SPOTLIGHT feature

Proteomics, Genomics & Microarrays

Exosomes: A Promising New Tool for Diagnostic and Clinical Applications

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Exosomes and extracellular vesicles (EV) are currently the subject of a lot of scientific interest; implicated in intracellular communication and with their ability to be used as a tool in biomarker discovery, they have the potential to enable advances in clinical settings. EVs are nanosized lipid membrane vesicles that are released from a vast number of different cell types into the extracellular space [1]. They are categorised into 2 broad areas of exosomes and ectosomes [2]. Exosomes are vesicles ranging in size of ~40-160nm in diameter and form through the endocytic pathway to be released from the cell by fusion of endosomal multivesicular bodies (MVBs) with the plasma membrane [2]. Ectosomes differ by directly budding from the surface of cells and include a number of subspecies; microvesicles, migrasomes, exophers, apoptotic bodies, and large oncosomes with a size range of ~50nm-5um [3].

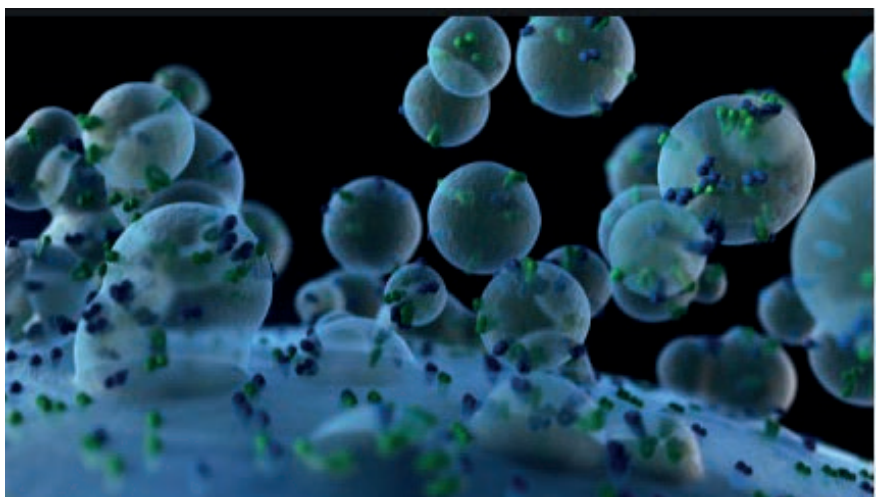

Figure 1. Illustration of cells secreting exosomes.

EVs are involved in various physiological and pathological processes, such as intercellular communication, immune regulation, and tumour progression. They contain various bioactive molecules, including proteins, lipids, nucleic acids, and metabolites, that can be transferred to recipient cells and modulate their functions. There is an abundant amount of evidence showing EVs are distributed widely in bio fluids including blood, urine, saliva, breast milk, cerebral spinal fluid, synovial fluid, and more [4]. There is also increasing evidence to demonstrate that the surface of an EV and its luminal content correspond with different pathophysiological states of the body [5]. The upshot is that EVs could potentially be a great source for potential biomarkers in the diagnosis and prognosis of a number of disease states. EVs also hold promising therapeutic applications for new drug discovery and drug delivery tools from the increasing evidence of their high biocompatibility, limited immunogenicity, cargo diversity, stability, and ability to cross the blood-brain barrier [6].

With their inherent properties of cell-to-cell signalling, EVs could offer considerable advantages within the medical and scientific fields. While this newly researched field is promising, it holds many questions and areas that need to be explored further before the clinical applications of EVs can be maximised. This includes research in mechanisms of EV biogenesis, cargo loading, and uptake, as well as their safety and efficacy in clinical settings.

Isolation Techniques

Working with EVs holds many challenges due to their broad heterogeneity and issues with contaminations from soluble proteins, lipoproteins, and other non-EV contaminants during EV isolation. This is why understanding the limits and advantages of different EV isolation techniques are important to determine the best EV isolation tool to match the research goals of the study. Different approaches to isolating EVs include ultracentrifugation (UC), size-exclusion chromatography (SEC), Tangential Flow Filtration (TFF), immunoaffinity, and precipitation methods.

A recent study [7] reviewed various isolation methods of EVs and found that UC is considered the gold standard for EV isolation, with newer techniques such as SEC and precipitation methods are becoming more popular.

UC is the benchmark for EV isolation techniques as it provides great purity and yield, as well as having established protocols for EVs studies. However, UC does have huge drawbacks in the field. Standard isolation protocols can take a full lab day for a set of isolations, it requires large sample starting volumes, and above all, UC needs an experienced EV isolation researcher to create isolation reproducibility. This method also has huge challenges in scalability which is why it is NOT suggested for clinical applications.

The increasing popularity of SEC has arisen from its ability to provide a simple and reproducible isolation protocol, the fact it is faster than UC, and because it can efficiently isolate out contaminants such as soluble proteins, lipoproteins, and nano sized non vesicular extracellular particles. Drawbacks to the SEC method are the tedious fractioning of isolated samples, volume constraints, and isolating based on size.

TFF, traditionally used on large biomanufacturing facilities, has gained a lot of interest in its use for EV isolation. This method uses ultrafiltration membranes with pores in a column that provides a tangential flow to avoid filter clogging and cake formation. This creates the ability to be scaled for different clinical applications. Drawbacks to this method includes co-isolating non EVs that have comparable size profile to EVs.

Immunoaffinity provides a different principle of isolation based on surface markers present on EVs. The key benefit of this method is the extreme purity of EVs that can be extracted with the ability to subtype EV populations based on their surface markers. Drawbacks to this method include the low yield from isolating a subpopulation of EVs, and the inability to get the targeting motif removed from the EV surface marker.

Lastly, the precipitation method provides a high yield of EVs by using polyethylene glycol (PEG) to decrease the solubility of the mixture to isolate EVs out of the solution. This method does have an extremely high yield of EVs, is cost effective, and has a simple protocol. However, in the isolation process impurities such as lipoproteins and other soluble proteins will contaminate isolated EV samples. The precipitation method cannot do large volumes of isolation, and so this method is most impactful for early-stage screening of EV populations.

Companies such as AMSBIO offer a variety of exosome isolation kits that are based on ultracentrifugation, size-exclusion chromatography and precipitation methods, that can help researchers to select the best method based on the parameters of their specific research or project.

EV Standards

Whilst standardised EV isolation methods can and will produce usable EVs for use in studies, by nature EVs are also inherently heterogeneous, due both to the vast number of cells producing EVs and the physiological conditions that these EVs are expressed from. This means that for the best results for diagnostic applications or when developing exosome-based therapies it can be better to use an externally produced set of EV standards. These standards can include purified exosomes from a specific cell type or a mixture of exosomes from multiple cell types. They can also include synthetic exosomes that have been engineered to contain specific biomarkers. The use of consistent, well-characterised exosome standards can facilitate the development of new diagnostic tests and therapies. A report in the Journal of Extracellular Vesicles [8] described the importance of using exosome standards to ensure the quality control of exosome-based diagnostics and therapeutics. AMSBIO offers a wide range of exosome standards that can be used in clinical settings, including purified exosomes from a specific cell type, exosome standards for diagnostic applications and synthetic exosomes that have been engineered to contain specific biomarkers.

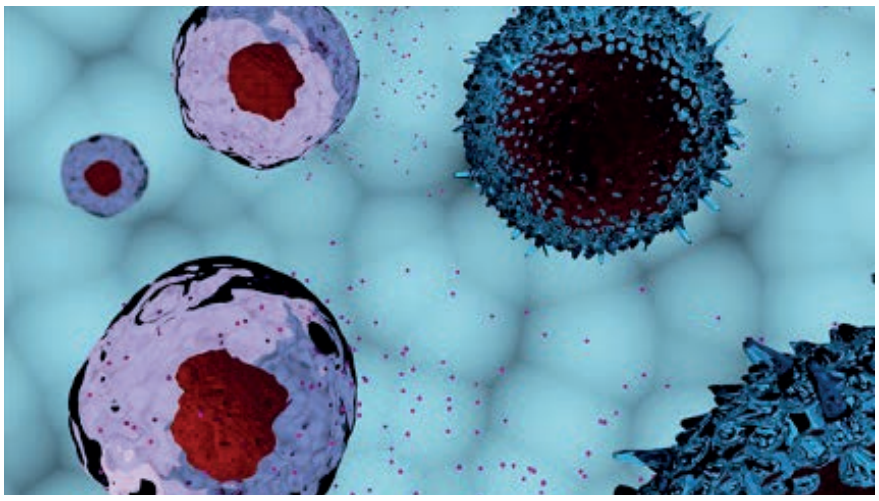

Figure 2. Illustration of cells secreting exosomes.

Characterisation Methods

Due to the numerous characterisation targets available for EVs, multiple characterisation techniques should be used during an EV study. Some characterisation targets include population size distribution, number of EVs, proteins, DNA, RNA, lipid structure, and more.

Basic tools used in EV analysis include: light scattering techniques such as nanoparticle tracking analysis (NTA) to determine the number of EVs and the size distribution of your population, identifying the amount of protein with a bicinchoninic acid (BCA) assay, and carrying out specific protein identification with western blotting.

Additional tools for EV characterisation include ELISA and flow cytometry. ELISA kits can identify and quantify specific protein markers of interest found on the surface of EVs. This method uses antibodies to specifically target markers of interest on EVs, then fluorescently activated markers will be added to identify the markers of interest.

The use of flow cytometry has increased in popularity among EV researchers as it gives the ability to quantify different subpopulations of EVs. By fluorescent labelling of target proteins with different colours in isolated EV populations, single cells can be analysed and compared to highlight differences in EV populations.

Recognising and analysing nucleic acids associated with EVs is another key area of importance for accurately characterising isolated EV populations. First, commercially available kits are used to lysate the EVs open and extract the nucleic acid from the solution. Then species of nucleic acid are sequenced and analysed. This can be done using a variety of different methods, including RNAseq.

Finally, mass spectrometry is widely used to analyse and characterise lipid content of EV membranes. Lipids have an important structural role in exosomal membranes as the lipid content of EVs are enriched in cholesterol, sphingomyelin, glycosphingolipids, and phosphatidylserine. They are also essential players in EV formation and release to the extracellular environment.

Custom Service

EV studies can be difficult, need a lot of scientific experimental nuance knowledge, and require a specific set of tools for completing a successful study. This is why companies like AMSBIO help customers by offering a variety of custom EV research services to help researchers with their exosome-related projects. These services include EV isolation, characterisation, and quantification, as well as custom EV production and engineering. Also provided are RNAseq and proteomics analysis.

The EV isolation services offered by AMSBIO include: ultracentrifugation, size-exclusion chromatography, and precipitation methods. These methods can be customised to fit the specific needs of the researcher's project and can be used to isolate exosomes from a variety of sample types such as blood, urine, and cell culture media.

AMSBIO also offers EV characterisation services which include exosome size and concentration analysis, as well as protein and RNA analysis. These services can be used to identify the specific exosomes present in a sample and to understand their biological properties.

Custom exosome production and engineering services are also available at AMSBIO, which can be used to generate exosomes with specific characteristics such as specific protein or RNA content. These custom exosomes can be used as standards or as tools for exosome-based therapeutics and diagnostics.

Overall, EV research services can be tailored to fit the specific needs of the researcher's project. These services can help researchers to isolate, characterise and quantify exosomes, as well as to generate and engineer custom exosomes for various applications.

Future Technologies and Applications

Biomarkers

With the potential ability to read the messages cells pass to other cells, EVs can be a great source for diagnostics. Currently, there is one EV product clinically approved for non-invasive screening for prostate cancer. This test uses urine taken from at-risk males 50 years or older for high grade prostate carcinoma (HGPCa). Urine samples are taken from these individuals to isolate exosomal RNA creating an EPI risk score. This determines if a further biopsy test is needed.

Due to the increased interest in precision medicine, new biomarkers have been an area of important research and development for pharmaceutical companies. The potential use of EVs as a biomarker to monitor the microenvironment in disease states can provide information on drug efficacy, enabling better precision-based medical treatment.

Therapeutics

Currently, there are no FDA approved EV based therapies on the market. Direct Biologics has entered phase 3 clinical trials for ExoFlo to treat all moderate to severe causes of acute respiratory distress syndrome (ARDS). ExoFlo is isolated from human bone marrow mesenchymal stem cells (MSCs) containing both growth factors and EVs for the treatment of ARDS.

Another exciting clinical application for exosomes is using them as a drug delivery vehicle as their membrane is biodegradable and has a high payload capacity. EVs can also improve biodistribution and can offer a controlled drug release. There are different techniques that can be used to load exosomes, including electroporation, sonication, transfection of cells that will then produce exosomes, treating exosomes with surfactants, and dialysis. EVs can be used for specifical drug delivery, by including targeting ligands specifically aimed at tumour cells.

Finally, EVs can also be used in imaging applications. For this, they need to be labelled with fluorescent dyes, nanoparticles or proteins. Additionally, bioluminescent molecules, such as luciferase can be used to track exosomes. For whole body imaging, gold or iron labelled nanoparticles need to be used to overcome detection issues.

Conclusion

Extracellular vesicles (EVs) hold enormous promise in the clinical landscape with their unique ability to serve as a vehicle for targeted delivery of therapeutic molecules and their use as diagnostic biomarkers. Although challenges remain in standardising isolation and characterisation protocols, the potential clinical benefits of EVs are undeniable. With further research and advancements in understanding EVs' biology, EV-based therapeutics and diagnostics could emerge as a game-changing clinical tool in personalised medicine, delivering safer, more effective, and precise treatments to patients.

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