Opinion

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Towards the Standardization of Exosome Collection and Purification

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ABSTRACT

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The discovery of the "cell-derived vesicles" dates back to the 1940s [1]. Yet, the recognition of exosomes as a distinct type of cell-derived vesicle, along with the term "exosome", only emerged during the 1980s [2]. Nowadays, this sub-type of extracellular vesicles is arising as a significant player in our normal and pathophysiology, instigating exosome-based research as an emerging field in medical research. At first, it was proposed that exosomes were released by reticulocytes to remove unwanted plasma membrane proteins before becoming erythrocytes [3]. Then, it was established that almost all mammalian cells release these exosomes to carry out several biological functions, with the most crucial being the facilitation of intercellular communication. Cells release exosomes with active components, including miRNAs, that when up taken by the recipient cell, causes initiation of specific cellular pathways [4]. Scientist growing interest in exosome-based research came as a result of their role in cell-to-cell signaling. Then, scientists started exploring with the idea of using these vesicles in clinical application. The most tested clinical applications for exosomes include tissue repair and regeneration. This idea came about when mechanism of action studies revealed that the administered regenerative cells, such as mesenchymal stromal cells, exert their tissue healing, and regenerative effects mainly through the release of paracrine factors including exosomes, and that the administration of exosomes alone is sufficient to generate the same effect [5,6]. Another clinical application being explored is the utilization of exosomes as a carrier vehicle for therapeutic agents such as antitumor miRNAs [7], or anti-inflammatory drugs [8]. Also, since cells change its exosome cargo depending on its state, examining the constituent of exosomes could be used as a diagnostic tool. For example, malignant cells release its exosomes, with its tumor-related miRNAs, into the patient circulation. Identifying these miRNAs in patient sera could be used as a biomarker for cancer diagnosis and development [9]. Because of their role in cellular communication, and their possible clinical applications, a large body of published literature in the field of exosome research is being generated. One major challenge, however, limiting exosome-based research is the issue of exosome isolation. Currently, there is no standardized method for exosome collection and purification. In all the methods used in the published literature, producing a consistent yield of exosomes that is suitable for *in vitro* or *in vivo* application is still a work in progress.

Earlier studies on extracellular vesicles and exosomes utilized an ultracentrifugation-based protocol for harvestation. The ultra-centrifugation protocol is based on the exosomes' characteristic density of 1.13–1.19 g/ml in sucrose. The protocol usually involves differential centrifugation and/or filtration to remove dead cells and debris, followed by ultracentrifugation coupled with a sucrose density gradient [10]. The ultracentrifugation method is considered, tell now, somewhat of the gold standard, to which the efficiency of other developed isolation techniques could be compared [11]. However, the protocol is quite time consuming and laborious. Preparation of the sucrose density gradient, sample loading, and vesicle collection requires a high level of technical skills that makes results hard to reproduce, over different repeats and among different technologist. Not to mention that not many laboratories

have access to an ultracentrifuge. Therefore, biomedical industries are developing other exosome isolation techniques using different principles. The first and most widely used commercial kits available in the market are based on the precipitation principle. The principle is quite simple; exosomes in a given sample are precipitated with a precipitation regent, and then collected via low centrifugal force. The Total Exosome Isolation Reagent by Thermofisher Scientific was among the first to become available. Now, the company has developed the original kit into separate kits for exosome isolation from different samples including; urine, plasma, serum, other body fluids, and cell culture media. The Original Exo Quick kit by System Biosciences is also one of the widely used exosomes isolation kit by precipitation; the company further developed this product to make one for Serum and Plasma, one for tissue culture media and another kit for both exosome isolation and exosome RNA Purification. The precipitation-based isolation techniques are fast, easy, does not require specialized equipment, ensure high exosome yield and relatively coast effective [12]. However, with this method, the exosome yield will probably be contaminated with the precipitin reagent and other precipitated proteins. This could affect the purity and the biological activity of the collected exosome or could have a cytotoxic effect on the cells to be treated. This should not be an issue if the study was focusing on analyzing the miRNA content of a given exosome population, for example. But, if the purity of the sample is essential for the study objective, there is the Immune-based isolation, where exosomes are collected via capture antibodies. MACS® Miltenyi Biotech utilized its well-known magnetic separation technique for exosomes purification. The company utilized the same MACS® technology used for cell isolation. The exosomes are first labeled with microbeads coupled with exosome specific antibodies. Then the sample is loaded into the separation column and placed in a magnetic field. As a result, the labeled and non-labeled fractions elute separately. While exosomes yield via immune capture is usually lower compared to other techniques, the yield is often highly pure [13]. This could be important if the end-use for the isolated exosomes was flowcytometric analysis for example. Nevertheless, in both these approaches a "reagent" or a "label" is added to the sample, this could be an issue in some studies. For example, if a study objective is to investigate the biological activity of a certain group of exosomes, these exosomes are expected to be intact for the results to be reliable. In this case, it is best to use a "reagent free" isolation approach. One such approach is the size exclusion chromatography (SEC), where the mobile phase, i.e. the sample passes through the stationary phase, i.e. the column. In the column, the constituents of the medium, including exosomes, travel at different speeds, allowing the separation and collection of exosomes. This method is believed to "minimally" alters the biological characteristics of the isolated exosomes compared to other techniques [14]. Like the ultracentrifugation method, the SEC method was quite tedious to perform. But nowadays, exosomes separation columns are available commercially. For example, a company called "izone" developed separation columns that are single and multiple use, for exosome isolation from biological samples and tissue culture media [15]. This approach is a bit more technical compared to the precipitation and its predicted that it provides less pure yield compared to the Immune-based approaches. Yet, since there are no reagents added, there is a good chance that the exosomes retain their biological activity for subsequent cell functional assay or for an animal study purposes.

All in all, there are many factors that dictates the selection of the exosome isolation method; these include the type of sample, availability of equipment and labor, budget, in addition to personal preferences. Still, in our opinion, the most crucial factor to consider before choosing the exosome isolation and purification approach is the purpose of isolation and the subsequent application. Currently, there is no standardized exosome isolation method for each application. In the published literature, different groups could adapt different isolation techniques, and end up utilizing the isolated exosomes in the same *in vitro* or *in vivo* application. This could lead to inconsistent reporting, especially if we consider the other additional variations such as method of sample collection and preparation in the case of biological samples, or type of media used and incubation period in the case of condition media. As the interest in exosomes grows, biomedical companies are racing to develop kits and instrumentations that revolve around extracellular vesicles research. With the current rate, we expect that companies will come up with even more efficient ways to produce exosomes in a standardized, large-scale manner. This is essential not only for medical research, to understand the role of these extracellular vesicles in health and disease, but also for successful utilization in possible clinical applications. Still, until we reach this point, it is of extreme importance for the published literature to adequately describe the process of sample or media preparation for exosome collection, in addition to the actual isolation protocol. This will not only improve the transfer of knowledge between different laboratories but also, it might explain the discrepancy in results between different research groups.

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