Exosome purification

Affinity tools for extracellular vesicle purification

Sandra Bezemer[1], Jaco Hazenoot[1], Frank Detmers[1], Pim Hermans[1], Chantelle Gaskin[2]

[1]Thermo Fisher Scientific, Emmy Noetherweg 2, Leiden, The Netherlands
[2]Thermo Fisher Scientific, 2 Preston Ct, Bedford, MA. 01730 USA

Abstract

Extracellular vesicles (EVs) are a promising platform for the delivery of therapeutic products, but the manufacturing process is challenged by their complexity and heterogeneity. Currently, purification strategies described in literature are based on time consuming and non-scalable options, like density gradient ultracentrifugation.

Leveraging the Thermo Fisher™ CaptureSelect™ affinity ligand technology, here we report the development of a novel affinity purification tool for EVs by immobilizing the Thermo Fisher™ CaptureSelect CD81 affinity ligand on a magnetic bead platform. EVs containing CD81 protein were specifically captured from cell-line derived and human plasma feed streams and eluted from the magnetic beads with arginine-based buffers. Efficient elution was achieved at mild pH which kept the EVs structurally intact.

Due to their complexity, orthogonal analytical methods were implemented to characterize and quantify EVs throughout the workflow, including phenotypic characterization, intactness, sizing, and titer determination. Results demonstrated an effective and efficient purification of CD81 bearing EV particles enabled by this magnetic bead platform. This simple purification method together with ligand-based analytical assays can help advance the development and manufacturing of new EV based therapies.

Affinity purification of EVs via magnetic beads

We developed a range of CaptureSelect affinity ligands that target and selectively bind different tetraspanin membrane proteins (CD9, CD63, and CD81) for various applications such as detection ELISAs.

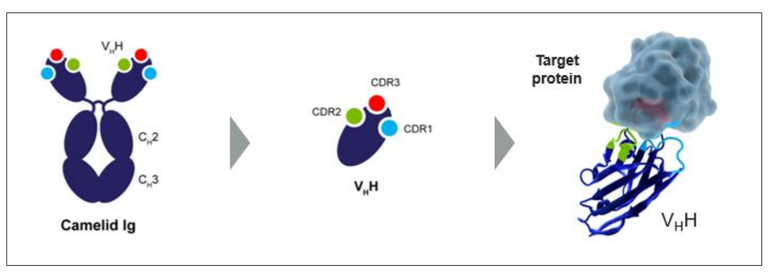


Figure 1: CaptureSelect ligands are V_HH fragments, the smallest antigen binding molecule. CaptureSelect technology, based on llama single domain antibodies, has been used to develop GMP-grade affinity resins with high specificity and mild elution conditions for a variety of new therapeutic targets.

The transmembrane protein CD81 is known to be the most widely expressed tetraspanin protein on EVs. Therefore, we created a purification tool for EVs by immobilizing the CaptureSelect CD81 affinity ligand that specifically recognizes CD81 on a magnetic substrate. The Thermo Scientific™ CaptureSelect™ CD81 Magnetic Agarose Beads are high-capacity, high-throughput magnetic affinity particles for exosome purification using manual or robotic magnetic separators

CaptureSelect CD81 Magnetic Agarose Beads enable:

- One-step exosome purification with high purity, from crude materials like cell clarified harvest samples and human plasma
- Manual and automated workflows (e.g., Thermo Scientific™ KingFisher™ instruments)
- Short operation time
- Elution with an exosome-compatible buffer (e.g. TBS, 1 M Arginine, pH 9)
- Non-animal-derived, free of animal components

Figure 2: The CD81 Magnetic Beads enable a rapid and easy purification process of CD81 bearing vesicles directly from cell clarified harvest samples or from biological fluids like human plasma in a single step with high purity and yield.

Simple EV purification protocol

EVs were purified from a wildtype HEK293 cell culture. Fractions were analysed and characterized by sandwich ELISA, SDS-PAGE, and Single-Particle Interferometric Reflectance Imaging (SP-IRIS)

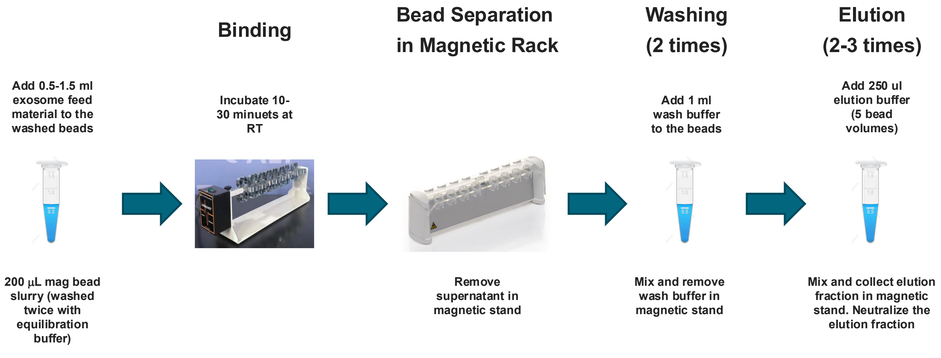
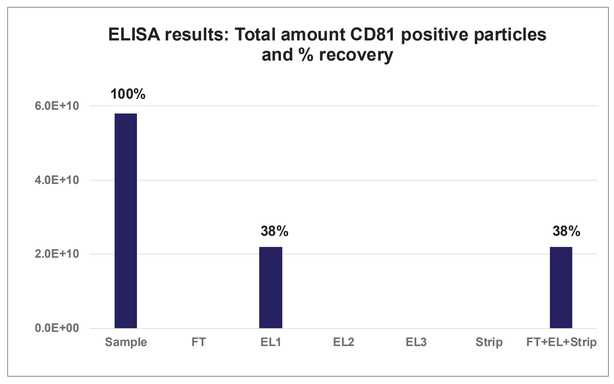


Figure 3: Clarified cell culture harvest (HEK293) containing ~8 x 10^9 EVs was applied to 200µL pre-washed magnetic bead slurry and washed twice with TBS, pH 7.4. Beads were incubated at RT before separating on a magnetic rack. Beads were washed twice more as previously and eluted three times with 1M Arginine/TBS pH 9. Elution fractions were neutralized with 1M Tris pH 7.

Results

Sandwich ELISA and SDS PAGE analysis



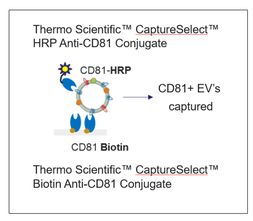


Figure 4:. Load, elution fractions, supernatant, and strip were analyzed by ELISA using Thermo Fisher™ CaptureSelect™ biotin anti-CD81 conjugate. Recoveries measured represent ongoing challenge in achieving mass balance in exosome purification.

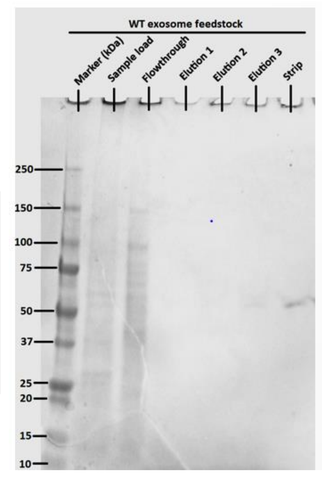
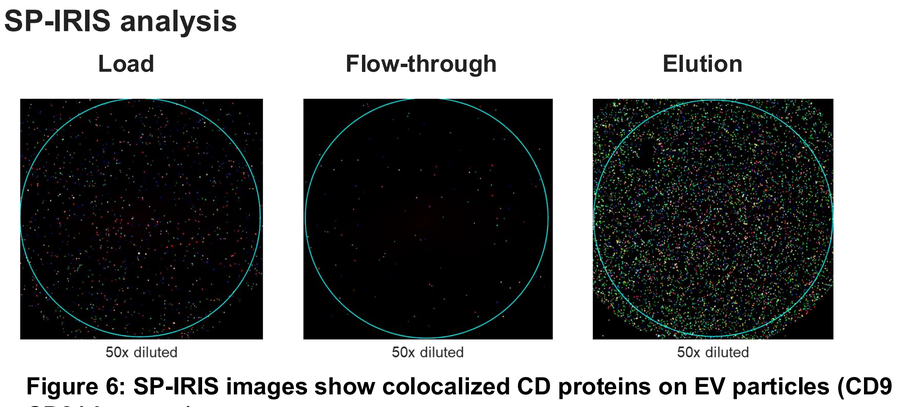


Figure 5: Non-reduced SDS PAGE shows protein detected in starting material and flow-through fraction, but removed in elution fractions

- ELISA results show efficient elution of CD81 containing EVs in the first elution fraction
- Protein analysis demonstrates near complete reduction of HCP and high level of purification in one-step.

SP-IRIS analysis



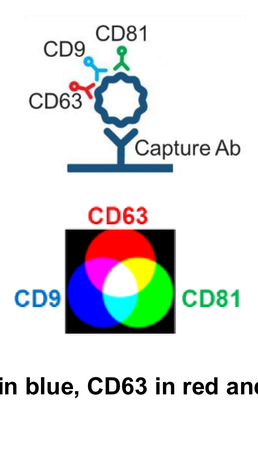
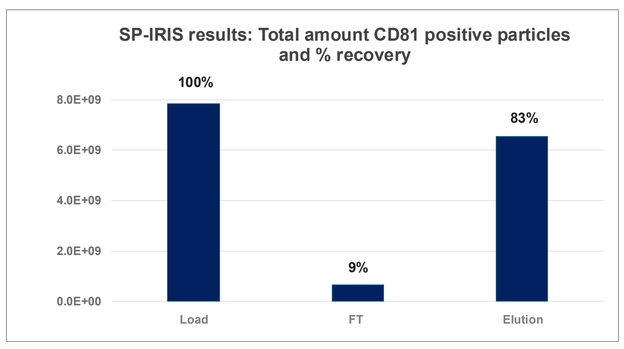


Figure 6: SP-IRIS images show colocalized CD proteins on EV particles (CD9 in blue, CD63 in red and CD81 in green).



- Recoveries higher than ELISA results and more in-line with expectations
- Closer to closing mass balance than ELISA results

Figure 7: Total amount of CD81+ particles recovered per fraction and corresponding step recoveries from SP-IRIS analysis. Elution fractions 2 and 3 omitted as data showed no presence of particles.

Colocalization of CD of proteins on EV particles

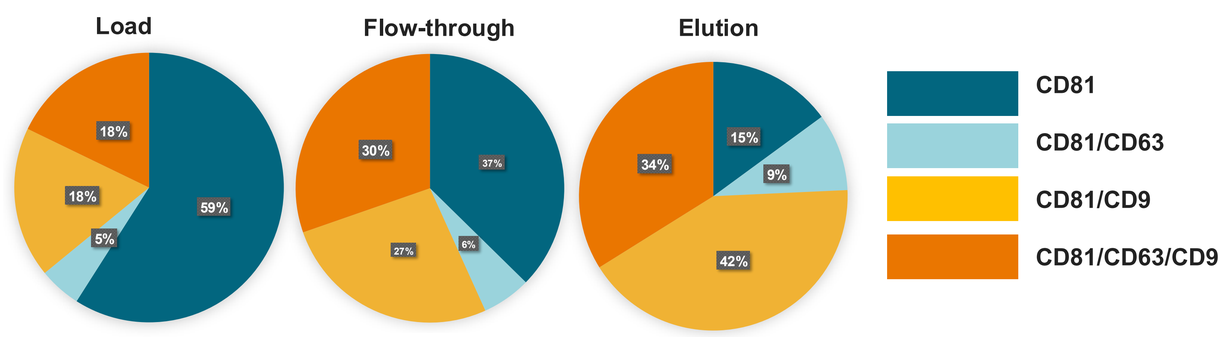


Figure 8:. Breakdown of single, double and triple colocalization ratios. SP-IRIS data suggest that there is an enrichment of double CD81/ CD9 and triple CD81/ CD63/ CD9 bearing EV particles in eluate pool.

Conclusions

- Thermo Fisher™ CaptureSelect™ CD81 Magnetic Beads offer a rapid and simple process for purification of CD81 bearing EVs, such as endogenous exosomes.
- High purity EVs can be obtained in a single step from clarified cell culture or biological fluid samples, with moderate to high recovery.
- Analytical methods such as ELISA and SP-IRIS allow for quantification of particles from in-process samples. Further work is needed to understand factors that impact discrepancies between assay methods.