ONI

Application Note

Extracellular Vesicles

Characterization of a protein corona formation on single EVs using super-resolution imaging

Based on the publication by Wolf M et al. JEV 2022, doi.org/10.1002/jev2.12207

SUMMARY

ONI's Nanoimager and software tools, including cloud-based CODI, offer a versatile platform to characterize individual EVs, profile surface tetraspanins, assess population heterogeneity and visualize EV-associated protein corona using multicolor dSTORM.

The Nanoimager and CODI enable:

- Characterization of surface markers on individual EVs to provide better understanding of vesicle subpopulations
- Visualization of protein corona associated with individual EVs, with 20 nm resolution
- Study of corona-associated EV populations and quantify the percentage of EVs containing the protein corona through co-localization of albumin with a pooled tetraspanin mix signal
- Distinction of signals obtained from individual EV over cell debris or protein aggregates

INTRODUCTION

Extracellular vesicles (EVs) are nanosized vesicular structures released from all cell types. EVs display biomarker signatures allowing specific cell targeting and cargo delivery. They play key roles in intercellular communication in both physiological and pathological conditions like cancer and neurodegenerative diseases. For these reasons, EVs have huge potential in clinical applications for biomarker discovery and targeted therapeutics.

In several studies, it has been shown that the biological activity of synthetic nanoparticles is determined by its protein corona, which is formed upon entry of these particles into a protein-rich environment, such as plasma. Recent findings show an equivalent protein corona formation on the surface of EVs. A recent publication by Wolf and colleagues from the Paracelsus Medical University (PMU), Salzburg, Austria, assessed the impact of the protein corona on EV biological functionality.[1] The team used the Nanoimager to study the functionality of therapy-grade placental-expanded (PLX) stromal cell-derived EVs, with or without protein corona in proangiogenic and immunomodulatory assays. EVs purified from PLX cells containing protein corona showed proangiogenic and immunomodulatory capability, whereas the same EVs after corona depletion (bare EVs), via ultracentrifugation, were no longer functional. In this study, super-resolution microscopy allowed direct visualization and characterization of protein corona associated with individual EVs.

CHALLENGE

Characterizing EVs by conventional light microscopy is challenging since a large portion of EVs are sized between 30-1000 nm, and fall below the diffraction limit of light. As a result, it is virtually impossible to visualize the protein corona associated with individual EVs for lack of ability to: distinguish between signals coming from the protein corona alone, from the EV membrane or from non-specific antibody binding, as these signals would overlap. This also hinders the user's ability to visualize directly and accurately characterize surface markers, internal cargo or determine EV size. Through multicolor dSTORM, the diffraction limit can be overcome to resolve single EVs and their associated protein corona at 20 nm resolution. Thus, gives users additional confidence in their analysis. Together with powerful clustering methods applied to super-resolution images, this allows for thousands of surface-bound EVs to be analyzed for accurate characterization of heterogeneous EV populations, biomarker positivity and sizing.

RESULTS

EVs derived from allogeneic placental-expanded (PLX) stromal cells were captured on a functionalized chip and EV surface markers were fluorescently labeled using ONI's EV Profiler Kit. Multicolor dSTORM was performed, which revealed unexpected highly heterogeneous EV subpopulations with respect to the colocalization of tetraspanins CD9, CD63 and CD81. Clustering tools on ONI's cloud-based software CODI were used to gain information on absolute tetraspanin counts, positivity and percentage of tetraspanin colocalization. Clustering analysis was performed on 99,209 captured EVs and it was found that 11.7% of EVs were positive for all three tetraspanins, 49.8% of the EVs were single positive for only one of the tetraspanins, and 38.6% were double-positive for CD9, CD63 or CD81 (Figure 1).

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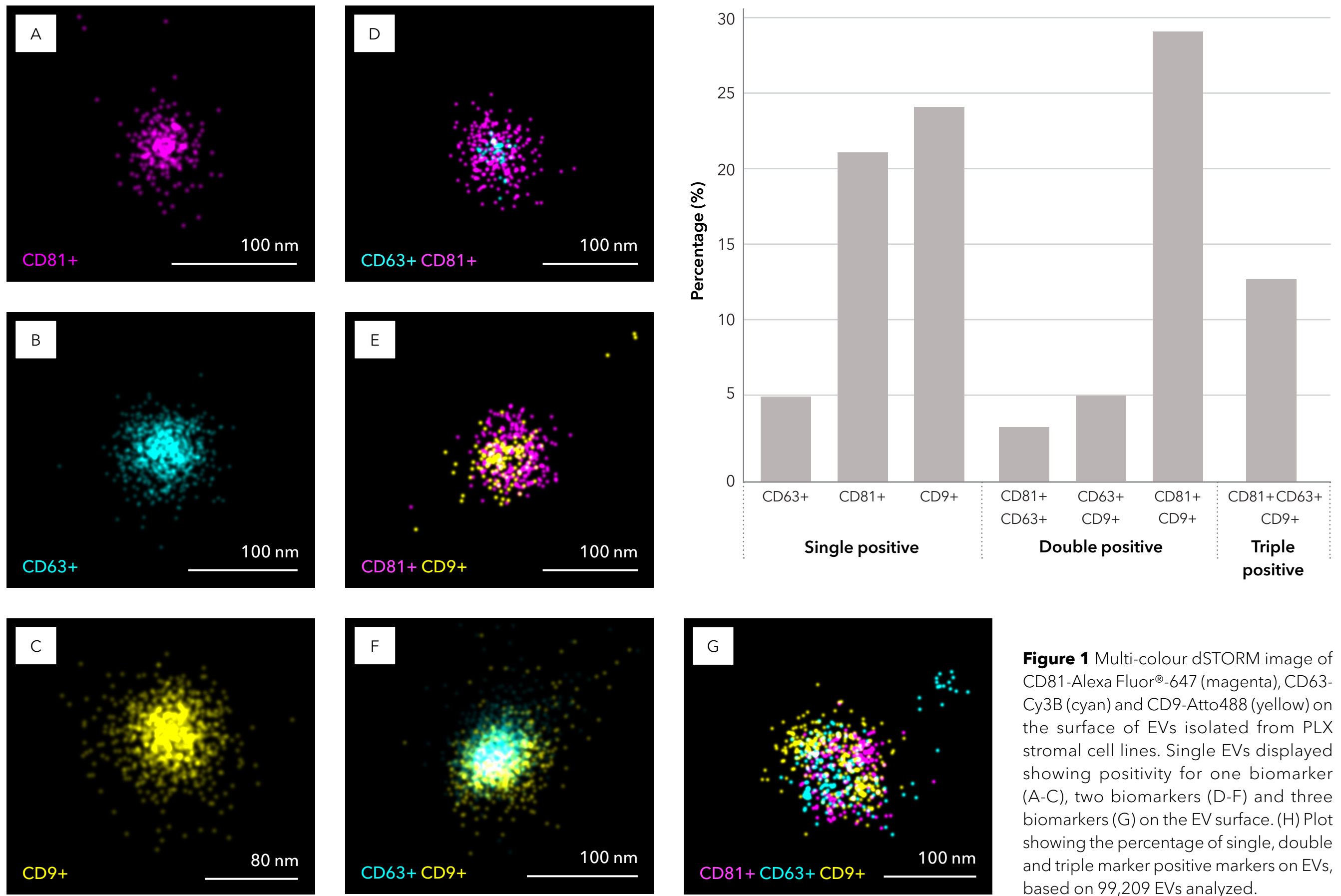
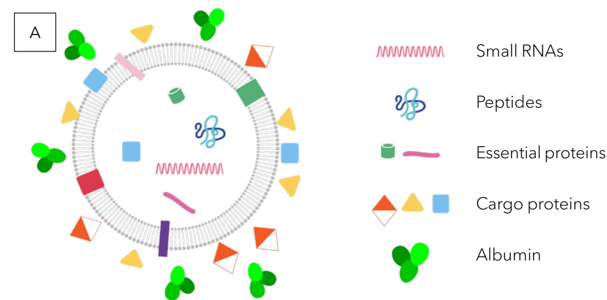


Figure 1 Multi-colour dSTORM image of CD81-Alexa Fluor®-647 (magenta), CD63-Cy3B (cyan) and CD9-Atto488 (yellow) on the surface of EVs isolated from PLX stromal cell lines. Single EVs displayed showing positivity for one biomarker (A-C), two biomarkers (D-F) and three biomarkers (G) on the EV surface. (H) Plot showing the percentage of single, double and triple marker positive markers on EVs, based on 99,209 EVs analyzed.

For the detection of the protein corona, the EV surface was labeled with tetramix (a mixture of antibodies against CD9, CD63 and CD81, all labeled with Alexa Fluor® 647). The protein corona on bare EVs was then reconstituted with fluorescent labeled albumin-AF488. Through multicolor dSTORM, confirmation of protein corona formation on the surface of EVs was observed through direct visualization of the albumin ring around the EV membrane (Figure 2). Further analysis using the CODI platform was performed to determine the percentage of EVs containing the protein corona through colocalization of albumin with the tetraspanin mix signal. In this study, it was observed that EVs with removed protein corona (bare EVs), did not have any therapeutic effects as compared to purified PLX-EVs with protein corona. This indicates the importance of the protein corona around EVs for the therapeutic function of PLX-EVs and hence the necessity to characterize it with high precision tools such as quantitative dSTORM.



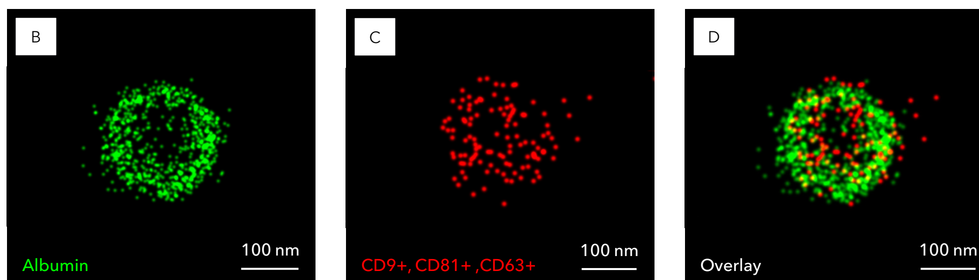
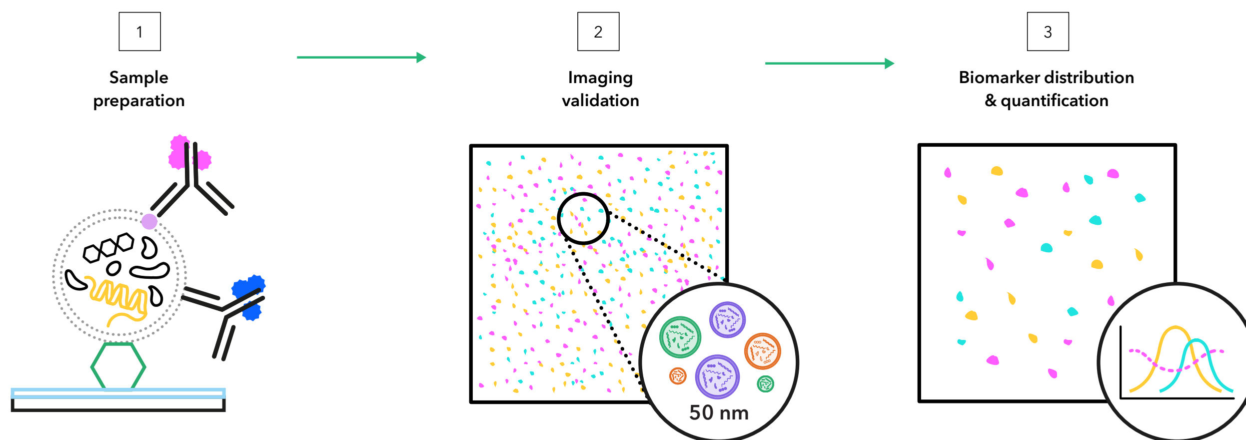


Figure 2 A) Two-color dSTORM image of reconstituted protein corona labeled with Albumin-AF488 (green) and surface tetraspanins CD9, CD63, CD81 labeled with Alexa Fluor® 647 (red) on bare EVs.[1] B) Schematic showing the EV protein corona model.

SOLUTION WITH THE NANOIMAGER

Protein corona formation is necessary for proper EV function, as shown by this study. It is, thus, important to understand the mechanism of protein corona formation and use EV isolation methods that preserve the corona. It is also crucial to use methods that allow robust characterization of the protein corona, and prevent misinterpretation of co-isolated protein aggregates as protein corona effects. The Nanoimager enables users to visualize the protein corona and EV membranes with 20 nm resolution. Automated clustering analysis can be used to provide further insights and quantify EV populations, EV size, biomarker positivity and marker colocalization.



References

1. Wolf M, Poupardin RW, Ebner-Peking P, Andrade AC, Blöchl C, Obermayer A, Gomes FG, Vari B, Maeding N, Eminger E, Binder HM, Raninger AM, Hochmann S, Brachtl G, Spittler A, Heuser T, Ofir R, Huber CG, Aberman Z, Schallmoser K, Volk HD, Strunk D. A functional corona around extracellular vesicles enhances angiogenesis, skin regeneration and immunomodulation. J Extracell Vesicles. 2022 Apr; 11(4): e12207.

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