Human Platelet-derived Extracellular Vesicles (EVs), a new EVs reference material from blood components.

Reetta Pusa, Milla Lampinen, Heikki Saari, Saara Laitinen; Finnish Red Cross Blood Service (FRCBS), Helsinki, Finland
Sirin Korulu Koc, Jevgenia Dering, Paolo Guazzi; HansabioMed Life Sciences, Tallinn, Estonia

Platelet Extracellular Vesicles

Platelet-derived Extracellular Vesicles (EVs) are involved in various physiological processes, including immune response, inflammation, and wound healing. In the last years, they have gained attention for their potential roles in diseases like cardiovascular disorders and cancer, as well as their therapeutic potential. Indeed, these EVs have shown promise in regenerative medicine, aiding in tissue repair, wound healing, and promoting angiogenesis [1,2]. Additionally, their ability to deliver therapeutic molecules, such as drugs or genetic material, makes them valuable for application in personalized and precision medicine.

In this technical note, we present the method used for the isolation of Platelet EVs, which ensures scalable manufacturing, reproducibility, and a consistent reduction in lipoprotein content compared to other commonly used methods.

[1] Torres, N. P., Moradi, Z., Martini, A. C., Bischoff, S. R., & Ran, H. (2020). Exosomes derived from activated platelets as novel therapeutics in tissue repair and regeneration. Journal of Extracellular Vesicles, 9(1), 1750205. doi:10.1080/20013078.2020.1750205.
[2] Aatonen, M. T., Ohman, T., Nyman, T. A., Laitinen, S., Grönholm, M., & Siljander, P. R. M. (2014). Isolation and characterization of platelet-derived extracellular vesicles. Journal of Extracellular Vesicles, 3, 24692. doi:10.3402/jev.v3.24692

Platelet EVs: purification workflow

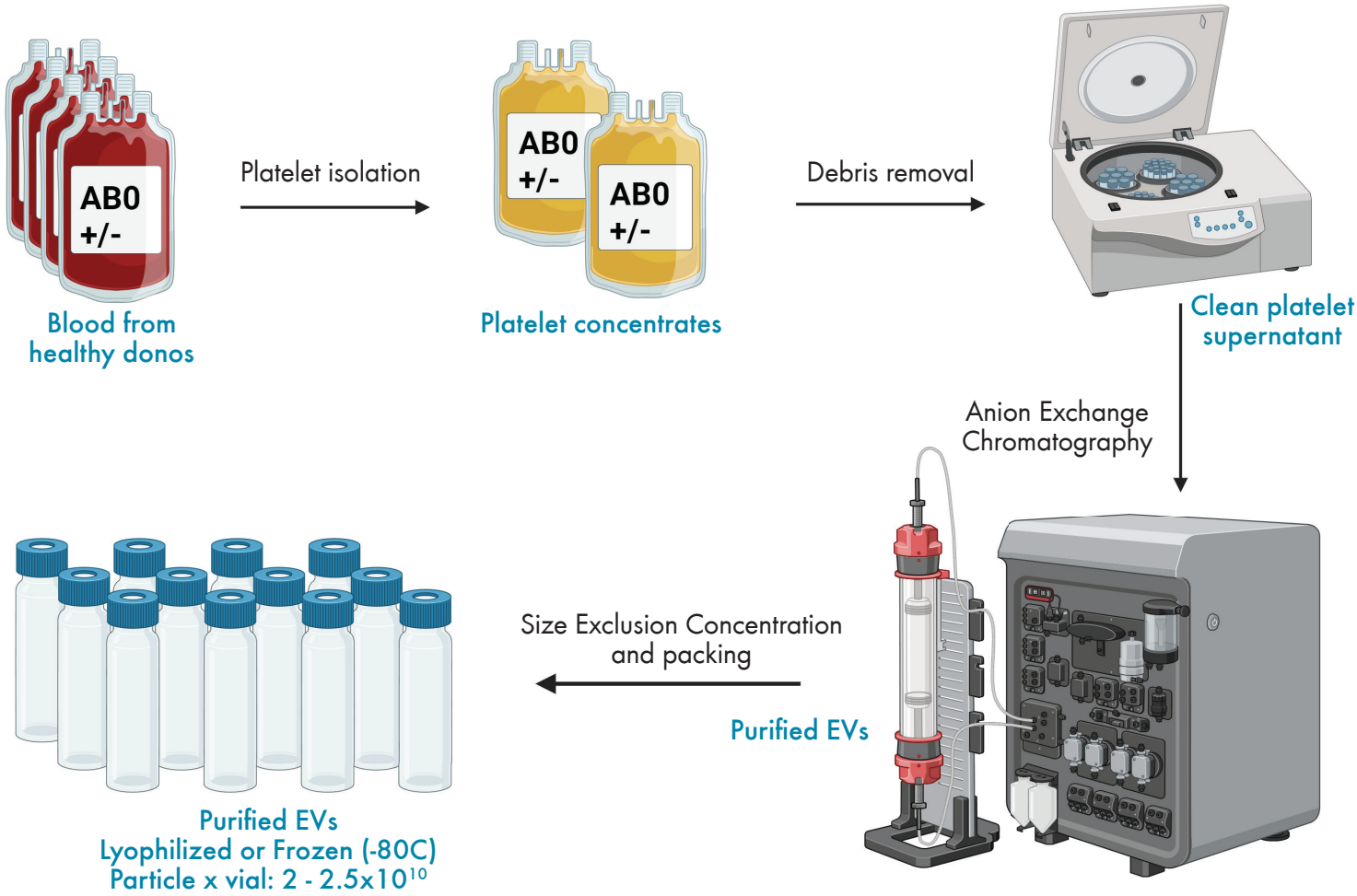
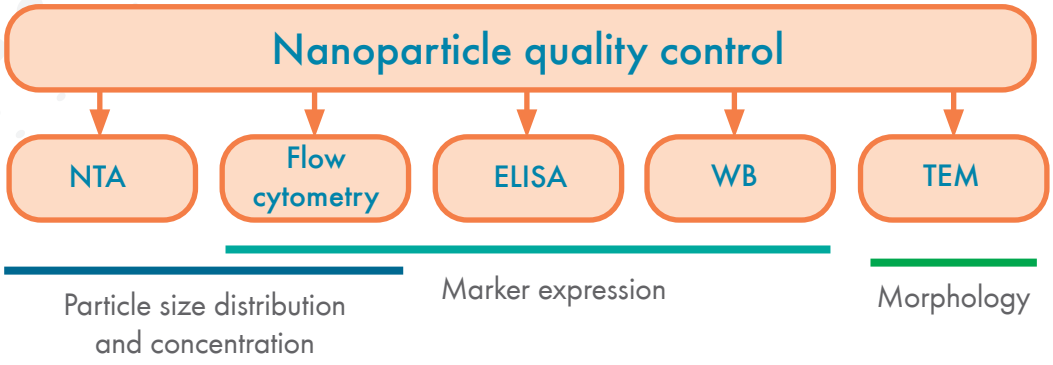
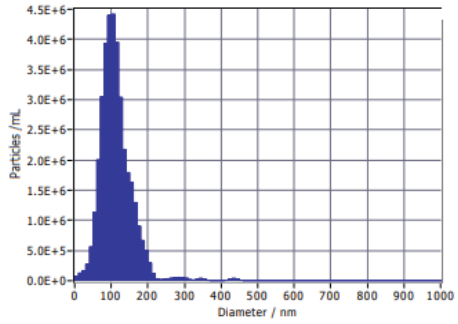


Figure made with BioRender.com

Platelet EVs: nanoparticle characterization



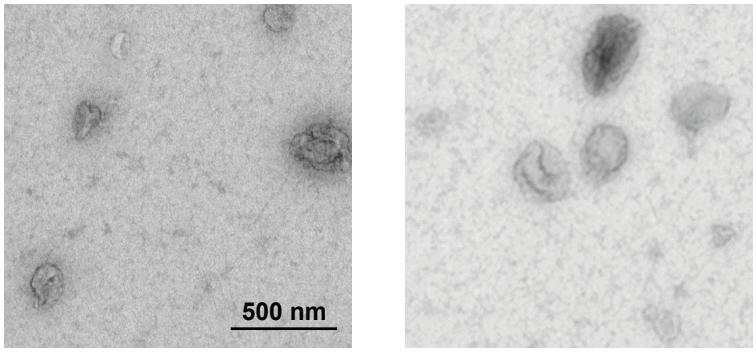
Nanoparticle Tracking Analysis



Particle concentration	2.2E11 +/- 2.1E10 part/ml
Particle size	100.7 +/- 5.7 nm

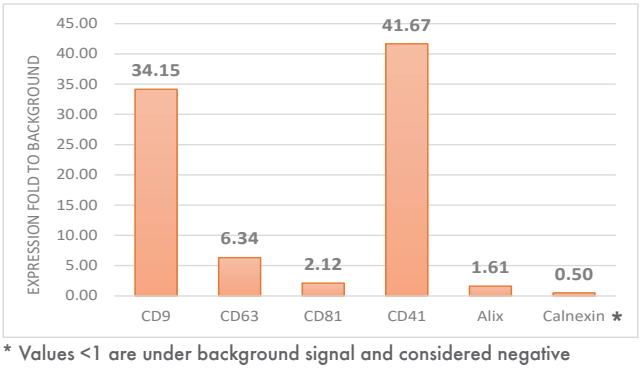
NTA performed with Zetaview analyzer (Particle Metrix)

Nanoparticle morphology

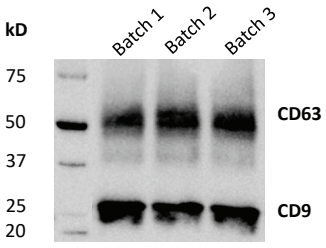


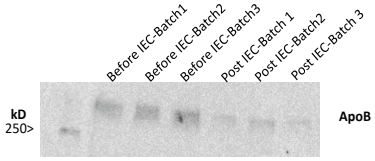
EVs viewed with transmission EM using TEM Hitachi HT7800

Marker Expression



* Values <1 are under background signal and considered negative





IEC method reduces the lipoprotein contamination

Platelet EVs: final format

- Format: 100 µl vials, each one containing 2 - 2.5x10^{10} particles, in PBS 1x buffer.
- Certificate of analysis: including particle size distribution and concetration, measuread by NTA (Zetaview, Particle Metrix), assessement of 4 membrane markers (CD9, CD81,CD63,CD41), 1 internal marker (ALIX), 1 negative marker (Calnexin).
- Lyophilized (shipping and storing at 4C) or frozen (-80C) on request.

HansaBioMed Life Sciences
Mäealause 2/1, 12618 Tallinn, Estonia
info@hansabiomed.eu
www.hansabiomed.eu

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