Extracellular Vesicles (EV) Array for Detection of Disease-Related Biomarkers

Author: Dr. Malene Møller Jørgensen & Rikke Bæk

Introduction to the EV Field

All living cells produce extracellular vesicles (EVs), which are **nano-sized compartments**. They are considered as a pivotal part of the intercellular environment and act as important players in cell-to-cell communication. The fact, that EVs are involved in the development and progression of several diseases, has formed the basis for the use of EV analyses in a **clinical setting** and envisions a great potential for using EVs as **disease-related biomarkers.**
EVs are a heterogeneous population of membrane-enclosed vesicles that can be divided into a number of subpopulations based on specific characteristics such as size, biogenesis, cellular origin, protein composition, and biological function. The two major subtypes of EVs are exosomes and microvesicles.

Advantages of the EV Array

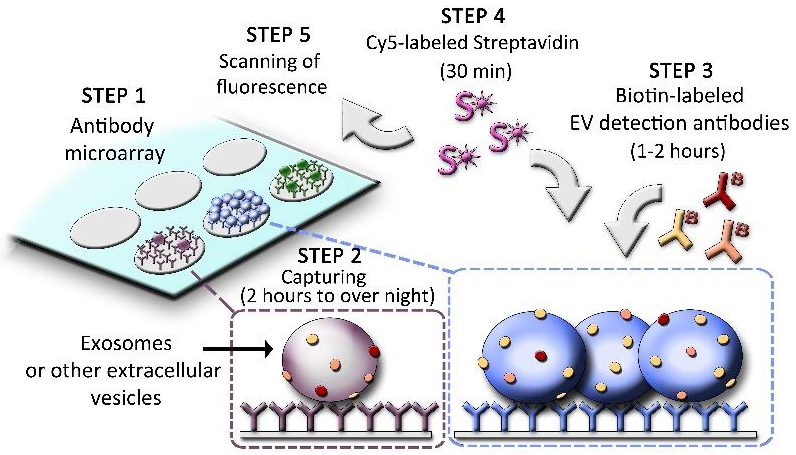
The research field of EVs has shown a great demand for commercially available technologies to analyze EVs for biomarkers. Currently, extensive and time consuming (> 24 hours) purification procedures is needed prior to analysis of single markers. **No other** existing technology phenotypes EVs in a multiplexed manner using unpurified material.
The EV Array consumes only 10 – 100 µL sample, whereas purification procedures use several milliliters. The EV Array is performed in multi-well cassettes in a high-throughput manner (up to 21 samples per slide) but is still easy to handle in a laboratory.

The EV Array has been tested and optimized for various body fluids:

Body Fluid	Optimal Volume	Body Fluid	Optimal Volume
Plasma	10 µL	Ascites	10-20 µL
Urine	50 µL	Bone Marrow	10 µL
Saliva	100 µL	Bronchoalveolar Fluid	50 µL
Synovial Fluid	50 µL	Cell Supernatant	100 µL
Cerebrospinal Fluid	50 µL		

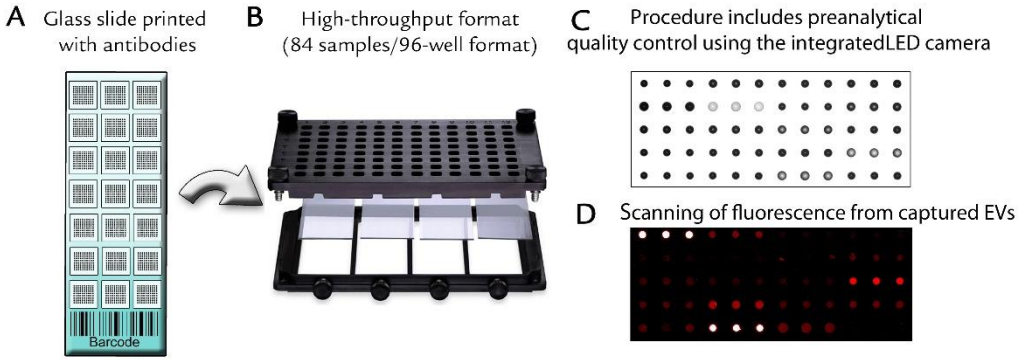
The use of microarray as a platform with spots of 1 nL volumes minimizes the cost of the EV Array as only small amounts of antibodies are needed.

Illustration: Principle of the Extracellular Vesicle Array (EV Array)



Step 1: The EV Array is composed of different capture antibodies printed in triplicates on a microarray slide.
Step 2: 10-100 µL plasma or other body fluids (urine, saliva, BALF, etc.) are applied in a 96-well setup and incubated 2 hours to overnight.
Step 3: The EVs are detected with a cocktail of biotinylated antibodies.
Step 4/5: The presence and thereby phenotype of EVs is visualized after incubation with Cy5-labeled Streptavidin using a fluorescence scanner.

Illustration: The EV Array in Practice



A. Barcoded microarray glass slides (7.5 x 2.5cm) printed in a 21-well setup.
B. Printed slides are placed in multiwell-cassettes for either one glass slide (up to 21 samples) or four glass slides (up to 84 samples).
C. The procedure is validated by a quality control using the integrated camera of the sciFLEXARRAYER S12.
D. Scanning after capture and detection of EVs can be performed on a normal laboratory fluorescence scanner or on a specialized microarray scanner for maximum sensitivity.

Summary & Conclusion

The research field and interest in EVs have increased tremendously during the past 5 years. This research field has shown a great demand for available technologies to anayze EVs for biomarkers. No other existing technology phenotypes EVs in a multiplexed manner using unpurified material.

Acknowledgement

This work has been conducted by Dr. Malene Møller Jørgensen, Senior Scientist and Rikke Bæk, Research Engineer at the Aalborg University Hospital. They would like to acknowledge the Toyato fundation, Vissings fundation and Doctor Sofus Carl Emil Friis and wife Olga Doris Friis' Fundation for financial support.

References

- Bhargava, P., et al., (2019) Altered Levels of Toll-like Receptors in Circulating Extracellular Vesicles in Multiple Sclerosis. Cells, 8, 1058.
- Brahmer, A., et al. (2019) Platelets, endothelial cells and leukocytes contribute to the exercise-triggered release of extracellular vesicles into the circulation. J Extracellular. Vesicles.,vol 8., 1615820.
- Mørk, M.,, et al., (2019) Elevated blood plasma levels of tissue factor-bearing extracellular vesicles in patients with atrial fibrillation. Thrombosis Research, vol 173, 141-150.
- Mørk, M., et al., (2018) Postprandial Increase in Blood Plasma Levels of Tissue Factor–Bearing (and Other) Microvesicles Measured by Flow Cytometry: Fact or Artifact?TH Open 2 (02), e147-e157
- Mørk, M., et al., (2018) "Prospects and limitations of antibody-mediated clearing of lipoproteins from blood plasma prior to nanoparticle tracking analysis of extracellular vesicles." J. Extracellular Vesicles, Vol 6, 1308779.
- Bæk, R., & Jørgensen, M.,(2017) Multiplexed Phenotyping of Extracellular Vesicles using Protein Microarray (EV Array) Meth Mol biol. 2017;1545:117-127.
- Revenfeld, A.L.S., et al., (2017) "Induction of a Regulatory Phenotype in CD3+ CD4+ HLA-DR+ T Cells after Allogeneic Mixed Lymphocyte Culture; Indications of Both Contact-Dependent and -Independent Activation Int. J. Mol. Sci. 2017, 18, 1603; doi:10.3390.
- Eitan, E., et al., (2017) "Age-Related Changes in Plasma Extracellular Vesicle Characteristics and Internalization by Leukocytes." Nature, Scientific Reports, May 2017.
- Sandfeld-Paulsen B, et al., (2016) Exosomal proteins as prognostic biomarkers in non-small cell lung cancer. Mol. Oncol. 2016 Dec;10(10):1595-1602
- Bæk, R., et al., (2016) The impact of various preanalytical treatments on the phenotype of small extracellular vesicles in blood analyzed by protein microarray. J Imm Meth. 438:11-20.
- Revenfeld A.L., et al., (2016) Presence of HLA-DR molecules and HLA-DRB1 mRNA in circulating CD4+ T cells. Scand J Immunol. 84(4), 44-52.
- Sandfeld-Paulsen B., et al., (2016) Exosomal Proteins as Diagnostic Biomarkers in Lung Cancer. J Thorac Oncol. 11 (10), 1701-10.
- Bæk, R., et al., (2016) Does smoking, age or gender affect the protein phenotype of extracellular vesicles in plasma? Transfusion and Apheresis Sciences (TRASCI), doi:10.1016/j.transci.2016.07.012
- Revenfeld, A.L.S., et al., (2016) Characterization of a Cell-culturing System for the Study of Contact-independent Extracellular Vesicle Communication. J. Circ. Biomark., 5, 3.
- Søndergaard, E.K.L., et al., (2016) Oxygen-related Differences in Cellular and Vesicular Phenotypes Observed for Ovarian Cancer Cell Lines. J. Circ. Biomark. 5,1.
- Jørgensen, M.M., et al., (2016) Phenotyping of Small Extracellular Vesicles from Clinically Important Body Fluids using Protein Microarray. Book Chapter in "Exosomes: Biogenesis, Therapeutic Applications and Emerging Research", edited by Arlando Meyers, Nova Publishers
- Jakobsen, K.R., et al., (2015) Exosomal Proteins as Potential Diagnostic Markers in Advanced Non-Small Cell Lung Carcinoma. J Extracell Vesicles ,4.
- Jørgensen, M.M., et al., (2015) Potentials and Capabilities of the Extracellular Vesicle (EV) Array. J Extracell Vesicles, 4.
- Jørgensen, M., et al., (2013) Extracellular Vesicle (EV) Array: microarray capturing of exosomes and other extracellular vesicles for multiplexed phenotyping. J Extracell Vesicles (2).