Platelet EVs: A New Reference Material from Blood Components with Superior Purity over Plasma EVs

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Introduction

Blood plasma is one of the most valuable biofluids for biomarker research, as it contains a complex and heterogeneous population of circulating extracellular vesicles (EVs) released by all cells and tissues [1,2]. Nevertheless, EV enrichment methods often fall short of achieving high yield and/or high purity, leading to the co-isolation of other non-vesicular extracellular particles (NVEPs), such as lipoproteins (LPs) [3,4]. These contaminating particles further complicate the labeling and detection of EVs, especially when high-resolution single-particle analyzers are employed [5].

In this tech note, we showcase the advanced technology of SPARTA Biodiscovery, which utilizes Raman spectroscopy for single-particle characterization, enabling molecular fingerprinting and identification of different particle subpopulations in a label-free non-destructive manner [6]. Moreover, we highlight the utility and superior purity of HansaBioMed's platelet EV standards compared to regular plasma EV samples. Since platelet-derived vesicles constitute a significant portion of the total plasma EV population, these standards serve as an excellent reference sample for plasma EVs without contamination issues typically associated with plasma EV preparation.

Materials and Methods

Platelet EVs (HBM-PET-100/2) were purified from healthy donor pool by combination of ion exchange chromatography and tangential flow filtration, whereas healthy donor plasma EVs were purified by size exclusion chromatography. Both EVs were further diluted 1:10 in PBS for analyzing on SPARTA AGIS I. 100 µL of this suspension was pipetted onto a SPARTA Sample Slide. Each particle spectrum was acquired for 10 s, with a laser shutter time of 1 s to allow particles to diffuse away from the trap. HBM buffer diluted 10x in PBS was used as a background. Data was analyzed using SPARTA Discovery software, with each spectra normalized to the area under the curve.

Results

Both plasma and platelet EVs exhibited the expected characteristic Raman peaks of lipids, proteins, and cholesterol (Figure 1). The protein peak at 1000 cm-1 is distinctive of EVs, while the signals detected at ~1250 and 1650 cm-1 correspond to particles containing unsaturated lipids, such as LPs. These observations are consistent with Raman signatures of LPs reported in the literature [7]. A stronger EV signal was detected in the platelet EV samples, whereas plasma EV samples displayed higher level of LP contamination (Figure 1).

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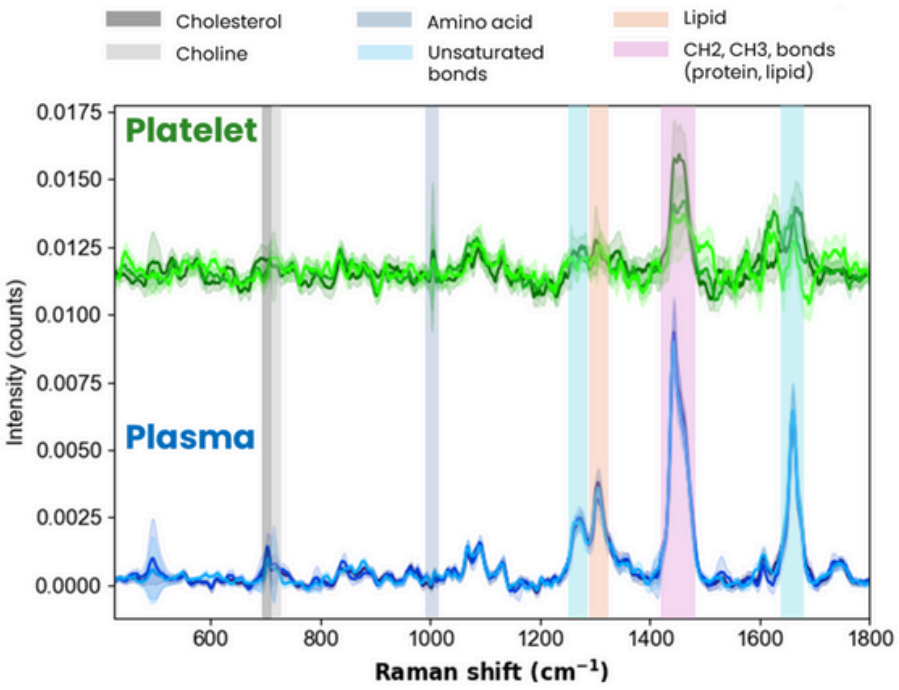

Figure 1: Raman spectra of platelet and plasma EV samples. Solid lines show mean spectra of all particles in 3 repeated measurements. Shading shows variation between particles. Highlighted areas represent Raman shifts of specific molecular components.

Furthermore, bivariate analysis of protein (~1000 cm-1) and lipid content (~1300 cm-1) revealed lower lipid signal and higher protein signal in platelet EV samples, indicating a greater EV-to-LP ratio compared to plasma EV preparations (Figure 2).

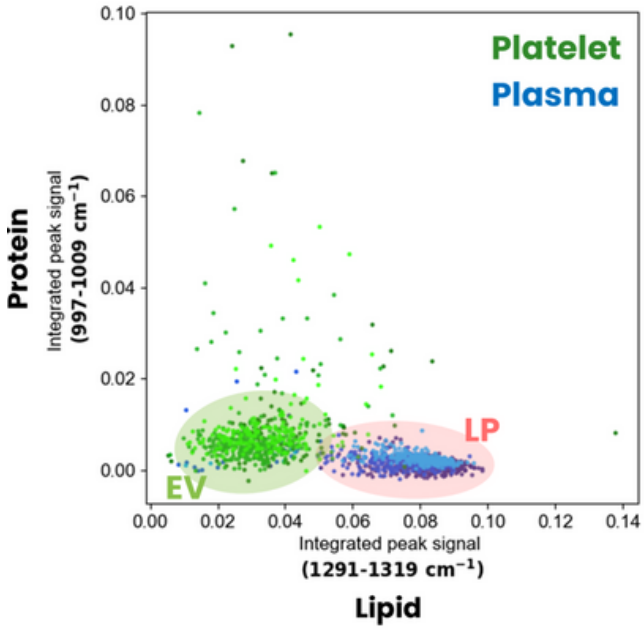

Figure 2: Bivariate analysis of protein (~1000 cm-1) and lipid content (~1300 cm-1) in platelet and plasma EV preparations.

Conclusion

- HansaBioMed's platelet EV standard demonstrate superior purity compared to plasma EV preparations making it preferred reference material for plasma EV research.
- SPARTA's AGIS I instrument is able to identify different particle subpopulations, assess their molecular fingerprint, and determine the sample purity in a label-free manner.

References

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