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EXOSOMES: A NOVEL BIOMARKER IN MEDICINE

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ABSTRACT
Exosomes are nano-sized vesicle secreted by nearly all cell and have received massive attention recently. In addition their roles in pathophysiological processes and diagnostic evaluation, recently. Several studies have applied in exosomes to design novel therapeutic application. Exosomes with an average diameter of ~100 nanometers and subset of EVs. The biogenesis of exosomes involves their originals in endosomes and subsequent interaction with other intracellular vesicles and organelles generate the final content of the exosomes. Their diverse constituent includes nucleic acids, protein, lipids, amino acids, other metabolites that can reflect their cell of origin. In various diseases exosomes offers s window in to altered cellular or tissue states and the detection in biological fluids potentially offers a multicomponent diagnostic readout the efficient exchange of cellular components through exosomes can inform their applied use in designing exosome based therapeutics. The clinical trials involving exosomes and its advantages hazards with difficulties involved during storage and large scale production with its application in nanotechnology .In this review, we tried to summarize the exosomal structure, composition, formation, and isolation process. We also discussed their active role in pathogenesis. Exosomes may have evolved early in the evolution of multicellular organisms and also seem to be important for tissue developmental processes.

KEYWORDS: Exosomes, extracellular vesicles, biomarkers, immune therapy, nanomedicine, diagnosis, surface stability, target drug delivery.

INTRODUCTION
Exosomes are with the diameter of around 40-100nm are biological nanoscale spherical bilayer vesicle secreted by cells, floating at a density of 1.13–1.19 g. mL^{-1} in a sucrose density gradient solution.[1] In 1981, Trams et al collectively referred to plasma membrane-derived vesicles as exosomes and first proposed the concept of "exosomes", which was regarded as membrane vesicles with 5'-nucleotide enzyme activity that may have physiological functions and originate from the exudation of various cell line cultures. The currently defined exosomes (40–100nm) were first found in sheep reticulocytes in 1983. Johnstone et al tracked transferrin receptors during the maturation of reticulocytes and found that the formation of exosomes is the mechanism for the loss of transferrin receptors in mature red blood cells. To distinguish them from other types of extracellular vesicles (EVs), they were named exosomes. However, it's worth noting that the term "exosomes", even if widely used, has been suggested to be replaced by the term "small Extracellular Vesicles (sEVs)" according to ISEV 2018 guidelines, due to methodological difficulties of separation.[2,3] Studies have found that exosomes contain nucleic acids, proteins, lipids, cytokines, transcription factor receptors and other bioactive substances.

Exosomes are associated with immune responses, viral pathogenicity, pregnancy, cardiovascular disease, central nervous system related diseases and cancer progression.[4] Exosomes can play a role in physiological and pathological processes, acting as mediators for intercellular communication and material exchange.[5] At the same time, exosomes can deliver a variety of bioactive substances and easy-to-deactivate or easily degradable ingredients (referring to therapeutic agents that have a short retention time in the body when administered alone) through multiple pathways and sites, and safely transfer them to target cells to participate in regulation, such as tissue repair, tumor diagnosis and treatment, and immune regulation and this review, we mainly focus on the classification, preparation and

characterization of exosomes, storage stability, biomarkers, targeted drug delivery systems and provides some insights.[7,8]

Formation of Exosomes[5,6]

Exosomes are formed with endocytic cellular pathway consisting with three main different stages as follows:

i) Plasma membrane investigation form the endocytic vesicles.

ii) Second stage inward budding endosomal membrane starts which give s multi vesicular bodies that is MVB's

iii) In third and last stage MVB's fused with plasma membrane and release the vesicular content that is exosomes.

The membrane proteins which undergo the endosomal pathway exhibit the same stages. Different types of lipid molecules are known for the involvement in exosome formation and release like phoshatidic acid and ceramides. Size of the exosomes is dependent on their site of origin as well as lipid belayered structure in the cell.

Composition of Exosomes[4,6]

Exosomes are due to its protein and lipid content which provide additional effect their identification. Exosomes mostly content their fusion proteins and transport proteins as well as phospholipases and lipid related proteins.[4,5] All the proteins can be use as positive markers. A complex by various proteins including receptors, transcriptions factors, enzymes, extra cellular matrix proteins and other components like lipids, nucleic acid, DNA, mRNA, miRNA inside and the surface of the exosomes.[7] More than 4400 proteins can be identified in association with exosomes by mass spectrophotometer and these proteins serves as cargo for intracellular communication. Along with proteins exosomes are enriched with lipid like cholesterol, sphingolipids and phosphogycerides, ceramides and short and long saturated fatty acid chain. Research indicates that exosomes serves to deliver the prostaglandins to the target cell and it has also been investigated the molecules of exosomes have saccharides.[6] Group in their structure and enriches with manose and glycan. It has also been reported that exosomes possess miRNA in the significant amount. The cargo function of exosomal RNA is entirely the different than that of cell of RNA.

Isolation Process of Exosomes[7,12,16]

Exosomes are isolated by ultracentrifugation method. The exosomes isolation is based on the size of exosomes. Exosomes extraction from blood or cell culture media is complicated because large number of micro sized particles is present in the media having the same size range as that exosomes.[5]

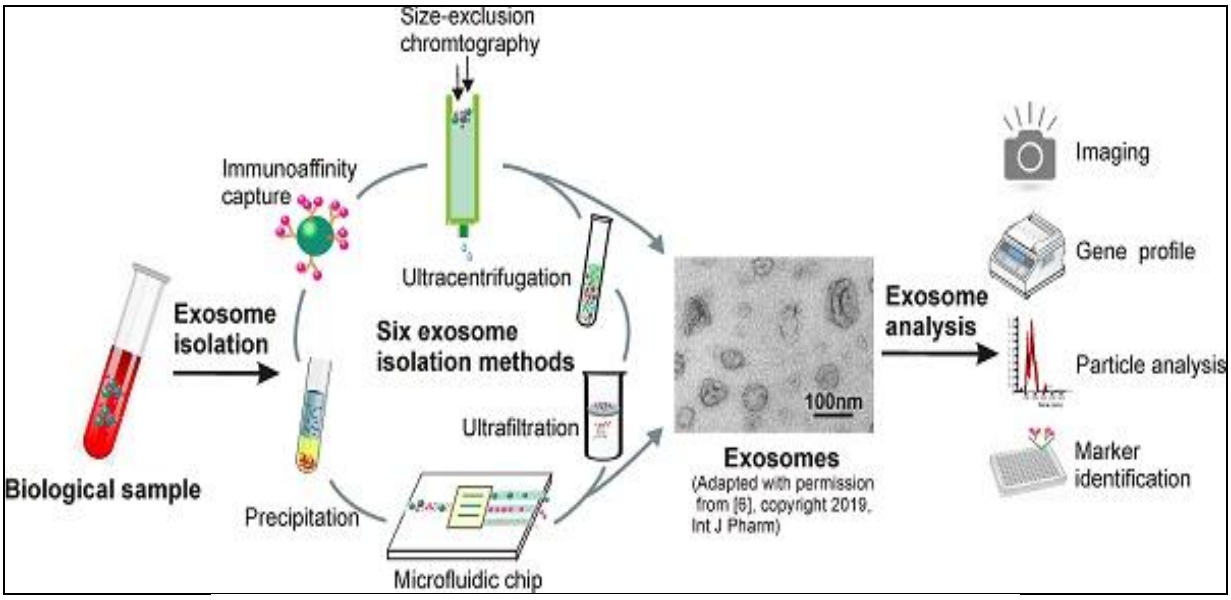


Fig: Isolation of Exosomes by Ultracentrifugation by biological sample.

METHODS OF ISOLATION

i) Ultra high speed method

This is mostly high speed performed this is the most highly performed process which is believed as a "gold standard" for exosome isolation. Based on variations in size and density among exosomes and other constituents, it requires a course of centrifugal forces on both low and high rotational rapidity, and a certain time for separation. Initially, little speed (300 g) is given to remove cells from the cell culture liquid. Then, the supernatant is applied to a larger centrifugal force to eliminate broken organelles and large cell debris.[9] Lastly, centrifugation of greater speed around 100,000–150,000 g is applied to accumulate pellet (containing exosomes) from the supernatant. It is widely used in CSF, urine, saliva, cell culture, plasma, serum. In addition to this, there are some cons regarding the technique like time consuming, cumbersome, high-equipment cost, and damage to exosomes because of high-speed rotation.[10]

ii) Size based isolation technique

Size-based exclusion chromatography (SEC) and ultra filtration are kinds of size-dependent separation. Exosomes are about tens of nanometers in dimension and bigger when compared to normal proteins. They could be effectively refined and segregated from the sample with ultra filtration membranes with divergent MWCO (mol. wt. cut off).[10] Pretreatment of sample is easy and no need for superior machinery. Here, all the molecules in the sample get separated in a porous stationary phase relying on its dimensions. Low size samples can be cleared through the pore another method that is dependent on SEC is acoustic fluid separation. Here, based on particle size, they are subjected to different acoustic forces and can be segregated consequently. It is label-free and contactless which wants validation and misused for diverse samples.[8]

iii) Polymer Precipitation

Polyethylene glycol (PEG), a hydrophilic polymer is employed for this technique for the separation of viruses and bio-macromolecules. This polymer has been used for more than a half-century. Water molecules are captured by the polymer, leading to the decline of exosome solubility and succeeding settling down in low-speed centrifugal circumstances. Comprising exosomes are incubated along with a PEG (Mol. wt: 8000da) solution, which gives rise to exosome precipitation At 4°C overnight incubation, this precipitation could be isolated or reprocessed using centrifugation or filtration.[9] Assume the eradication of ultracentrifugation requirement, so many commercial exosome isolations kits have developed such as miRCURY™, Exo-spin™, Exo Quick™, and Pure Exo ®. These kits use polymeric additives (for special reagents purpose) to induce exosome precipitation and separations are to be achieved within 30min utilizing a standard centrifuge (10,000g). Quite a lot of studies have related to the exosome precipitation efficiency methods with conventional ultracentrifugation, which revealed that these commercially available kits normally produce higher purity and yields. for therapeutic applications, adding precipitation reagents can inhibit intact exosome recovery from the matrix of polymer which is a serious disadvantage because the matrix could have an impact on biological characteristics and activities of exosome.[11]

iii) Immuno-affinity technique

It is used to separate the exosomes from biological fluids that are present in exosomes' outer surfaces such as many proteins, polysaccharides, receptors, and lipids. This procedure exploits the vast specific affinity interactions b/w antibody and antigen. Outer surface components are considered potential ligands. In an ideal world, Exosome biomarkers are greatly concentrated or solely occur on their membrane and don't have free counterparts. Some of them are HSP70, Alix, EpCAM, CD63, CD81, CD10, CD9, FLT1, AQP2, TSG101, and CD24.[11, 12]

iv) Di-electrophoretic segregation (DEP)

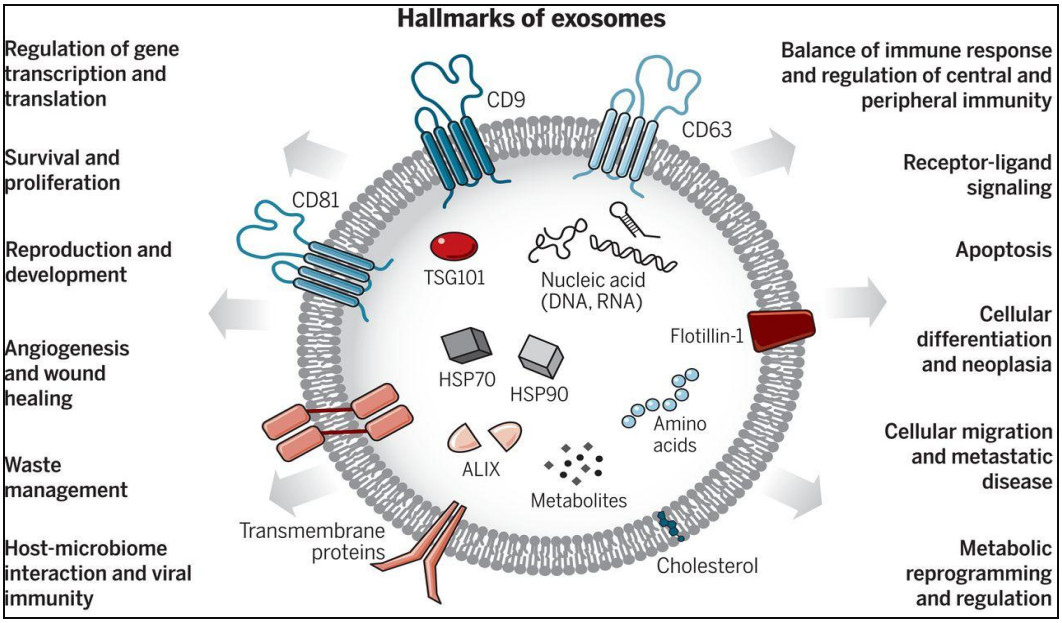
This process is based on the principle that particles (polarized ones) experience a dielectric force when placed in a non-uniform electric field. High electric field regions usually attract exosomes whereas low electric field regions receive particles large in size. In DEP, factors such as intrinsic dielectric properties applied electric field frequency and magnitude of the cell impact the magnitude of the forces employed on particles. The positive aspect of this method could be the high and rapid throughput characteristics while the often necessity of electro-thermal heating is a limitation.[12, 13, 15]

Method of Preparation

Dilute the plasma or Serum (1:1)
↓
Centrifuge it at 2.000 x g, 30min, 4 degree C

Supernatant was collected
↓
Sediments cells were discarded
↓
Centrifuge the supernatant at 12.000 x g, 45min, 4 degree C
↓
Supernatant
↓
Stored overnight in the glass tube at 4 degree C
↓
Dilute the plasma with 10ml 30% Sucrose solution
↓
Centrifuge at 110.000 x 2gm, 2Hrs, for 4 degree C
↓
Sedimentation
↓
Exosomes and sediment contaminating protein obtained
↓
Wash with PBS and transfer into clean ultra tube
↓
Centrifuge it at 110.000x g, 70min, 4 degree C
↓
Sedimentation = Exosomes
↓
Shake it with to 2000uL PSB and resuspended
↓
Stored at 80 Degree C and pure exosomes is obtained

Structure of Exosome



General Mechanism of Exosome Biogenesis[7, 11, 14]

Exosomal biogenesis starts within the endosomal system and early endosomes mature into the late endosome or MVB's, during this process the endosomal membrane invaginates to general ILV's in the lumen of the organelles. Depending on the final destination of their ILV, two main kind of MVE's are defined as degradative MVE's which fuse with lysosomes to promote the breakdown of their intraluminal content, and secretary MVE, which fuse with the plasma membrane to release their cargo into the extracellular space. In addition, MVE are implicated in the formation of specialized endosomal compartment such as melanosome in the pigment cell. Independently of their final destination, ILV's formation relies on the recruitment of specified lipid and proteins of limiting membrane of MVE. After the lateral segregation in order to promote the organization of specified sub domains from ILV inward budding occurs. The ESCRT machinery involve in the formation of ILV in MVE and the recruitment of ubiquinated protein to target them to degradation in lysosomes.

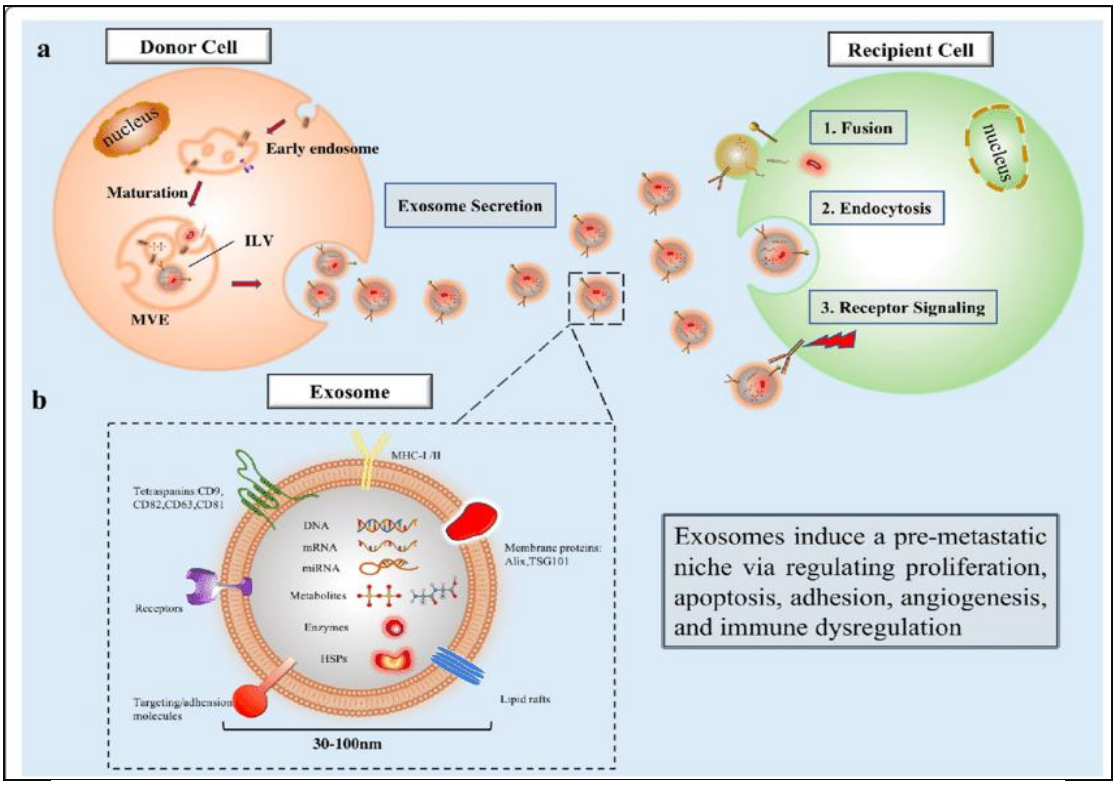


Fig: The biogenesis of exosomes and mechanism involves in intracellular communication.

Biological functions of exosomes[12,16,17]

Multiple cell lines that release the exosomes in vitro like neuronal cells, fibroblast cells, adipocytes, intestinal epithelial cells and tumor cell lines have been described. *In vivo*, exosomes are found to be present in many biological fluids like: synovial fluid, breast milk, blood, urine and saliva, amniotic liquid and malignant effusions of ascites. In blood serum, exosomes are almost present in a quantity of 3,000,000 per microliter. The first reported biological function of exosomes is as proteins, which are expelled out from reticulocytes during the process of maturation in erythrocytes. Authors believed that particles, which sediment from plasma at 10,000 ◊ g, are circular in nature and name them as exosomes. Further research showed that exosome secretion is just like an excretion process to get rid of unnecessary proteins and RNA. With the passage of time, more research was being conducted on exosomes discovering that exosomes are found to be secreted by many cell types. On the basis of their origin, exosomes perform a variety of functions. Extensive studies have been done on the facilitator effect of exosomes in immune response and its antigen presenting role has also been extensively reported. Exosomal role in coagulation, inflammation and angiogenesis were also reported. After the activation, platelets secrete exosomes as well as other shedding micro vesicles. In this case, exosomes did not perform any role in coagulation. It has been reported that exosomes are involved in dictyostelium cells migration by using chemo-attractant signals. Another group of researchers studied the level of miRNA in exosomes of human breast milk for several months during lactation. Recent studies demonstrate that the exosomes are not only involved in triggering downstream signaling but they also specifically target the recipient cells and Exchange proteins. Exosomes also deliver the specific nucleic acids and work as cargo. The unique function of exosomes is cell to cell communication, especially between the far distance cells in the body. Similarly, exosomes play a unique role in spreading various pathogens like virus and prion from one cell to another.

Exosomes in diagnostics[8,10,12]

For last few years, much research has been done on diagnostic aspect of exosomes and it was discovered that almost all the body fluids (blood, saliva, milk, and urine) contained exosomes. Because of unique structure of the exosomes, which possess proteins, lipids and RNAs, it may be useful for the diagnostic purposes. In late 1970s, micro vesicles (MVs) were derived from the cancer cells in person suffering from Hodgkinis disease. Since that day to-date, considerable efforts have been done to use the MVs as diagnostic tool. It was reported that MVs levels were elevated in serum, urine and blood in the cancer patient. However, microvesicular components may provide important information regarding a disease. For example, mucin bearing MVs are used as diagnostic marker for the diagnosis of adenocarcinoma. A proteomic investigation of urine identified eight proteins, which act as an important diagnostic tool in bladder cancer. Thus it can be said that protein portion of the exosomes are the useful tool for the diagnosis of the diseases. In addition, recent studies have showed that cancer patients exhibit different patterns of RNA and miRNA. In cancer patients, RNA and miRNA have been found in circulating MVs form. The PCR of miRNA is a sensitive and stable method for the diagnosis and detection of miRNA in patients serum, which is a new promising approach to detect disease in early stages. Down-regulation of miR-92a in plasma is the biomarker of hepatocellular carcinoma and leukemia.

Commercialized Exosome Products[9,16]

There are lots of companies doing research on exosomes and creating products for therapeutic applications as shown in Table:

Table No. 1: Companies doing research on exosomes and creating products for therapeutic applications.

Company Name	Product Description
TYPE I: naïve exo Avalon Globocare	Development of clinical grade Tissue-specific exosome (ACTEX™)
Aegle Therapeutics	Phase ½a clinical trial of MSCs-exos as a remedy for dermatological disorders
Exocobio	Developing exosome-based biomedicine and regenerative aesthetics
Capricor	Exosomes for the cure of clinical range rare, severe inflammatory illnesses
TYPE II: engineered exo Exo-pharm	Advancement of LEAP (ligand-dependent Exo affinity purification) for separation of exosomes with affinity chromatography
Codiak Biosciences	For precise targeting. Trade value and scale of exosomes for medical purposes
Evax	Targeting expertise, producing, and refining approaches for transformational medicine
Ilias Biologics	Development of therapeutic exosomes, Exo-targets as potential treatments for sepsis, pre-term birth, and various hard-to-treat diseases in inflammatory and metabolic areas
Carmine Therapeutics	Using red blood cell EVs (RBCEVs), developing next-generation gene therapies that overcome the current limitations of viral-based gene therapies such as immunogenicity, small transgene capacity, and manufacturing challenges
Aruna Bio	Neural exosomes to cure several CNS and neurodegenerative ailments

Therapeutic Application[6,15,22]

The fewer amount of exosomes can be naturally secreted from their origin point, limiting the clinical trials and research. Last few years, the arrival of synthetic exosomes (a new platform for the delivery systems) produced by cell extrusion or cell membrane cloaked nanoparticles have the potential to be generated in a high amount, which will also have the potential to be produced in high amounts. Without any additional modification, native exosomes can be used for downstream purposes, can be engineered as well, and used as carriers of drugs or biomolecules to change the properties of target cells. By direct loading of exogenous agents into exosomes or transfecting them into exosome secreting cells through some techniques such as calcium chloride, incubation, repeated freeze-thaw, lipofection, sonication, electroporation, and saponin permeabilization produces exosomes with non-native and selective therapeutic contents. A vital step in the engineering of exosomes and their application is the loading of desired agents into exosomes. Delivering of exosomal cargo is done by three possible mechanisms. They are, exosomes may (i) adhere to target cells via either surface adhesion molecules and/or receptors thereby inducing the receptor activation and downstream signaling in the target cells; (ii) Direct fusion with the target cells and deliver their cargo; (iii) Internalized by endocytosis, affecting the target cells after processing the exosomal cargo in the endosomal pathway. The delivered cargo could eventually up or down-regulate some specific genes and proteins in target cells and eventually change their properties. Hence, they have the potential to function as therapeutic tools against various illnesses like kidney, cardiovascular, liver, and lung diseases.

Liver disease

In a mouse model, liver regeneration is stimulated by treating the liver with liver-derived MSCs- conditioned media. Moreover, HUCMSCs-exos inhibited the CCl4-induced LF damage or partly reverse it. Several reports have shown that the MSCs-EVs have an anti-inflammatory effect normally, particularly about exosomes. EVs proteins and nucleic acid have therapeutic effects for liver problems. In vitro, the anti-tumor response can be increased by the HSP (heat shock protein) enriched exosomes. In tissue regeneration, angiogenesis, and immune regulation by delivering paracrine factors and supervising the intracellular micro-communication, MSCs-exos has a contribution to their therapeutic potential. Hence, the ideal cell-free therapy for many liver disorders could be the MSCs-exos administration. MSCs transplantation alone is not enough for the LF treatment. For improving theMSCs therapeutic potential, exosomes are induced with gene cargos. On the contrary, MSCs-exos must be aimed for the transport of HSCs. There is no report available on the ligands or homing peptides fusing to transmembrane proteins on the surface of exosomes permitting them to aim HSCs. Researchers used phage display technology to find out the ligands like cell-penetrating peptide (CPP) and peptide-431. In Hepatocellular carcinoma (HCC), malignant cells the microenvironment for invasion and proliferation and is also controlled by other cells through cell-cell communication by means of MVs and exosomes.

Endothelial cell-exosomes could stimulate the process of hepatic stellate cell signaling and movement through S1P. MVs might also work for therapeutic application. Liver stem cell-MVs prevent the growth of HCC, which is facilitated by many miRNAs present in those MVs.

Kidney Disease

In normal environments, nephron cells discharge EVs. EVs relocate biomolecules after being obtained by recipient cells and then produce a cellular reaction EVs quantity and contents are majorly based on the body state. In the kidney, the major function of EVs is removing waste materials from cells, be able to proficiently remove injurious chromosomal DNA fragments, prion, and misfolded proteins. Even though exosomes can be produced from systemic circulation in a very low quantity, many kidney cells like collecting duct cells, podocytes, distal/ proximal epithelial cells, and glomerular epithelial cells secret exosomes. Thereby, we can presume that a greater portion of the urinary exosomes is obtained from the kidney. These exosomes can act as potential means that might aid the researchers in recovering important information regarding renal diseases. Some of the important applications wherein exosomes were used as therapeutic agents for kidney diseases. The regulation of B-catenin by exosomes was reported by et al. Zhang et al. (downstream signaling molecule of Wnt signaling route) By administering exosomes, urine and microalbuminuria volume got decreased and the apoptosis of tubular epithelial cells induced by heavy glucose range got prevented in SD rats after continuous administration of exosomes for kidney injury. Exosomes were also able to restrain the caspase-3 over expression and amplifies the transmission of the glomerular endothelial cell Exosomes inhibited the next progression of the disorder to chronic kidney disease by hindering glomerulosclerosis, blocking the tubule interstitial fibrosis in an AKI-rat model.

Heart Disease

A research team from Singapore separated the buoyant HESCs-derived MSCs, said that the Particles that corresponded to exosomes are given to a MI/R injury mice model which then extraordinarily decreased the infarct size. They understood that ATP tissue levels and nicotinamide adenine dinucleotide (NAD) were increased, whereas reactive oxygen species (ROS) levels were considerably reduced after giving exosomes. Then, Akt and Gsk-3 phosphorylation (Glycogen synthase kinase 3) (have anti-apoptotic effects) notably reduced in cardiac tissue, which revealed that exosome administration extensively decreased macrophage and neutrophil infiltration in the heart next to reperfusion, signifying that anti-inflammatory effect is possible with

exosome treatment. Exosomes released by cardiomyocytes could reduce cardiac fibrosis caused by the pressure through releasing of huge miR-378. Likewise, iPSCs-exos prevent them OR pathway resulting in the improvement of cardiomyocyte survival and increment of autophagic flux. Cardio sphere derived cells (CDCs) derived exosomes (tailored) have more potential as a therapeutic tool than the parent ones. MSC-derived exosomes can raise the macrophage's division from M1 to M2 level, lead to the increase of cardiac tissue inflammation and thereby decreasing the infarct size. By stimulating AKT protein and hindering the JNK3/caspase-3 pathway, it is also shown that MSC-derived exosomal miR-19a show anti-apoptotic effects. CDCs-derived exosomes have the capacity to decreasing infarct size and increasing cardiac hypertrophy induced by angiotensin-II. Geoffrey et al. have revealed that this exosomal miR-18 b was able to block the expression of PKCδ.

Exosomes as a Targeting Drug Delivery System

Exosomes are small in size, which can effectively avoid the phagocytosis of mononuclear macrophages, and can freely cross the blood vessel wall and extracellular matrix. The expression of CD55 and CD59 on its surface avoids the activation of opsonin and coagulation factors, so it can be widely distributed and stable in the biofluids. Compared to liposomes and other nano-delivery systems which are synthesized in vitro, exosomes originate from the body, and have better biocompatibility and lower immunogenicity in theory. In fact, due to the heterogeneity of exosomes, they carry various proteins on the surface, which enter the cells in a variety of ways after contacting with cells. Among them, receptor-mediated endocytosis is one of the main ways of information communication between exosomes and target tissues, which optimizes the endocytosis process of exosomes and promotes the internalization of the encapsulated drug and facilitates the continuous and stable transport of the contents in the blood with high transport efficiency. Moreover, exosomes have strong ability to homing target tissues or cells and penetrate biological barriers (like the blood-brain barrier), so they have the advantage of natural drug delivery and are promising targeted drug carriers, which can be used to deliver genetic drugs, traditional Chinese medicine, western medicine, and so on. However, natural exosomes may have problems such as weak targeting and susceptible to be quickly cleared in the body, resulting in poor treatment effect. At this time, they are usually modified to form engineered exosomes. Engineered exosomes refer to natural exosomes loaded with therapeutic agents or modified. In the following part, the applications of targeted delivery system of exosomes will be explained mainly from the perspectives of drug loading and surface modification.

Table 2: Circulation exosomes as potential diagnostic markers for various diseases as follows.

	Sample type	Markers	Disease
Quantity	Plasma	MPS's Level	Gastric Cancer
	Serum	MPS's Level	Prostate Cancer
Protein Expression	Ascites	CD24, EpCAM	Ovarian Cancer
	Serum	Tissue Factor	General Cancer
	Plasma	Tissue Factor	Breast Cancer
	Pleural infusion	SNX 25, BTG1	Misothelioma
	Urine	Fetuin- A	Acute Kidney Injury
miRNA and mRNA Expression	Serum	Glioblastoma	Glioblastoma
	Serum	MAGE-1, HER-2	Gastric Cancer

Application of Nanotechnology in Exosome Research[26, 27]

Nano-fibers act as drug delivery system, cell- tissue regeneration and application, scaffolding for wound dressing and as carrier in cell or tissue reconstruction. Nanorods are engaged as computer components, Nano electric component, gas sensors and in solar energy, conversions and microelectromachanical system and video displayed. Electrospining outshined various methodologies such as rotary jet spinning, sol-gel methods and melt-blown protocols, electro spraying, template synthesis and self assembly and phase separation in terms of efficacy and highly effective method for nano-fibers production. It is continuous and scalable, can be applied to various polymers and produces outstanding control over nano-fibers and its orientation.

Tumor derived exosome have great have potential as biomarkers and even they capture and release methods remain challenging. For prostate cancer diagnosis, prostate specific antigen (PSA) has presented adequate sensitivity by which tumors reactions to treatment can be concluded. The interconnected micro-pores of nano-fibrous membrane (structure and modification) has a great surface area for immobilization of specific antibody for proficient exosome capturing which isolate above 87% of exosomes present in cell mediamaking thema validmethod for isolation of exosomes Using Nano-fibrous PCL–gelatin membrane, from the blood serum the exosomes were isolated which presented good sensitivity for isolating exosomes.

For the fabrication of the immune-sensor to show great target selectivity, Graphene Quantum Dots (GQDs) were used as signaling agent. The carboxyl groups of GQDs were used to bind them to anti-prostate specific antigen

(anti-PSA) using EDC/NHS coupling chemistry enabling them to aim the exosomes having the PSMA (prostate specific membrane antigen), is precise for cancer-derived exosomes and the GQDs– antibody complex identified the separated exosomes with high sensitivity.

Table 3: Overview of the application of exosomes as a nanoscale cancer vaccine.

Exosome Type	Cancer Type	Exosome Purification	Exosome Derivation And Delivery	Result Overview
DEX	Melanoma	Centrifugation up to 100.000 g, 30% Sucrose by D2O cushion treatment with PBS	Exosomal MHC peptide complex pulsed with a myeloma antigen CTL pulsed with melanoma primed exosome	DC release exosome to other native Dc for T cell priming exosome required, Myeloma effective T cell producing IFN-γ effectors lymphocytes
AEX	Various	Centrifugation up to 100.000 g, 30% Sucrose by D2O cushion treatment with PBS	DC pulsed with AEX PBL stimulated with AEX pulsed DC	Release of IFN-γ by PBL tumor cell lysis
DEX	Toxoplasma gondii	Centrifugation up to 100.000g, treatment with PBS	DEX pulse with T gondii derived antigen	Murine models resistant to brain cyst formation normally caused by T gondii specific antibody increased production of cytokines.
TEX	Leukemia	Centrifugation up to 100.000 g, 30% Sucrose by D2O cushion treatment with PBS	Direct vaccination of murine models with TEX-1	Inhibition of tumor growth CTL induced lysis of cancer cell.
DEX	Melanoma	Centrifugation up to 100,000 *g* 30% sucrose /D_2O cushion Treatment with PBS	Exosomal MHC–peptide complex pulsed with melanoma antigen CTL pulsed with melanoma-primed exosomes + CpG adjuvants.	CpG adjuvants + exosome elicit immune response toward cancer cells
TEX	Lymphoma Leukemia colon	Centrifugation up to 100,000 *g* Treatment with PBS	Direct vaccination of murine models with TEX and heat-shocked TEX[1]	Heat-shocked TEX more efficacious than TEX alone Immune response mostly mediated by CTL

Challenges and Future Perspectives

Although it has been established from the results in the clinical trials hat exosomes can be safely administered, their potency terms of eliciting appropriate responses to kill cancer cells kill much leaves much to be desired. The small numbers of the patients involve in the clinical trials calls into the questions the satisfactory results. The most experimental reviewed here involves around solid tumors and it has not been sufficiently demonstrated that non-solid tumors like hematological malignancies can be treated using exosome technology. And furthermore taking into the account that exosomal immunotherapy relies heavily on the immune system. The remains to be address of the issue of cancer patients those who are immune-compromised and or immunosuppresed due to chemotherapy and radiotherapy regimen and therefore might not be able to sufficiently surmount cancer cells with their immune system alone. The next major goal to increase the biological magnitude of immune response and this could be achieved by artificially coating and engineering exosome with tumor antigen to make it more recognizable to immune system. It has also been shown that heat shocked TEX alone as it confers a greater immunogenicity and elicits a greater response there by potentially making it more effective cancer vaccine. It has also been shown that when CpG adjutants are added alongside exosome therapy. T cells are more effectively primed. Exosomes can also carry cytokines, DNA, RNA, adjutants, labels, constimulatory signals, and gene therapy vectors ultimately it makes Trojan horse. Therefore engineering the production of exosome using nanotechnology embodies what we believe is the new platform for cancer vaccine of the production.

CONCLUSION

Compared with liposomes, nanoparticles, microspheres, micro emulsions and other synthetic drug loading systems, the endogeneity of exosomes is a natural and

unique advantage. The superiority of exosomes makes it an important medium for cell-to-cell communication, and it plays unique biological functions in regulating the normal life activities and in the diagnosis and treatment of diseases. Exosomes, as current research hotspots, have received extensive attention from researchers at home and abroad. Research on the biology and function and the potential application of exosomes increased exponentially the over the past decade. The superiority of the exosomes makes it an important medium for cell-to-cell communication and it plays unique biological function in regarding the normal life activities and in the diagnosis and the treatment of disease. Exosomes as a current research hotspot have received extensive attention from researchers at home and abroad. Exosomes can be used clinically as soon as possible depends largely on the results of optimization and improvement on the existing exosome problems. The utility of exosomes may further expanded since they are not found in mammalian cells but also in the different pathological microorganisms such as gram negative bacteria, eukaryotic parasites of the kinetoplast lineage and opportunistic fungal pathogens. Finally coupling the use of exosome in nanotechnology will most likely form the basis where novel nanoscale vaccines will be developed in the future.

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