenerative disease research.

Exo-spin™ mechanism

Exo-spin™ Technology combines **precipitation and size exclusion chromatography (SEC)**, making it superior to techniques that rely solely on precipitation which result in co-purification of large amounts of non-exosomal proteins and other material as well as carryover of the precipitant.



1. Remove cells and cellular debris
2. Precipitate exosomes containing pellet
3. SEC column purify exosomes

- **Comparative data:**

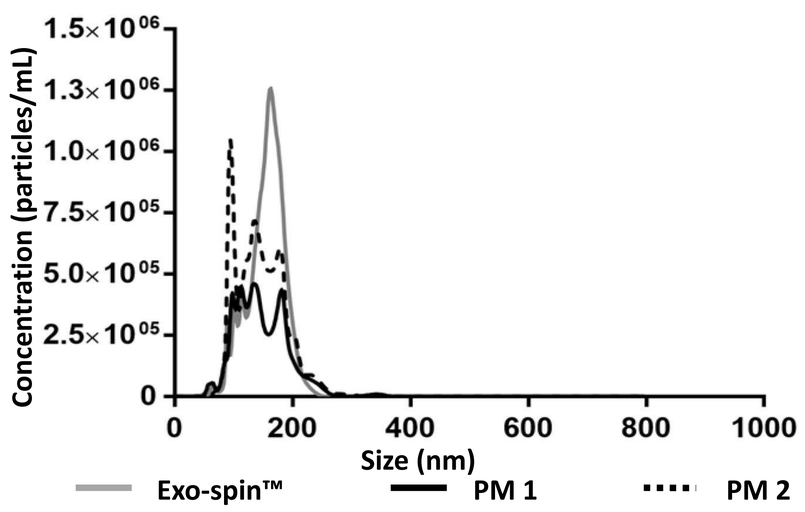


Figure 1: Data determined by nanoparticle tracking analysis (NTA). Each curve represents the average of 3 technical replicate measurements for each exosome isolation method and biofluid triplicate experiment. (PM = Precipitation Method)

Reference: Soares Martins, T., Catita, J., Martins Rosa, I., A. B. da Cruz e Silva, O. and Henriques, A. (2018). Exosome isolation from distinct biofluids using precipitation and column-based approaches. PLOS ONE, 13(6), p.e0198820.

Exosomes isolation from CSF

Method

5 ml of CSF starting volume was obtained from consenting patients between 20–40 years of age and processed using Exo-spin™.

Subsequently, a proteomic study comparing EVs isolated from CSF (EVs-CSF) from relapsing remitting (RR) multiple sclerosis (MS_ patients and non-demyelinating disease controls (Idiopathic intracranial hypertension; IIH) was performed.

Results

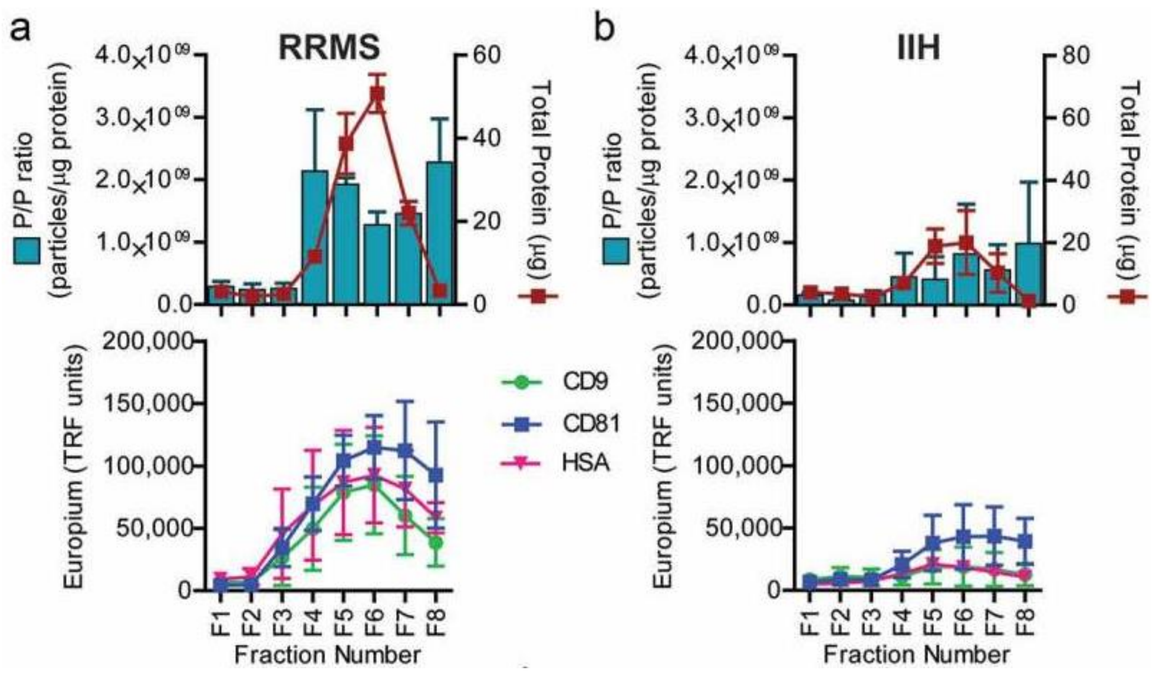


Figure 2: EV isolate sample characterization for RRMS or IIH patients. 8 fractions of 100 µl were collected and analyzed. The protein concentration was estimated by NanoDrop™ and the particle concentration measured by NTA. The ratio of particles to protein (particles/µg) was calculated and plotted (left axis: blue bars), with total protein (µg/ml) on the right axis (red line) (±SEM). A proportion of each fractions was also immobilized onto high-protein-binding microplates. The wells were stained with primary antibodies against CD9, CD81 or HSA and detected using TRF as a readout (arbitrary TRF units shown).

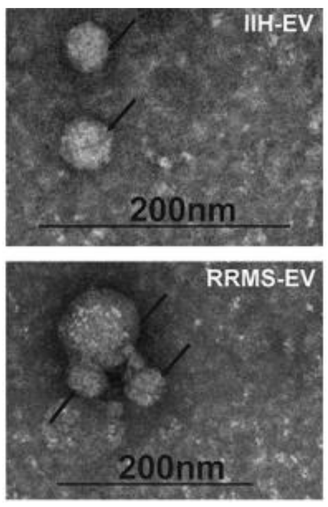


Figure 3: EVs-CSF imaging by transmission electron microscopy. Representatives of the heterogeneous EV populations are highlighted with black arrows.

Reference: Welton J., Loveless S., Stone T., Ruhland C., Robertson NP., and Clayton A. (2017). Cerebrospinal fluid extracellular vesicle enrichment for protein biomarker discovery in neurological disease; multiple sclerosis. J Extracell Vesicles 6(1): 1369805.

Conclusions

- Exo-spin™ offers a fast and easy protocol (< 10 min/sample) unlike ultracentrifugation or larger SEC columns available.
- Successful isolation of EV-enriched fractions can be achieved with Exo-spin™, as demonstrated by the presence of EV-associated markers, tetraspanins CD9 and CD81.