Exploring the Potential of Exosomes in Drug Delivery: A Review Paper

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***Abstract*- Exosomes are nanosized, small extracellular vesicles that are released by cells and contain bioactive components that are involved in both physiological and pathological processes in the body, such as proteins, lipids, and nucleic acids. The endogeneity and heterogeneity of exosomes afford them extensive and distinctive benefits in the realm of illness diagnosis and therapy when compared to synthetic carriers like liposomes and nanoparticles. Exosomes' clinical applicability is nevertheless constrained by their poor targeting, low production, low purity, and storage durability. Extracellular vesicles were originally identified as such 30 years ago and have since been associated with the transfer of disease states and cell-to-cell communication. As a result, more research is required to optimise the aforementioned issues and facilitate future treatment discovery.But there are still unanswered fundamental issues concerning their biochemistry. Here I discuss the current definition of exosomes and some of the unresolved problems in exosome biology. I also emphasise the challenges in investigating exosomes. regarding exosome functional research. In-depth knowledge of the subcellular elements and mechanisms involved in exosome synthesis and precise cell-targeting will shed insight on their physiological functions. Exosome research is still in its infancy**.

Keywords: **Exosomes, Biogenesis, Composition, Novel ,Vesicular.**

Introduction:

Exosomes are biological nanoscale spherical lipid bilayer vesicles released by cells that have a diameter of approximately 40–100 nm and float at a density of 1.13–1.19 g mL1 in a sucrose density gradient solution.Exosomes were first described as membrane vesicles with 5'-nucleotide enzyme activity that may have physiological functions and originate from the exudation of various cell line cultures in 1981 by Trams etal They collectively referred to plasma membrane-derived vesicles as exosomes and first proposed the term "exosomes". In 1983, it was discovered that sheep reticulocytes had the first known exosomes (40-100nm).The loss of transferrin receptors in adult red blood cells is caused by the creation of exosomes, according to research by Johnstone et al who monitored the development of reticulocytes and transferrin receptors. They were given the name exosomes to set them apart from other extracellular vesicles (EVs). It's important to note, however, that while being extensively used, the term "exosomes" has been advised to be replaced by the term "small Extracellular Vesicles (sEVs)" in ISEV 2018 guidelines due to methodological challenges with separation. Exosomes have been revealed to contain a variety of bioactive compounds, including cytokines, transcription factor receptors, proteins, lipids, and nucleic acids, according to studies.Exosomes are widely distributed, and among them, exosomal protein components can be broadly divided into two groups: the public components, which take part in the vesicle formation and secretion process, and which include Heat shock proteins and proteins related to membrane transport and fusion. (like HSP70, HSP90), members of the four-transmembrane protein superfamily (like CD63, CD81), ESCRT complex-related proteins (like Tsg101, Alix), integrins, etc.; the second category is composed of specific elements that are closely related to their progenitor cells, or cell-specific, such as CD45 and MHC-II derived from antigen-presenting cells. Exosome research is being conducted in great detail, and its applications are expanding. Exosomes can operate as media- tors for intercellular communication and material exchange, contributing to physiological and pathological processes[1].

Exosomes have the ability to safely transfer a variety of bioactive substances, as well as ingredients that are quick to degrade or easily inactivate in the body when administered alone, to target cells through a variety of sites and pathways.Transferring them to target cells securely allows them to take part in regulatory processes like immune control, tumour detection, and tissue healing The classification, preparation, and characterisation of exosomes, storage stability, biomarkers, and tailored drug delivery methods are the key topics of this review, along with some observations[2].

What is an exosome now defined as?

That is a really excellent question. Since exosomes were first described more than 30 years ago, the term has been amorphously applied to a variety of extracellular vesicles, confusing the issue and often fueling mistrust towards the research. Exosomes are best described as extracellular vesicles that are released from cells as a result of the fusion of

the multivesicular body (MVB), an intermediate endo-cytic compartment, with the plasma membrane. Exosomes are the vesicles that are released as a result of this liberating intraluminal vesicles (ILVs) into the extracellular milieu[3] .
There are additional cell-derived microvesicle forms, such as apoptotic bodies and ectosomes. respectively going through apoptosis and loosing their plasma membranes. Although apoptotic bodies, ectosomes, and exosomes are all around the same size (usually 40–100 nm) and all include "gulps" of cytoplasm, they are distinct species of vesicles, and understanding the differences between them is crucial yet sometimes disregarded[4].

Structure and Composition

Due to their small size, exosomes are not visible to the naked eye or under light microscopes and can only be visualized under an electron microscope. They appear as fattened spheres, most likely due to the preparation process for elec-tron microscopy, which involves extreme dehydration resulting in the exosomes collapsing In recent years, structural studies of exosomes have revealed that they contain certain lipids that help to maintain their biological activity, many of which are recorded in the exosome database ExoCarta. Exosome membranes are composed of a lipid Composition and Structure[5]
Exosomes can only be seen with an electron microscope since they are so minute that neither the human eye nor a light microscope can see them. Most likely as a result of the intense dehydration required during the electron microscopy preparation process, which causes the exosomes to collapse they look as fattened spheres. Exosomes include certain lipids that support the maintenance of their biological activity, many of which are listed in the exosome database ExoCarta, according to structural investigations conducted in recent years. A lipid makes up the exosome membranes. ExoCarta. In addition to other biomolecules including unsaturated lipids, cholesterol, phosphatidylserine, sphingomyelin, and gangliosides, exosome membranes are made up of a lipid bilayer It's possible that the exosome membrane's high concentration of unsaturated lipids and sphingomyelins is what gives it its strength and rigidity, making it less prone to degradation outside the cell and more stable as a carrier[6].
A group of proteins called the endosomal ESCRT is necessary for the formation of MVBs during membrane Exo-somal proteins are made up of substances that are connected to the endocytic pathway in the cytosol or plasma membrane but not in the mitochondria, endoplasmic reticulum, or Golgi complex[7]
Exosomes are also known to contain significant amounts of DNA and RNA along with lipids and proteins. The size of EV-DNA, or DNA that is transported inside extracellular vesicles, varies from 100 base pairs to 2.5 kilobases (kb) . It is believed that transfer RNAs (tRNAs), microRNAs, and Based on sequencing of all RNA produced from isolated EVs in serum, RNA repeats make up 50% of EV-RNA . Some RNAs are present in higher concentrations than those seen in the origin cells, such as those produced from exosomes of mesenchymal stem cells. Although numerous studies have demonstrated that RNA may be transferred within exosomes from one cell to another [m, it is still unknown if this transferred RNA is functional in the destination cells and how much of it was fragmented and transferred[8].

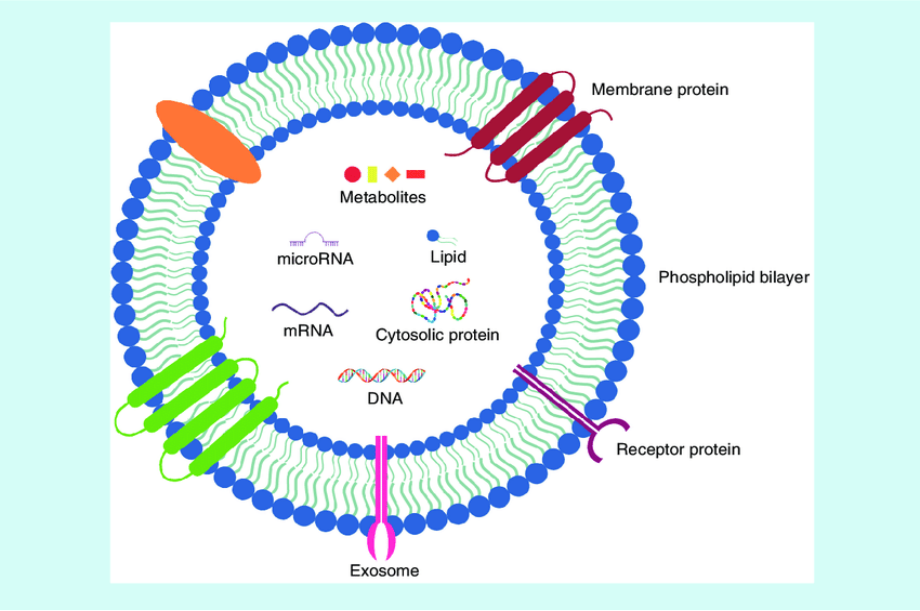

Fig.1 Structure of exosome

Classification

Depending on whether they have undergone artificial modification, exosomes are classified as either natural exosomes or designed exosomes. Ultimately, animal-derived exosomes and plant-derived exosomes are separated under the category of natural exosomes. Exosomes are further separated into normal exosomes and tumour exosomes since they

are produced in both normal and tumour environments.Nearly every type of healthy cell, including human umbilical vein endothelial cells, mesenchymal stem cells (MSC), T cells, B cells, macrophages, dendritic cells (DC), and natural killer (NK) cells, is capable of producing exosomes. For instance, mesenchymal stem cells (MSCS) are pluripotent stem cells that can differentiate in multiple directions and self-renew. In addition to their ability to adapt to the tumour microenvironment, MSCs also exhibit potent paracrine activity and produce a significant quantity of exosomes. Studies have shown that exosomes containing paclitaxel (PTX) have the ability to serve as drug carriers and have a sizable inhibitory effect on the proliferation of the human pancreatic cancer cell line CFPAC-1 in vitro. Additionally, it has been demonstrated that MSC exosomes contribute to the emergence of numerous illnesses[9].

They not only take part in tissue healing and injury, and have some therapeutic effects on cardiovascular (like myocardial infarction) and neurological (like Parkinson's disease) disorders but they can also lessen liver harm and be used to cure liver diseases.Macrophages are mononuclear phagocytes that are involved in angiogenesis, immunological control, wound healing, and inflammation. Macrophages can be polarised into M1 or M2 macrophages and are often found throughout the body. Exosomes produced by macrophages have been found to influence the immune system and inflammatory signals in the lung tissue microenvironment. In addition, Exo-PTX significantly inhibits lung cancer spread in the Lewis model[10].

The almost complete co-localization of the respiratory pathway-delivered macrophage-derived exosomes with cancer cells' metastasis suggests that these exosomes may have unique surface proteins that cause them to accumulate preferentially in cancer cells, though the precise mechanism is still unknown.Exosomes have the capacity to inherit several particular biomolecules . One of the factors contributing to the heterogeneity of exosomes is their capacity to inherit a wide variety of particular biomolecules from parent cells. Additionally, there are some variances in the yield, contents, functions, and drug loading of exosomes from various sources. Different therapy outcomes could arise as a result. Doxorubicin (DOX) was successfully loaded onto pancreatic cancer cells (PCCs), macrophage-derived exosomes, and pancreatic stellate cells (PSCs) using co-incubation, according to Kanchanapally et al. Exosomes generated from macrophages have the strongest anticancer effect, whereas PSC-derived exosomes have the highest yield and drug loading rate, demonstrating the specificity of exosomes from various origins[11].

Additionally, the previously described normal exosomes can be found in large quantities in biofluids like saliva, plasma, urine, ascites, milk, and bile. The chemotherapeutic medication paclitaxel has been successfully delivered using milk-derived exosomes, and standards for bioavailability, stability, safety, and toxicity have been tested. Exosomes found in biofluids can also exhibit specific diagnostic and therapeutic qualities. For instance, a thorough examination of the circRNAs found in exosomes found in cerebral fluid or blood can help to shed light on the underlying mechanisms underlying the emergence of neuropsychiatric disorders. Additionally, spinal cord injury (SCI) can be diagnosed and its prognosis determined using miRNAs transported by serum exosomes[12].

Likewise, it was discovered that miRNAs with differential expression can serve as biomarkers for the diagnosis of endometrial cancer (EC) by analysing the expression profile of miRNAs in EC and urine-derived exosomes from suspicious patients. Exosomes can be produced in great quantities by tumour cells, and the unique antigens on their surface may reveal information about the origin of donor cells. Tumour exosomes have so garnered a lot of interest in cancer research. In addition to being crucial for the growth, metastasis, and immunological control of tumors, tumour exosomes also monitor disease progression and act as diagnostic markers for various illnesses.For instance, Wu et al. discovered that exosomes produced by colorectal cancer (CRC) cells that overexpress CAPS1 can improve the FHC cell migration in healthy colonic epithelium. As a result, patients with metastatic CRC may benefit from treatment that prevents the release of tumour exosomes. TKIs are tyrosine kinase inhibitors that target the BCR-ABL1 p210 oncoprotein and have considerable therapeutic effects on chronic myeloid leukaemia (Ph+ CML), according to a study. The presence of residual active leukaemia cells that cannot be detected by the traditional MRD monitoring system can be determined by the tumour exosomes in TKI-treated CML patients who are in the chronic phase of the disease. This new monitoring method can be utilised to stop CML from recurring in the future. In addition, food-derived exosomes also have good development prospects. In recent years, studies have found that plant-derived exosome-like nanoparticles (ELN) have similar structures to mammalian exosomes. Ginger-derived particles can prevent the development of liver-related diseases, and ELN derived from grapes, carrots, grapefruit and ginger have anti-inflammatory effects and can maintain intestinal homeostasis At present, exosomes are classified mainly on the basis of sources[12]. This classification does not analyze the characteristics and functional applications of various types of exosomes in detail. In the future, further subdivisions from the aspects of organophilicity, biological distribution and immunogenicity may be considered. Exosomes generated from food also have promising development prospects. Exosome-like nanoparticles (ELN) produced from plants have been reported to resemble mammalian exosomes in recent years. Particles made from ginger can stop the progression of liver-related illnesses, while ELN made from grapes, carrots, grapefruit, and ginger has anti-inflammatory properties and can keep the gut flora balanced[13].

Exosomes are currently categorised primarily based on their sources. The characteristics and functional applications of the various types of exosomes are not thoroughly examined in this classification. Future classifications based on organophilicity, biological dispersion, and immunogenicity may be taken into consideration[14].

Clinical trials

It is well-established that exosomes are an ideal candidate for the treatment of many diseases. Before clinical trials, many preclinical experiments have confirmed the advantages of exosomes for the treatment of many diseases spanning the field of regenerative medicine to cancers. Over the recent years, exosomes were investigated in clinical trials, collectively two types of exosomes are used in clinical trials, specifically, exosomes of human cells/samples and plants specimen. A survey on Clinical Trials shows the major applications of exosomes are biomarkers, exosome-therapy, drug delivery systems, and cancer vaccines. Besides, some trials have been aimed to analyze exosomes from human samples under different conditions[15]. An analysis shows that a total of 116 trials have been recorded, of which 58 (50%) belong to biomarker applications. In the case of exosome therapy, 33 (28.44%) studies have been registered . For drug-delivery system trials 6 (5.17%) studies, while for exosomes basic analysis 17 (14.66%) studies have been registered. Finally, 2 clinical trials (1.72%) are related to exosome vaccine studies. Exosomes in clinical trials need to comply with good manufacturing practice (GMP). A GMP grade exosome production method comprises the type of cells, culture environment, cultivation system, and culture medium. Further purification is essential after production, usually divided into three-step process. Te third subject in GMP of exosomes is the establishing of characterization and identification method, comprising physical configuration and bioactivity function characteristics Furthermore, European Medicines Agency has released scientific recommendations on classification of advanced therapy medicinal products. Tis agency describes that new scientific progress in cellular and molecular biotechnology has led to the development of advanced therapies, such as gene therapy, somatic cell therapy, and tissue engineering. Tis nascent field of biomedicine offers new opportunities for the treatment of diseases and dysfunctions of the human body. In addition, it provides guidelines, recommendations, and criteria for researchers to advanced therapy medicinal products and amending directive and regulation[16].

Biogenesis of exosomes

Exosomes are generated constitutively from late endosomes, which are created when the membrane of the restricted multivesicular body (MVB) buds inward. Intraluminal vesicles (ILVs) occur within big MVBs as a result of the invasion of late endosomal membranes While the cytosolic components are absorbed and contained within the ILVs, some proteins are incorporated into the invaginating membrane during this process. Upon fusing with the plasma membrane, the majority of ILVs are discharged into the extracellular space and are known as "exosomes" .As an alternative, lysosomes are used to transport these components for destruction. When created by artificially drying during preparation, canonical exosomes exhibit a certain biconcave or cup-like form, although they seem spheroid in solution under transmission electron microscopy . On sucrose gradients, their densities typically vary from 1.13 g/mL (B cell-derived exosomes) up to 1.19 g/mL (epithelial cell-derived exosomes)[17]

The endosomal sorting complex required for transport (ESCRT) is necessary for ILV development, according to evidence. Four distinct protein ESCRTs (0 through III) make up the complex protein machinery, which cooperates to promote MVB development. Protein cargo sorting and vesicle budding . Ubiquitin-binding subunits of ESCRT-0 recognise and sequester ubiquitinated proteins to specific areas of the endosomal membrane to begin the ESCRT process. The complete complex will merge with ESCRT-III, a protein complex involved in encouraging the budding processes, following interaction with the ESCRT-I and -II complexes. The ESCRT-III complex then separates from the MVB membrane with the help of energy provided by the sorting protein Vps4 after cleaving the buds to create ILVs. Exosomes separated from numerous cell types have already been found to include multiple ESCRT components and ubiquitinated proteins, notwithstanding the debate over whether exosome release is an ESCRT-regulated mechanism. Additionally, it has been shown that the typical exosomal protein Alix, which is linked to numerous ESCRT (TSG101 and CHMP4) proteins, is involved in exosomal cargo selection via interaction with syndecan as well as endosomal membrane budding and abscission These observations gave rise to a theory linking ESCRT activity to exosomal biogenesis[18].

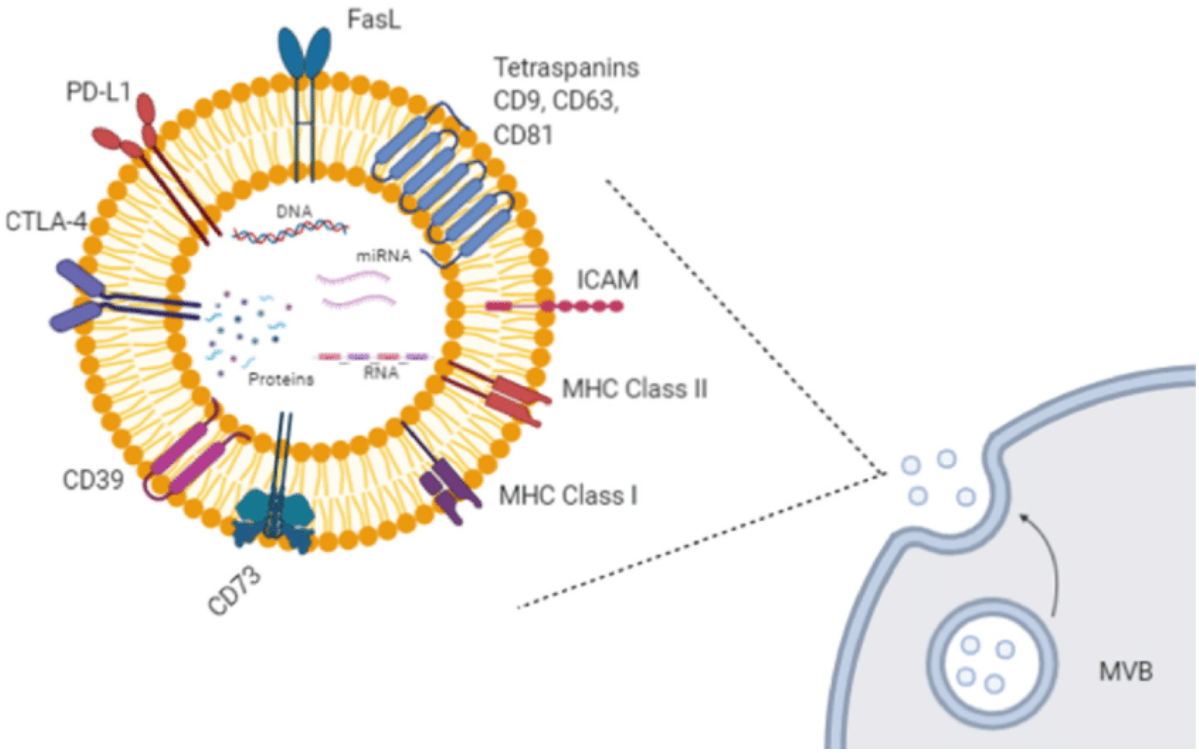

Fig.2 Biogenesis of Exosome

It's interesting to note that recent research supports a different mechanism that appears to depend on raft-based microdomains for the lateral segregation of cargo within the endosomal membrane and sorts exosomal cargo into MVBs without the need for ESCRT. Sphingomyelinases, from which ceramides can be produced by hydrolytic removal of the phosphocholine moiety, are predicted to be greatly abundant in these microdomains . It is known that ceramides cause the lateral phase separation and coalescence of microdomains in model systems. Additionally, the endosomal membrane's natural negative curvature may result from the cone-shaped structure of ceramide, which would encourage domain-induced budding[19].

In light of this ceramide-dependent process, exosomal lipids play a crucial role in exosome formation . Tetraspanins and other proteins take part in exosome formation and protein loading. Tet- raspanin-enriched microdomains (TEMs), which are widely used specialised membrane platforms for compartmentalising receptors and signalling proteins in the plasma membrane , are a common cellular structure. It has been demonstrated that TEMs and tetraspanin CD81 are essential for sorting target cells.exosomes are attracted to receptors and intracellular components[20]. It appears that different specialised processes, either ESCRT-dependent or -independent (involving tetraspanins and lipids), may work differently depending on the origin of the cell type to ensure the specific sorting of bioactive compounds into exosomes. Apoptotic bodies and plasma membrane-budded microvesicles (MVs) are two additional forms of membrane vesicles that cells can make in addition to exosomes. MVs are diverse populations of membrane vesicles produced by the plasma membrane's outward budding[21].

They are classified mostly as products of platelets, endothelial cells (ECs), and red blood cells and range in size from 100 to 1000 nm and shape. According to reports, MVs have a density of between 1.25 and 1.30 g/mL . In the late stages of apoptosis, apoptotic bodies are only released from the plasma membrane. They are similar in size to platelets and range in size from 1 to 5 mm. They contain a variety of intracellular fragments, cellular organelles, membranes, and cytosolic components[23]. Closed structures known as apop- totic bodies have higher sucrose gradient densities than MVs, ranging from 1.18 to 1.28 g/mL . Table 1 includes a list of the traits and traits of these cell-derived MV types. Finally, exosomes successfully avoid being cleared by the mononuclear phagocyte system because to their comparably smaller size and unifed structure, which not only increases the amount of time they spend in circulation indicating their superiority in cell-to-cell communication, but also[24].

Storage Stability

Exosomes are a promising cell-free treatment, however they cannot be kept in storage for an extended period of time. Therefore, research into exosome preservation technology is required to safeguard their biological activity and make them practical for clinical application and transit[25].

Currently, freezing, freeze-drying, and spray-drying are the main protective strategies used[26].

Cryopreservation :-

Usually used at temperatures between 4 °C to 196 °C, cryopreservation is a storage technique that lowers the temperature below the temperature needed for biochemical processes tomaintain the functional stability of the biological particles[27]. However, "frostbite" can happen when using this storage strategy. The creation of ice crystals inside the biological particles and the imbalance of osmosis duringthe freezing process are the main causes of the "frostbite" discussed here. One or more antifreezes with the proper concentrations are frequently added selectively to make up for thisshortfall in order to increase the shelf life. Permeability and non-permeability are the two categories that antifreeze is typically categorised into[28]. Among these, the permeable antifreeze, such as dimethyl sulfoxide and ethylene glycol,has a tiny molecular weight and can enter the cell membrane into the cell to prevent the formation of ice crystals. Studies have demonstrated that while DMSO-added exosomes incryopreservation are identical to fresh exosomes, direct freezing alters the stability of exosomal membranes and accelerates their disintegration. Additionally, short-term cryopreservation (within 2 months) did not dramatically alter the biological activity of exosomes. Hydrogenbonds can occur between non-permeable antifreeze and water, typically Other carbohydratesinclude trehalose and sucrose[29]. Through a contact between the phospholipid head group and theOH portion of the sugar, water molecules around the lipid head group are replaced by sugar. The sugar's glass matrix can stop vesicles from gathering and lessen the harm that ice crystals do.The greatest disaccharide antifreeze for exosomes, trehalose, is described as the mosteffective disaccharide antifreeze when safety is taken into account. To maintain a balanced state and avoid unneeded harm, it is important to consider the proper antifreeze concentration before usage[30].

Freeze-Drying :-

In order to meet storage requirements, a process known as freeze-drying chills materials containing moisture in advance, freezes them to a solid below -zero degrees, directly sublimates
the ice under vacuum, and then eliminates the water vapour. Technology for freeze-drying is
primarily separated into three stages: step prior to freezing, sublimation drying analytical drying step and stage. Exosomal lyophilized powder that has been freeze-dried can significantly improve storage conditions. It can keep its original activity and minimise harm to biological tissues and cell bodies since it completes the dehydration and drying of items at low temperature and vacuum settings[31]. The substance can be easily maintained in a constant
storable condition and is quickly reconstitutable with just a little water. It is an effective technique for protecting heat-sensitive items like proteins, vaccines, and EVs. Due to the forces created by freezing and dehydration during the freeze-drying procedure, the biomolecule's molecular structure may be disrupted. In order to safeguard the biological material, antifreezes must be added selectively[32]. Exosomes from B16BL6 melanoma were divided into three parts, each of which was freeze-dried with trehalose added, one of which was stored at 80°C, and one of which was freeze-dried directly. compared the morphology, protein content, and impact on pharmacokinetics of the three parts of exosomes. The findings demonstrate that trehalose can successfully promote colloidal stability, inhibit exosome aggregation during the freeze-drying process, and do not alter exosome morphology. The lyophilized exosomes' protein content was comparable to that held at 80°C, and the pharmacokinetic effects were barely noticeable[33].

Spray-Drying :-

Spray drying is a method that is used consistently to dry materials. The moisture immediately evaporates in contact with the heated air after the EVs solution is atomized in the drying room to produce dry particles. The stability of exosomes is influenced by the atomization pressure and outlet temperature during the process. Spray drying is a continuous method that can achieve one-step milling, which is more efficient and less expensive than lyophilization. extensible and has the ability to modify product particle size.80 °C frozen storage is now the most effectiveall-around storage method[34]. However, the choice of temperature for exosomes' long-term storage stability is influenced by a variety of sources and experimental methods. According to studies, storing exosomes at 80 °C for 4 days will alter their shape compared to newly extracted exosomes, and storing them there for 28 days will decrease their biological activity days. The loading rate of paclitaxel also stays constant, and other investigations have demonstrated that milk-derived exosomes may be maintained at 80 °C for four weeks without losing any of their physical characteristics.Therefore, it is crucial to examine exosome storage stability from a variety of angles, particularly the long-term stability. As an illustration, consider the source, drug delivery method, application technology, and future research focus[35]. In essence, it is advantageous to complete the following functional Exosome studies speed up the experimental procedure and broaden the range of possible applications.

Drug-delivery application

Exosomes as developed biological nanoplatforms for delivering therapeutic agents have been examined in preclinical and clinical experiments. In the field of nanotechnology, designing smart carriers for targeted drug delivery is a gold standard. Exosomes may be a good applicant for the clinical translation of several drug-delivery platform formulations[36]. From a drug delivery standpoint, exosomes are analogous to liposomes, regarding phospholipid structure. However,

exosomes can be collected from various body fluids and cells with a complex structure of different lipids and surface proteins and receptors; these biomolecules Flowchart for current Good Manufacturing Practices (GMP) manufacturing of exosome therapeutic products. These distinctive protein-decorated phospholipid membranes may likely have the definite barcodes desirable to interact with their target both neighboring and at distant locations. Even though extensive investigations, the superiority of exosome-based drug delivery system over delivery by other synthetic nanocarriers, like liposomes, and the related beneft-risk ratio remain problems of debate[37]. Therapeutics agents, including nucleic acid, proteins, and drugs, can be inserted into exosomes at least in two general methods and then carried to a specific target . These methods are direct methods or exogenous approaches where drugs are loaded into isolated exosomes via several methods; and indirect methods or endogenous approaches in which parental cells are loaded with drugs or genetically modifed for expressing optional proteins, receptors, and RNAs. The endogenous approaches has a lower level of complication if the parent cells right produce exosomes with the chosen molecule. Alternatively, there is a method known as exosomesinspired liposomes in which a desired liposome is fused with exosome to construct a hybrid carrier. Ongoing clinical trials show that exosomes are being used for delivering therapeutic agents, for example, there are six clinical trials registered In two clinical trial, authors aimed to encapsulate curcumin into plant derived exosomes for treatment colon cancer and Irritable Bowel Disease (NCT01294072 and NCT04879810) by direct methods. In addition, three clinical trials aimed to modify mesenchymal stem cells (MSCs) for producing overexpressing exosomes[38] . None of these trials is completed and results are not available until now. It is worthy to note that some clinical trials used microparticles from tumor cells for delivering drugs (e.g. NCT02657460 and NCT01854866). These studies used the term microparticles not exosomes and are recorded before the ISEV guidelines for EVs-based studies. Although researchers have endeavoured to harness the exclusive properties of exosomes to advance smart drug delivery systems that show considerable profits in pharmacokinetics, targeting, and safety against those of synthetic nanocarriers, clinical translation of these results faces challenging . Because of the characteristic complexity of the exosomes themselves, size heterogeneity, and natural (batch-to-batch) discrepancies run throughout their assembly, the intrinsic risks of the biogenesis procedure are higher than those of virginally synthetic fabrication approaches[39]. Furthermore, technologically and reproducible available approaches are required to load them with therapeutic drugs. Even though loading approaches for liposomes are optimized and used in an industrial context, such methods for exosomes are still needed. Current cell culture and exosome purification methods limit the implementation of standardized and large-scale production of exosomes . Therefore, for exosomes to be considered as a reliable therapeutic carriers, scalable manufacturing processes are required to produce exosomes in a fast, cost-effective, and reproducible way[40].

CONCLUSION :-

Exosomes have several advantage over conventional nanocarriers, including less immunogenicity, less toxicity, and greater translocation through biological barriers. Exosomes have demonstrated significant value in nucleic acid delivery based on these benefits. and can guard against immune system clearance and host immune system deterioration of therapeutic compounds. Additionally, exosomes have the potential for tailored delivery due to the innate targeting capacity inherited from their parents cells. This enhanced targeting ability allows exosomes to cross the tumour vascular barrier and accumulate at tumour locations, considerably strengthening their therapeutic efficacy. Additionally, various preclinical researchinvestigations and clinical trials have looked into the medicinal uses of exosomes as drug delivery vehicles. So exosome-based delivery methods have special benefits for treating cancer. The use of exosomes to transfer various nucleic acids (DNA, mRNA, miRNA, siRNA, and others) is discussed in this article. circRNA, etc.) to combat different types of cancer are outlined. Although significant progress has been made, there are still certain obstacles to theuse of exosomes as a therapeutic tool. The synthesis of exosomes on a big scale for clinical studies is the first challenge. Bioreactors, 3D scafolds, and microfuidic devices are used to boost exosome production. For instance used tangential flow filtration (TFF) in conjunction with 3D culture to 140-fold increase exosome production when compared to 2D or 3D cultures alone. According to another study, utilising a hollow fber bioreactor could 40-fold boost exosome yield ..The outcomes showed that CNP produced upto 50 times as many exosomes as the conventional approaches (bulk electroporation and Lipo2000 transfection). The technology for separating and purifying exosomes using microfluidic devices also demonstrated encouraging outcomes. To address the shortcomings of conventional approaches, the second task is to create innovative techniques for incorporating nucleic acids into exosomes. Current exosome-nucleic acid loading techniques, including as electroporation, incubation, and transfection, have a low loading efficiency that restricts their use.

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