Analytical and Bioanalytical Chemistry (2023) 415:1235–1238
https://doi.org/10.1007/s00216-023-04536-7

EDITORIAL

Advances in extracellular vesicle analysis

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Accepted: 11 January 2023 / Published online: 5 February 2023

Extracellular vesicles (EVs) are nanometric objects involved in cell-cell communication [1]. They can be secreted by all types of cells, travel through the body while protecting their cargos from degradation until they are taken up by recipient cells [2]. Because their molecular composition could mimic their cells of origin, and their functional cargos can alter biological processes once they enter other cells, EVs carry high potential as disease markers and therapeutics [3–5].

However, comprehensive understanding of EV's physiological and pathological functions requires enormous efforts in EV purification, characterization, visualization, and molecular profiling. As pointed out by the International Society for Extracellular Vesicles (ISEV) in their guidance [6, 7], all of these four aspects are needed to ensure high reproducibility and reliability of EV studies and lead to responsible conclusions about the functions of EVs or a sub-population of EVs, as well as about the biomarker potential of EV cargos.

Since EVs are small and highly heterogeneous in their physical appearance, biochemical nature, and biological functions, it is challenging to (1) rapidly isolate specific EV populations with high efficiency and purity; (2) fully characterize EVs by size, morphology, and/or proper markers (including both positive and negative) with low sample consumption; and (3) comprehensively profile molecular cargos in EVs or EV sub-populations.

These challenges also present opportunities for analytical scientists. More and more novel and powerful analytical techniques have been and are being developed to address these challenges. Advanced analytical platforms have been developed, employing diverse techniques, including column or other formats of separation, spectroscopy, spectrometry, microscopy, electrochemistry, and microfluidics [8, 9]. These developments not only have helped achieve high sensitivity, high specificity, and fast analysis in study of EVs, but also have advanced analytical science. Therefore, this topical collection has included reviews that summarize the recent developments in analytical technologies [10, 11] and research articles [12–14] that report new methodologies to address these challenges.

Because of the importance of EVs in circulation systems for monitoring pathological development, Martins et al. review the current progress in the isolation of EVs from biofluids, including plasma, serum, saliva, and urine [11]. In particular, the authors have focused on the isolation of exosomes, which have been found to carry significant information from the parental cells and important signaling molecules.

While the other critical review included in this collection also summarizes technologies for EV purification, it concentrates on the advancements and obstacles of microfluidic devices for such a purpose, categorizing the systems as being "Passive" and "Active" based on the mechanisms of fluidic handling [10]. Passive devices include those using filtration, functionalized surface, hydrodynamic force, lateral displacement, and viscoelastic interface for separation. On the other hand, active devices apply magnetic, electric, or ultrasonic fields to drive the separation. Most of the devices separate EVs by their hydrodynamic sizes and surface protein contents, except for dielectrophoresis that separates by their dielectric constants.

Published in the topical collection *Advances in Extracellular Vesicle Analysis* with guest editors Lucile Alexandre, Jiashu Sun, Myriam Taverna, and Wenwan Zhong.

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Effective isolation technologies can render EVs with higher purity and increased concentration, simplifying the subsequent EV content profiling. This is well implemented in the work reported by Turko and Nguyen [12]. Recently, comprehensive analysis of the highly complex EV cargos has revealed the presence of sub-cellular organelle components in EVs, like mitochondrial proteins and DNA. The EV subpopulations that carry mitochondrial components could be important for intercellular mitochondrial transfer. To better differentiate the mitochondrial components enclosed in EVs and those originated from the free extracellular mitochondria, Turko and Nguyen tested the EV fractions prepared from pooled human plasma using a series of separation processes, including ultracentrifugation, size exclusion chromatography, and the heparin-based affinity chromatography. The contents of selected mitochondrial proteins, plasma membrane-specific, cellular membrane-specific, and soluble proteins were quantified by a multiple reaction monitoring (MRM) mass spectrometry (MS) assay using several ^{15}N-labeled quantitative concatamers (QconCATs). The authors concluded that the combination of these isolation steps was effective at separating the EV-containing mitochondrial contents from the EV-free counter parts.

Undeniably, the substantial potential of EVs as disease biomarkers has attracted enormous efforts to develop sensitive and specific sensors for detection of the low-abundance, disease-relevant EV populations in biofluids. Electrochemical detection can provide ultrahigh sensitivity and is the method of choice for portable sensors. Still, surface fouling is one big obstacle for direct analysis of biomarkers in complex biofluids for electrochemical sensors. Therefore, the research team led by Jun Luo developed the peptide-anchored biomimetic interface to facilitate highly sensitive detection of the cardiomyocyte-derived EVs in serum [13]. In their approach, a lipid bilayer was formed on the surface of the electrode, which effectively reduced non-specific protein adsorption on the electrode; and the peptide that can recognize the specific protein marker on EV surface was labeled with a palmitoyl group at the N-terminus, which anchored the peptide into the lipid bilayer. The method permitted detection of EV concentrations ranging from 1×10^3 to 1×10^8 particles/mL and was able to directly detect the target EVs in serum. The same surface modification and anchoring strategy should be useful for EV detection targeting other surface markers.

Besides being significant biomarkers or sources of them, the capability of EVs as drug carriers for enhanced delivery has been widely explored. Thus, the Paper in Forefront of our topical collection from Yan et al. reports the assessment of six different drug loading strategies for incorporating doxorubicin into small EVs, employing the nano-flow cytometry (nFCM) developed by their group [14]. This instrument can detect the fluorescently stained EVs with sizes as small as 40 nm and has been applied for multiparameter characterization of lipid particles, including EVs, and evaluation of drug loading efficiency at the single particle level. In the present work, nFCM enabled rapid measurement of the EV concentration, the percentage of drug-loaded EVs among all EVs, the drug content inside the EVs, and the membrane protein level, which could influence the drug loading efficiency. This work provides solid proof for the power of this instrument in assisting the development of EV-based drug carriers for therapeutic applications.

These excellent reviews and research reports included in our topical collection showcase the power of analytical science in advancing our understanding on EV functions and in recognizing their biomarker or therapeutic potentials. They reflect the diversity of approaches of scientists that are shaping the world of EVs. The advanced techniques reported in this collection are also useful for analysis of other biological substances, such as virus and cell organelles, moving forward the field of analytical and bioanalytical chemistry.

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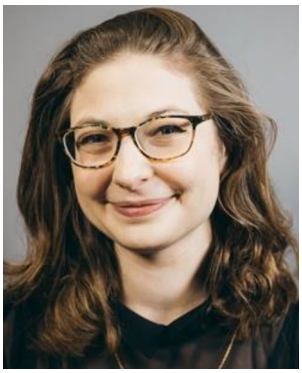

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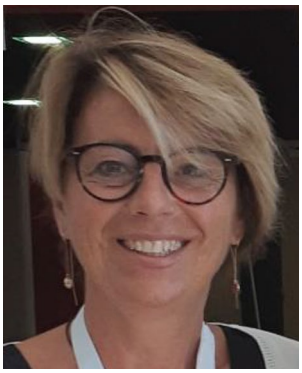

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