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Standardizing cell culture methods is critical for reliable and reproducible exosome isolation

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Abstract

Increasing evidence has shown that Extracellular Vesicles (EVs), such as exosomes (EXOs), have attracted considerable interest due to their unique biological functions as drug delivery vehicles, non-invasive tools for disease diagnosis, and their clinical potential, most prominently in the fields of oncology and cell-based therapy. Although significant advancements have been made in EXO isolation techniques, standardizing cell culture methods remains an important challenge that must be overcome to ensure specific, pure, and effective EXOs for diverse life science and clinical applications.

Editorial

EVs are lipid bilayer-enclosed structures produced and released by living cells, including EXOs (30-150 nm), microvesicles (150-1000 nm), and apoptotic bodies (1-5000 nm) (1-3). EXOs are formed through the fusion of multivesicular bodies (MVBs) with the plasma membrane in the endosomal compartment and are released into the extracellular space by direct budding from the plasma membrane (1,4,5). Pan and Johnstone initially identified and named EXOs when they investigated the maturation of sheep reticulocytes (6). For many years, EXOs were thought to be responsible for carrying cellular metabolic waste (7). Zhang et al. confirmed the spherical shape of EXOs using electron microscopy (8). Their biological importance and relevance to disease were understood many years later, when researchers explored their roles in cell-to-cell communication and disease (9,10). Many cellular functions, such as apoptosis, cell growth, and cell division, are regulated by EXOs through the delivery of active biomolecules, including nucleic acids, proteins, and lipids, to target cells (11,12). EXOs have been found in various body fluids, such as blood, urine, saliva, sweat, tears, vaginal fluid, and cerebrospinal fluid (1), as well as in cell culture supernatant (13).

Several studies have now been designed on EXOs and have shown that EXOs play an important role in cancer biology and immunoregulation (14-18). One of the main challenges in EXO research is the lack of an efficient standardized isolation method for precise EXO subpopulations because of their small size and heterogeneity (19-22). In addition, the isolation of intact and pure EXOs is necessary, especially when they are intended to be used as vehicles for drug delivery in the treatment of human disease (4). Biofluid-isolated EXOs unavoidably have a mixed cellular origin (20). Pure EXOs can be obtained from the conditioned medium (CM) of a cell line. However, flow cytometry analysis has shown that not all components of a cell line have the same phenotype (17). Therefore, it may be necessary to use a subpopulation of a cell line with specific surface CD marker. EXOs are usually isolated from culture medium conditioned by specific cells for 24-48 h (20), although conditioning times of 15-60 min have also been reported (23,24). Hence, the conditioning time of culture medium in specific cells must be standardized.

The use of serum supplements that contain their own EXOs in cell culture medium can contaminate EXOs secreted by specific cells. Ultracentrifugation and serum-free media (SFM) are recommended to eliminate serum-derived EXOs (25); however, both approaches have limitations, such as the need for efficient elimination of serum-derived vesicles and serum protein quantification in ultracentrifugation, and the secretion of EXOs with different compositions in the SFM strategy (20). To overcome external serum-derived EXOs, it is mandatory to develop precise and robust antibody-based methods for the complete elimination of external EXOs or defined SFM that mimic human serum composition. Dead or dying cells release different vesicles that contaminate live cell-derived EXOs. Also, the composition of EXOs may change due to the uptake of soluble materials released from dying cells (20). It seems that separating live cell-derived EXOs from dead cell-derived EXOs is a challenge that requires the development of methods that can detect and isolate live cell-derived EXOs. However, standardization of conditioning time and washing dead cells before EXO isolation may be helpful. Any microbial contamination, such as mycoplasma or viruses, can contaminate the exosomal content of conditioned medium (20). Hence, cell lines should be checked for microbial contamination before EXO isolation.

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Conflicts of interest

None.

Author contributions

All parts of the manuscript were written and submitted by Ayyoob Khosravi.

Data availability statement

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