Assessment of Extracellular Vesicle Corona with ExoTEST ELISA and MP-SPR

Abhishek Sharma, Justas Svirelis, Matias Jokinen, Annika Järvinen, BioNavis, Tampere, Finland
Danilo Mladenović, Paolo Guazzi, Tayfun Tatar, HansaBioMed Life Sciences, Tallinn, Estonia

Introduction

Extracellular vesicles (EVs) in complex matrices associate with different biomolecules (e.g., proteins, nucleic acids) and non-vesicular extracellular particles (NVEPs), such as lipoproteins, forming a composite and dynamic shell known as EV corona. In this application note, we showcase how HansaBioMed's ExoTEST ELISA kit can be utilized to assess the formation, stability, and biochemical composition of EV surface corona. Simultaneously, BioNavis MP-SPR technology enables analyses of dynamic corona changes by measuring the thickness of the EV nanolayer on the surface of the sensor. The two complementary methods provide the opportunity for highly informative EV corona research.

Workflow

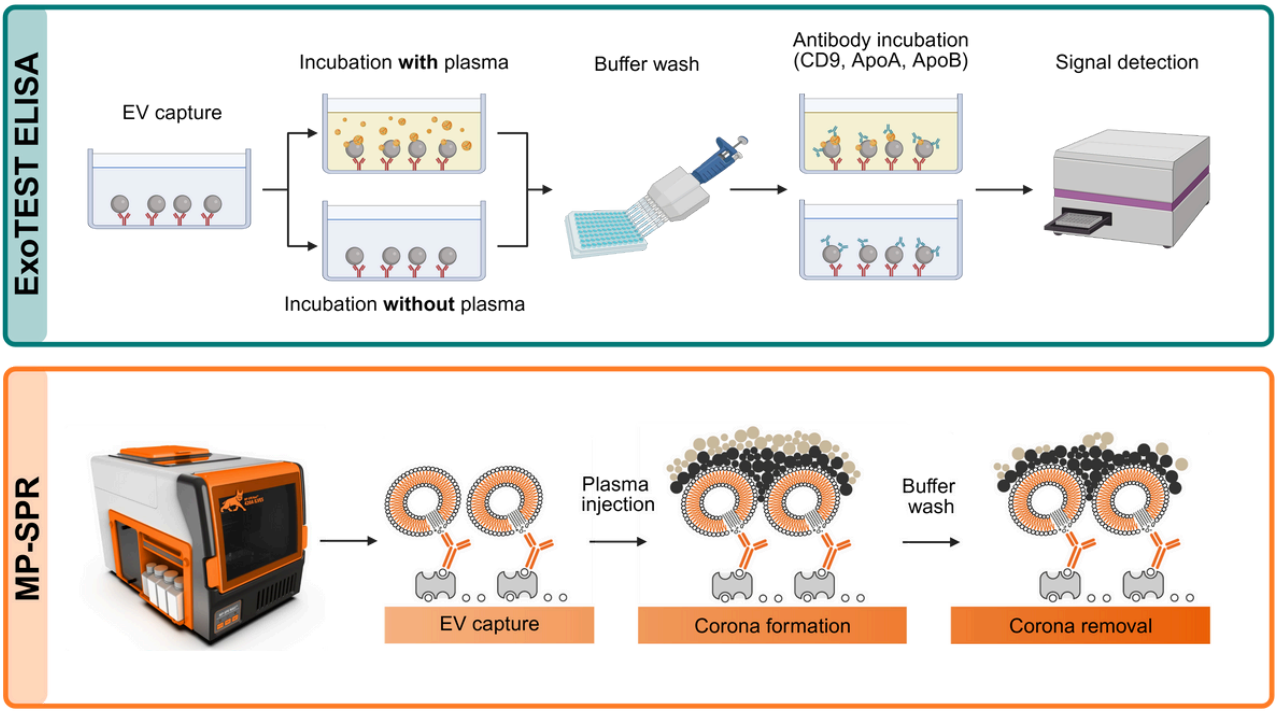


Figure 1: COLO1 EVs were captured in ExoTEST ELISA plate or on BioNavis biotin sensor slide using anti-CD9 antibodies. The thickness of the EV nanolayer was measured with MP-SPR before and after incubation with blood plasma, and after each buffer wash (0.01%, 0.1%, and 1% Tween 20) to assess corona stability. At the same time, EV and corona markers were analyzed in sandwich ELISA to evaluate compositional changes.

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

Results

ExoTEST ELISA

- Lipoproteins formed a corona on top of the bound COLO1 EVs (Figure 2).
- Subsequent washing with different detergent concentrations gradually removed the corona.
- Low detergent concentrations improved the detection of CD9 by stabilizing the antigen and facilitating the antibody-antigen interaction.
- High detergent concentrations had detrimental effect on EV stability.

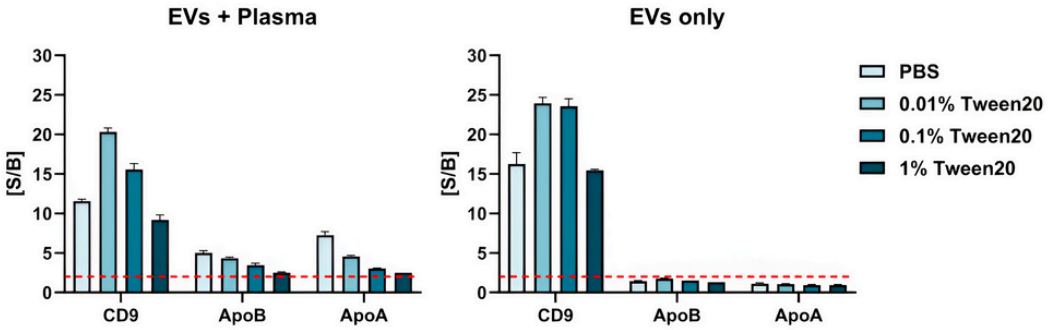
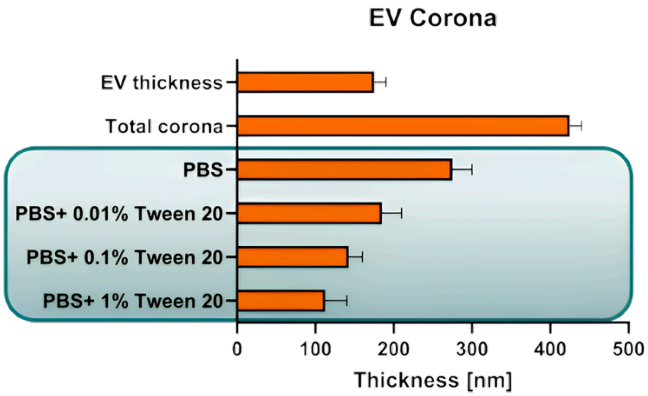


Figure 2: ExoTEST sandwich ELISA showing EV and corona marker coexpression after different buffer washing steps. Red dotted line represents the signal threshold. [S/B] - signal-to-background ratio.



MP-SPR

- MP-SPR confirmed EV corona formation after incubation with blood plasma (Figure 3).
- Corona progressively decreased after each buffer washing step.
- Like in ExoTEST ELISA, the highest detergent concentration likely affected EV integrity.

Figure 3: EV and corona thickness measured with MP-SPR before and after buffer washing.

Conclusions

- HansaBioMed's purified COLO1 EVs demonstrate a very high expression of the CD9 marker and are free of lipoprotein contaminants.
- Lipoproteins interact with the EV surface forming a corona, which exhibits differential detergent sensitivity.
- ExoTEST ELISA kit and MP-SPR can be used as complementary methods to assess the EV corona formation, stability, thickness, and biochemical composition.
- In real-time MP-SPR can determine EV thickness as well as corona formation and removal. And in the same measurement, EV stability and integrity can be determined.
- MP-SPR can be used to perform precise EV size and concentration measurement.
- MP-SPR provides real-time insights into EV-antibody interactions, making it a powerful tool for EV-based diagnostic development.

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