Monitoring Protein Corona Formation on Extracellular Vesicles Using MP-SPR

This Application Note demonstrates how Multi-Parametric Surface Plasmon Resonance (MP-SPR) measures protein corona formation on extracellular vesicles (EVs) derived from COLO-1 cells when exposed to human plasma (Figure1). MP-SPR quantifies the adsorption and partial removal of the protein corona in real-time using buffer and detergent washes. Dual-wavelength measurements enable direct determination of EV and corona layer thickness throughout the interaction.

Introduction

Multi-Parametric Surface Plasmon Resonance (MP-SPR) is an advanced optical sensing technique that enables real-time, label-free analysis of molecular interactions at surfaces. MP-SPR captures the complete SPR curve and multiple wavelength-dependent responses at the same measurement spot, enabling advanced analysis beyond conventional methods. These attributes make MP-SPR particularly suitable for studying extracellular vesicles (EVs), including their size and refractive index. Furthermore, multiple wavelengths facilitate the measurement of protein corona thickness without the need for external references. In this study, MP-SPR was employed to monitor the formation and removal of protein corona on COLO-1 EVs during their interaction with human plasma.

Materials and methods

EV Capture

The BioNavis EV Size and Concentration Measurement Kit (SPR102-EV) was used for EV capture and characterization *in situ* MP-SPR Navi™ 210A VASA. The standard protocol of the kit was employed. The BioNavis Biotin sensor slide was activated using Regeneration solution (Reg1), followed by the addition of Regenerable avidin (AVD) in running buffer (1× PBS, pH 7.4).

- Biotinylated anti-CD9 antibody (HBM-CD9B-100, HansaBioMed) in PBS was bound for EV capture.
- COLO-1 EVs (HBM-COLO-30/2, HansaBioMed) in PBS were subsequently captured on the sensor slide.

Protein Corona Measurement

Human plasma (diluted 1:1) was introduced onto the COLO-1 EVs to allow corona formation. To investigate corona removal, sequential injections of PBS and PBS supplemented with Tween-20 (at concentrations of 0.01%, 0.1%, and 1%) were performed.

MP-SPR Monitoring

Real-time MP-SPR measurements were conducted to track the sequential immobilization of Regenerable avidin, biotinylated anti-CD9 antibody, COLO-1 EV capture, protein corona formation, and corona removal (Figure 2). These measurements were performed using a BioNavis MP-SPR equipped with multi-wavelength detection (670 nm and 785 nm) (Figure 3).

Results and discussion

In this study, MP-SPR successfully captured COLO-1 EVs using biotinylated anti-CD9 antibodies on BioNavis Regenerable avidin kit sensor slides (Figure 2).

The thickness of COLO-1 EVs was determined using MP-SPR dual wavelength method (as described in SPR-102-EV kit). The average thickness of EVs were calculated to be 140 nm (Figure 4). Notably, the biotinylated surface, regenerable AVD, and capture IgG contribute approximately 20 nm to the overall thickness 160nm determined.

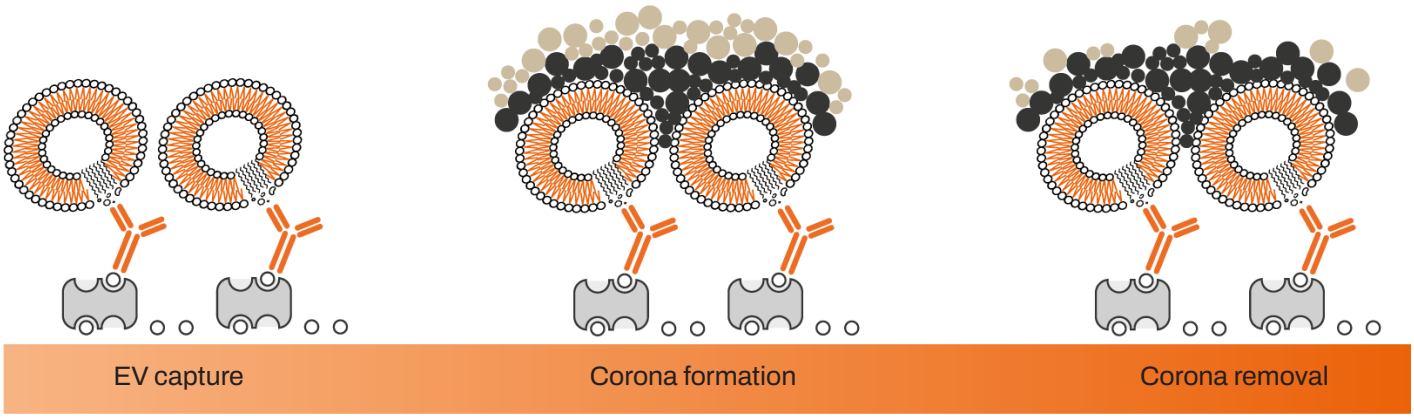

Figure 1. MP-SPR measures protein corona formation on extracellular vesicles (EVs) derived from COLO-1 cells when exposed to human plasma. MP-SPR quantifies the adsorption and partial removal of the protein corona in real-time using buffer and detergent washes.

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email: info@bionavis.com
www.bionavis.com

Upon injection of human plasma, a sharp increase in the SPR signal was observed, indicating protein corona formation on the EVs (Figure 2). This confirms that EV-plasma interactions lead to corona formation, as previously reported [1]. The protein corona formed on top of the nanoparticles or EVs can be termed as soft (which can be easily removed) and hard corona [1]. The introduction of PBS alone resulted in partial removal of the soft corona, while subsequent washes with PBS containing Tween 20 progressively reduced the corona thickness. These findings align with previous reports indicating that certain proteins within the EV corona are easily displaced, whereas others exhibit strong interactions with EVs [2,3] (Figure 4). Additionally, detergent introduction alters EV integrity, as shown by the reduction of the total size of EV+ protein corona (Figure 4). This has been previously documented on EV-detergent interactions [4].

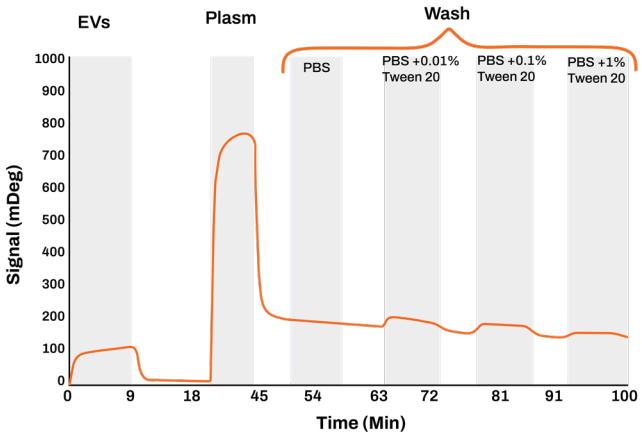

Figure 2. Real-time monitoring of EV capture, corona formation on EVs when exposed to human plasma and partial removal of corona during washes.

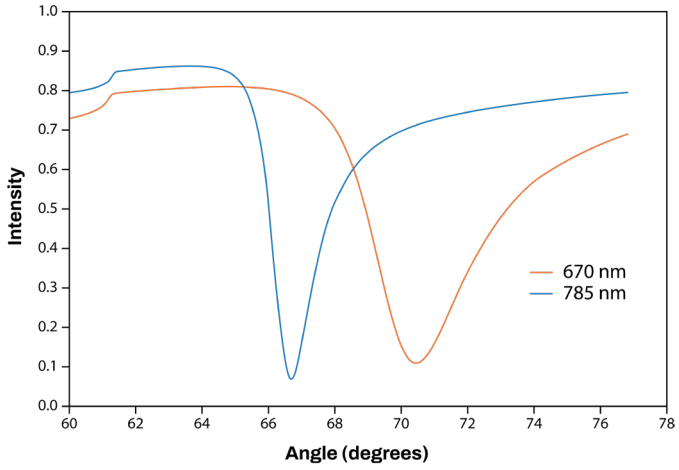

Figure 3. MP-SPR measures complete SPR curves with multiple wavelenghts (670nm and 785nm). This enables thickness calculation of the EVs and corona layers.

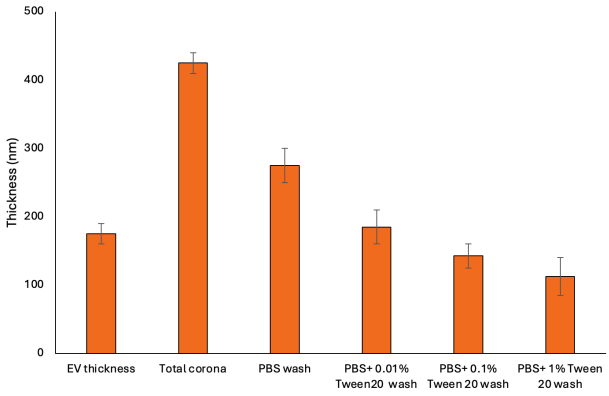

Figure 4. MP-SPR calculated thicknesses of the EV and corona layers.

Conclusions

MP-SPR is a powerful label-free technique for investigating EVs and their associated protein corona. In this study, MP-SPR was used to successfully capture COLO-1 EVs via anti-EV antibodies, determine the thickness, and monitor protein corona formation and depletion in response to PBS and detergent washes. The ability of MP-SPR to capture the complete SPR curve and utilize multiple wavelengths was instrumental in these analyses. Beyond corona studies, MP-SPR has broader applications, including EV-kinetics studies using antibodies or aptamers, EV-cell interaction analysis, and EV-based diagnostic development.

References

1. Kari *et al.*, Nanoscale 2020, 12 (3).
2. Wolf *et al.*, Journal of Extracellular Vesicles 2022, 11 (4).
3. Reinsalu *et al.*, Int. J. Mol. Sci. 2021, 22(10).
4. Cimorelli *et al.* PLoS One 2021, e0249603.

Thank you to HansaBioMed Life Sciences OÜ for providing the antibody and EV samples!

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 220A NAALI or 210A VASA

Sensor surface: EV Size and Concentration Measurement Kit SPR102-EV

Software: MP-SPR Navi™ Control, DataViewer and LayerSolver for MP-SPR Navi™

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email: info@bionavis.com
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