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

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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 [1,2]. Depending on the nature and strength of these interactions, we can differentiate between soft (weak associations) and hard corona (strong associations). Due to its intricate composition, and implications it may have in altering EVs' functionality and biodistribution, surface corona has become increasingly popular subject of many EV studies.

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.

Materials and Methods

ExoTEST ELISA

Experiment was performed according to the scheme in the Figure 1A. ExoTEST ELISA plate coated with anti-CD9 antibodies was used to capture COLO1 small EVs (HBM-RTK-POC/TC, HansaBioMed Life Sciences). Wells incubated with PBS only (without EVs) were used as a blank. Upon capturing to the bottom of the well, EVs were incubated with human blood plasma (diluted 1:4) to allow corona formation, after which the plate was washed with either PBS or PBS with varying detergent concentrations (0.01%, 0.1%, or 1%) to assess the stability of the corona. In parallel, second ELISA plate was prepared with EVs only (without incubation with plasma), as a negative control. Afterwards, plates were incubated with different antibodies in order to detect EV and corona molecules in a sandwich ELISA fashion. Namely, anti-CD9 was used to detect EVs captured in the anti-CD9 plate, while anti-ApoB (for VLDL, IDL, and LDL) and anti-ApoA (for HDL) were used to assess lipoprotein association to these EVs after incubation with plasma.

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MP-SPR

The BioNavis EV Size and Concentration Measurement Kit (SPR102-EV) was used for EV capture and characterization. Briefly, COLO1 EVs (HBM-COLO-100/2, HansaBioMed Life Sciences) were captured on BioNavis biotin sensor slide using biotinylated anti-CD9 antibody (HBM-CD9B-100, HansaBioMed Life Sciences) (Figure 1B). To study protein corona formation, human plasma (diluted 1:1) was introduced onto the immobilized COLO1 EVs. Corona removal was assessed using sequential injections of PBS and PBS supplemented with Tween 20 at concentrations of 0.01%, 0.1%, and 1% (Figure 1B). Real-time MP-SPR measurements were conducted using a BioNavis MP-SPR Navi™ 210A VASA system equipped with multiple wavelengths (670 nm and 785 nm) to track the sequential immobilization of regenerable avidin, biotinylated anti-CD9 antibody, COLO1 EV capture, protein corona formation, and corona removal (Figure 1B).

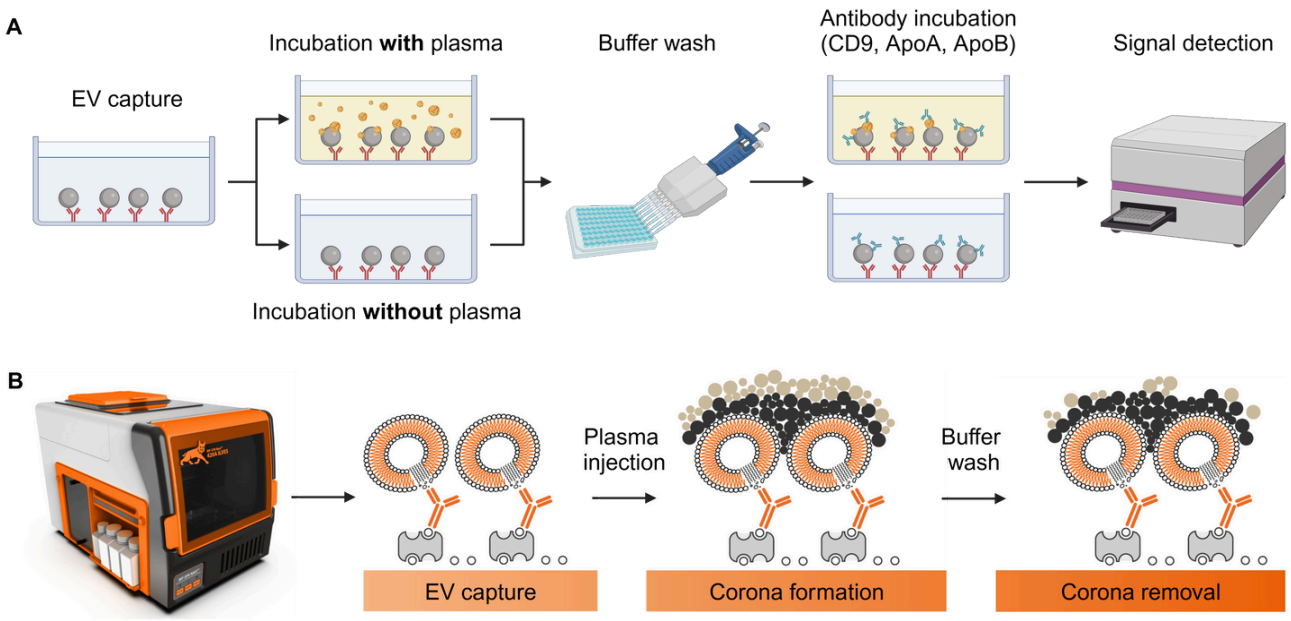

Figure 1: Experimental workflow. ***A)*** *ExoTEST anti-CD9 ELISA workflow* ***B)*** *MP-SPR workflow*

Results

ExoTEST ELISA

Coexpression of CD9/CD9, CD9/ApoB, and CD9/ApoA (Figure 2) was detected in ELISA plate incubated with EVs and plasma, demonstrating the efficient capturing of EVs to the bottom of the wells, and lipoprotein (VLDL, IDL, LDL, and HDL) corona formation on top of the captured EVs. As expected, the ApoB and ApoA signals were absent in the plate incubated with EVs only, while the CD9 signal was very high, confirming the specificity of the assay and purity of the EV preparation. Furthermore, the detected CD9 signal in both plates was higher in the wells washed with low concentration of Tween 20, which can be explained by the non-ionic detergent's ability to stabilize the antigen and facilitate the antibody-antigen interaction, as previously reported [3].

However, the highest Tween 20 concentration (1%) had a detrimental effect on CD9 detection, likely due to the partial EV degradation by the detergent [4]. At the same time, in the plate incubated with EVs and plasma, both ApoB and ApoA signals were showing a steady decline even after washing with the lowest concentration of detergent (0.01%), while the highest detergent concentration (1%) completely abolished the signal, demonstrating the effective removal of the EV corona. The effect was more prominent with ApoA marker, suggesting a weaker association of HDL with EVs.

MP-SPR

MP-SPR is a highly surface-sensitive technique widely used to analyze biomolecular interactions in real time, including protein-protein and protein-drug interactions, as well as monitoring biomaterial layer thickness. In this study, MP-SPR effectively captured COLO1 EVs via biotinylated anti-CD9 antibodies on Regenerable Avidin-coated sensor slides.

The thickness of COLO1 EVs determined by MP-SPR was approximately 140 nm (Figure 3). Upon human plasma injection, a sharp increase in the SPR signal confirmed corona formation on the EVs (Figure 3), supporting earlier findings on liposomes [5]. When PBS alone was introduced, only partial removal of the soft corona was observed. In contrast, subsequent washes with PBS containing Tween 20 progressively decreased corona thickness.

These findings corroborate previous studies suggesting that some proteins in the EV corona are easily removed, while others remain tightly bound [2,6]. Moreover, detergent treatment not only removes the corona by disrupting hydrophobic interactions but may also affect EV structural integrity [4].

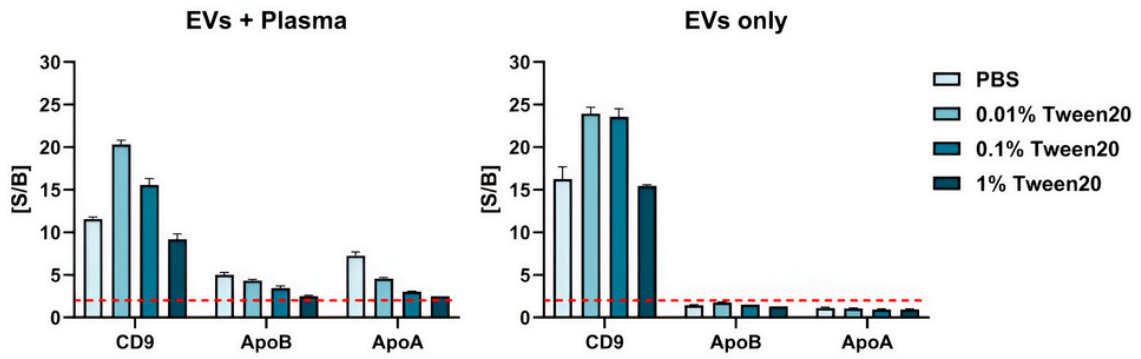

Figure 2: Sandwich ELISA showing CD9/CD9, CD9/ApoB, and CD9/ApoA coexpression for plates incubated with EVs and plasma or EVs only. Data is shown as signal-to-background ratio (S/B). Red dotted line represents the signal threshold

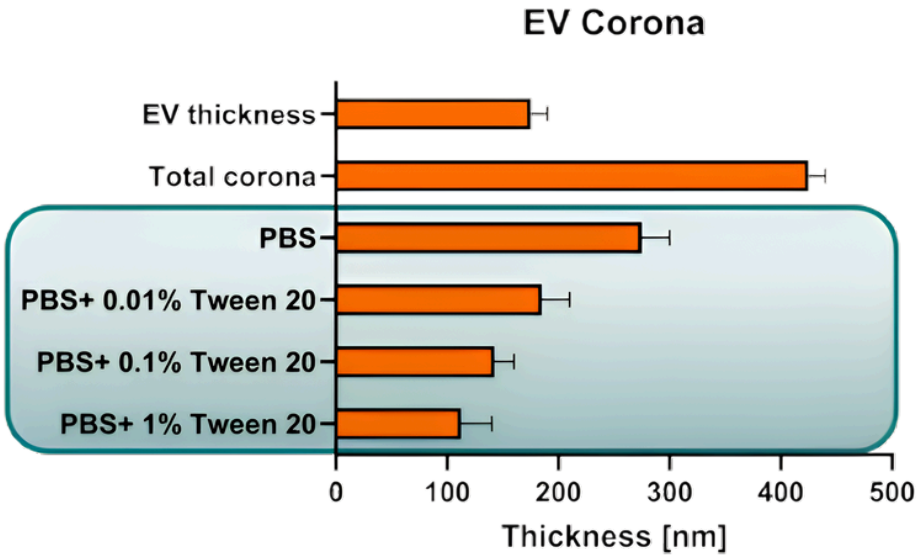

Figure 3: EV thickness and protein corona formation and depletion of the protein corona with PBS and PBS supplemented with detergent (Tween 20)

Conclusion

- HansaBioMed's purified COLO1 EVs demonstrate a very high expression of the CD9 marker and are free of lipoprotein contaminants.
- VLDL, IDL, LDL, and HDL lipoproteins expressing ApoB and ApoA, respectively, 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.

References

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