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A Review on Exosome Based Drug Delivery System

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ABSTRACT

Because of their natural origin, superior biocompatibility, and inherent capacity to facilitate intercellular communication, exosome-based drug delivery systems have become a new and promising platform in nanomedicine. Extracellular vesicles [30 to 150 nm] called exosomes are released by almost every type of cell and can be found in a variety of bodily fluids, such as urine, saliva, and blood. They transport a complex cargo of lipids, proteins, and genetic elements like miRNA and mRNA transcripts, which are essential for controlling biological and disease-related processes. These biological vesicles have distinct benefits over manufactured nanoparticles, such as their high stability, minimal immunogenicity, effective cellular absorption, and capacity to pass through biological membranes that provide protection. Exosomes have drawn a lot of attention lately as natural carriers of therapeutic molecules, like as proteins, nucleic acids, and tiny medicines, for the treatment of diseases like cancer, neurological disorders, and cardiovascular disorders. Exosomes' targeting effectiveness and therapeutic potential have been improved by sophisticated methods in exosome extraction, purification, and engineering, including as surface modification and cargo loading. All things considered, exosome-based drug delivery systems offer a state-of-the-art, biocompatible, and adaptable approach to precise, targeted, and customized therapy in contemporary biomedical research. Current review focuses on types of exosomes, the biology and biogenesis of exosomes, isolation and characterization of exosomes.

Keywords: Exosomes, Isolation, Characterization, Biogenesis

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INTRODUCTION

One of the most important fields in the modern medicine is drug delivery. Not only to transport drugs to the body is the aim of drug delivery systems but also to make sure that the drug will reach the correct site, in the right amount, and with minimal side effects. The traditional drug delivery systems such as micelles, polymeric nanoparticles and liposomes are useful, but they often face challenges like poor targeting, low stability, toxicity and inability to cross barriers such as the blood–brain barrier.[1]

The exosomes, which are natural extracellular vesicles secreted by the cells, are now considered as the powerful and new option for delivery of drug. The size of the exosomes is very small [30-150 nm] and they are found in breast milk, saliva, urine, blood and many other body fluids.[2]

Naturally they carry nucleic acids, proteins and lipids from one cell to the another, and thus play an vital role in the cell communication. Exosomes are more biocompatible, stable in circulation, and less immunogenic compared to artificial nanoparticles due to their biological origin.

Additionally, they can be engineered to carry genes, drugs, and other therapeutic molecules, which makes them eminently suitable for the targeted therapy in diseases like neurodegenerative disorders, cancer, inflammation, cardiovascular diseases. Though exosome based drug delivery shows great potential, challenges such as standardization, large scale isolation, safety evaluation, loading efficacy and still remain.[3]

TYPES OF EXOSOMES

I. Cell Source

The exosomes are named according to the type of cell from which they originate;

a) Exosomes that are Immune cell-derived: Released by cells like dendritic cells, macrophages, or T-cells. They help in antigen presentation and immune signaling.
b) Exosomes that are tumor cell-derived: These are secreted by cancer cells and carry often the oncogenic molecules that help in metastasis and growth of tumor.
c) Exosomes that are stem cell-derived: These are known for their regenerative and healing properties from mesenchymal or other stem cells.
d) Neuronal exosomes: It is released from nerve cells and play roles in the communication between the brain cells.
e) Endothelial or Epithelial exosomes: It help in cellular communication and maintaining tissue barriers.

II. Size and Density

The exosomes are generally 30-150 nm in size, but depending on isolation methods they can differ slightly;

a. Small size exosomes [Exo-S]: Around 30-90 nm.
b. Large size exosomes [Exo-L]: Around 90-150 nm.

Both carry proteins, lipids, and RNA, but their composition may vary slightly.

III. Function

Based on their biological roles the exosomes can be;

a. Exosomes that are signaling: Between cells they transfer protein and genetic information.
b. Exosomes that are diagnostic: For diseases such as neurological disorders or cancer they are used as biomarkers.
c. Exosomes that are therapeutic: For treatment, these are engineered to deliver drugs, RNA, or bioactive molecules.[1]

THE BIOLOGY AND BIOGENESIS OF EXOSOMES

Description and Categorization of the EVs

The EVs refers to the miscellaneous population of the membrane-bound fragments discharged by the cells, surrounding the subtypes like microvesicles, exosomes and the apoptotic bodies [each differing by the cargo, size and biogenesis] [4]. The concord in the field is that the term exosome must be reserved for the EVs of the endosomal origin, specifically for those formed inside the MVBs [multivesicular bodies] and released by the exocytosis, typically between the 30 nm to 150 nm in size [occasionally expanded to the ~200 nm in broader definitions]. In contrast the microvesicles [also known as ectosomes] arise via the direct budding from a plasma membrane [size ~100 to 1000 nm] and the apoptotic bodies are released from the dying cells during a programmed cell death [>1 μm]. The classification of EV may also consider characteristics such as biomolecular profile, density, or the cellular origin. Though, due to the measurement limitations and overlapping properties the strict biochemical or biogenetic verification is often lacking. In such cases, the broader term “EV” is preferred unless the clear origin is demonstrated [5]. For the purposes of this review, the “exosomes” will refer to EVs of an endosomal origin, recognizing that experimentally challenging is the strict separation.

The mechanisms of exosome formation [Endosomal Pathway]

The ILV (Intraluminal Vesicle) formation within MVBs (multivesicular bodies)

With the inward budding of the endosomal membrane, the exosome biogenesis begins, creating ILVs [intraluminal vesicles] within the late endosomes or the multivesicular bodies. The classic endosomal sorting complex, which is necessary for the transport [ESCRT-dependent pathway] and

involves the sequential action of the ESCRT-0, -I, -II, and -III complexes along with associated ATPase VPS4 for the membrane scission, can sort the cargo into these intraluminal vesicles. The ESCRT-0 initially binds the ubiquitinated proteins & clusters them, ESCRT-I/II drive the membrane deformation; The ESCRT-III mediates the membrane neck formation and constriction which ultimately leading to a vesicle budding. The process is completed by the VPS4-mediated disassembly. In many cell types this is broadly conserved and operates which includes immune, epithelial and tumor cells. The experimental depletion of the ESCRT components such as ALIX, TSG101 or VPS4A markedly Reduces the secretion of exosome which confirms their central role in the vesicle biogenesis.

This pathway tends to produce exosomes enriched in RNA-binding Proteins, ubiquitinated proteins and specific miRNAs-cargo profiles, functionally that are often more uniform and simpler to manipulate for the therapeutic engineering [6]. However, on lipid microdomains and tetraspanin rich membranes, the ESCRT-independent pathways rely. One of the key mechanism involves the neutral sphingomyelinase 2 [nSMase2], which generates the ceramide, the bioactive lipid that induces negative membrane curvature and facilitates the budding of Intraluminal Vesicle in the absence of the ESCRT machinery. The Tetraspanins such as CD81, CD63, CD9 and also participate by organizing the microdomains that promote the selective cargo clustering & vesicle formation. These lipid-driven routes are particularly active in certain neuronal and tumor cells, fibroblasts and may favor inclusion of the signaling lipids and specific proteins rather than the ubiquitinated cargo A relative contribution of the ESCRT-dependent versus ESCRT-independent mechanisms varies among the cell types and the physiological conditions. For eg; in the dendritic cells the inhibition of nSMase2 reduces the ceramide-dependent vesicle formation, although TSG101 knockdown the primarily affects ESCRT-dependent sorting of the MHC-II-containing vesicles. Such a diversity implies that the different donor cells may naturally bias the composition of exosome, and influencing their downstream biological effects and suitability for the therapeutic applications. Thus, understanding these mechanistic differences is therefore essential for optimization of yield, rational design of engineered exosomes and control of the composition of cargo in clinical manufacturing. [6,7].

Multivesicular bodies Fate: Secretion vs. Degradation

Once Intraluminal Vesicle have formed, Multivesicular bodies face 2 main fates; first fusion with the autophagosomes/lysosomes for the degradation or second is fusion with the plasma membrane to release the Intraluminal Vesicle as exosomes. The factors such as the cargo composition, Multivesicular bodies [MVB] -associated proteins, and the cellular signals influence the routing.

Especially the different types of cargo may bias Multivesicular bodies [MVBs] toward the secretory pathway thus creating the heterogeneity among exosomes that are released and reflecting the functional specialization.

The regulation and heterogeneity

Within the single cell, multiple pathways can operate sequentially or simultaneously leading to the diverse subpopulations of the exosomes even from a same donor. The regulatory mechanisms depend heavily on cellular stresses, cell type and activation state and thus, the exosome output is context-dependent and dynamic [7].

The molecular cargo and surface markers:

The common surface markers-

The exosomes are enriched in tetraspanins such as CD81, CD63, & CD9 and are widely used as the canonical exosome markers and critical for the target-cell adhesion, vesicle formation, and cargo selection. The additional surface proteins include the heat-shock proteins [Hsp90 & Hsp70], adhesion molecules, integrins, the receptors [For example; transferrin, MHC molecules in immune contexts, primary entry receptor for Coxsackievirus A21& ICAM] and other membrane associated components depending on the cell of origin [8].

The Internal cargo: Proteins, Lipids, DNA, RNA

The exosomal cargo comprises the rich repertoire of the biomolecules-

a. **The proteins:**

   The evolutionarily conserved proteins which includes the cytoskeletal elements [tubulin, actin] chaperon proteins, metabolic enzymes, signaling molecules and a membrane transport/fusion protein. The exact profile is the cell- and the context-specific.

b. **The nucleic acids: microRNAs, mRNAs**

   The [miRNAs] and the ncRNA [non-coding RNA] are abundant in the exosomes, and in some of the cases the double-stranded DNA has also been detected. These RNAs can be delivered to the recipient cells and translated or exert the gene-regulatory activities. The packaging of miRNAs may follow sequence-specific motifs [For example; The GGAG EXOmotif is recognized by sumoylated hnRNPA2B1], thus suggesting active sorting rather than a passive loading.

c. **The lipids:**

   The exosomes are rich in lipid, specially in sphingomyelin, cholesterol, saturated phosphatidylcholine, ceramides, and the phosphatidylethanolamine lipid composition distinct from the whole-cell membranes. These lipids not only provide the structural

integrity but also may contribute to the vesicle formation through membrane curvature and the sorting [9]. This diverse cargo reflects the physiological condition of the parent cell and this can be selectively enriched making the exosomes functionally potent and biologically informative.

The natural role in intercellular communication

The modes of Interaction

Through 3 primary modes the exosomes mediate the intercellular signaling:

a) The direct membrane fusion or the endocytosis by recipients, thus delivering cargo internally,

b) The receptor mediated binding, where the surface markers interact with the receptors on the recipient cells, and

c) The paracrine or endocrine transport by allowing the exosomes to travel via bodily fluids and to act at distant sites [10,11]. These mechanisms underline the versatility of the exosomes as vehicles for information exchange which is capable of modulating physiological functions across tissues.

The physiological functions

Exosomes play key roles in immune modulation, tissue development, homeostasis, and waste disposal, in the normal physiology. For eg: In the hematopoietic system, the exosomes regulate antigen presentation & communication between the dendritic cells and the B cells; in the reproductive context, embryo-maternal signaling via exosomes which contributes to implantation and pregnancy maintenance [11]. The exosomal RNA and protein signals also help in maintaining tissue health, coordinate regenerative processes and mediate oxidative stress responses. In the cancer the tumor cell-derived exosomes deliver the mutated receptors [For e.g: EGFRvIII] oncogenic proteins and miRNAs to the other cancer or stromal cells promoting angiogenesis, organotropism, invasion and immune evasion. The Integrins and other surface molecules on the exosomes can the direct metastasis to specific organs [“organ-tropic” patterns] [12]. The host or the pathogen-infected cell-derived exosomes can carry the inflammatory mediators [For e.g: LPS, cytokines], microbial antigens, or the pathogen-derived RNAs, by modulating immune responses in the target cells or the tissues, in infection [11]. The exosomes contribute to aging and inflammation in the systemic disease. For eg: the senescent cells release EVs enriched in a specific miRNA that drive the inflammatory signaling in a recipient cell, contributing to the tissue deterioration and age-related pathologies [4].

The regulatory and translational insights

The recognition of the exosomes as a major mediator of intercellular crosstalk has transformed thinking into a cell biology and the disease models. The EVs are now recognized as the potential biomarkers, reflecting disease states through their cargo and as the biological vehicles that can be harnessed for a therapy. As expected, this awareness has stimulated interest in the bioengineering exosomes for the targeted drug delivery as their natural functions suggest advantages in biocompatibility, stability and targeting [13]. The exosomes are the distinct EV subtype defined by their endosomal origin, small size [~30 nm–150 nm] and specific cargo in profile. Both ESCRT-dependent and -independent mechanisms control the development of intraluminal vesicles within multivesicular bodies during their biogenesis.

The exosomes are enriched in the conserved surface markers notably in tetraspanins and carry diverse molecular cargo including lipids, proteins and nucleic acids often sorted selectively. Practically, the exosomes serve as the versatile mediators of a intercellular communication, influencing immunity, physiological homeostasis, disease progression, development and including infection and cancer. By understanding these fundamental biological properties lays the groundwork, to leverage the exosomes capabilities as a natural, programmable drug delivery vehicles in therapeutic applications.

The immunogenicity of exosomes

Although exosomes are widely recognized as low immunogenic vehicles [i.e. they themselves do not provoke adaptive immune rejection or strong innate] the tumor-derived exosomes can nonetheless mediate the active immunosuppression through their cargo. The difference is that “low immunogenicity” refers to the absence of the strong immune responses against a vesicle itself, since the biological content may modulate the immune responses. Certainly, the tumor exosomes often carry the immunosuppressive molecules such as PD-L1, TGF-β, FasL, miRNAs, and the others which can inhibit the T-cell activation, promote the regulatory T cells, induce apoptosis of effector cells, or the polarize myeloid cells toward a suppressive phenotype [For example: M2 macrophages] [14]. In the particular, the exosomal PD-L1 has been extensively studied. It binds the PD-1 on the T cells, induces T-cell exhaustion or the apoptosis, and impairs the antitumor immunity in vivo. The suppression of exosomal PD-L1 led to systemic antitumor responses and immunological memory in a mouse model, supporting its functional significance [15]. Also, in mouse/ melanoma models the administration of the exosomes with PD-L1 promotes the tumor growth and reduces the tumor-infiltrating CD8+ T cell [16].

On the other side, the exosomes derived from the mesenchymal stem cells [MSCs] or other non-tumor sources tends to be well tolerated and also have low immunogenicity, making them as safer

for the therapeutic use. The MSC-derived exosomes are well tolerated and has the low immunogenicity as compared to the parent MSCs or the viral vectors [17]. Thus clinically the MSC-EVs are being evaluated in the regeneration/immunomodulation precisely because of this conducive immunological profile [18]. Hence, the anomaly is resolved by appreciating that the exosomes are not immunologically inert in the functional sense: their origin and the cargo determine whether they are neutral, immunostimulatory or immunosuppressive. For the translation, this implies-

a. by avoiding or modifying a tumor-derived exosomes as the therapeutic vectors,
b. using "benign" source [For example: immune cells, MSCs] and
c. widely characterizing the immune effects [T cell activation, cytokine response etc.] in preclinical safety studies.

The comparative of Exosome Sources

The exosomes can be derived from the large variety of the types of cells and the choice of the donor source profoundly influences their bioactivity, translational potential and composition. Among the mammalian sources the mesenchymal stem cell [MSC]-derived exosomes due to their low immunogenicity, regenerative and anti-inflammatory properties, and scalable culture systems are most widely investigated. [19]. The immune cell-derived exosomes, particularly from the T cells and dendritic cells, are valued for their antigen-presenting capacity and their ability to stimulate or to modulate immune responses, thus forming the basis for several cancer vaccine platforms [20]. On the contrary the tumor-derived exosomes exhibit natural tropism toward the tumor tissues and high intrinsic loading of the oncogenic and signaling molecules while useful as a delivery models or the biomarkers, as their clinical translation is limited by potential immunosuppressive and the pro-tumorigenic effects [21]. The epithelial and endothelial cells derived exosomes are advantageous for the vascular and the barrier-related delivery, by providing the high yields and the reproducible secretion [22]. Lately the non-mammalian sources such as the plant-derived exosome-like nanovesicles and the bacterial outer membrane vesicles [OMVs] have emerged as the favourable alternatives. The plant vesicles, obtained from sources like grapefruit, broccoli or ginger offer the biocompatible, safe and potentially scalable platform for the oral drug delivery [23]. On the other hand, the bacterial OMVs, while immunogenic can be engineered to serve as a vaccine carriers or the adjuvants [24]. The diversity of the exosome sources not only provides a versatile toolkit for the biomedical applications, but also it underscores the need for the rigorous source selection functional validation and purification to ensure the reproducibility and safety.

ISOLATION OF EXOSOMES

Presently the exosomes are isolated by the differential centrifugation, filtration, size-exclusion chromatography, and polymer precipitation. To obtain a ultrapure exosome or isolation of the potential subpopulation of exosome, an immunomagnetic isolation strategy can be applied by targeting the exosomal markers [25]

The filtration method

To isolate cells and large EVs in biological samples, the commercial membrane filters or polycarbonate can be used. The filtration methods are often combined with the ultracentrifugation, where the membranes are used to sieve cells and large EVs, after which the separation of exosomes from the proteins is achieved via ultracentrifugation. A number of research groups have explored commercial ultrafiltration, in order to eliminate the need for ultracentrifugation as the means to separate the exosomes from the protein contaminants [26]. By using matrices with defined molecular weight or size exclusion limits the exosomes can be separated from other soluble proteins and aggregates. These vesicles can be selectively isolated based on the molecular weight greater than two million Daltons, followed by the isolation with the diameter less than two hundred nm. This allows the separation of the smaller aggregates and the soluble components from the exosomes [27].

The polymer precipitation

To isolate and purify polymers, this technique is used. On the formation of the mesh-like net that embeds EVs with the size ranging from 60 to 180 nm, the methods of polymeric precipitation are based. To culture media or to bodily fluids, these methods may be applied. In the detection of the biomarkers in vesicles derived from the small biological samples, in particular the polymeric precipitation methods may have the advantage [28].

The differential centrifugation

The differential centrifugation is the most prevalent approach for exosome isolation. [29], for isolating exosomes from biological fluids, it is adopted as the dependable technique [30]. The method consists of various steps, including [a] To remove cells and apoptotic debris, a low-speed centrifugation [b] To eliminate larger vesicles, a higher speed spin and lastly [c] To precipitate exosomes, a high-speed centrifugation. When viscous biological fluids such as plasma and serum are used for analysis, although the efficiency of the method is lower [31]. The quantity of the collected EVs is affected by centrifuging time and speed, so these parameters must be optimized for each type of rotor [32].

The immunomagnetic isolation

The exosomes are observed in the most bodily fluids containing typical exosomal markers such as CD81, and CD63 and CD9. By targeting these markers using magnetic beads, the potential subpopulations of the exosomes can be captured. For the exosome isolation and downstream analysis, the magnetic beads are versatile tools. [33]. The immunomagnetic isolation uses the antibody-labeled magnetic beads which can specifically capture the exosomes and a magnetic field will separate the captured exosomes from the other substances [34].

The size-exclusion chromatography

Also known as the molecular sieve chromatography, the size-exclusion chromatography, [35] is a method where the separation of the different compounds occurs according to their size [hydrodynamic volume] measured by how efficiently they penetrate the pores of a stationary phase. The size exclusion chromatography has 2 basic versions. It is called GPC [gel permeation chromatography] when performed using organic solvents. The main application field of GPC [gel permeation chromatography] is in the polymer analysis. When the size-exclusion chromatography is performed by using aqueous solvents, it is known as gel filtration [36]. This method is superior with 43.00 % stable recovery of EVs, with almost complete removal of the contaminating proteins, as compared to the ultracentrifugation method, which is having highly varying EV yields [02–80%]. The demerits of this method are (iii) the post-isolation analysis of each fraction may be rather time consuming, contrarily to the simplicity and time effectiveness of the separation protocol, (i) the accessibility of the chromatography column to the contamination, accordingly aseptic working conditions should be ensured, especially if the isolated EVs are intended for the therapeutic use; (ii) in order to make sure a complete separation of EV subtypes and contaminating proteins, the large number of fractions should be collected and analyzed [37].

THE CHARACTERIZATION TECHNIQUES

After the isolation of the exosome, the exosome samples would be characterized thoroughly by using the set of a combination methods to validate the method of isolation. The accuracy of the methods in measuring the quantity and purity of exosomes is one of the major challenge in the exosome biology. The characterization methods used for measuring the purity of exosome are categorized into imaging-based, marker-based, and biophysical methods.

The Imaging

The imaging tool is the qualitative technique that is used to determine the morphology of the exosomes. The microscopic techniques may not show the sufficient resolution to image exosomes, owing to the size of the exosome vesicles. The TEM [transmission electron microscopy], AFM

[atomic force microscopy] and SEM [scanning electron microscopy] are commonly used imaging techniques that can allow the high-resolution exosome imaging.

The Atomic force microscopy [AFM]

The AFM [Atomic force microscopy] imaging technique detects & measures the force between the probing tip and the sample surface in order to produce the topological map of the sample. The Atomic force microscopy [AFM] uses the surface scanning with the sharp tip on the cantilever to scan over the surface of sample. The attractive force between the surface and the tip makes the cantilever deflect towards a surface and provides the sub-nanometer, high-resolutions imaging at less than 01 nm, when the tip approaches the sample surface [38]. In brief, the sample containing the exosome vesicle is placed on the mica substrate and dried at room temperature then this dried samples are thereafter washed and allowed to dry in to the presence of liquid nitrogen. At this moment the dried samples can be viewed under the AFM [Atomic force microscopy] by using the silicon probe and then it is analyzed with software [39]. The atomic force microscopy technique requires a minimal sample preparation and it can be used to measure the exosome vesicle in the native conditions with the non-destructive mode of the operation. A useful information related to the biomolecular characteristics, morphology, biomechanics of the exosomes is provided by this technique. Various studies had reported that how to use Atomic force microscopy effectively in characterizing the mechanical properties, membrane composition, morphologies and sizes of the various types of cell-derived exosomes [40].

The transmission electron microscopy [TEM]

To characterize the existence of the exosomes in the solution and to study the size, structure and morphology in order to assess the quality of the exosomes, TEM is used. [41]. The transmission electron microscopy, uses an accelerated electronic beam with the smaller wavelength than that of the light to determine the morphology characteristics and structure. Here the principle involved is the generation of an image as the beam of electrons that passes through an sample whereby the secondary electron is generated [40]. Concisely, the suspension of the exosome vesicles is fixed with the paraformaldehyde [02% w/v], then it is deposited onto formvar-carbon-coated grids and incubated for twenty minutes. The carbon-coated grids are then washed with phosphate buffered saline then incubated with the glutaraldehyde, which is crosslinking agent, as well as washed with the water. Lastly, the exosomes vesicles are stained with the uranyl acetate solution [02% w/v] followed with air-drying. [42]. During transmission electron microscopy [TEM] sample preparation, the morphology of the exosomes may experience changes due to the involvement of the multiple steps and often the electron beams may induce the changes in the morphology of the

exosomes. Therefore, the transmission electron microscopy technique may be upgraded (Cryo-transmission electron microscopy) to eliminate the effects related to the sample preparation by utilizing the different protocol [43].

The scanning electron microscopy [SEM]

In this scanning electron microscopy technique the accelerated electrons carry the significant amount of kinetic energy, which is decadent as the different signals produced by the interactions between the electron samples, while the incident electrons retards in the solid sample. In short, the exosome samples are fixed on the carbon-coated or the copper grid with the glutaraldehyde and dehydrated with ethanol. The grids are then dried with the help of air and the sputter-coated with gold at the thickness range of the 2 to 10 nm then the samples are analyzed by using scanning electron microscopy. In one of the study, it was reported that the images of the exosomes were round and expanding in appearance when observed using scanning electron microscopy [44,45,46].

The DLS [dynamic light scattering]

The dynamic light scattering is also known as the photon correlation spectroscopy which works on the principle of the time dependent fluctuations in the scattering intensity caused by the Brownian movements of the particles within the sample [47]. For measuring the monodisperse suspensions (one type of particle in a suspension) the dynamic light scattering is the most suitable technique. When the large vesicles are present in a suspension even at the low quantities which may be difficult for the detection of the small particles [48], to determine the vesicle size of the exosomes this technique can be used. Inspite of that it does not provide the information about the source or the biochemical data for the exosomes [49]. Although the dynamic light scattering method has some barriers in the characterization of the exosomes, which are as follows: a) this technique requires the high sample [particle] concentration which may be challenging to make exosomes; b) the presence of the larger particles in the sample results, mask the exosome population due to influence of the larger particles on the intensity distribution; c) this technique is unable to determine accurately the concentration of particle; d) the low scattering properties of a exosome can make the measurements inaccurate [45].

The Flow Cytometry

The flow cytometry is a technique that passes the individual cells through the laser beam at the specific wavelength & detects the emitted fluorescence or the scattered light. This technique allows the measurement of the structure and size of the exosome which is used to characterize the surface proteins of exosome, and thus has the potential to determine the cellular origin of the single

exosome vesicle [40,50]. By using this technique, the Melo et al. [51] reported that the exosomes isolated from the non-tumorigenic cells carries less glypican-1 when compared to the exosomes obtained from the pancreatic cancer cells. The detection limit of the conventional flow cytometry is 200–500 nm which limits its use in measuring the free exosomes. Upon optimization of the flow cytometry technique to detect the small particles it can then be used to detect the exosomes, hence if the detection limit is low [approximately 100 nm] it indicates the insufficient sensitivity of this technique. Though, the sensitivity of the instrument in detecting the exosomes can be improved by decreasing the wavelength of a laser beam to 405 nm [50,52].

The NTA [Nanoparticle Tracking Analysis]

This technique relies on the same basic principles as that of dynamic light scattering. Though, in nanoparticle tracking analysis, the microscope is used to capture the individual particles in the Brownian motion. The nanoparticle tracking analysis can measure the concentration of exosome and the size distribution in the range from 10 nm to 02m. It permits one to quantify a path of movement of exosomal by tracking the individual particles through a analysis of image then, further this movement can be correlated to estimate the hydrodynamic diameters. As the individual particle is imaged in the different regions, this technique can detect the particles in the sample of different sizes. Furthermore, nanoparticle tracking analysis can also have fluorescence capability, means on the exosome, it can be used to detect the antigen present by applying the fluorescently labeled antibodies [53]. The pre and the post-processing setting used for nanoparticle tracking analysis, such as the changes to the sensitivity of the camera and the detection threshold for the particle, the results can be influenced [54]. The correct dilution factor and sample preparation are 02 important parameters for the success of the nanoparticle tracking analysis. Its speed, with the diameters as low as 30 nm and the ease of the preparation of sample and the recovery in their native form after the measurements, these abilities of the nanoparticle tracking analysis to detect exosomes makes this technique more attractive [55].

The TRPS [Tunable Resistance Pulse Sensing]

This is the biophysical technique which involves, the passing of single particles through an nanoscale pores. When the particles pass through the pores, the frequency and duration of resistance pulses are determined. To determine the zeta potential, concentration and size this information is used. The tunable resistance pulse sensing can detect the colloidal particles with diameters ranging from the 50 nm up to the cells size, which is crucial while investigating the cellular functions and uptake. The exosomes concentration of particles and size distribution are more closely resemble to the true distribution as measured by the tunable resistance pulse sensing

which when compared to the nanoparticle tracking analysis, while making the measurements using tunable resistance pulse sensing, the repeated pore blocking by the particles and the susceptibility to system suitability, issues have been reported [52,56].

The Protein Characterization

To confirm that the exosomes that are isolated contain the low levels of the potential contaminants and do not possess high levels of the exosome markers, this characterization methods based on proteins or the markers can be used. The entire protein content of exosome can be quantified by measuring an total protein assay. While using this method, although an discriminating the exosomal proteins from non-exosomal proteins is the challenge, which is mainly because of the co-isolation of the exosomes with non-exosomal proteins, during the process of isolation. As is it originated from endosome, the isolated exosomes from most of the sources will contain the proteins [intraluminal proteins or membrane] which are involved in the formation of endosome. By using western blot or enzyme linked immunosorbent assay [ELISA] techniques, the characterization of the protein markers can be performed [57,58].

The ELISA [Enzyme Linked Immunosorbent Assay] Technique

This technique used for the detection and the quantification of content of protein of the exosomes. some of the demerits of this technique are its low sensitivity and the large sample volume is needed. To measure with accuracy the exosome counts, this enzyme linked immunosorbent assay technique can be used. [59].

The Western Blotting Technique

To detect the presence of target proteins associated with exosomes, this technique is most commonly used. Concisely the purified exosome samples are first treated with buffered lysis solution containing the denaturants or the protease inhibitors, followed by dodecylsulfate-polyacrylamide gel electrophoresis is used to sort out the protein lysates before being transferred onto the membrane for immunoblotting of the particular protein targets. In determining the sizes of different proteins, this western blotting technique is useful. Although, the processing times involved and preparation is lengthy these are the major demerits of this method. Both western blotting and enzyme linked immunosorbent assay techniques have similar detection limits. Nevertheless, the enzyme linked immunosorbent assay for high-throughput measurements can be scaled up [60,61].

FUTURE SCOPE

The use of exosomes in the delivery drug provides the new ways for developing targeted and safe treatments with therapeutic efficiency which is high. The scientists are developing the engineered

exosomes that can target a specific cells or organs more effectively. To improve the quality and production the new technologies such as genetic engineering, microfluidics and 3D cell culture are being used. The synthetic exosomes that mimic natural ones, in the future may also be used for the large-scale delivery of drug.[62]

The exosomes may play an crucial role in the personalized medicines also delivering the treatments specifically designed based on their disease profile for each patient.[63]

CONCLUSION

The exosome-based DDS (drug delivery systems) have been emerged as one of the most biocompatible and optimistic nanoplatforms for the targeted therapy. These naturally derived vesicles possess solitary properties such as the low immunogenicity, nanoscale size and the ability to cross the biological barriers making them an ideal candidate for the transporting therapeutic agents like the small-molecule drugs, nucleic acids and proteins. Their capability in the regenerative medicine, cancer therapy and neurodegenerative diseases highlights their comprehensive biomedical significance.[64]

Although there are certain exception; such as (a)the lack of the standardized characterization protocols (b) the limited yield during the isolation and (c) difficulties in the large-scale manufacturing and still, restrict their clinical translation. Addressing these challenges through the advancements in the exosome engineering, the regulatory frameworks and purification technologies will elevate their therapeutic applicability.[65]

Concluding that the technological innovation and continued interdisciplinary research will cover the way for the exosomes to evolve into the clinically viable, efficient and safe drug delivery systems, eventually contributing to the future of the precision and personalized medicine.

REFERENCES

1. Johnsen KB, Gudbergsson JM, Andresen TL, Simonsen JB: What is the blood concentration of extracellular vesicles? Implications for the use of extracellular vesicles as drug delivery systems. J Control Release: 2019: 316: 404-412.
2. Zhang Y, Liu Y, Liu H, Tang WH. Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci: 2019: 9: 19.
3. Liang Y, Duan L, Lu J, Xia J: Engineering exosomes for targeted drug delivery. Theranostics, 2021: 11(7): 3183-3195.
4. Yu, J.; Sane, S.; Kim, J.E.; Yun, S.; Kim, H.J.; Jo, K.B.; Wright, J.P.; Khoshdoozmasouleh, N.; Lee, K.; Oh, H.T.; et al: Biogenesis and delivery of extracellular vesicles: Harnessing the power of EVs for diagnostics and therapeutics. Front. Mol. Biosci: 2023: 10,1330400.

5. Gurung, S.; Perocheau, D.; Touramanidou, L.; Baruteau, J. The exosome journey: From biogenesis to uptake and intracellular signalling. Cell Commun. Signal: 2021, 19, 47.
6. van Niel, G.; D'Angelo, G.; Raposo, G.: Shedding light on the cell biology of extracellular vesicles. Nat. Rev. Mol. Cell Biol: 2018, 19,213-228.
7. Jin, Y.; Ma, L.; Zhang, W.; Yang, W.; Feng, Q. Wang, H.: Extracellular signals regulate the biogenesis of extracellular vesicles. Biol. Res: 2022, 55, 35.
8. Xu, K.; Feng, H.; Zhao, R.; Huang, Y.: Targeting Tetraspanins at Cell Interfaces: Functional Modulation and Exosome-Based Drug Delivery for Precise Disease Treatment. ChemMedChem: 2025, 20, e202400664.
9. Moeinzadeh, L.; Razeghian-Jahromi, I.; Zarei-Behjani, Z.; Bagheri, Z.; Razmkhah, M.: Composition, Biogenesis, and Role of Exosomes in Tumor Development. Stem Cells Int: 2022, 2022, 8392509.
10. Isaac, R.; Castellani, F.; Ynig,W.; Olefsky, J.M. Exosomes as mediators of intercellular crosstalk in metabolism. Cell Metab.: 2021, 33, 1744-1762.
11. Bischoff, J.P.; Schulz, A.; Morrison, H.: The role of exosomes in intercellular and inter-organ communication of the peripheral nervous system. FEBS Lett.: 2022, 596, 655-664.
12. Su, S.A.; Xie, Y.; Fu, Z.; Wang, Y.; Wang, J.A.; Xiang, M.: Emerging role of exosome-mediated intercellular communication in vascular remodeling. Oncotarget: 2017, 8, 25700-25712.
13. Van Niel, G.; Carter, D.R.F.; Clayton, A.; Lambert, D.W.; Raposo, G.; Vader, P.: Challenges and directions in studying cell-cell communication by extracellular vesicles. Nat. Rev. Mol. Cell Biol. :2022, 23, 369-382.
14. Hao, Q.;Wu, Y.;Wu, Y.;Wang, P.; Vadgama, J.V.: Tumor-Derived Exosomes in Tumor-Induced Immune Suppression. Int. J. Mol. Sci.: 2022, 23, 1461.
15. Poggio, M.; Hu, T.; Pai, C.C.; Chu, B.; Belair, C.D.; Chang, A.; Montabana, E.; Lang, U.E.; Fu, Q.; Fong, L.; et al.: Suppression of Exosomal PD-L1 Induces Systemic Anti-tumor Immunity and Memory. Cell: 2019, 177, 414-427.e13.
16. Liu, J.; Peng, X.; Yang, S.; Li, X.; Huang, M.; Wei, S.; Zhang, S.; He, G.; Zheng, H.; Fan, Q.; et al.: Extracellular vesicle PD-L1 in reshaping tumor immune microenvironment: Biological function and potential therapy strategies. Cell Commun. Signal: 2022, 20.
17. Tang, Y.; Zhou, Y.; Li, H.J.: Advances in mesenchymal stem cell exosomes: A review. Stem Cell Res. Ther.: 2021, 12, 71.

18. Lotfy, A.; AboQuella, N.M.;Wang, H.: Mesenchymal stromal/stem cell (MSC)-derived exosomes in clinical trials. Stem Cell Res. Ther: 2023, 14, 66.
19. Marote, A.; Teixeira, F.G.; Mendes-Pinheiro, B.; Salgado, A.J.: MSCs-Derived Exosomes: Cell-Secreted Nanovesicles with Regenerative Potential. Front. Pharmacol.: 2016, 7, 231.
20. Luo, S.; Chen, J.; Xu, F.; Chen, H.; Li, Y.; Li,W.: Dendritic Cell-Derived Exosomes in Cancer Immunotherapy. Pharmaceutics: 2023, 15, 2070.
21. Whitesie, T.L.: Tumor-Derived Exosomes and Their Role in Cancer Progression. Adv. Clin.Chem.: 2016, 74, 103-141.
22. Ren, Y.; Zhang, H.: Emerging role of exosomes in vascular diseases. Front. Cardiovasc. Med. :2023, 10, 1090909.
23. Mu, J.; Zhuang, X.; Wang, Q.; Jiang, H.; Deng, Z.-B.; Wang, B.; Zhang, L.; Kakar, S.S.; Jun, Y.; Miller, D.; et al.: Interspecies communication between plant and mouse gut host cells through edible plant derived exosome-like nanoparticles. Mol. Nutr. Food Res.: 2014, 58, 1561-1573.
24. Gerritzen, M.J.H.; Martens, D.E.;Wijffels, R.H.; van der Pol, L.; Stork, M.: Bioengineering bacterial outer membrane vesicles as vaccine platform. Biotechnol. Adv.: 2017, 35, 565-574.
25. Pedersen KW, Kierulf B, Manger I, Oksvold MP, Li M, Vlassov A, Roos N, Kullmann A, Neurauter A.: Direct isolation of exosomes from cell culture: simplifying methods for exosome enrichment and analysis. Transl Biomed.: 2015;6:2.
26. Contreras-Naranjo JC, Wu HJ, Ugaz VM.: Microfluidics for exosome isolation and analysis: enabling liquid biopsy for personalized medicine. Lab Chip.: 2017;17:3558-77.
27. Taylor DD, Shah S.: Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes. Methods.: 2015;87:3-10.
28. Deregibus MC, Figliolini F, D'Antico S, Manzini PM, Pasquino C, De Lena MD, Tetta C, Brizzi MF, Camussi G.: Charge-based precipitation of extracellular vesicles. Int J Mol Med.: 2016;38:1359-66.
29. Livshts MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, Generozov EV, Govorun VM.: Isolation of exosomes by differential centrifugation: theoretical analysis of a commonly used protocol. Sci Rep.: 2015;5:21447.
30. Helwa I, Cai J, Drewry MD, Zimmerman A, Dinkins MB, Khaled ML, Seremwe M, Dismuke WM, Bieberich E, Stamer WD, Hamrick MW, Liu Y.: A comparative study of

serum exosome isolation using differential ultracentrifugation and three commercial reagents. PLoS ONE.: 2017;12:e0170628.

31. Yakimchuk K. Exosomes: Isolation and characterization methods and specific markers. Maker Methods.: 2015;5:1450.
32. Kang H, Kim J, Park J.: Methods to isolate extracellular vesicles for diagnosis. Micro Nano Syst Lett.: 2017;5:15.
33. Oksvold MP, Neurauter A, Pedersen KW.: Magnetic bead-based isolation of exosomes. Methods Mol Biol.: 2015; 1218:465-81.
34. Li P, Kaslan M, Lee SH, Yao J, Gao Z.: Progress in exosome isolation techniques. Theranostics.: 2017;7:789.
35. Berg JM, Tymoczko JL, Stryer L.: Biochemistry. 5th ed. New York: National Center for Biotechnology Informationõs Bookshelf: 2002. p. 93-126.
36. Vekey K, Telekes A, Vertes: A. Preface-medical applications of mass spectrometry. Medical applications of mass spectrometry, vol. 18.: 2008:1262-71.
37. Kotmakçı M, Akbaba GE.: Exosome isolation: is there an optimal method with regard to diagnosis or treatment?, chap 8. In: Wang J, editor. Novel implication of exosome in diagnosis and treatment of cancer and infection disease. London: Intech: 2017. p. 163-82.
38. Paolini, L.; Zendrini, A.; Di Noto, G.; Busatto, S.; Lottini, E.; Radeghieri, A.; Dossi, A.; Caneschi, A.; Ricotta, D.; Bergese, P.: Residual Matrix from Different Separation Techniques Impacts Exosome Biological Activity. Sci. Rep.: 2016, 6, 23550.
39. Parisse, P.; Rago, I.; Ulloa Severino, L.; Perissinotto, F.; Ambrosetti, E.; Paoletti, P.; Ricci, M.; Beltrami, A.P.; Cesselli, D.; Casalis, L.: Atomic Force Microscopy Analysis of Extracellular Vesicles. Eur. Biophys. J.: 2017, 46, 813-820.
40. Gurunathan, S.; Kang, M.-H.; Jeyaraj, M.; Qasim, M.; Kim, J.-H.: Review of the Isolation, Characterization, Biological Function, and Multifarious Therapeutic Approaches of Exosomes. Cells: 2019, 8, 307.
41. Wu, Y.; Deng, W.; Ii, D.J.K.: Exosomes: Improved Methods to Characterize Their Morphology, RNA Content, and Surface Protein Biomarkers. Analyst: 2015, 140, 6631–6642.
42. Jung, M.K.; Mun, J.Y.: Sample Preparation and Imaging of Exosomes by Transmission Electron Microscopy. J. Vis. Exp.: 2018.
43. Patil, S.M.; Sawant, S.S.; Kunda, N.K.: Exosomes as Drug Delivery Systems: A Brief Overview and Progress Update. Eur. J. Pharm. Biopharm. 2020, 154, 259–269.

44. Gebeyehu, A.; Kommineni, N.; Meckes, D.; Sachdeva, M.: Exosome Vehicles as Nano-Drug Delivery Materials for Chemotherapeutic Drugs. CRT: 2021.
45. Sokolova, V.; Ludwig, A.-K.; Hornung, S.; Rotan, O.; Horn, P.A.; Epple, M.; Giebel, B.: Characterisation of Exosomes Derived from Human Cells by Nanoparticle Tracking Analysis and Scanning Electron Microscopy. Colloids Surf. B Biointerfaces 2011, 87, 146-150.
46. Brinton, L.T.; Sloane, H.S.; Kester, M.; Kelly, K.A.: Formation and Role of Exosomes in Cancer. Cell. Mol. Life Sci. 2015, 72, 659-671.
47. Butreddy, A.; Janga, K.Y.; Ajjarapu, S.; Sarabu, S.; Dudhipala, N.: Instability of Therapeutic Proteins—An Overview of Stresses, Stabilization Mechanisms and Analytical Techniques Involved in Lyophilized Proteins. Int. J. Biol. Macromol.: 2021, 167, 309-325.
48. Hoo, C.M.; Starostin, N.; West, P.; Mecartney, M.L.: A Comparison of Atomic Force Microscopy (AFM) and Dynamic Light Scattering (DLS) Methods to Characterize Nanoparticle Size Distributions. J. Nanoparticle Res.: 2008, 10, 89-96.
49. Gercel-Taylor, C.; Atay, S.; Tullis, R.H.; Kesimer, M.; Taylor, D.D.: Nanoparticle Analysis of Circulating Cell-Derived Vesicles in Ovarian Cancer Patients. Anal. Biochem.: 2012, 428, 44-53.
50. Pospichalova, V.; Svoboda, J.; Dave, Z.; Kotrbova, A.; Kaiser, K.; Klemova, D.; Ilkovics, L.; Hampl, A.; Crha, I.; Jandakova, E.; et al.: Simplified Protocol for Flow Cytometry Analysis of Fluorescently Labeled Exosomes and Microvesicles Using Dedicated Flow Cytometer. J. Extracell. Vesicles: 2015, 4, 25530.
51. Melo, S.A.; Luecke, L.B.; Kahlert, C.; Fernandez, A.F.; Gammon, S.T.; Kaye, J.; LeBleu, V.S.; Mittendorf, E.A.; Weitz, J.; Rahbari, N.; et al.: Glypican-1 Identifies Cancer Exosomes and Detects Early Pancreatic Cancer. Nature: 2015, 523, 177-182.
52. Van der Pol, E.; Coumans, F.A.W.; Grootemaat, A.E.; Gardiner, C.; Sargent, I.L.; Harrison, P.; Sturk, A.; van Leeuwen, T.G.; Nieuwland, R.: Particle Size Distribution of Exosomes and Microvesicles Determined by Transmission Electron Microscopy, Flow Cytometry, Nanoparticle Tracking Analysis, and Resistive Pulse Sensing. J. Thromb. Haemost.: 2014, 12, 1182-1192.
53. Thane, K.E.; Davis, A.M.; Hoffman, A.M.: Improved Methods for Fluorescent Labeling and Detection of Single Extracellular Vesicles Using Nanoparticle Tracking Analysis. Sci. Rep.: 2019, 9, 12295.

54. Vestad, B.; Llorente, A.; Neurauter, A.; Phuyal, S.; Kierulf, B.; Kierulf, P.; Skotland, T.; Sandvig, K.; Haug, K.B.F.; Ovstebo, R.: Size and Concentration Analyses of Extracellular Vesicles by Nanoparticle Tracking Analysis: A Variation Study. J. Extracell. Vesicles: 2017, 6, 1344087.
55. Szatanek, R.; Baj-Krzyworzeka, M.; Zimoch, J.; Lekka, M.; Siedlar, M.; Baran, J.: The Methods of Choice for Extracellular Vesicles (EVs) Characterization. Int. J. Mol. Sci.: 2017, 18, 1153.
56. Anderson, W.; Lane, R.; Korbie, D.; Trau, M.: Observations of Tunable Resistive Pulse Sensing for Exosome Analysis: Improving System Sensitivity and Stability. Langmuir: 2015, 31, 6577-6587.
57. Wang, J.; Chen, D.; Ho, E.A.: Challenges in the Development and Establishment of Exosome-Based Drug Delivery Systems. J. Control. Release: 2021, 329, 894-906.
58. Théry, C.; Witwer, K.W.; Aikawa, E.; Alcaraz, M.J.; Anderson, J.D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-Smith, G.K.; et al.: Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018): A Position Statement of the International Society for Extracellular Vesicles and Update of the MISEV2014 Guidelines. J. Extracell. Vesicles: 2018, 7, 1535750.
59. Rissin, D.M.; Kan, C.W.; Campbell, T.G.; Howes, S.C.; Fournier, D.R.; Song, L.; Piech, T.; Patel, P.P.; Chang, L.; Rivnak, A.J.; et al.: Single-Molecule Enzyme-Linked Immunosorbent Assay Detects Serum Proteins at Subfemtomolar Concentrations. Nat. Biotechnol.: 2010, 28, 595-599.
60. Shao, H.; Im, H.; Castro, C.M.; Breakefield, X.;Weissleder, R.; Lee, H.: New Technologies for Analysis of Extracellular Vesicles. Chem. Rev.: 2018, 118, 1917-1950.
61. Coumans, F.A.W.; Gool, E.L.; Nieuwland, R.: Bulk Immunoassays for Analysis of Extracellular Vesicles. Platelets: 2017, 28, 242-248.
62. Bunggulawa EJ, Wang W, Yin T, Wang N, Durkan C, Wang Y, et al.: Recent advancements in the use of exosomes as drug delivery systems. J Nanobiotechnology: 2018; 16(1): 81.
63. Li S, Li Y, Chen B, Zhao J, Yu S, Tang Y.: Exosomes in cancer therapy: a novel experimental and clinical strategy. Int J Cancer: 2021; 148(8): 1778-1788.
64. Kalluri R, LeBleu VS.: The biology, function, and biomedical applications of exosomes. Science: 2020; 367(6478): eaau6977.

65. Vader P, Mol EA, Pasterkamp G, Schiffelers RM.: Extracellular vesicles for drug delivery. Adv Drug Deliv Rev: 2016; 106: 148-156.