Applications And Biological Functions of Exosomes: A Comprehensive Review

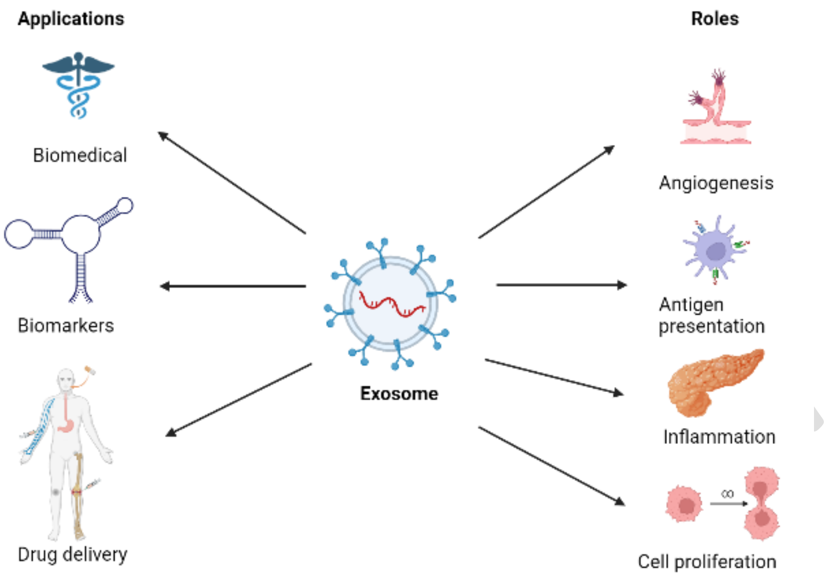
Comment [R1]: Small letter

Abstract:

Exosomes are also known as extracellular vesicles (EVs) which is bounded by a membrane mostly seen in eukaryotic cells secreted within the endosomal compartment along with some of the selected composition of RNA, proteins, lipids and DNA. They are capable of transferring signals among cells therefore it is used as a mediator for cell-to-cell communication. Exosomes helps in the excretion of cellular waste from the body. Exosomes possess various widespread activity in many of the biological functions such as transferring the biomolecules like enzymes, proteins, ribonucleic acid, lipids and also in the regulation of various pathological and physiological process in various diseases. Exosomes are released in to the in *vitro* growth medium with the help of cultured cells. They are said to be identified in coined matrix and tissue matrix. They are also identified in some of the biological fluids such as cerebrospinal fluid, urine, blood. Exosomes are considered as promising biomarkers in identification and treatment of many diseases as they contribute a lot in the diagnosis of various therapies. The efficacy and stability of imaging probes and therapeutics are enhanced by its biocompatible nature. Exosomes play a major role because of their use in the field of clinical application. It is important to understand the molecular mechanism behind their function and transport in order to explore more about exosomes. Here we discuss about the review and advancement done in the field of exosomes along with their biomedical applications, isolation techniques and biological functions.

Keywords: Exosomes, extracellular vesicles, inflammation, isolation

Graphical abstract:



Introduction:

Exosomes have the capacity of interacting with receiver cells and also to deliver and exchange the intercellular messages from one cell to another due to which they are noticed widely in the therapeutic platform [1, 2]. Communication between cells and with the other organ takes place using chemical materials with the help of extracellular vesicles (EV). Exosomes carry out organic compounds such as non-coding RNAs, RNA, genomic DNA and proteins [3]. The exosomal release takes place in various cell types when the combination of multivesicular bodies and plasma membrane takes place [7]. Exosomes are commonly seen in B cells, dendritic cells, T cells, mastocytes and platelets and they can be isolated from various body fluids such as plasma, urine, semen, saliva, cerebrospinal fluid, epididymal fluid, amniotic fluid, malignant and pleural effusions of ascites, bronchoalveolar lavage fluid, synovial fluid, and breast milk [8]. The method of isolation of EVs are more standardized and improved nowadays [13]. Whereas immune-blotting, protein staining and proteomic techniques are some of the techniques that are used for analyzing the vesicles that are isolated from EVs. Similarly exosomes are isolated by using some of the conventional techniques such as immune-affinity separation, precipitation, size exclusion, ultrafiltration, differential and buoyant density centrifugation [14]. The most commonly used method for isolation of exosomes in the cell culture media and the body fluids is differential and buoyant density centrifugation. Several companies have come up by developing quick, reliable and easy isolation kits in order to meet the need of huge sample volume of exosomes for conventional methods. The purity of exosomes are analyzed with the help of several validation and characterization methods so that it can be used for both clinical and research purpose. Some of the methods used for validation are enzyme-linked immunosorbent assay (ELISA), nanoparticle tracking analysis (NTA), resistive pulse sensing, scanning electron microscopy (SEM), dynamic light scattering (DLS), transmission electron microscopy (TEM), fluorescence-activated cell sorting (FACS), electrochemical biosensors and enzyme-linked immunosorbent assay (ELISA).[13,15] The proteins that originates from different cells of exosomes share a similar structure and function such as Rab GTPase, annexins, flotillin, tetraspanins, glycosylphosphatidylinositol-anchored molecules, SNAREs, cholesterol, Alix, Tsg101, sphingomyelin, and hexosylceramides.[14,16] The ATP-mediated activation of purinergic receptors[19], activation by lipopolysaccharides [20] and thrombin receptor activation [21,22] helps in the stimulation for releasing EVs [17,18,].

Comment [R2]: Correction

Exosomes are present in many of the bodily fluids which is indicated by their biological functions. Exosomes play a important role in viral infections and cancer as well as in neurodegenerative disease like Alzheimer's [9]. Cell communication, cell signaling for regeneration, differentiation and immune responses are said to be some of the unique properties of exosomes due to which they are considered to be a unique biomarkers. Exosome signaling is said to be found in viral replication [10]. Diseases like ovarian cancer, melanoma, glioblastoma, colon and prostate cancers consider exosomes as their ideal biomarkers [11]. Based on their ability of transferring certain elements they are considered as biomarkers in various infectious diseases. For example, the presence of hepatitis C virus infected exosomes is observed in human hepatocytes that was isolated from Huh 7.5 cell lines [12]. Exosomes are used in the process of diagnosing as they exhibit the property of natural shedder and their presence in various physio-pathological and biological actions. Here in this review we discuss the role of exosomes in the field of exosomes along with their biomedical applications, isolation techniques and biological functions.

ISOLATION OF EXOSOMES

Ultracentrifugation

Exosomes are isolated using different methods such as affinity capture on antibody-coupled magnetic beads, ultrafiltration, ultracentrifugation, polymer-based precipitation and chromatography.[23] Based on the type of sample the isolation technique is adopted which is explained in figure 1 [24]. As the process of isolation of exosomes, understanding their applications and mechanism in biomedical sciences is comparatively difficult. Whereas isolation of exosomes cannot be done by ultracentrifugation because it includes multiple overnight centrifugation steps, time-consuming, requires costly instrumentation and labor-intensive but Gurunathan et al isolated vesicles that are of high and low density using ultracentrifugation and density gradient ultracentrifugation [27]. Where he was able to isolate the purest exosome population with the help of density gradient ultracentrifugation when compared to precipitation-based and ultracentrifugation methods [25]. The exosomes are isolated by centrifugation methods because of their difference in size between each cell, different sub units of extracellular vesicles and the proteins present in it. Differential ultracentrifugation and density gradient ultracentrifugation are the two different types of preparative ultracentrifugation methods.

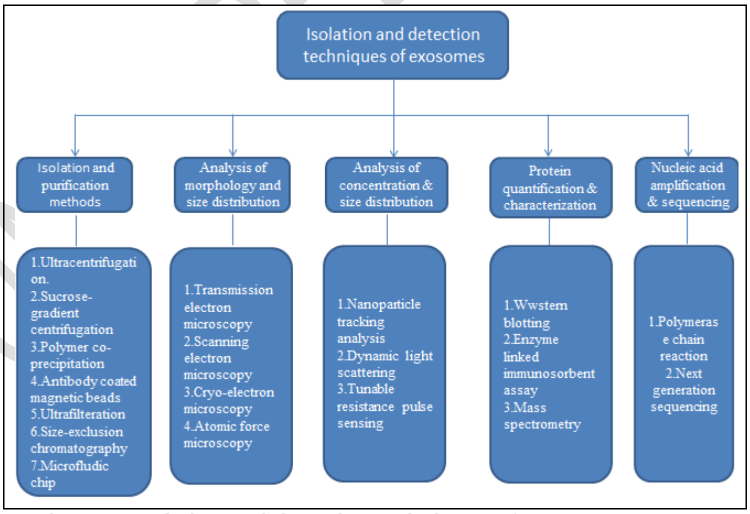


Figure 1: Isolation and detection techniques of exosomes

Where it is really hard to alter the separation process of exosomes because of their molecular density. Only large volumes of samples can be tested by ultracentrifugation. High speed centrifugal forces up to 1,000,000× g are required for the process of ultracentrifugation [26] Analytical and preparative are the two types of ultracentrifugation. Biological components, such as extracellular vesicles, viruses, bacteria and subcellular organelles are segregated with the help of preparative ultracentrifugation whereas the physico-chemical properties of selective materials is investigated with the help of analytical ultracentrifugation. This technique is used for isolation of exosomes from urine. Where the urine sample undergoes centrifugation for 10 mins for 2000 g in 4˚C then for 45 minutes for 17000 in 4˚C. Later the pellets are gained by ultra-centrifuging 110,000 g for 2 hrs in 4˚C. These pellets are stored at -80˚C after re-suspending it in PBS. This method is also used in isolation of EVs from milk. Where human milk and bovine milk are centrifuged at 3000 and 12000 rcf in order to remove the somatic cells, casein, fat globules, cellular debris which is later centrifuged at high speed. Once the fat and casein are removed the supernants collected are filtered at 0.2 µm and then centrifuged finally at 100,000 rcf. Later the pellets holding the EVs are resuspended in 600 µl PBS which is introduced to sterilized qEV size exclusion chromatography column.100 nm latex beads is used to test the single representative chromatography column and exosomes are separated into fractions using QC [28].

Size-Exclusion Chromatography, Polymer Precipitation and Size-Based Filtration.

Ultrafiltration (UF) is considered as the most essential method used for isolating exosomes based on their size which depends on the factors like molecular weight and size. Exosomes with standard exclusion limits for size and with standard molecular weight are isolated using membrane filters [29]. Ultrafiltration is said to be faster when compared to the process of ultracentrifugation where any costly equipment is not required for carrying out this process. Ultrafiltration (UF) and size-exclusion chromatography (SEC) techniques are used for preparing exosomes and EMVs of high purity[30]. Fractionation is done for the isolation of exosomes whereas it is difficult to remove the contaminating protein in ultrafilteration. Exosomes are isolated by segregation with the help of SEC with the help of columns packed with CL-4B- or Sepharose 2B-. Large extracellular vesicles and biological sample cells can be isolated with the help of membrane filters such as PVDF or polycarbonate that is of 50-450 nm pore size [31].

Exosomes can be isolated from proteins with the help of ultracentrifugation after sieving the large EVs and cells through the membranes by combining filtration method along with ultracentrifugation [32]. SEC allows proteins for the isolation of exosomes but they don't allow MVs, lipoparticles, protein aggregates or macromolecules for the isolation of exosomes. As the EVs passes through the physical barriers in chromatography or filtration technique their separation is done due to the difference in size. Combining SEC with ultracentrifugation results in great yield of enriched urinary exosomes when compared to the yield that is obtained by ultracentrifugation or ultrafiltration [33]. Exosomes can be instantly isolated from mesenchymal stem cells with the help of size-exclusion fractionation technique which shows great intact in TEM analysis. Exosomes holding sixe between 50 and 150 nm is collected and captured and then isolated by precipitation methods using slow, medium, high speed centrifugation upto 1500× g in ''polymer nets'',. Costly or specialized equipment are not required for these precipitation methods of exosomes. Hence, it is very easy to use and large sample sizes can be scaled using this technique as it exploits the existing technologies therefore it pays way for easy integration [29]. The polymer that is used for the isolation of exosomes should not react with the immune responses in-vitro or in-vivo hence it has to be made out from inert and harmless material eg: Polyethylene glycol (PEG). One of the major disadvantage of exosomes are there are other materials such as protein aggregates which are also mixed up with the exosomes which also gets isolated [34].

Immuno-Affinity Purification of Exosomes

Large quantities of proteins are present in exosomal membranes. By exploiting the interaction between receptors and their ligands as well as the interactions between the antibodies and the antigen (proteins) the exosomes are isolated using immunoaffinity (IP) method.[40] The proteins which lack the soluble compartments in the surface of exosomes can be isolated using this method. The isolation of exosomes are also done in biological components containing mixed population of biological fluids, cell culture and tissues. In this method the exosomes are isolated based on certain surface markers so that isolation of non-exosomal materials can be avoided from the complex mixture of overall population. Regular laboratory equipment are enough for this technique and it is easy and rapid. Antibody such as anti-CD9, -CD81 and -CD63 are captured and coupled with streptavidin coated magnetic beads in high affinity. These antibodies are used for isolating the exosomes. Exosomes where isolated from cancer cells of colon by Tauro et al using this technique which is found to be well organized than density gradient isolation and ultracentrifugation method [36,37]. Specific antibodies are used for quantifying and capturing exosomes in serum, urine and plasma. The yield obtained from immunoaffinity and ultracentrifugation where almost similar thus in orders to increase the yield submicron-sized magnetic particles are used in order to capture the exosomes. This resulted in showing 10-15 times higher percentage of yield when compared to morphology and biological activity. This may be beneficial for diagnosing and prognostic application for patients affected with acute myeloid leukemia (AML) one of the advantage of this method is they don't have limitation to volume [39]. High capturing efficiency and high sensitivity is seen in this immunoaffinity method done by using magnetic beads [40].

Microfluids-Based Isolation Techniques

Different kind of new techniques are used for producing exosomes of high purity for clinical purposes. Standard techniques face challenges like poor yield, low purity, expensive and difficult to standardize. Micro fluids dependent methods are used for the micro isolation, analysis and detection of exosomes by considering the biochemical and physical properties of exosomes. This method considers the separation properties such as immunoaffinity, size and density as well the new shorting mechanism such as nanowire-based traps, electromagnetic and electrophoretic manipulation. Nano sized displacement and viscoelastic flow are considered to be fast and consumes low quantity of samples and reagent [40, 41-43]. Immunoaffinity capture increases the specificity and capability in the micro fluid chip [44]. To differentiate exosomes and other cellular debris and EVs porous silicon nanowire on a micro polar structure was developed [45]. Cells 2019, 8, 307 8 of 37 exosomes with a diameter between 40 and 100nm were trapped by microfluidic device by filtering the proteins along with other cellular debris and extracellular vesicles. Microfluidic dependent immunoaffinity capture target the specific markers of EV subpopulation [43]. Exochip platform catches the circulating EVs. Micro surface coated with antibody efficiently removes the exocytic vesicles from the plasma membrane [47]. Sha0 et al [48] separated exocytic vesicles with the help of magnetic capturing beads and magnetic MF-IAC system.

Comment [R3]: Space mistake

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APPLICATIONS

Biomedical Applications

It has been studied that exosomes are one of the safest drug delivery system when compared to others including the exosomes that are derived from fruits and bovine milk [49]. Many studies are also done with murine milk and porcine in addition to bovine milk. The delivery vehicle that can be used in chemo preventive treatment is bovine milk [50]. Studies have proved that exosomes isolated from blood has the capacity to predict joint diseases in the early stage and the progression of the disease can be delayed and the joints can be repaired in addition to the stem cells.[51] Joint disease such as osteonecrosis of the femoral head (ONFH) along with femoral head ischemia is caused due to excess alcohol consumption and hip trauma which leads to dissociation of the femoral head and necrosis of the chancellors bone.[52] Later, it was observed that they gets vanished in the case of patients having rheumatoid arthritis (RA) and osteoarthritis (OA) which leads to the progression of joint diseases and the degeneration of cartilage. Kato et al. evaluated exosomes having a important role in the communication of articular chondrocytes and synovial fibroblasts (SFB) [54]. Normal chondrocytes is treated with interleukin 1 beta (IL-1β) after inducing exosomes which is derived from SFB which helps in down regulating the nucleus pulpous cells when compared to uninduced SFB exosomes. Several changes where considered in in vitro and in vivo models when IL-1β induced SFB is used to stimulate osteoarthritis [55]. Zhangh et al observed the characterization as well as the analysis of exosomes in patients who are affected from rheumatoid arthritis and osteoarthritis [56]. He observed that exosomes having membrane-bound TNF-α are seen only in RA patients but not in osteoarthritis patients. Which shows that TNF-α increases the production of SFB exosomes leaving a positive loop in the pathogenesis of rheumatoid arthritis (RA). The production of exosomes are said to vary with gender in case of OA patients. When Kolhe et al analyzed this gender variation between OA patients and in normal individual it was observed that men and women had varied content of miRNA present in the exosomes.[57] The therapeutic importance of exosomes in case of joint diseases are proved in many studies. It was reported by Cosenza et al. that in collagenase induced OA model a protective effect is exhibited by exosomes in the joint damage shown in figure 2 [58].

Comment [R5]: Correct it year ?

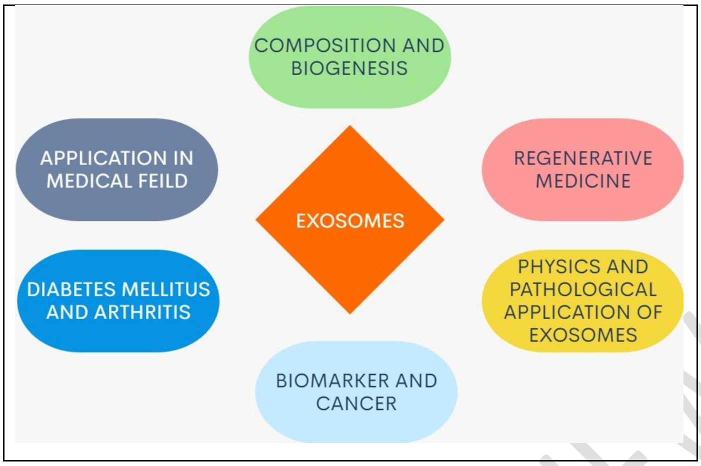

Figure 2: Role of exosomes in biomedical industries

Application of Exosomes in Drug Delivery

The mandatory characteristics of vesicles used for drug delivery are they should be capable of encapsulating the required amount of drug in order to meet the therapeutic effect, must be stable enough to hold on with their size, structure and properties while circulating in the blood stream until they reach the target organ and should not undergo any interaction which possesses toxicity, non-immunogenic and biocompatible with the immune response shown in figure 3 [59]. Exosomes which meets the above criteria can be used in biomedical application. It was observed that the exosomes that are isolated from brain-specific proteins are capable of transferring drug across the blood-brain barrier [60]. Exosomes loaded with activator of transcription and signal transducer inhibitor or delivery of curcumin (Exo-cur) from nose to brain is used in the therapy for brain inflammatory diseases [61]. Investigators have concluded that the exosomes loaded with the drug protects them from the inflammatory brain diseases in animal models. Exosomes that are derived from bone marrow MSC where tested for delivery of drug in functional anti-miRNA-9 to tumor cells and it was observed that the relation between the brain glioblastoma cells and MSCs where mediated by the exosomes [62,63]. Exosomes are said to possess high longevity but they do not show effective immune response in mice. Exosomes can be easily identified from the other microvesicles, heat shock proteins, tumor-induced gene 101 (Tsg 101), apoptotic body, lysosomal proteins, annexin, flotillin, and various receptors in the endosomal pathway due to the presence of some specific protein [64]. The unique liquid composition is seen in exosomes due to the presence of dominant cholesterol and diacylglycerol content which helps in their easy identification [65].

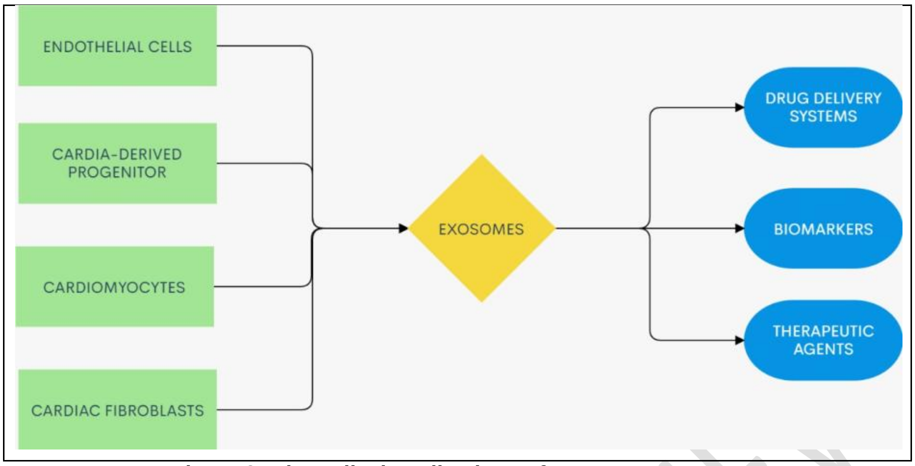

Figure 3: Biomedical applications of exosomes

Applications of Exosomes as Biomarkers

Exosomes reflects the actual characteristics if the cells from which they originate and they act as a cargo carrier which also involves in cell communication. The stability of exosomes in biological fluids such as urine, cell culture media blood and saliva is maintained and the biodegradation of the content is prevented by the presence of lipid bilayer. Exosomes carries proteins and nucleic acid from the cells of the host which is used as an indicator in pathophysiological conditions. Various diseases including ovarian cancer, melanoma, glioblastoma, prostate and colon cancers along with neurodegenerative disorders consider exosomes as their ideal biomarkers.

Therapeutic Applications of Exosomes

The actual ability of exosomes where not predicted during the time of discovered at 1983. Whereas today, they are looked up for their infinite potential in the field of biomedical application. They can transfer signals from recipient cells and deliver their content, thus mediating a standard mechanism in the communication between the cells either having direct contact or without any direct contact with the cells. The primary features of exosomes make them capable of reducing inflammation across the blood brain barrier, increase the neural and motor functions and also helps in multiple dosing without any side effects. They have the capacity of influencing the pathological and physiological processes. Various studies proves that exosomes are considered to be novel therapeutic reagents [66]. Where models having diseases such as respiratory, neurological, cardiovascular, hepatic, musculoskeletal, dermatological, renal and gastrointestinal disease are tested with the exosomes that are derived from MSCs [66]. Anti-inflammatory action is exerted by inhibiting the pro-inflammatory cytokines expressions in MSC derived exosomes which enhances the extracellular remodeling by promoting tissue regeneration [67-71]. Similar therapeutic effects are also observed in exosomes that are isolated from induced embryonic stem cells, cardiac progenitor cells and pluripotent stem cells [72-74]. The EVs derived from MSC resembles the cytoprotective and immunomodulatory of their parent cells [75]. Whereas exosomes that are derived from bovine milk reduces arthritis [76]. A cancer-specific T cell response is seen when exosomes that is derived from DC incubated with cancer antigen is used [77,78]. Exosomes derived from bone marrow MSC protects from various disease conditions such as reperfusion injury or myocardial ischemia, brain injury and hypoxia-induced pulmonary hypertension [79]. EVs derived from the umbilical cord of human MSC protect from sudden liver fibrosis and renal injury [80,81]. Tumor cell-derived exosomes helps in the stimulation of immunosuppression by promoting the apoptosis of T cell, suppressing the NK cytotoxicity and dendritic cell differentiation by stimulating the immunosuppressive regulatory T cells and myeloid suppressor cells [82,83].

BIOLOGICAL FUNCTIONS OF EXOSOMES

Fibroblasts, neurons, tumor cells, adipocytes, and intestinal epithelial cells are the cells from which exosomes are released which is seen in many of the biological fluids such as malignant effusions of ascites, synovial fluid, breast milk, saliva, urine, blood and amniotic fluid. The origin of the cell, process of the originating tissue or the time at which the exosomes gets generated decides the function, heterogeneity and biology of exosomes. Expelling out the nonfunctional or excess cellular components is said to be major function of exosomes. Signaling molecules and cell surface proteins are recycled by involving endocytic vesicles in it [84,85]. Exosomes plays a major role in many of the biological processes such as coagulation, intercellular signaling, cellular homeostasis, antigen presentation, apoptosis, inflammation and angiogenesis which helps them in the transfer of lipids, RNA, enzymes, and proteins which affects pathological and physiological processes in various diseases that includes neurodegenerative diseases, cancer, autoimmune diseases and infections.

Role of Exosomes in Angiogenesis

New capillaries are formed from the existing blood vessels and mediation is done by cellular events after several complex multistep process which is termed as angiogenesis [86,87]. Multicellular eukaryotes are integrating by complex signals due to the significance of intercellular communication. The T lymphocytes and vascular endothelial cells interact closely during the trans-endothelial migration and recirculation process. Angiogenesis is promoted by positive CD105 MVs which is released from stem cells of renal cancer in humans. Cell proliferation [90], angiogenesis [88], endothelial cell migration [89] and tissue vascularization [91] is simulated by exosomes that is released from progenitor cells. The exosomes derived from stromal cells/mesenchymal stem (MSCs) is loaded with many types of miRNAs, such as miR210, miR132, miR21 and miR126 which plays a major role in angiogenesis [92]. Proliferation and progression of cell cycle like miR-21, miR-222, let-7a, miR-191, induce EC differentiation like miR-6087 and modulate angiogenesis like miR-21, miR-222, let-7f is regulated by exosomes that is derived from MSC which is loaded with multiple miRNAs [93]. The second-degree burn injury in the skin is repaired by exosomes that is derived from mesenchymal stem cells of human umbilical cord that enhances angiogenesis [94]. Atienzar-Aroca et al. reported that retinal pigment epithelial cells are said to release large numbers of exosomes in high levels species of oxygen which induces the angiogenic processes containing expression of VEGFR mRNA at high levels [96]. Angiogenesis, drug resistance, and metastasis is promoted by stem cells of cancer which are resident in stem cells of tumor and non-homing whereas the tumor progression and growth in various cancer types are regulated by various cancer cell populations through local interactions. Physiological processes such as proliferation, vascular smooth muscle cells, tube formation of endothelial cells and migration undergoes angiogenesis [97,98,99]. The current therapeutic impact in the treatment of ischemic diseases shows the involvement of exosomes in differentiation, enhanced blood flow restoration, capillary network formation and neovascularization [101].

Role of Exosomes in Apoptosis

Inflammation, infection, autoimmunity, and cancer are some of the diseased condition in which apoptosis gets regulated in normal healthy cells. Cell viability is caused in malignant disease in which oncogenic mutations leads to the imbalance of homeostatic conditions. The excretion of the dying cell happens when morphological changes happens in apoptotic cells which is recently classified into three main morphological steps such as formation of a thin membrane protrusion, where the size of the apoptotic bodies ranges from 1 to 5 µm [102]. An apoptosis vesicle which is dependent and in which a wide variety of cellular organelles and bioactive molecules can be encapsulated is known as apoptotic body [103]. Key signals send helps the neighboring tumor cells in promoting proliferation, inhibit apoptosis and metastasizes with the help of EVs(<100 nm) derived from stromal cell that is released due to cell stress[104]. The Bcl-2 pathway is affected by the exosomes that is isolated from liver metastases in colorectal cancer (CRC), carrying miR-375[105]. It is proven that exosomes derived from BM-MSC induces cell cycle arrest and apoptosis in HepG2 cells and suppression of tumor in SCID mice [106].

Role of Exosomes in Antigen Presentation

The inflammatory response is mediated by the signaling of exosomes in bio-macromolecules which also includes short and long non-coding and coding proteins, lipids and RNAs. The exosomes which is released from the APCs have the capacity to take over the therapeutic benefits in which the immune response is stimulated followed by presenting and carrying major peptide, histocompatibility functional complexes that modulate T cell responses which is antigen-specific. Exosomes derived from the dendritic cell (DC) as well as from macrophages, and DCs exhibits immune stimulatory and immunosuppressive properties respectively by T and B cells activation [107]. The damage-associated or pathogen-associated molecular partners is recognized by the immune system that is activated by limited receptors [108,109]. The inflammatory gene expression course is regulated by temporary epigenetic regulations in the recipient cells which is induced by exosomes having pre-miRNAs and siRNAs [110]. Noncoding RNA populations and differential bimolecular signatures is seen in mouse macrophages and a human-derived monocytic cell line such as THP-1 cells , RAW 264.7, lipopolysaccharide (LPS) are seen after stimulation. The physiological condition of the originating cells and the species influences the secretion of exosomes. RAW 264.7 cells stimulated by LPS derived exosomes exhibits high chemokines and cytokines levels due to the release of extracellular vesicles into the immune synapse [111]. Both MVs and exosomes are released from activated DCs facilitates the DC-T cell interactions and EVs that are derived from DC contains tumor- specific antigens which is loaded in both MHC-I and MHC-II and stimulatory molecules that is found to make the cancer immunotherapy more effective[112,113,114].

Role of Exosomes in Inflammation

Tissue homeostasis is restored by white blood cells which initiates the immune response such as inflammation against any infection. Any of the untreated or uncontrolled inflammation leads to chronic inflammat1ory states resulting in tissue damage or diseases[115]. Where systemic inflammation has an major role in the pathogenesis of several diseases. Exosomes play a vital role in the inflammatory process of many pathologic states such as cancer, type 2 diabetes, inflammatory bowel diseases, rheumatoid arthritis, neurodegenerative diseases and obesity [116]. Tumor development, resistance to therapy and immune surveillance shows exosome-mediated inflammatory responses in all the stages[117,118]. Gastric cancer cells derived exosomes can promote the proliferation and tumor cell migration is increasing the level of inflammation factors followed by the stimulation process of NF-κB pathway in macrophages[119]. The secretion of pro-inflammatory cytokines, such as interleukin 6 (IL-6), granulocyte colony- stimulating factor, chemokine C-C motif ligand 2 (CCL2) and tumor necrosis factor alpha (TNFα) is induced by the circulating exosomes. A toll-like receptor mediates the pro-metastatic inflammatory response which is triggered by the exosomes containing miR-21 and miR-29a. Abusamra et al. observed that proliferation of T-cell is inhibited and apoptosis of T-cell is induced through the FasL pathway by the exosomes derived from prostate cancer cells in human. The T cell differentiation and proliferation is influenced by the exosomes that are derived from tumor when the MARK1 signaling pathway is down regulated. Improper maintaining of intestinal homeostasis may lead to chronic disorders such as Inflammatory bowel diseases (IBDs). The pro- inflammatory cytokine level and the level of IL-8 is increased when exosomes are introduced to human colonocyte cell line. The presence of EVs promotes thrombosis, immune-mediated disease and inflammation. Increased level of mRNA in the CCL2 and inflammatory chemokine in exosomes can lead to inflammation. Tubulointestinal inflammation along with enhanced macrophage migration and inflammatory response is seen in mice when CCL2 is injected to it but injecting CCL2 to humans resulted in proteinuria in nephropathy patients.

Role of Exosomes in Cell Proliferation and Differentiation

Ligand concentration, the integration of diverse signaling pathway and receptor expression takes place with the help of cell-cell communication[120]. Where exosomes that are secreted acts as mediators in the communication between the cell in case of pathological and physiological situations. They play an important role in the growth of tumor and in capturing angiogenesis associated with tumor. Thrombus formation and metastasis is promoted along with which expression of angiogenic factors, tumor chemotaxis, proliferation and invasion is induced by the exosomes that are derived from platelet. The role of exosomes in various biological activities such as immune function, the development and differentiation of stem cells as well as in intercellular communication is vital.

Exosomes and Receptor Mediated Endocytosis

Physiological and pathological functions are generally mediated by exosomes by transferring information among the cells and by carrying different types of functional molecules. Since exosomes exhibits various biological functions they are still observe as their role in case of receptor-mediated endocytosis and their need in uptake mechanisms is still unknown. Phagocytosis, clathrin-mediated endocytosis, macropinocytosis, clathrin and caveolae-mediated endocytosis are some of the pathways involved in receptor-mediated endocytosis. The internalization of the erythroleukemia derived exosomes is done by phagocytosis or macropinocytosis whereas internalization of glioblastoma derived exosomes is done by lipid raft-mediated endocytosis which is regulated negatively by caveolin-1. Delivery of miR21 is done by exosomes which is derived from PC12 cell through clathrin-mediated macropinocytosis and endocytosis. It was reported that vasorin that is transferred through exosomes is said to promote migration in the umbilical vein of humans in endothelial cells through receptor-mediated endocytosis of exosomes. Exosomes derived from dendritic cell delivers the content with the help of fusion or semi-fusion of both the membranes after binding with the plasma membrane.

CHALLENGES FACED WHILE USING EXOSOMES

Large numbers of benefits are obtained from exosomes in the field of therapeutic application at the same time exosomes possesses various biological and technical challenges. One of the primary challenges of exosomes are producing sufficient quantities of exosomes where minimum of 10-100 µg of exosomes is necessary to produce an optimized dose. But, the yield obtained from the culture medium is 1 µg which is very less on comparative bases. Whereas the exosomes that is isolated from biological fluids are observed to be contaminated. Variable as well as the yield obtained is also very low. Due to which standardizing low quantity of EV analysis is considered as the biggest challenge. During the process of exosomal production and isolation factors such as cell culture matrices and plastics, mycoplasma, cell passage, the culture medium composition, cell confluency and viability along with volume and other microbial contamination status should be considered for optimization. Bioreactor is used to obtain exosomes in large scale up to 5-10 folds. It has been proved that the production of exosomes can be increased by inducing physical, chemical, and biological stress but in this case the therapeutic safety and efficacy has to be completely evaluated because contamination with apoptotic bodies is possible. Certain conventional methods such as ultracentrifugation, ultrafiltration, SEC, immunoaffinity, aqueous two-phase systems, microfluidic devices and polymer-based precipitation are modified or new techniques are developed in order to enhance the purity and production of exosomes. Selecting the culture media has a major role while developing the exosomes production method. For example, several impurities are seen in exosomes that are isolated from a culture media having serum in it but altered EV secretion in observed when stress is induced to the cells in serum free conditions. Though large quantity of exosomes can be produced with the help of the current technologies one of the major challenge faced is their uniformity and quality. The method used for isolation determines the physicochemical properties and purity. For example, ultracentrifugation and precipitation are the most commonly used methods but due to various factors such as varying composition, size, subpopulations, aggregations this method is not used in case of therapeutic applications. It is very challenging to store the isolated exosmes for biological activity so exosomes are stored at −80 °C after suspending it in phosphate-buffered saline and trehalose is added in order to prevent exosomes from cryodamage. Optimization is an important technique in order to isolate pure exosomes so that specific subpopulations can be reduced and side effects that is caused due to contamination can be reduced so that therapeutic efficacy can be enhanced by modifying the overexpression of miRNAs.

CONCLUSION

Extracellular vesicles possess various roles in pathological conditions where exosomes have a important role in the communication within the tumors and the targeted cells. The exosomal release is required for certain process such as targeting therapeutic agents to particular cells and gene transfer. The exosomal composition, content and release depends on the nature of the origin cell. The standard existing procedures that are used for the isolation and characterization of exosomes are mostly preferred inspite of having several new technologies that are developed for diagnostic and prognostic purpose. Here, we have reviewed the isolation techniques, biological functions, applications in various diseases and the challenges that are faced during the use of exosomes. The biogenesis of exosomes uses various lipid and protein compounds which is based on the homeostasis of cell and cell type. The exosomal parameters such as the number and size is analyzed using NTA and DLS methods. The method that was considered to be ideal for analyzing the structural features of exosomes is electron microscopy. Where inspite of this several other procedures are to be followed for the characterization and isolation of exosomes. Thus, the selection of the isolation methods plays an important role in order to obtain exosomes that are of standard quality based on which the results are validated. One of the common challenge that is faced in the detection of exosomes is differentiating the exosomes that is isolated from normal cells and the one which is isolated from cells under pathological conditions. Inherent heterogeneity is one of the another challenge that has been faced. Developing various quantification techniques is necessary to differentiate various subtypes of exosomes in heterogeneous samples which can make the process of quantification and detection easier. Where one main feature that is to be considered during the development of new method should be the ability to isolate different vesicular subpopulation that are present in exosomes. These are some of the limitations that are to be overcomes that can helps us to understand exosomes better and facilitate the development of new novel therapeutic techniques.

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