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Methods of Isolation and Purification of Extracellular Vesicles from Different Biological Matrixes: Special Issue at a Glance

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Editorial

In the last decade, there has been intensive research on the role of extracellular vesicles (EV) as diagnostic and prognostic biomarkers, therapeutic targets, as drug delivery vectors or even as candidates for reparative or regenerative medicine in a plethora of diseases. EV are a composite of heterogeneous phospholipid bilayer-surrounded particles ranged between 30 and 5000nm in diameter, namely exosomes, microvesicles and apoptotic bodies, which conform a singular cluster of mediators of cell-to-cell communication, thanks to their selective packaging of bioactive molecules (such as lipids, proteins, RNA and metabolites). As almost all cells (prokaryotic and eukaryotic) release EV, they might be involved in the pathophysiology of virtually all diseases. In fact, the molecular cargo of EV released after a specific stimulus, determines its mechanism of action and reflects the state of its parental cell.

Despite all scientific efforts and technical progresses, the research in the properties, structure, composition, pathophysiological activity and metabolism/clearance of EV is still technically challenged by the EV extraction and purification methodology. In this context, this method collection is aimed at reporting methods of isolation and purification of EV, for further downstream applications.

In this issue, El Itawi and colleagues[1], describe the isolation of splenocyte-derived EV in a murine model of infection by *Porphyromonas gingivalis*. Authors isolate the EV by differential centrifugation from a splenocyte suspension, and afterwards harvest the EV to study its *in vivo* effects. However, authors state that their method should be adapted for adherent cells.

Jones and coauthors[2], isolate EV originated by cultured oligodendrocytes combining size exclusion chromatography (SEC) and 3-KDa ultrafiltration, thus ensuring a proper EV purity with minimal protein contamination in their extracts. This semi-automated method can be used for virtually all downstream applications such as functional assays, molecular characterization of EV or EV imaging.

SEC is also proposed by Ryan *et al.*[3], to isolate EV from *Mycobacterium tuberculosis*, in order to understand the bacterial physiology, the interaction with the host, and the EV molecular cargo. In this methodological manuscript, authors compare the performance of density gradient ultracentrifugation and SEC, and conclude that the EV recovery and purity of EV is improved when performing SEC. Authors also suggest that the methodology described can be applied to other mycobacteria-derived EV.

Valle-Tamayo *et al.*[4], describe an easy two-steps method to obtain astrocyte-derived EV by a polymer-based precipitation and immunocapture with astrocyte cell surface antigen-1 (ACSA-1) antibody conjugated to magnetic micro-beads. Authors suggest that immunocapture can be combined with SEC instead of polymeric precipitation, and that magnetic micro-beads could be conjugated to other antibodies to isolate EV from other cell types. The recollection of these EV from human plasma or other biofluids might be of high value in the field of neurological diseases or related disorders.

In the last manuscript of the issue, Koh and colleagues[5] propose an ultrafiltration method to isolate small EV (sEV) from cultured human mesenchymal stem cells. The authors state that the ultrafiltration-isolated EV extracts can be used for therapeutic applications and other downstream analyses. While this is a simple and fast method that could be applied to other biofluids and different cell types of origin, authors acknowledge that it might not be suitable at large scale for industrial applications.

Overall, this Special Issue provides different relatively simple and easy laboratory methods to isolate EV from different sizes, and from a variety of specimens, such as bacteria, animal organs and human cells. The extraction yield and purity differs among methods, and should be considered to select the most cost- and time-effective method according to the final use of the isolated EV.

Finally, I would like to end by thanking all authors and reviewers for their contributions, and the Editorial staff of the journal of Visualized Experiments for their invaluable assistance in such process. I sincerely hope this special issue will be useful to EV investigators in their future research.

Disclosures

The author has nothing to disclose.

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