EXOSOMES

Exosomes are membrane bound extracellular vesicles produced in the endosomal compartment of eukaryotic cells. The multivesicular bodies fuse with the cell surface to release the intraluminal vesicles as exosomes. The exosomes act as mediators of near & long distance intercellular communication. They can carry nucleic acids, proteins, lipids & metabolites to the distant cells. They are discovered in biological fluids including blood, urine & cerebrospinal fluid and also in tissue matrix (matrix bound nanovesicles).

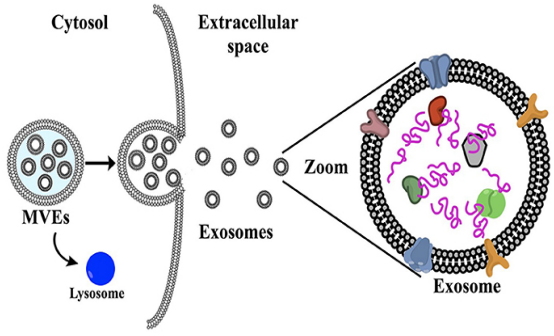
History behind the discovery of exosomes:

The concept of "exosome" came in concern of modern biological science in the year 1983 by the experiment done by Harding C & Hauser J. According to their publication in the 'Journal of cell biology', the authors cultured the immature red blood cells (reticulocytes) with labelled transferrin receptors to trace the movement of transferrin receptors from plasma membranes into the reticulocytes. it was observed that the labelled transferrin receptors are internalized within the reticulocytes, and then repackaged into small (~50 nm) vesicles inside them. These vesicles, originally thought to be extracellular to be trafficked to lysosomes for destruction, are subsequently secreted out of the maturing blood reticulocytes into extracellular space. The experiment done by Pan BT et al also support this statement.

Later, in the year 1989, Johnstone et al. coined these vesicles as "exosomes".

Structure function relationship:

Exosomes belong to a large family of membrane vesicles referred to as extracellular vesicles, which generally include microvesicles (100–350 nm), apoptotic blebs (500–1000 nm), and exosomes (30–150 nm). Their pathophysiological roles are in various medical conditions and diseases including cancer. When examined under an electron microscope, exosomes show characteristic cup-shaped morphology, appearing as flattened spheres with diameters ranging from 30 to 150 nm. The cup-shaped morphology is most likely originated from the sample preparation process of conventional electron microscopy during which the exomes are extremely dehydrated, thus leading to the collapse of the exosomes. In contrast, the exosomes remain fully hydrated in cryo-electron microscopic examinations, round-shaped morphology is observed.



exosomes have a characteristic lipid bilayer which has an average thickness of ~5 nm. The lipid components of exosomes include ceramide, cholesterol, sphingolipids, and phosphoglycerides with long and saturated fatty-acyl chains. The outer surface of exosomes is rich in saccharide chains, such as mannose, polylactosamine, alpha-2,6 sialic acid, and N-linked glycans. The proteins are found on the surface and in the core of exosomes. As one of the most important cargoes that exosomes carry, the proteins can provide information associated with the physiological states of the parental cells of exosomes. Many exosomes contain proteins that are common among all exosomes regardless of the types of cells which secrete them, whilst only a small fraction of proteins is cell-specific, reflecting the type and pathophysiological conditions of those secreting cells. Proteins typically found in exosomes include platelet derived growth factor receptor, lactadherin, transmembrane proteins and lysosome associated membrane protein-2B, membrane transport and fusion proteins like annexins, flotillins, GTPases, heat shock proteins, tetraspanins, proteins involved in multivesicular body biogenesis, as well as lipid-related proteins and phospholipases. These characteristic proteins therefore serve as good biomarkers for the isolation and quantification of exosomes. Another key cargo that exosomes carry is nucleic acids including deoxynucleic acids (DNA), coding and non-coding ribonucleic acid (RNA) like messenger RNA (mRNA) and microRNA (miRNA).

Importance of exosomes:

Exosomes have tremendous importance in recent years due to the fact that the biological fingerprints of exosomes practically mirror those of the parental cells they are originated. Although the exact biological functions of exosomes are yet to understand, but, increasing evidence has indicated that exosomes play a vital role in many cellular processes like cell-cell communication, coagulation, antigen presentation, waste management, as well as transfer of proteins and nucleic acids. Cell-cell communication is crucial in all multicellular organisms and were previously thought only to be possible via contact dependent (direct contact between adjacent cells), paracrine (binding of short-ranged signalling molecules on receptors of nearby cells), endocrine (binding of long-ranged hormones on far away cells via bloodstream), or neuronal (electrical impulses travelling along neurons) signalling. Recent research has been highlighted possible exosome-mediated cell-cell communication in many processes, for example immune signalling, angiogenesis, senescence, proliferation, differentiation, and implicated in many human diseases like neurodegenerative disorders, cancer, and AIDS. More importantly, growing evidence has also suggested that exosomes play a key role in facilitating tumorigenesis by regulating angiogenesis, immunity, and metastasis. Circulating exosomes in body fluids and blood in particular are potentially non-invasive or minimally invasive biomarkers for early diagnosis and prognosis of various types of cancer. As such, exosomes not only contribute to a greater understanding of cell physiology and pathology, but also have a great potential to be translated to various clinical applications, ranging from diagnosis and prognosis to nucleic acid delivery and cancer therapeutics.

Isolation techniques:

The isolation and quantification of exosomes have become a major initiative in both basic research and clinical applications. As the first step towards improving human health, exosomes have to be reliably and efficiently isolated from complex biological matrices like blood, urine, and cerebrospinal fluid since they are currently tested as next-generation biomarkers in those body fluids. To date, five groups of exosome isolation techniques have been developed. They are differential ultracentrifugation-based techniques, size-based techniques, immunoaffinity capture-based techniques, exosome precipitation, and microfluidics-based techniques.

Ultracentrifugation based isolation technique: Due to the heterogeneity of exosomes and considerable overlap in size of extracellular vesicles, differential ultracentrifugation often suffers from contamination and exosome losses. Ultracentrifugation is often coupled to isopycnic or moving-zone techniques to allow

the exosomes of relatively low densities to float and to further purify the exosomes.

Size-based isolation techniques:

One of the popular size-based exosome isolation techniques is ultrafiltration. The fundamentals of ultrafiltration are no different from conventional membrane filtration in which the separation of suspended particles or polymers is primarily dependent on their size or molecular weight. Therefore, based on their size, exosomes can be isolated using membrane filters with defined molecular weight or size exclusion limits. Ultrafiltration is faster than ultracentrifugation and does not require special equipment. However, the use of force may result in the deformation and breaking up of large vesicles which may potentially skew the results of downstream analysis.

Immunoaffinity capture-based techniques: The presence of plenty of proteins and receptors in the membrane of exosomes offers an excellent opportunity to develop highly specific techniques for the isolation of exosomes by tapping on immunoaffinitive interactions between those proteins (antigens) and their antibodies, and specific interactions between the receptors and ligands.so, immunoaffinity capture-based techniques have been developed for the isolation of exosomes.

Exosome precipitation:

By altering their solubility or dispersibility, exosomes can be settled out of biological fluids. For this purpose, water-excluding polymers such as polyethylene glycol (PEG) is engaged. Generally, samples are incubated with a precipitation solution containing PEG with a molecular weight of 8000 Da. After incubation at 4°C overnight, the precipitate containing exosomes is isolated by means of either low speed centrifugation or filtration. Exosome precipitation is easy to use and does not require any specialized equipment. This allows easy integration into clinical usage by exploiting existing technologies and is scalable for large sample sizes.

Microfluidics-based isolation techniques: The fast advances of microfabrication technology have offered a rare opportunity for the fabrication of microfluidics-based devices to rapidly and efficiently isolate exosomes, tapping on both the physical and biochemical properties of exosomes at microscales. In addition to the usual approaches like size, density, and immunoaffinity, innovative sorting mechanisms such as acoustic, electrophoretic and

electromagnetic manipulations can be implemented. With the use of such devices, significant reductions in sample volume, reagent consumption, and isolationtime are expected.

Synthetic exosomes:

The clinical transformation of preparations based on nanotechnology brings new hope for drug selection and targeted delivery. Traditional drug delivery systems (DDSs), including polymeric nanoparticles (NPs), solid lipid NPs, nanoemulsions, and liposomes etc., have been used as effective tools to target the brain. The development of cellular-specific therapy also opens new possibilities for the treatment of neurological diseases, but the use of living cells may induce an autoimmune response or rejection, which may increase the risk of sustained stimulation. Considering the complexity of production process, safety and biocompatibility, naturally occurring nano vesicles are superior in this aspect.

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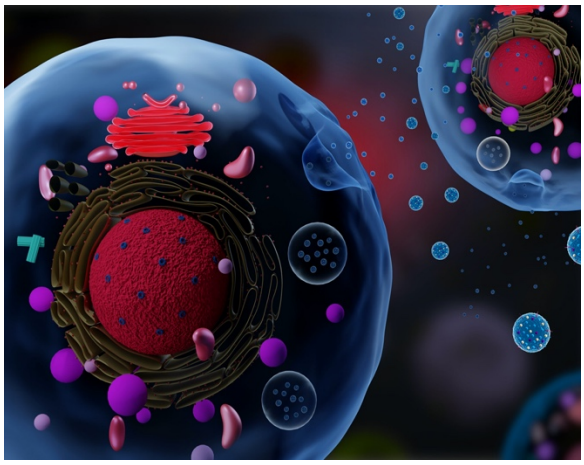

Contributor:

Dr Sanchayan Sinha
Department of Biochemistry, College of Medicine & Sagore Dutta Hospital, Kolkata

Moderator:

Dr Manali Sinharay
Associate Professor
Department of Biochemistry, CNMC, Kolkata

Illustrator:

Dr Abhra Ghosh
Associate Professor
Department of Biochemistry, DMCH, Ludhianna