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Commentary

Nickel affinity: A sensible approach for extracellular vesicles isolation?

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Extracellular vesicles (EVs) are of great interest as disease biomarkers since these secreted vesicles contain a variety of molecules that can indicate the phenotype of their parent cells at the time of their secretion. EVs are also abundantly released by most cells to accumulate in the circulation where they can provide information about the disease status of specific cell types or tissues that might otherwise require a tissue biopsy. However, the development and clinical translation of EV-based diagnostic assays has been hampered by the current lack of validated procedures that can rapidly and reproducibly isolate high-purity intact EV samples for biomarker analysis studies in research and clinical settings.

Current EV isolation methods rely on a variety of procedures that can differentially fractionate EVs from other serum or plasma factors based on their physical properties through the use of density separation, size selection, affinity enrichment, or precipitation approaches, each of which has its own distinct advantages and limitations. Ultracentrifugation remains the most common means used to isolate EVs [1], but requires expensive equipment, has limited throughput, and isolates EV samples with variable yield, purity, and membrane integrity. Recently, however, it has been reported that an anion-exchange chromatography approach can isolate EVs from clarified conditioned media to produce EV isolates with yields, size distributions, and biomarker expression levels that closely resemble those produced by ultracentrifugation [2], while offering several advantages, including the ability to perform scalable purification, rapid process input samples, and avoid the co-isolation of protein aggregates found in ultracentrifugation samples.

In this article of *EBioMedicine*, Notarangelo et al. reported a variation of this method, in which they demonstrate that nickel-binding beads can be employed to rapidly capture EVs directly from patient plasma samples by anion exchange due to the negative charge conferred by the zeta potential of the EVs [3]. This approach differs from the previously reported anion exchange method in that EVs can be captured and released under physiologic conditions [2], which may serve to maintain the stability and integrity of EV isolates relative to conventional anion exchange where buffer changes required for EV isolation may subject EVs to osmotic stresses that may negatively impact EV integrity and stability. Notarangelo et al. recovered EVs captured on nickel-binding beads by a wash step employing PBS supplemented with low millimolar amounts of chelating agents (EDTA and citric acid). EVs isolated on nickel charged beads demonstrated greater integrity and stability than those collected by ultracentrifugation but were not compared to EVs isolated by anion exchange chromatography so the relative benefit of isolating EVs by a chelating versus high salt elution buffer approach is unknown.

Nonetheless, the proposed method exhibits several advantages that render it suitable for use in research and clinical settings, is rapid, scalable, employs a simple workflow suitable for high throughput operation, does not require expensive materials or equipment, and does not subject EVs to forces or buffers likely to destabilize the resulting EV preparations. Currently, improved EV isolation procedures primarily benefit research laboratories, since the FDA has yet to approve an EV assay or EV-associated biomarker for any clinical application, although a small number of EV assays are now performed as laboratory developed test in clinical laboratories that have CLIA certification for these assays. Results presented by the authors do, however, imply strong potential for the future clinical utility of this EV capture approach in future clinical applications, as their data indicate that it can be readily adapted for use with digital droplet PCR assays and purification-free ExoScreen assays, two ultra-sensitive biomarker quantification approaches [4,5].

One potential disadvantage of this approach, shared by several other EV isolation methods, is that it isolates vesicles that overlap the size range of exosomes and microvesicles, which may exhibit some overlap in their markers but which are generated by different biogenesis mechanisms and may exert distinct regulatory effects [6]. As the authors indicate, however, exosome and microvesicle components of these EV preparations could be further resolved by size fractionation.

Despite these potential concerns, the ability to capture EVs directly from human serum samples for isolation using a precipitation based method or direct analysis represent a significant advance, and further analytical validation studies are warranted to better define the performance and reproducibility of this approach.

Disclosure

The author declares no conflicts of interest.

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