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Stem Cells and Exosomes-associated Therapeutic Applications

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Abstract: Exosomes are extracellular vesicles with sizes from 30 to 150 nm in diameter and modulate the transport of multiple intracellular biological molecules including proteins, nucleic acids, lipids, and metabolites. They regulate a large number of cells and are involved in different pathological and physiological activities including carcinogenesis, viral infection, cell-cell communication and immune responses as well. Stem cell-derived exosomes carry many benefits over simple stem cells in the form of easy access, freedom from tumourigenic capabilities, non-infusion toxicity, effortless preservation, and immunogenicity. Exosomes have almost the same properties and perform functions effectively in the same way as their parental cells do like adult stem cells and embryonic stem cells. Due to their pluripotent or multipotent abilities, stem cells (SCs) transform into several types of cells. In addition to other secretions, SC also give exosomes, which in turn shows therapeutic significance for many disorders, including cancer, diabetes mellitus, skin allergies and regenerative medicine. Exosomes originating from mesenchymal stem cells (MSCs) have miRNAs, lipids, and proteins that trigger diabetes and cancer situations in humans. Exosomes from SCs (sc-exos) are preferred to SC as there are fewer side effects and other challenges, including effectiveness, drug delivery, lower immunogenicity and tumourigenicity. In the current review, we summarize the data from the last 5 years' articles about exosomes and stem cell-derived microvesicles for the therapeutic potential of various diseases such as cancer, Alzheimer's disease, diabetes, and Parkinson's disease with clinical challenges and future aspects.

Keywords: Stem cells; Exosomes; Therapeutic; Cancer; Parkinson; Diabetes

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1. Introduction

Exosomes are lipid bi-layered, round, extracellular vesicles that measure between 30 and 150 nm in diameter. They function as communicators between cells. Because exosomes from a particular cell type produce sets of biomolecules that are exclusive to that cell or distinct to that cell, exosomes have been viewed as miniature replicas of their parent cells. Exosomes are little membrane vesicles that develop inside of cells known as multivesicular bodies. These multivesicular entities merge into the cell membrane, releasing them. Proteins, lipids,

different RNA molecules (mRNA, microRNA), and even DNA are among the many biological components they contain. They can transfer their payload to target cells through physiological fluids like blood and cerebrospinal fluid [1]. As a result, cells can communicate with one another and affect the actions of faraway cells. The particular chemicals that exosomes carry can affect several processes within receiving cells. This involves controlling the immune system, development, distinction, and even the spread of cancer. Exosomes are promising biomarkers for many disorders due to their distinct molecular profile [2]. Exosome transport analysis performed on body fluid isolates may provide information on how diseases develop and how well treatments work. Exosomes are being investigated as potential drug delivery carriers because of their cellular targeting capabilities and biocompatibility. Therapeutic compounds can be directly delivered to damaged cells using engineering them [3]. An interesting strategy for gene therapy is provided by exosomes. They could be used to treat hereditary illnesses by being injected with corrected genetic material that targets particular cells. Exosomes have been shown in studies to support tissue renewal and healing [4]. Conditions like wound healing, neurological illnesses, and cardiovascular disorders can be treated with them.

A collection of undifferentiated cells known as stem cells possesses the extraordinary capacity to grow themselves and regenerate functioning tissues. Stem cells could be divided based on totipotent, pluripotent, multipotent, or oligopotent. There are unavoidable drawbacks to stem cell therapy, including infusion toxic effects, immunogenicity, tumourigenic possibility, and ethical difficulties [5]. Exosomes, which are released by nearly every type of cell, including stem cells, have been proposed as an improved, adaptable and safe substitute for stem cell treatments. Exosomes need to be processed and modified in terms of synthesis, cleansing, and modification before being used in therapeutic settings. In recent years, a large number of medical studies examining these upstream exosome therapeutic approaches have been published. However, several research directions are still unexplored. For example, there is a dearth of systematic studies devoted to downstream therapeutic applications, particularly from a surgical standpoint [6]. Surgery is an ideal setting for stem cell-derived exosome therapy because injured tissues react both inflammatory and regenerative, depending on whether the harm was caused by an illness. Exosomes generated from stem cells bear therapeutic benefits akin to those of their parent cells, such as immunomodulation, tissue repair, and anti-inflammatory properties [7].

2. Exosome's composition, structure, storage, and mechanisms of action

Exosomes are produced by late-stage endosomes and result in the formation of intraluminal vesicles (ILVs) inside big multivesicular bodies (MVBs). Certain proteins get incorporated into the budding membrane during ILV development, and cytosolic elements get enclosed in ILVs. ILVs fuse with the plasma membrane and are then discharged as exosomes into the extracellular environment. The late endosomal sorting complex required for transport (ESCRT) must be present for ILV production to occur within late endosomes. The ESCRT system, which consists of four separate complexes (ESCRT-0 to ESCRT-III), works together to support vital functions such as vesicle budding, protein transport organizing, and MVB production [8,9].

Lipids are well known to be important for the structure and shape of cell membranes. Neutral sphingomyelinase, the enzyme that produces ceramide, and exosomes released from mouse oligodendrocytes are related. The research demonstrated that the introduction of synthetic ceramide can promote MVB budding through metabolism into sphingosine 1 phosphate (S1P), which then attaches to the S1P receptor on MVBs to induce the formation of ILVs. Additionally, phospholipase D2 was shown to be the effector molecule linked to the small GTPase ADP-ribosylation factor 6 (ARF6). ARF6 is essential for MVB budding and the subsequent production of exosomes [10].

Tetraspanins are also involved in the exosome-generating procedure, which is independent of ESCRT. These proteins are known to play a part in the organization of cellular membrane microdomains. Tetraspanins group collectively and interact with proteins located in the cell's internal signalling system as well as the membrane to bring about this arrangement. Tetraspanin-enriched microdomains are these specialised regions that work well as cargo platforms [11]. Interestingly, β-catenin exosomal secretion is enhanced when tetraspanins CD9 and CD82 work along with e-cadherin. Another ESCRT-independent mechanism functions via the receptor for epidermal growth factor (EGFR). According to a recent study, EGFR was detected in exosomes extracted from cancer patients' serum, suggesting that the ESCRT complex may not be the only entity in charge of exosome production. Furthermore, the study demonstrated that cells could import ubiquitin particles that lacked EGFR when they were experiencing serum deprivation. Surprisingly, it was shown that these EGFR molecules colocalized with CD63-positive MVBs that were influenced by *RAB31* [12].

2.1. Structure and composition of exosomes

Exosomes are incredibly extensive and versatile, comprised of a wide range of constituents such as 4400 proteins, 1639 messenger RNAs (mRNA), 764 miRNAs, and 194 lipids. Among the important proteins that are widely found in exosomes are tetraspanins, specifically CD9, CD63, CD81, and CD82. These proteins have significance for activities such as cell entry, expansion, and fusion. Furthermore, exosomes contain heat shock proteins (HSP70 and HSP90) that support stress responses by attaching and presenting antigens. In addition, several proteins that are abundant in MVBs-like tumour susceptibility gene 101, heat shock protein 70, CD81, and CD63 could be used as markers to distinguish various exosomes [13,14].

Exosomal elements are constantly altering based on the state of the cell meaning that they do not remain constant. For example, the exosomes of several cancer types including pancreatic, breast, renal, melanoma, laryngeal, gliomas, colorectal, oral, and ovarian cancers, showed a significant increase in a specific microRNA, microRNA (miR-1246). A different study demonstrated the 100% sensitivity and 80% specificity of miR-1246 in the diagnosis of colorectal cancer (CRC). Notably, miR-1246 performs better than conventional CRC tests like cancer antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA), both of which have far poorer precision and accuracy [15]. Certain proteins enclosed within exosomes have been linked in studies to the detection and prognostic of breast cancer. When comparing small EVs (sEVs) from breast cancer individuals to normal individuals and those with benign breast tumours, a meta-analysis revealed considerably increased quantities of carrier compounds, including human epidermal growth factor receptor 2 (HER2), CD47, Del-1, miR-1246, and miR-21. Tumour relapse and distant organ metastasis have been linked to several proteins, including HER2, CD49d, KDR, and CD44. Depending on where they originate, some exosomal elements may be glycosylated. There are notable distinctions between exosomes formed from tumour versus non-tumour cells due to the glycosylation patterns of exosomes reflecting those of their original cells. Making use of these variations, exosomal glycosylation variations can be employed as extremely unique and easily identifiable biomarkers for illnesses like cancer [16].

2.2. Storage of exosomes

Currently, cryopreservation, lyophilization, and spray-drying are the three primary retention techniques employed for the long-term storage of exosomes. In cryopreservation, temperature and antifreeze are the two most crucial components. The storage of exosomes at 4 ℃ may cause a decrease in their biological function and protein material, while -80 ℃ is thought to be the ideal temperature that has the least effect on their morphology and content. The best option is non-permeable disaccharide antifreeze, particularly trehalose since it inhibits

cryodamage and exosome polymerization. Heat-sensitive substances could be readily preserved and regenerated with water, such as exosomes and vaccines, which are processed by lyophilization or freeze-drying [17]. According to the latest research, even if maintained at normal temperature, lyophilization with cryoprotectant might maintain the activity of exosomal proteins and RNA for about four weeks. Finally, because spray-drying is a one-step procedure as opposed to freeze-drying, it requires less expensive machinery and time-consuming multi-step milling. Exosome protection and carrier integrity, however, can be impacted by key spray-drying factors such as exosome feeding rate, atomization pressure, and outlet temperature.

2.3. Uptake and internalization of exosomes by cells

Exosome uptake happens quickly and is temperature-dependent, with lower temperatures causing less absorption. Furthermore, a variety of common endocytic mechanisms, such as lipid rafts, phagocytosis, macropinocytosis, and clathrin-mediated endocytosis (CME), are used to carry out internalization. The formation of transmembrane receptors and ligands is a characteristic of CME. This method entails the involvement of a triskelion scaffold called clathrin in the production of clathrin-coated vesicles. After the vesicles are ingested, they uncoat and then combine with endosomes to make it easier for them to enter into cellular processes. A variety of kinds of cells demonstrate the importance of CME in exosome absorption. This demonstrates how this route is involved in both normal and pathological settings [18]. Additionally, cancer cells that overexpress the transferrin receptor, an attached protein linked to CME, show increased exosome absorption, suggesting that changes in receptor activity may affect how well cancer cells internalize exosomes.

The capacity of phagocytosis to absorb several kinds of particles, including bacteria, is well known. But this biological activity can also involve the absorption of tiny organizations, such as exosomes, and is not just confined to bigger fragments. Immune cells that ingest exosomes mostly use phagocytosis, especially dendritic cells and macrophages. The bending of the cell membrane, which encloses the extracellular particles to form phagosomes, is the first step in the phagocytic mechanism. The internalized product is then guided in the direction of lysosomes. Notably, phospholipase C (PLC) and phosphatidylinositol-3-kinase (PI3K) are important enzymes that aid in phagosome closure [19]. A crucial biological process known as macropinocytosis uses actin-driven lamellipodia to start the plasma membrane's forward penetration, which creates intracellular spaces known as macropinosomes. It's noteworthy to note that macropinocytosis is crucial for exosome internalization as well. Furthermore, studies have shown that the uptake of modified exosomes intended to target oncogenic kirsten-RAS is facilitated by rat sarcoma virus RAS-mediated macropinocytosis. These results advance our knowledge of the various roles and regulatory processes of macropinocytosis, including its involvement in the import and absorption of exosomes [20].

3. Exosome-mediated intercellular communication

Because parent cells may discharge such vesicles, which can interact and affect the intended cell activity, exosomes have lately come to light as a unique form of interaction between cells. The transfer of genetic material happens via two main pathways: (1) direct internalization and (2) receptor-ligand interactions, which primarily facilitate this interaction. When it comes to receptor-ligand interactions, exosomes have surface proteins that attach to particular target cell receptors and start signalling pathways that alter biological processes. For example, TGF-β-loaded exosomes can influence cell multiplication by activating the receiving cells' suppressor of mothers against the decapentaplegic (SMAD) signalling pathway. Providing another option, direct internalization enables the functional proteins, RNAs, mRNA, miRNA, and transcription factors found in exosomes to be transported to the cytoplasmic of the target cell. This may result in the amplification of signalling networks

that are essential for cell survival, advancement, and division, such as the PI3K/protein kinase B (Akt) and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways [21]. Moreover, exosome-mediated delivery of miRNAs can modify the integrity and translation of mRNA in recipient cells, as demonstrated by the control of PTEN expression by exosomal miR-21, which triggers the PI3K/Akt pathway. Exosome-derived bioactive chemicals influence target cells via several mechanisms, such as the transfer of activated receptors and the production of epigenetic modifications, highlighting the intricacy and precision of exosome-mediated communication [22].

4. Exosomes from adipose-derived stem cells

Adipose-derived stem cells (ADSCs) are well known for their various differentiation capacities and readily available tissue sources. These stem cells possess multipotency, meaning they can differentiate into multiple cell types such as chondrocytes, myocytes, adipocytes, and osteoblasts. Exosomes from ADSCs are, therefore, extremely useful for tissue regeneration, particularly in the repair of bone fractures and limb ischemia. An adipose tissue sample is initially obtained by liposuction to extract ADSCs. After that, an enzymatic procedure employing collagenase is used to extract these cells from the adipose region. Following this digestion, the tissue is separated from the ADSCs by centrifugation. Surface markers, including CD29, CD44, CD73, CD90, and CD105, are commonly utilized as positive indicators to verify the authenticity of ADSCs, while hematopoietic markers like CD31, CD34, and CD45 are frequently indicated as negative indicators. After being separated, ADSCs are grown in specific growth media and gradually release exosomes into the medium. After that, the exosome-rich media can be collected and centrifuged to get rid of any leftover cells or debris. To ensure optimal exosome production, the pelleted exosomes are resuspended and refined utilizing methods such as size-exclusion chromatography, owing to the assistance of an ultracentrifugation procedure [23].

The size range of exosomes obtained from ADSCs is usually between 30 and 200 nm. When examined under a transmission electron microscope (TEM), they have a characteristic cup-like shape. Studies investigating these exosomes in the setting of lower limb ischemia revealed the absence of the exosomal indicators GM130 and β-tubulin, as well as the existence of the exosomal markers CD9, CD63, and CD81. Remarkably, new research has demonstrated the function of the microRNA miR-125b-5p, which is present in ADSC-exos, in the restoration of ischemia-damaged muscle tissue. In ischemia situations, miR-125b-5p functions as a critical regulator, principally by directing and regulating the function of a particular protein called alkaline ceramidase 2 (ACER2). When elevated, ACER2 has been associated with increased generation of ROS, as seen in models of diabetic hindlimb ischemia [24]. Thus, miR-125b-5p's modulation of ACER2 raises the possibility of a treatment option for the therapy of muscle damage in ischemia patients, particularly those with diabetes. Moreover, adding miR-125b-5p to C2C12 cells (a myocyte variant) promotes both cell migration and proliferation. This action is especially noteworthy since it directly inhibits the detrimental overexpression of ACER2, laying the groundwork for miR-125b-5p's ability to target ACER2 and treat ischemic muscle injury in patients with diabetes. Additionally, ADSC-exos stimulate angiogenesis in human umbilical vein endothelial cells (HUVECs) and facilitate the proliferation and migration of C2C12 cells. ADSC-exos have been demonstrated in clinical trials on living beings to protect ischemic skeletal muscle, promote vascular renewal, and speed up the recovery of muscle injuries. According to these procedures, miR-125b-5p is the key component [25]. Another research study that looked at the impact of ADSC-exos on the recovery of nonunion bone fractures in diabetic rats discovered that ADSC-exos significantly accelerated the bone recovery mechanism. The Wnt3a/β-catenin signaling pathway, which encourages osteogenic development in bone marrow-derived stem cells (BMSCs), maybe the cause

of this increase. Nevertheless, the specific exosomal element and method underpinning this remain to be determined [26].

5. Exosomes from bone marrow-derived mesenchymal stem cells

Like other MSCs, BMSCs are multipotent, which allows them to differentiate into a variety of mesodermal cell lineages. A bone marrow sample requires a demanding and unpleasant aspiration procedure, and the sample volume needed is usually rather large, between 20 and 50 mL. Due to new developments, this demand has been significantly reduced to about 6 mL. This progress can be attributed to an extraction technique called red blood cell (RBC) lysis, which boosts productivity by permitting the capture of the same, if not higher, quantities of MSCs with a significantly reduced volume of blood marrow. Additionally, the duration required to produce the therapeutic range of MSCs from the aspirate was consistent for both large and small-volume contributors. Ammonium chloride is used in RBC lysis, a quicker and more uniform method that separates MSCs from RBCs without changing the stem cells' makeup. Research has indicated that low-volume aspirates are linked to fewer problems, including less haemoglobin loss, from the patient's perspective. Moreover, this provides a more effective approach to producing more MSCs. After the bone marrow is extracted, usually from the iliac crest or the metaphysis of the proximal tibia and distal femur, enzymatic digestion and centrifugation are used to isolate the BMSCs among different kinds of cells [27]. The identification of BMSCs is verified by particular surface indicators, namely lower expressions of CD34 and CD45 and elevated levels of CD73, CD90, and CD105. It is possible to collect exosomes for additional analysis after culture BMSCs. Nanoparticle tracking analysis (NTA) has been applied to analyze isolated exosomes. The average diameter of these BMSC-derived exosomes, or BMSC-exos, was 90 nm, with a range of 30 to 100 nm. TEM showed their discoid form. Exosome markers (TSG101, CD9, and CD63) were shown to be highly expressed in BMSC-derived exosomes, in addition to elevated expression of miR-96. The impact of BMSC-derived exosomes on doxorubicin-induced cardiac damage was examined in an in vivo rat investigation. Because BMSC-exos were injected into a tail vein, mice treated with doxorubicin showed improved cardiac systolic and diastolic function and decreased cardiac damage on echocardiography. Reduced dosages of collagen fibers and cytokines that cause inflammation, tumour necrosis factor-alpha (TNF-α), interleukin 1 beta (IL-1β), and IL-6, were observed after BMSC-exos successfully attenuated inflammatory reactions caused by doxorubicin-induced myocardial toxic effects [28].

The presence of miR-96 in BMSC-exos was suggested to be the cause of this beneficial activity. Increased heart output, decreased oxidative stress, inflammation, and myocardial fibrosis were all associated with overexpression of miR-96. On the other hand, the protective effects of BMSC-exos were neutralized by blocking miR-96. This research emphasized the critical role that the *Rac1*/nuclear factor (NF)-κB signalling pathway plays in the protective effects of miR-96. *Rac1* and NF-κB signalling were elevated after doxorubicin-induced cardiotoxicity, while they were both downregulated after BMSC-exos therapy. These results imply that miR-96 directly suppresses *Rac1*. Moreover, proteins linked to the NF-κB signalling pathway, which acts as a key modulator of inflammation which may stimulate apoptosis in doxorubicin-induced cardiotoxicity, were found in lower concentrations when *Rac1* was inhibited. Thus, the research proposed that miR-96 attenuates the myocardial harm generated by doxorubicin by blocking *Rac1*, which in turn downregulates the downstream NF-κB signalling pathway [29].

6. Exosomes from induced pluripotent stem cells

The three main germ layers that can differentiate from induced pluripotent stem cells (iPSCs) are endoderm,

mesoderm, and ectoderm. First, somatic cells like fibroblasts are treated as hosts for the specific transcription factors (TFs) that are needed to create induced pluripotent stem cells (iPSCs). To make it easier for these TFs to enter the cell nucleus, viral vectors are usually used. The TFs bind to specific DNA sequences within the nucleus, triggering pluripotency genes such as *OCT4*, *SOX2*, *NANOG*, *KLF4*, and *c-MYC*. For stem cells to maintain their embryonic status and intrinsic characteristics, these genes must be activated. This process is essential for somatic cells to undergo to transform into pluripotent cells. After being reprogrammed, these cells multiply and produce colonies that resemble embryonic stem cells in terms of development features and shape [30]. iPSCs can then be cultivated to promote exosome production and cell development. Ultracentrifugation can be used to separate exosomes from the cell culture. The extracted exosomes, as identified by NTA, have a mean diameter of 143.5 nm and a characteristic cup-shaped morphology that is visible on TEM. According to NTA analysis, the isolated exosomes have a mean diameter of 143.5 nm and a TEM-visible cup-shaped morphology. Exosomes also positively produce Tumour Susceptibility Gene 101 (*TSG101*) and CD9, two exosome indicators, in addition to the iPSC-specific signal SSEA-1. It's important to note that a variety of miRNAs, including the long miR cluster of miR-17-92, miR-19b, miR-20a, miR-126-3p, miR-130a-3p, and miR-210-3p, are included in these exosomes. These miRNAs are linked to significant biological functions such as angiogenesis, cell cycle regulation, and hypoxia damage reaction. Exosomes have been discovered to have the capacity to transport the Splicing Factor Proline- and Glutamate-Rich (SFPQ) protein to Müller cells, which led to an improvement in the levels of histone deacetylase 1 (HDAC1). An increase in HDAC1 activity within Müller cells causes the protein hypoxia-inducible factor (HIF-2α) to be deacetylated, which in turn suppresses the protein linked to hypoxia-induced damage and retinal disorders. Increased HIF-2α levels are associated with consequences like retinal neovascularization in ischemic retinal disorders brought on by low oxygen levels [31].

7. Role of exosomes in clinical applications

Many biomarkers, including those with rather limited specificity, have been created and used as medical indicators in the past. Only 32.4% of biopsied people had a true-positive result for the Prostate-Specific Antigen (PSA) test, a widely accepted detection technique used for initial screening and determining the necessity of transrectal ultrasound-guided prostate biopsy. The benign prostatic hyperplasia that affects the remaining 67.6% of people usually goes away on its own without treatment. In light of the high incidence of false-positive results and the possible negative consequences of these intrusive methods, researchers are always looking for accurate non-invasive health indicators. In comparison to the free serum PSA antigen test, one study found that exosomes expressing CD81 and PSA antigen on the outside had better clinical accuracy for detecting prostate cancer. This finding could significantly improve the ability of doctors to make decisions, which would make transrectal ultrasound-guided prostate biopsies easier to perform, especially on individuals who show clear signs of the symptoms [32]. Exosomes represent the molecular characteristics of the source cells, which means that they can provide important information for illness identification, monitoring, and the development of individualized therapy plans. Even while some exosomes have shown promise as indicators of disease on their own, adding them to well-established assays like the PSA assay may improve both the specificity and precision of the tests. Conventional physical biopsy has been the gold standard for confirming tests for several disorders, particularly malignancies, for a long time [33]. Moreover, several studies have proved the association of stem cell-derived exosomes significant involvement in multiple diseases such as cancer, skin injuries, and diabetes mellitus etc. as shown in **Figure 1**.

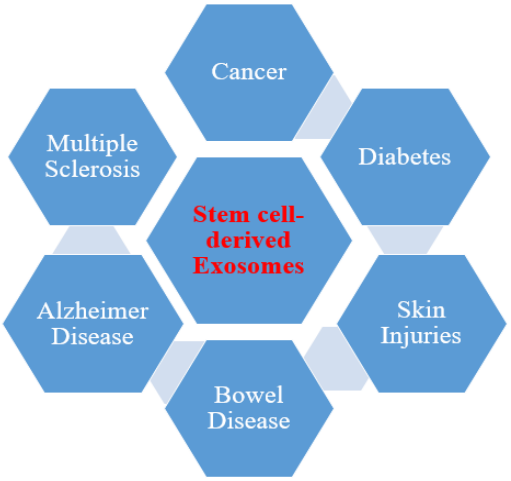

Figure 1. The key clinical applications of stem cell-derived exosomes in various human conditions such as cancer, diabetes, Alzheimer's disease etc.

A biological biopsy can yield extremely trustworthy results, but it requires actual possession of the area of interest and an excellent specimen. For relatively inaccessible areas, such as brain tumours, this becomes difficult. In these situations, the available options either require surgery to remove the lump for examination or rely on imaging methods, which frequently lack sensitivity for particular kinds of cancer. Given the developments in medical science, both methods are not ideal, and here is where liquid biopsies come into play. Exosomes are constantly released by cells and may be useful as indicators for diagnosis. Exosomes provide the least-invasive and reliable method for assessing disease status, which gives them a substantial benefit compared to conventional tissue biopsies. For instance, it was discovered that serum exosomal-miR-122 may diagnose non-alcoholic fatty liver disease just as well as tissue biopsy [34].

7.1. Cancer

Cancer stem cells (CSCs) have become essential in tumour biology because of their capacity to multiply rapidly and exist in large quantities, both of which promote to the spread and relapse of cancer. These cells have the rare capacity to regenerate themselves and differentiate, which promotes tumour development and heterogeneity, just like their counterparts that are not malignant. The specific and niche microenvironment plays a critical role in controlling the activity of CSCs. This niche environment's biochemical details can affect CSC distinction, resistance processes, and growth. This niche ecosystem is made up of several parts that are not cells [35]. These elements work together intricately to provide a microenvironment that is favourable to CSCs. Among these, CSC-derived exosomes, or CSC-exos, are becoming known to play a significant role in how deadly cancer is. According to research, exosomes released by oesophagal cancer stem cells (ECSCs) included a protein known as O-GlcNAc transferase (OGT), which allowed the cells to escape immune monitoring and enhance their potential for regeneration. By transferring this exosomal OGT to nearby CD8+ T cells, the immune cells' expression of PD-1 increased. The immunosuppressive process known as the programmed cell death protein (PD-1)/programmed death ligand-1 (PD-L1) pathway effectively reduces the function of cytotoxic CD8+ T lymphocytes. The persistence and growth of ECSCs were enhanced by upregulating PD-1 expression. In a different study, lung cancer stem cells (LCSCs) improved the surrounding non-stem cells' capacity to spread [16]. Exosomes carrying the bioactive miR-210-3p are released by LCSCs, and these exosomes, when transferred to other lung cancer cells, enhance the metastatic characteristics of the recipient cells by suppressing the expression of FGFRL1. The expression of N-cadherin, vimentin and matrix metalloproteinase (MMP-9 and MMP-1)

was elevated by those exosomes, which enhanced the invasiveness and migratory capacity of lung cancer. Because CSC-exos contribute significantly to the aggressiveness of cancer, they can be utilized to identify cancer and monitor its progression and resistance, which can aid in estimating the disease's diagnosis. They might also assist in customizing the course for therapy for each patient [16].

7.2. Diabetes mellitus

It has been demonstrated that MSC-exos can slow the development of diabetes mellitus, especially type 1 diabetes (T1DM). In the case of transplanted islet cells, MSC-exos are believed to possess protective properties that prevent the autoimmune destruction of pancreatic B islet cells. This is probably because they can stimulate angiogenesis through increased VEGF expression, allowing islets to revascularize [36]. Additionally, their activity has been observed to improve the achievement rate of transplantation and encourage the revascularization of the transplanted donor cells in hosts. According to molecular research, this is caused by the exosomes' capacity to transfer miRNA, which also improves regulatory T cells' (Tregs') capacity to counteract inflammatory cytokines and prevents peripheral blood mononuclear cells' (PBMCs') capacity to proliferate. MSC-exos are abundant in miR-3075, which can raise insulin sensitivity, and they also increase the AMP-activated protein kinase (AMPK) pathway, which may significantly improve glucose metabolism. These findings further support the beneficial effects of MSC-exos in the treatment of diabetes. The advantages of MSC-exos, however, rely on what's inside them because exosomes with different RNA variations can have an adverse impact [37].

7.3. Inflammatory bowel disease

As the prevalence of inflammatory bowel disease (IBD), additional study efforts are being made to manage it, including the administration of sc-exos. Because they can polarize M2b macrophages, which reduces inflammation and prevents the formation of fibrosis, BMSCs are very important because they can improve the mucosal obstacle. These exosomes' cargo includes several proteins, particularly metallothionein-2, that have anti-colitis effects. Other pertinent cargo include many miRNAs, including miR-146a and miR-155, which are essential for immunity integration because they control T cells and NF-κB pathways [38]. Moreover, exosome therapy for IBD has been shown to restore normalcy to the intestinal flora, particularly when IPSC-derived exosomes are used. In animal models, HUCMSC-exos have also been established to have an impact on the therapy of IBD in frequently years. This is especially evident in their ability to mitigate intestinal fibrosis, a late-stage consequence of IBD. This was principally accomplished by blocking the activation of the extracellular signal-regulated kinase (ERK) pathway, which resulted in a reduction in fibrotic components, including collagen I and fibronectin [39].

7.4. Skin injury

Skin injuries are primarily typical. The four steps that usually coexist with skin regeneration include inflammation, angiogenesis, the production of new tissue, and remodelling. Recent research has demonstrated that by modifying relevant signalling pathways, human-derived MSC-exos can successfully treat skin lesions and speed up wound recovery. Another study used an intriguing combination therapy to administer hADSC-exos both intravenously and locally to speed up the healing of skin wounds. It is noteworthy that exosomes produced from fetal dermal mesenchymal stem cells (FDMSC-exos) have been demonstrated to stimulate adult dermal fibroblasts (ADFs) through activating the Jagged 1 ligand in the notch signalling pathway, promoting cell proliferation, migration, and secretion and eventually speeding up wound recovery [40]. Comparable outcomes have also been noted for MSC-exos produced from humans who carry miRNAs. Interestingly, research showed that hBMMSCs and jaw bone marrow MSCs (JMMSCs) might stimulate macrophages to become M2 polarized and aid in the healing of wounds. The theory proposed that donor-secreted exosomes could control macrophage

polarization by delivering miR-223 that targets *Pknox1*. However, additional research is required because scientists are unable to determine whether other miRNAs or substances transported by these exosomes contribute to the development of M2 polarization. Similarly, one of the previous study created a functional exosome by treating BMSC-exos with 50 μg/mL Fe3O4 nanoparticles and 100 mT SMF. Notably, miR-21-5p was overexpressed in mag-BMSC-exos and, by targeting SPRY2 to activate the PI3K/AKT and ERK1/2 signalling pathways, enhanced angiogenesis both in vivo and in vitro to speed up the healing of skin wounds [41]. A research reported that UCMSCs-EVs are substantially enriched in miR-27b, which targets the Itchy E3 ubiquitin-protein ligase (ITCH) and promotes the expression of JUNB and IRE1α, thereby speeding up the regeneration of cutaneous wounds. Furthermore, a collection of microRNAs (miR-21) delivery that nanosized exosomes, which are also packed with powerful biomolecules, are quite likely to overcome [40]. The majority of SC-exo therapies address amyloid-β (Aβ), which is central to the pathogenesis of AD.

The first efforts were directed toward removing the pathological protein Aβ peptide from aggregates [42]. In the early stages of a preclinical model of AD, Elia and colleagues found that intracerebral injection of MSC-derived exosomes decreased the burden of Aβ plaque and dystrophic neurites in both the cortex and hippocampus. Additionally, the authors used immunoblotting to establish that the lysates and mRNA of the exosome contained neprilysin, a neutral endopeptidase that may degrade Aβ. Some groups have concentrated on reducing oxidative stress and synaptic dysfunction. Wang J *et al.* (2017) discovered that exosomes produced from MSCs may alleviate exogenous Aβ-induced inducible nitric oxide synthase (iNOS) production, repair synaptic damage, and enhance mental activity in APP/PS1 mice [43]. Li J *et al.* (2022) used NSC-derived exosomes rather than MSC-derived exosomes, and this improved mitochondrial operation, upregulated sirtuin 1 activation, increased synaptic activity, and corrected cognitive disorders [44]. Huber CC *et al.* (2022) used different techniques and found that exosomes produced by heat shock that were derived from NSCs showed increased neuroprotection against oxidative stress and Aβ-induced neurotoxicity.

Energy homeostasis has been the focus of study for some groups. Using 18F-FDG PET/CT imaging and NOR testing [45]. Chen YA *et al.* (2021) discovered that MSC-derived exosomes could enhance brain glucose metabolism and cognitive function in AD transgenic mice, respectively. To slow the course of AD, some groups have concentrated on neuronal cell death and neurogenesis [46]. When MSC-derived exosomes were administered to AD mice by Hernández-Sapiéns MA *et al.* (2020), the neurogenesis in the subventricular zone was enhanced and the Aβ-induced cognitive impairment was lessened. These outcomes are similar to what the MSCs have demonstrated. A few groups have concentrated on the BBB, whose breakdown results in enhanced flexibility, microbleeds, poor glucose transport, and the deterioration of endothelial and pericyte cells [47].

7.5. Multiple sclerosis

The most prevalent neurodegenerative, debilitating, non-traumatic CNS disease affecting young individuals is multiple sclerosis (MS). Demyelinating lesions with an inflammatory and autoimmune component arise in the brain and spinal cord, which is the pathophysiological hallmark of MS. Licensed disease-modifying medicines at the moment include immunomodulatory, interferon-based, immunosuppressive, and immunological reconstitution medications. The potential of MSC-derived exosomes for treating multiple sclerosis has been addressed by a few preliminary investigations. An animal experiment utilizing rats with experimental autoimmune encephalomyelitis (EAE) to demonstrate that SC-exos treatment greatly lowered neural behavioural scores, diminished the penetration of inflammatory cells into the central nervous system, and lessened demyelination. Furthermore, via controlling microglia polarization, exosome therapy increased M2-related cytokines while downregulating M1-related ones. A study investigated that SC-exo treatment might induce remyelination by acting both directly

on oligodendrocyte (OL) progenitors and indirectly on microglia. The study used two mouse models of demyelination, namely the EAE model and the cuprizone diet model. Exosomes produced from MSCs may enhance neurological results, boost the quantity of freshly formed and mature OLs, reduce the density of Aβ precursor protein, reduce neuroinflammation by transitioning from M1 to M2 phenotype, and obstruct the TLR2/IRAK1/NF-κB pathway [47].

Table 1. Data on stem cell-derived exosomes in therapeutics of various diseases.

Diseases	Mechanisms of action	Advantages of exosome therapy
Alzheimer's disease	They promote neuroprotective elements, regeneration of neurons and neuroinflammatory substances	Neuron growth is promoted and inflammation is reduced
Skin injuries	Enhancing angiogenesis, skin cell division, and ultimately inflammatory reactions	Inducing skin regeneration, lessening inflammations and boosting wound healing
Inflammatory bowel disease	Regulating inflammatory reactions and increasing immune resistance	Modulating the immune system and diminishing inflammation
Diabetes	Promoting insulin signalling cascade and stimulating immune reactions in the pancreas	Induce the pancreatic beta cell growth and regeneration
Cancer	Transporting tumour suppressor molecules, triggering apoptosis in tumour cells, and promoting anti-cancerous immune reactions	Inhibition of cancer growth and development and promoting immune reactions against tumourigenesis

8. Challenges and future directions

Given their tiny size, low density, and similar composition in biological fluids, it is critical to investigate the methods for separating exosomes. Being separated successfully is severely hampered by these reasons. The upkeep of extracellular vesicles (EVs) remains a difficulty because research indicates that these vesicles are most stable when stored for an extended period at temperatures close to -80 °C. This presents a further problem because low temperatures can reduce protein translation. An approach to address this is the freeze-drying of exosomes, which involves extracting water from a frozen sample while applying a vacuum. This can reduce the stringent storage needs of exosomes and increase their lifespan. The EVs' tendency to aggregate during the freeze-drying procedure can reduce their effectiveness in acting on specific regions, which presents another problem. Nevertheless, stabilizers like glucose or starch can be introduced to reduce the amount of EV clustering throughout the procedure. Sterile conditions can also be a problem. Because exosomes and different viruses have similar sizes, viral material may infect exosomes. This is especially true during the biogenesis phase when research has demonstrated that retroviruses, like the human T-lymphotropic virus type 1 and the human immunodeficiency virus-1, can employ exosomes as biocarriers to travel across the body and evade the immune system. Before applying, any pathogens should be closely monitored to reduce this danger. Another issue is that there are no established protocols for isolation. Since the field of sc-exos is new, there is no established method for separating these vesicles, which can have pleiotropic effects due to differences in amount and quality. Along with raising safety issues and increasing the chance of contamination, this also poses a risk to the exosomes. Future studies are needed to develop safe, standardized procedures for further purifying exosomes and to enhance the separation technique.

Additionally, to prevent causing platelet aggregates downstream during blood collection, it is advised to use a needle with a gauge that does not produce shear stresses. The time and fed/fasting state of the blood sample is another crucial uniformity component, as it affects the exosome content. The recipient's characteristics, including age, gender, race, comorbidities, and associated treatments, are also significant. Exosomes must go through a characterization process to ascertain their size, shape, and possibly even content after they have

been effectively isolated from the sample. In this discipline, many techniques are being developed; among the most common ones are scanning electron microscopy (SEM), transmission electron microscopy (TEM), and non-thermal analysis (NTA). Although TEM gives details about the geometry of the exosome, NTA provides helpful data regarding size and concentration.

Further analyses, including reverse transcriptase quantitative polymerase chain reaction (RT-qPCR), dynamic light scattering (DLS), liquid chromatography with tandem mass spectroscopy (LC-MS-MS), two-dimensional gel electrophoresis (2DGE), and fluorescent microscopy (FM), can be used based on the purpose of the investigation. Another concern is the activation of antigen-specific major histocompatibility complex T cell responses upon exosome extraction from human B cells. Additionally, oncogenes are promoted when cancer cells and the tumour stroma exchange oncogenic miRNAs. Furthermore, exosome activation may trigger inflammatory reactions in the recipient cell.

9. Conclusions

Recently, exosomes have been investigated as a cell-free substitute for stem cell treatment. Exosomes generated from ESC, iPSC, HSC, MSC, NSC, and EPC are particularly interesting since their parent cells exhibit pluripotency or multipotency. Stem cell-derived exosomes, after undergoing synthesis and purification with or without modification, have shown great promise in addressing a variety of disorders found in the field of surgery. Wound recovery and neurosurgical issues provide good examples of this. Mechanistically, disease-specific cellular and tissue responses and tissue-specific molecular signaling pathways-exos are responsible for the various curative properties of stem cell-derived exosomes. Altogether, stem cell-derived exosome therapy has demonstrated itself to be a highly effective and adaptable substitute for stem cell treatment in the medical setting.

Prospects for understanding sc-exos in regenerative medicine seem bright. Expanding research endeavours are anticipated to uncover novel treatment uses and enhance existing methodologies, thereby facilitating the acceptance of extensive clinical implementation. Furthermore, the combination of regenerative healthcare with scaffold-exos can improve precision medicine and enable customized care for a variety of illnesses. To evaluate the use of this unique modality in a human population, more investigation is required. Clinical uses of exosomes produced from stem cells should focus in future years on different points in this treatment pathway. As the first step, a high-throughput cellular source, a repeatable and sustainable synthesis and isolation methodology, and an emphasis on the therapeutic large-scale creation of exosomes are needed. Therefore, the bioreactor's operating characteristics need to be improved to enable the large-scale generation of exosomes produced from stem cells. Second, transport strategies other than systemic dosing should be investigated to target the therapeutic potential of exosomes. Exosomes can be administered locally to circumvent their quick removal from blood circulation after being supplied through the venous system, where they then gather in the lungs, spleen, and liver. Recently, a variety of biomaterials have been employed to enhance, support, and preserve locally delivered exosomes to optimize their therapeutic benefits. These biomaterials could be created with their sources in consideration. Stem cell-derived exosomes will find faster therapeutic uses in a continuously increasing range of health conditions if efforts are made to speed exosome synthesis in tandem with multimodal exosome delivery.

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Disclosure statement

The author declares that they have no conflict of interest.

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