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Review article: An overview of exosomes in biology and their potential applications in regenerative medicine

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ABSTRACT

Extracellular vesicles (EVs), commonly acknowledged as Exosomes, are tiny, single-membrane, secreted organelles that range in size from 40 to 150 nm. They are noticeably abundant in various proteins, lipids, nucleic acids, and glycoconjugates and share the same structure as cells. Numerous and non-hematopoietic cell types continuously manufacture and release stable, less toxic, and biocompatible exosomes with many complex compounds (in the form of various signaling molecules, miRNA, and mRNA) in the liquid parts of the body. Exosomes help in intercellular communication/transfer of proteins, RNA, cell differentiation, immune signaling, delivering antigens, angiogenesis, and stress response. In recent studies, researchers found that Mesenchymal Stem cells (MSCs) generate exosomes that symbolize biological processes like tissue regeneration by encasing and delivering active biomolecular species to the infected/damaged cells and tissues. The most extensive research in regenerative medicine has focused on MSCs-Exosomes. Regenerative medicine plays a crucial role in restoring the damaged/lost parts of organs and tissues and aiding in wound healing. Immunomodulation and tissue repair are possible by introducing Mesenchymal Stem cell (MSC) exosomes, which have triggered remodeling reactions. They produce local anti-inflammatory and healing signals crucial for regeneration and tissue repair. The primary goal of this review is to highlight the MSCs-exosome's mechanism of action and its therapeutic uses in clinical settings. Also, it highlights new developments in employing MSCs-exosomes to treat various ailments and disorders.

Introduction

Mesenchymal stromal/stem cell (MSCs) investigations for immunomodulation and regenerative medicine are widely used in cellular treatment.[1] Mesoderm-pluripotent embryonic stem cells (MPSCs) can be discriminated into diverse cell forms.[2] The reassuring characteristics of MSCs-exosomes, such as their outstanding capacity for cell differentiation and regeneration, have generated much study attention over the past few decades. MSCs, which may be extracted from several organs, are the adult stem cells most frequently used in regenerative medicine, they have a high potential for replication in culture and can develop into different cells such as adipocytes, chondrocytes, and osteoblasts.[3] They are predominantly located in the perivascular spaces encompassing nearly every human tissue and system. Bone marrow, fatty tissues, umbilical cord, and maternal tissues are the primary sources of MSCs, as shown in Figure 1.[4.] Under this, some studies claimed that MSCs-EVs/exo, i.e., exosomes isolated from MSCs have superior curative benefits to general MSCs.[5]

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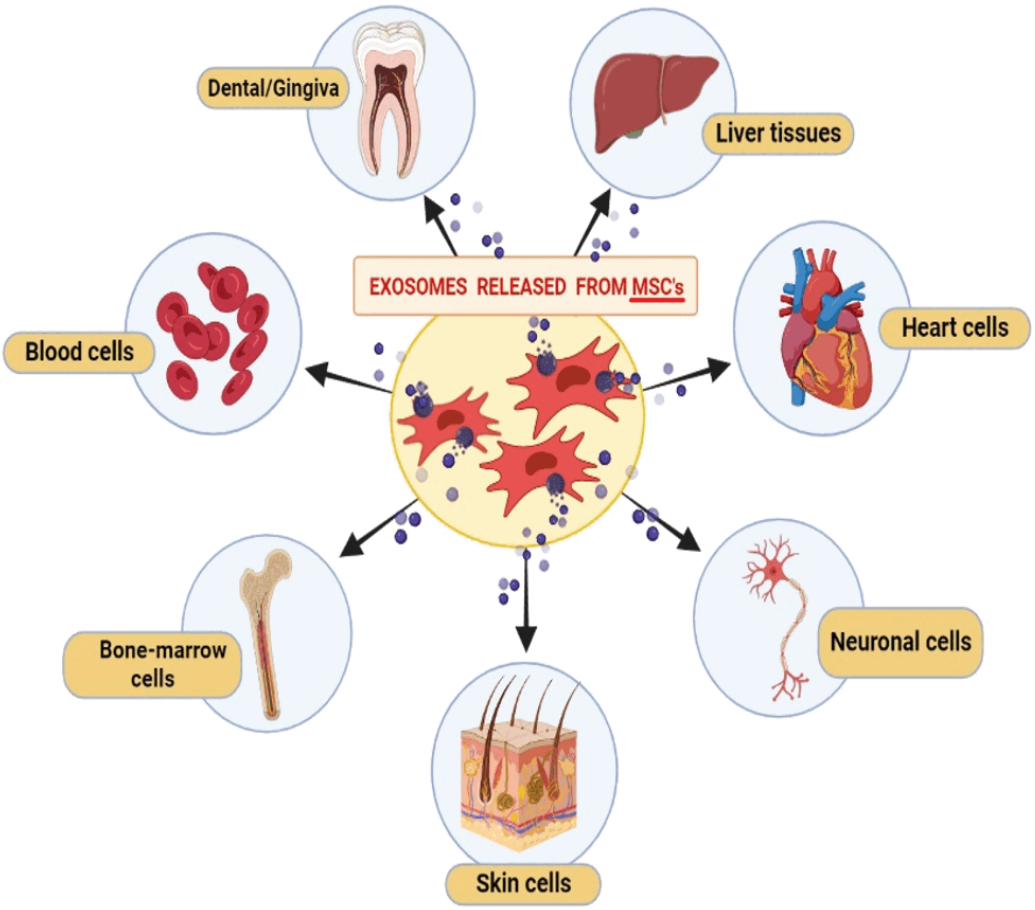

Figure 1. *Mesenchymal stem cells (MSCs) termed exosomes isolated from diverse organs/ tissues (bone marrow, adipose tissue, dental pulp, umbilical cord blood, placenta, liver, and neuronal cells).*

Since MSCs have receptors for various factors expressed on their outermost cell layer, they can go to the site of cancer or inflammation and release inflammatory factors, including IL-6, IL-8, and MCP-1, into the environment of such inflamed areas. These elements support MSCs' instructed movement.[6,7] Exosomes produced from MSCs (MSCs-Exo) have been the subject of numerous studies, and it has been found that these exosomes share many of the same processes as MSCs, including the ability to repair tissue damage, suppress inflammatory responses, advance tumors, and promote angiogenesis.[8] Extracellular vesicles (EVs) or MSCs-Exos, which are released by the majority of MSCs cells, as well as secretory vesicles, which are produced by some MSCs cells and allow the vesicular transport of cargo like neurotransmitters or hormones, are among the membrane vesicles that MSCs cells can produce.[9] It was initially believed that the production of EVs served as a mechanism for the cell to get rid of waste products in the form of "platelet dust/cellular debris".[10] The ability of EVs to carry cargo between cells, such as DNA, lipids, and proteins, is the main area of interest in this subject because it has become clear that EVs are more than merely trash carriers.

The EVs are crucial for maintaining or developing cell pathologies as cellular communication agents. EVs can be split into two significant groups according to their origin: Micro- and exo-vesicles (MVs).[11] According to the International Society for Extracellular Vesicles (ISEV), extracellular vesicles might fall into 3 categories: exosomes, microvesicles, and apoptotic structures. These groups' location, size, and substance are crucial.[12,13] The smallest of these are exosomes, with a size assortment of ~ (40-150) nm on the nanoscale [14]. Furthermore, their biogenesis is another distinguishing trait of exosomes that sets them apart from other EVs. Exosomes have an endosomal origin in contrast to microvesicles, which form directly from the plasma membrane, and apoptotic bodies, which form during apoptosis (Figure 2).[15] Exosomes are formed by the fusion of plasma membrane with multivesicular bodies (MVB).[16] Multivesicular bodies (MVB), a type of internal multivesicular compartment, are created when the endosomal membrane invaginates.[17,18] Exosomes have been characterized as having a "cup-shaped" structure with varying sucrose densities between 1.13 and 1.19 g/mL.[19]

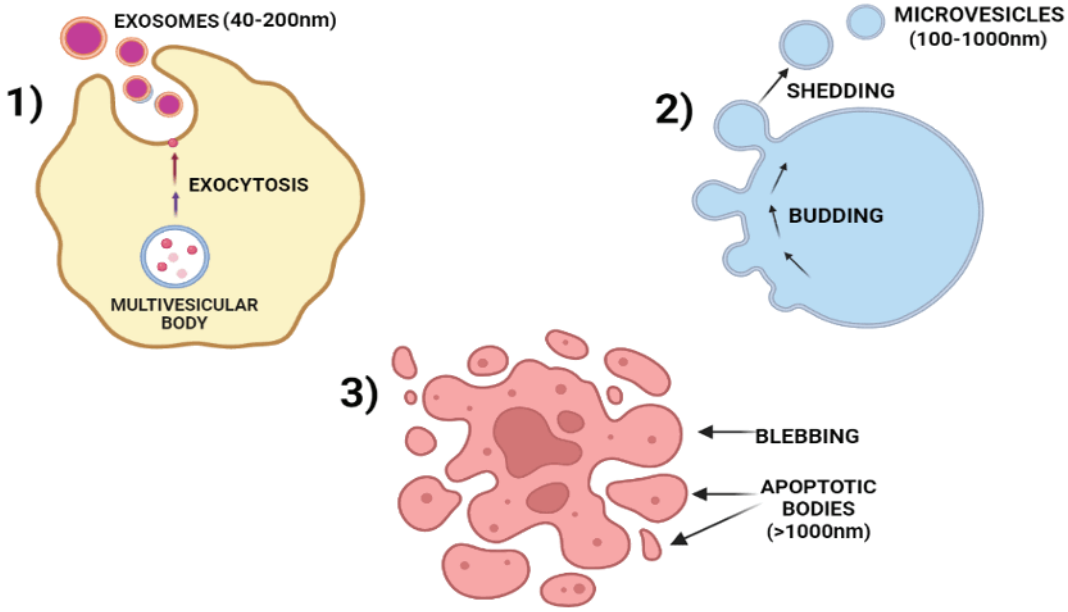

***Figure 2**. Extracellular vesicles (EVs) classification.*

A few of the cells that produce exosomes are immune cells, platelets, smooth muscle cells, and endothelial cells. When the extracorporeal membrane of the polyvesicles coalesces with the plasma membrane. Exosomes are produced inside cells and released into the extracellular matrix by lysosomal polyvesicles.[20] Exosomes, which are generally released into receptor cells by host cells, regulate the biological functions of receptor cells by supplying substances that affect how receptor cells behave. These substances include proteins, lipids, nucleic acids (NAs), and other added substances. These outcomes sustain the use of exosomes as biomarkers and in immunotherapeutic strategies. Our study presents a brief overview of the function of exosomes in connection to their origin and roles, and it addresses the possible use of exosomes to treat various disorders, such as diabetes, immunomodulation, wound healing, and hair follicle growth.[21]

Three distinctive classes of EVs are portrayed above where: 1) Exosomes, which are 40-200 nm in diameter, are produced by endocytic pathways and are let out into extracellular spaces by a process called exocytosis; 2) Microvesicles are irregular shaped bodies that are of diameter size 100-1000 nm and are generated by budding from the cytoplasmic membrane; and 3) Cells that undergo apoptosis produce apoptotic bodies which are of diameter size >1000 nm by the process of cell blebbing.[22] In Table 1 depicts the diverse forms of exosomes.

Table 1. Depicts the origin, size, markers and different types of exosomes

Different types of vesicles	Origin	Size (nm)	Markers
Microvesicles	Plasma membrane and many other cell types	20-1000	Broad variety of non-specific markers such as selectin, integrins and CD40 ligand[23,24]
Apoptotic bodies	Cell membrane from Endoplasmic reticulum	1000-5000	Phosphatidylserine, DNA & its products, Histones[25,26]
Membrane fragments	Epithelial cell membrane	50-80	Prominin-1 (CD133) [27]
Exosomes	Endosomes from various cell types	40-100	other endosomal-related indicators, such as lipid rafts, Tetraspanins, flotillin, Alix, TSG101, sphingomyelin, and Rab5b. All markers, however, specifically target exosomes. [28]

Discovery of exosomes

Exosomes were once believed to be apoptotic bodies for the minimally disruptive discharge of cellular debris from cell injury or byproducts of cell homeostasis.[29] Harding *et al.*,[30,31] and Pan *et al*.,[32] were the two scientists who first described exosomes in their research papers on two separately different animal studies, and this was published a week apart in 1983. In their research, scientists noticed how sheep Transferrin Receptors (TfRs) were released from the plasma membrane and entered

growing human reticulocytes. It was discovered that transferrin receptors interact with cells and are then packed into tiny (~50 nm) vesicles.[33,34] Schirrmacher and Barz, a year after this event, noticed that tumor-derived exosomes (TDEs) had antigens that were comparable to those of the matching tumor cells.[35] These vesicles were subsequently named "exosomes" in 1987 by Johnstone *et al*.,[36] which were initially believed to be on their way to lysosomes for destruction before being secreted into the extracellular area by maturing blood reticulocytes.[37]

Extensive study and research on exosomes increased due to the development of more sophisticated approaches to understand functional biology clearly. They extended therapeutic applications of exosomes, such as techniques for tissue regeneration, immunomodulation, and cell differentiation [38]. The initial findings from clinical tests on exosome-based immunizations were published in the mid-2000s.[39] In-vitro conditions are a viable therapeutic option for several illnesses with tumor-specific biomarkers. Nevertheless, MSCs-Exos applicability in orthopedics, dentistry, and COVID-19 therapy, as well as liposome-mediated drug delivery in cardiovascular disorders and cancer using tumor-specific biomarkers.[40-43]

Structure and composition of exosomes

Following diverse proteomics techniques like mass spectrometry, exosomes are said to contain more than 4,000 distinct types of biomolecules, involving particular groups of lipids, proteins, nucleic acids, mitochondrial DNA (mtDNA), microRNAs (miRNAs), mRNAs such as transfer RNA (tRNA), long noncoding RNAs (lncRNAs), piwi-interacting proteins RNAs (piRNAs), cytokines, transcription factor receptors, and other bioactive compounds are among the different kinds of RNA. Exosomes from progenitor cells transport these RNAs to target cells, where they can perform specific functional tasks. Exosomes vary in constituent parts due to various physiological and pathological circumstances and specific cell types; the cell of origin significantly influences the exosomal contents.[44-49]

Exosome protein components are classified into two categories; one category consists of elements that are involved in the creation, release, and exosome biogenesis of intraluminal vesicles (ILVs) regularly, like programmed cell death 6 interacting protein (PDCD6IP or ALIX), and another category made up of the tumor susceptibility gene 101 (eg. tetraspanins-8 (TSPAN 8), Vacuolar protein sorting-associated protein 4 (VPS4), TSG101, SD106), whose release is mainly reliant on proteins like Rab GTPases.[50-53] Additionally, exosomes contain non-specific proteins like cytoskeleton proteins (such as myosin, actin, and tubulin), cell-specific antigen-presenting components like CD45 and Major Histocompatibility Complex proteins (such as MHC I and MHC II), which are typically seen in donor cells, heat shock proteins (Hsp) (such as Hsp70, Hsp90, Hsp110), membrane integrating transfer proteins (such as flotillins, annexins, and Rab), ESCRT, and CD63.[54,55] Compounds such as sphingomyelin, cholesterol, phosphatidylserine, glycosphingolipids, and ceramide, which impact signaling, exosome release, structure, and cargo separation, are also seen as abundant in exosomes. These lipid components are stable and necessary for the preservation of exosome structure, biogenesis of exosome, and homeostatic control of the target cell.[56] Additionally, these proangiogenic proteins, like basic fibroblast growth factor (bFGF), interleukin 6 (IL-6), monocyte chemokine protein-1 (MCP-1), migration-promoting chemokines, vascular endothelial growth factor (VEGF), towards the inflammation site, proteins involved with the mitochondria, proteasomes, and endosomal reticulum, are strongly expressed in MSCs-derived microvesicles (MVs) (Figure 3).[57]

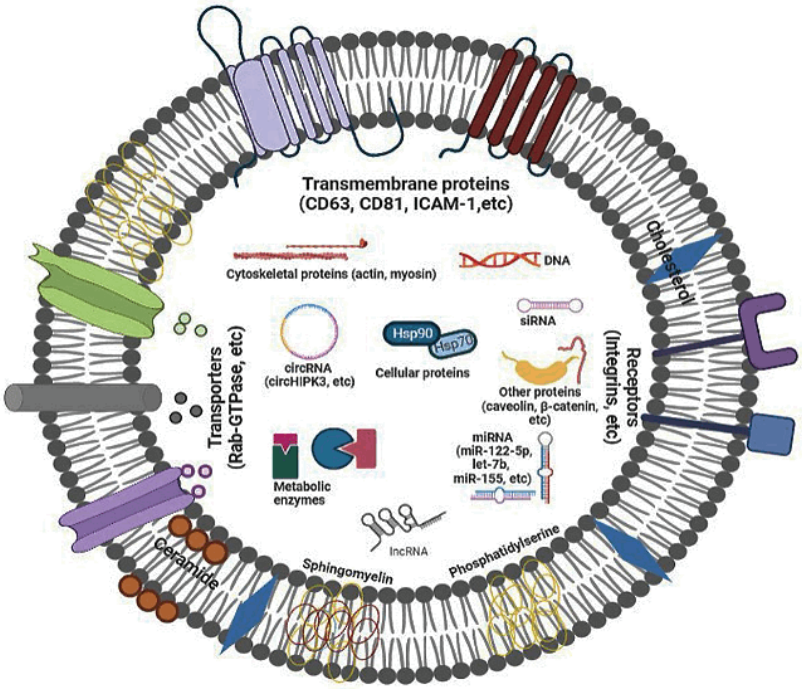

Figure 3. *Structure and composition of exosome.*

Exosome biogenesis, release, and uptake

Initiation, endocytosis, multivesicular body growth, and secretion are the four stages of exosome maturation.[58] When the plasma membrane invaginates, early endosomes are created that include membrane protein and multivesicular bodies (MVBs), which are exosomes. Sometimes, it is also referred to as late endosomal structures comprising several ILVs with a changing subcellular structure.[59] Finally, MVBs are fused with the plasma membrane to release exosomes into the extracellular environment or carried to lysosomes to degrade all transported compounds. They are also transferred to the trans-Golgi network (TGN) for endosome recycling.[60] Early endosomes (EEs), created by merging endocytic vesicles, are the first stage in biogenesis.[61] Later, the formed EEs are packaged into late endosomes (LEs) or return the cargo proteins outside the cell.[62] To move proteins, the packing of proteins inside multivesicular bodies and intraluminal vesicles (ILVs) relies on the endosomal-sorting complex (ESCRT). Typically 4 ESCRT protein structures such as ESCRT0, ESCRTI, ESCRTII, and ESCRTIII, in conjunction with AAA, ATPase, Tsp101, ALIX, and Vps4 accessory protein complex that relies on ubiquitin protein for further mechanism or go through ubiquitin independent mechanism of action for selection of required cargo, and the production of target exosomes.[63-65] Both the Vps4 complex and the ESCRT-III complex work together to cause vesicle neck scission; ESCRT-III multifaceted dissociation and regeneration occur. The multifaceted ESCRT-0 also arranges ubiquitinated cargo proteins into the lipid area. ESCRT-I, as well as ESCRT-II, drive the deformation of the membrane to develop the membrane-stable neck.[66] The first stage is the creation of ESCRT-0 complex, a heterodimer made up of the ubiquitylated cargo-identifying proteins STAM1/2 and HRS.[67] The FYVE domain covers 3 retained components such as C-terminal RVC motifs, which form phosphatidylinositol 3-phosphate (PtdIns-3) binding site, N-terminal WxxD, the central RR/KHHCR, cytoplasmic protein HRS, and early endosome antigen 1 (EEA1) results in formation of endosomes,[68,69] which connects to phosphatidylinositol 3-phosphate (PtdIns3P). This lipid is plentiful on the surface of pre-MVB endosomes.[70] Then, HRS employs Clathrin,71, who assists in the corralling and ubiquitylated freight congregating.[72] At the site of ILV creation, ESCRT-0 interacts with ESCRT-I and ESCRT-II. Since both contain ubiquitin-interaction domains, a sorting domain with elevated avidity for ubiquitylated cargo must be created.[73-76] ESCRT-III, which promotes membrane deformation along with tightening of the collar through consequent intussusception,[77] further is also recruited at that precise moment.[78,79] [Then, ubiquitin is eliminated from the payload by Deubiquitinating enzymes (DUBs),[80] and the ESCRT complex is dissociated so that its constituent parts can be repurposed by the ATPase VPS4 and co-factor VTA1.[81] Theoretically, suppression of any of these factors should prevent the production of ESCRT-dependent exosomes, yet without having an impact on other procedures like lysosomal targeting.[82]

RNA entry into exosomes seems to be lipid-mediated, unlike proteins organized by ESCRT that rely on self-regulating lipid and cargo locations. Nucleotide sequences demonstrate the increased attraction for the phospholipid bilayer and largely depend on elements like lipid structure (lipid rafts), hydrophobic alterations, and sphingosine at an average proportion in rafted membranes (Table 2).[83] The plasma membrane's fatty rafts are regions that are abundant in proteins that are glycosylphosphatidylinositol (GPI)-anchored, sphingolipids, and cholesterol. ILVs are formed spontaneously by the budding-in process while ceramide, lysophospholipid, and glycosphingolipid molecules accumulate on the limiting membrane. In the presence of ceramidase and sphingosine kinase, the ceramide is transformed to sphingosine and sphingosine-1-phosphate (S1P), and continual stimulation of sphingosine-1-phosphate receptors on the limiting membrane facilitates the organization of tetraspanin into ILVs.[84] Tetraspanins organize membrane microdomains, defined as tetraspanin-enriched microdomains (TEMs) with many transmembrane and cytosolic signaling proteins. Four transmembrane domains set tetraspanins apart as a protein superfamily of transmembrane proteins linked to cell surfaces.[85] According to a 2009 study by Stuffers, Susanne *et al*., the absence of the ESCRT tool in mammalian cells did not prevent the development of MVB vesicles. Still, it did result in abnormalities in the amount and size of ILVs and in the cargo-sorting into ILVs. This suggests that exosome biogenesis may be a synchronized process with both ESCRT-dependent and ESCRT-independent pathways (Figure 4).[86]

The first step in producing and generating exosomes is the creation of early endosomes, which include RNA and cytosolic proteins. These early endosomes later develop into active subcellular structures called MVBs (Multivesicular Bodies). MVBs can then be destroyed by lysosome fusion or released by plasma membranes to create exosomes. Lastly, there are numerous methods by which exosomes deliver their cargo, which includes proteins, DNA, and microRNA, to the target cell.

Table 2. The process of synchronization of exosomes and their release.

ESCRT-Dependent exosome

Protein	Cell lines used for *In vitro* studies	ESCRT-dependent exosome proteins used
Syndecan	MCF-7	HSP70, Alix, CD63 (membrane cargo binding to syntenin-1)[87,88]
Hepatocyte growth factor receptor tyrosine kinase substrate (HRS) an endosomal protein	HeLa-CIITA, DCs	VPS4B, MHC-II, CD63, Tsg101[89-91]
Tsg101 (VPS23)	MDCK, MCF-7, HeLa-CIITA, DCs	CD81, MHC-II, CD63, syndecan-1, ALIX, HSC70[92]
Syntenin	MCF-7	HSP70, CD63[93]
VPS4	MCF-7, DCs, HeLa CIITA	syndecan-1, HSC70[94]
Alix	MCF-7, DCs, HeLa, CIITA	syndecan-1, TSG101, RAB5, HRS, HSC70[95]
CHMP4C (SNF7C)	HeLa-CIITA	CD81, HSC70, CD63, MHC-II[96]
CHMP4B (SNF7B)	HeLa-CIITA	HRS, TSG101, RAB5[97]
STAM1	HeLa-CIITA	MHC-II, HSC70, CD63, CD81[98]

ESCRT-independent exosomes

Protein	Cell lines used for *In vitro* studies	ESCRT-Independent exosome proteins used
RAB31	HeLa, HEK-293T	Tsg101, CD81, Alix, CD9, CD63[99]
nSMase2	HEp-2, Oli-neu	Tsg101, Hrs, PLP, Alix, Syntenin[100,101]
DGKα	J-HM1–2.2	B-Actin, Fasl, CD63[102]
PLD2	RBL-2H3, MCF-7	SDC1CTF, CD63[103,104]
CD9	BMDCs, HEK293	Flotillin-1, β-catenin[105]
CD63	DG-75, HEK293, HK1, Rat1, MNT-1, HeLa	Calnexin, CD81, HSC70[106]
CD82	HEK293	β- Catenin[107]

Release of exosome

Protein	Cell lines used for In Vitro studies	Release of Exosome
Tetherin	HeLa	ALIX, TSG101, CD63[108]
RAB11	Drosophila S2, K562	HSC70, Evi (WB), Lyn, Transferrin receptor[109,110]
YKT6	A549	Tsg101[111]
RalA, RalB	4T1	HSC70, ALIX, TSG101, CD63[112]
VAMP7	K562	Acetylcholinesterase activity[113]
RAB27a/b	Human peripheral blood, HeLa-CIITA	Tsg101, Hsc70, Hsp70, CD63[114]

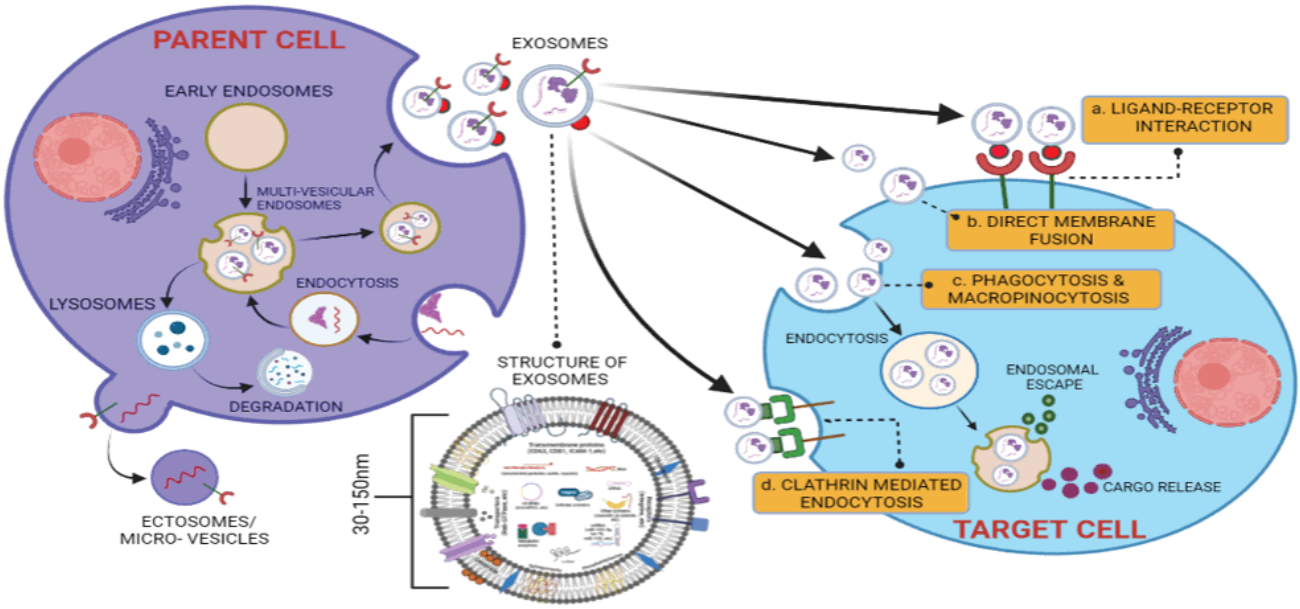

***Figure 4**. Exosome biogenesis, release, and uptake.*

Uptake of exosome

Exosomes communicate with target cells via three different mechanisms: (a) receptor-ligand contact, (b) direct membrane fusion, and (c) endocytosis using phagocytosis. Additionally, several proteins function as ligands to stimulate the absorption of the exosome, such as ICAM-1 for Tim 1/4 for B-cells and cells that present antigens (APCs).[115-117] Exosomes perform an integral part in pathological and physiological methods based on their capability to communicate with target cells once discharged outside the cell and transmit proteins, lipids, and nucleic acids. Even though numerous techniques have been proposed.[118-120]

Applications of exosomes

Immunomodulatory activity of exosomes

MSCs are extremely important in the field of regenerative medicine and are found to interfere with numerous immune response mechanisms and exhibit immunomodulatory functions. MSCs have broad immunoregulatory capabilities through interactions with immune cells in both the adaptive and innate immune systems, resulting in immunosuppression of diverse effector functions.[121] MSCs regulate immunomodulation by reducing B-cell proliferation and activation, hindering NK cell cytotoxicity and proliferation, decreasing dendritic cell maturation and suppressing T-cells, and also by facilitating the generation of regulatory T-cells through cell-cell contact or with the help of several soluble factors such as prostaglandin E2 (PGE2), hepatocyte growth factor (HGF), transforming growth factor-β1 (TGF-β1), etc.[122] Exosomes and microvesicles generated from MSCs have been found to have similar immunosuppressive properties.[123]

Zhang *et al*. noticed that MSC-derived exosomes persuaded the phenotype of monocytes to M2-like promoted T-cells, which further evolved into regulatory T-cells.[124] Nonetheless, lowered immunological actions *in vivo* augment the viability of mice allogeneic skin grafts.[125] Morrison *et al*. demonstrated that MSCs-derived EVs promote a highly phagocytic and anti-inflammatory macrophage phenotype through mitochondrial delivery.[126] Responses via T-cell mediated immunomodulation is a powerful strategy for controlling autoimmune and inflammatory disorders. *In vitro* experiments with T-cells treated with MSCs-derived EVs revealed a significant reduction in T-cell-driven proliferation and decreased production of specific proteins, including IFN-γ and TNF-α.[127] Animal models were utilized to investigate the immunomodulatory role of MSCs-derived EVs, with results revealing immunological activity and changes in the production of pro-inflammatory and anti-inflammatory cytokines.[128] The impact of immunomodulation by the exosomes produced from MSCs in animal models of antigen-driven tissue damage exhibited a variety of effects, including a reduction in the numbers of synovial joint lymphocytes and reduced expression of TNF-α mRNA in the synovial joints increased in survival of induced lung injury as well as an increase in regulatory T-cells after concanavalin-A-induced liver damage.[129-131] According to these findings, MSC-derived EVs preserve the biological functions performed by the mother cells and can be used as an immunosuppressive tool.[132]

MSCs have recently been proposed as a promising treatment for SARS-CoV-2. MSCs suppress viral infections by releasing certain cytokines; these features are inherent in MSCs even before they are separated from their parent tissue. As a result, when these MSCs and their exosomes (MSCs-Exo) are transplanted into a patient with proven SARS-CoV-2 infection, they are likely to survive.[133]

Exosomes in hair follicle regeneration

The skin, the biggest organ, is a vital bodily barrier, playing a major role in detecting impulses and defending against infections and other environmental substances.

The skin appendices also contain hair follicles (HF) and glands; these hair follicles originate from the first two layers of skin, epidermis and dermis, respectively. The HF is generally made of layers of cells within the layers of the skin.[134] The cycle of HF development occurs continuously throughout the life of the organism and occurs in phases, which are telogen (rest phase), anogen (growth phase), and catagen (regression phase). Epithelial, melanocyte, and mesenchymal stem cells (MSCs), which self-renew, differentiate, control hair growth, and uphold skin homeostasis, are among the numerous skin stem cell groups found in the bulge section of the HF.[135] The skin has drawn much interest as a potential target for regeneration treatment owing to the improved access and knowledge about the localization action of skin stem cells, in general, the skin, and the HF in specific.[136,137]

Wnt factors are well-known primary controllers of hair growth and HF morphogenesis.[138] Wnt ligands are critical in de novo hair creation and are induced by adult skin wounds. Active Wnt factors, on the contrary, have been revealed as molecules that are secreted by the exosomes that can be both housed within and released from these vesicles.[139,140] Wnt4 and Wnt11, obtained from the MSCs of human umbilical cord exosomes, have been proven to promote the revitalization of a cutaneous layer of skin in a burnt skin rat model. Wnt4 improved the skin's angiogenesis and Wnt/- β-catenin signaling processes.[141,142] Additionally, it was discovered that different subsets of vesicles containing Wnt could be isolated in a specific pattern in epithelial cells of the polarised type. Moreover, a mechanism for the discharge of Wnt3a and Wnt11 from MDCK cells has also been shown to be crucial for the structural organization of the HF and interfollicular epidermis.[143] These findings show that enhanced expression of Wnt3a and Wnt5a was linked to stimulation of hair formation in mouse skin treated with intradermally injected EVs generated from MSCs.[144] When injected into the skin of mice, exosomes derived from human Dermal Papilla (DP) cells were found to extend the anagen phase of the hair cycle by promoting the production of β-catenin and Sonic Hedgehog (Shh). The Hedgehog pathway is a crucial pathway that plays a major role in homeostasis, repair, and the development of the HF epidermis and the maintenance of HF bulge stem cells.[145] MicroRNAs (miRNAs) can also be transported in EVs, and it has been found that these molecules also have a major role to play in the regulation of skin and HF development through Wnt signaling modulation.[146] MiR-181c, detected in the exosomes of MSCs from the human umbilical cord, has been proven in studies to be an important factor in lowering inflammation brought on by burn in a rat model.[147] Additionally, synovium-MSCs release exosomes that overexpress miR-126-3p, which has been shown to trigger increased P-AKT and ERK1/2 production in HMEC-1 endothelial cells and support diabetic rat skin wound healing.[148]

Subcutaneous administration with conditioned media produced from MSCs derived from human amniotic fluid improved hair regrowth and expedited wound healing in a wound model in rats.[149] Intradermally injected MSCs-EVs improved the telogen-to-anagen transition in a mouse model.[150] Exosomes from human DP were injected into mice's skin to promote hair growth, possibly simulating the paracrine effects that DP cells naturally have on epithelial cells. The outer root sheath cells of epithelial hair follicles taken from human scalps and cultured with DP exosomes showed elevated Shh and β -catenin levels in the treated skin. In response to exosomes, these findings repeatedly showed how the Wnt/ β -catenin and Shh pathways regulate hair growth (Figure 5).[151]

EVs are natural hair cycle regulators and potential delivery systems for enhancing hair and skin regeneration. The precise physiological significance and contribution of exosomes to the HF cycle in vivo and the therapeutic implications of using exosomes to enhance hair growth in clinics require further investigation.

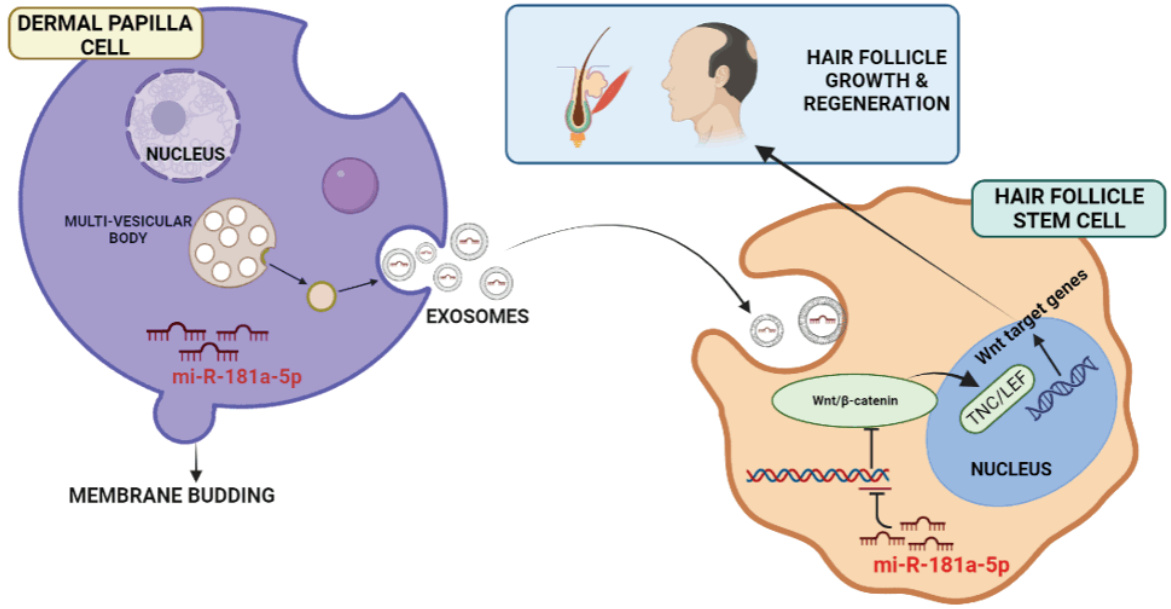

***Figure 5**. Exosomes used in Hair follicle regeneration.*

Exosomes in wound healing

Mesenchymal stromal cells (MSCs) and their exosomes have recently emerged as game-changing tools in tissue engineering and regenerative medicine. Exosomes are generally known to transport functional cargos like miRNA, cytokine, growth factor, etc., from MSCs to the respective target cells, thus influencing the biological processes in the recipient skin cells like proliferation, migration, and ECM component secretion (e.g., collagen, fibroblasts, and keratinocytes).[152] Wound healing is a significant difficulty in certain cases like pathological disorders like diabetic foot ulcers (DFUs), plastic surgery, and others. Persistent wounds cause significant patient morbidity, negatively impacting patient quality of life and exacerbating pain, stress, and despair.[153,154] The current therapeutic procedures are prohibitively expensive, ineffective, and time-demanding, and more than half of infected wounds display strong resistance to topical therapy, which cannot reduce scarring.[155] Mesenchymal stromal cells (MSCs) have emerged as an essential strategy for restoring skin wound healing. MSCs show their therapeutic efficacy primarily through numerous actions on numerous cell types and at all stages of wound healing, from hemostasis through remodeling.[156] Exosomes generated from MSCs have biological functions like the parental cells and can thus help tissue regeneration by moving their contents to neighboring cells.[157] Although clinical trials show that MSCs-based treatments are safe, feasible, and helpful, the limited sample size and absence of long-term follow-up make these trials unsatisfactory.

The cell and metabolic processes throughout the wound repair process are classified into four major phases: hemostasis, inflammatory, proliferative, and remodeling (or maturation phase).[158] As previously noted, MSC-derived exosomes (MSCs-exosomes) will have resource cell characteristics, encouraging tissue regeneration and self-healing of cells, reestablishing tissue homeostasis, and accelerating wound healing in damaged regions.[159] Emerging data suggests that MSCs produce bioactive substances to target tissues and cells via endocrine and paracrine pathways, lowering wound inflammation and enhancing tissue healing.[160] Exosomes produced by various MSCs can attenuate the inflammation by downregulating proinflammatory enzymes such as COX-2, inducible nitric oxide synthase, and chemokines such as chemoattractant protein (MCP)-1, TNF-α and IL-1β. Also, MSCs-exosomes can induce anti-inflammatory cytokine production called IL-10, which is known to be significant in regulating inflammation of cutaneous wounds and scar formation in numerous disease types.[161] Cell proliferation and re-epithelialization of the skin are critical for cutaneous regeneration. Skin fibroblasts are involved in the contraction of wounds, deposition of extracellular, remodeling of tissues, and other aspects of skin tissue healing and regeneration.[162]

It has been demonstrated that MSCs-exosomes control the expression of the genes linked with growth factors, which in turn controls the production and fibroblast migration. Therefore, it promotes the development of granulation tissue and collagen, facilitating structural support for wound healing.[163] Exosomes derived from Human fibrocytes include miRNAs and proteins along with various biological functions, and these exosomes improve the healing of wounds in diabetic rat models by promoting skin migration and proliferation of cells.[164] Studies also have demonstrated that, when transplanted to wound sites, exosomes derived from human amniotic epithelial cells (hAEC-exosomes) were shown to fasten the process of re-epithelialization and wound closure.[165]

One of the mesenchymal stem cells (MSCs) is adipose-derived stem cells (ADSC), which have a variety of origins, are simple to isolate and amplify, and are less immunogenic. It is commonly recognized that ADSC can accelerate wound healing by controlling the activity of several effector cells involved in the healing process. Exosomes that are derived from ADSCs (ADSC-exosomes) have also shown that they can control the synthesis of collagen at various stages of the wound healing process by simply speeding up the healing of a wound by an increase in type I and type III collagen production during the early stage and preventing the synthesis of collagen in a late stage, and thus decreasing the formation of scar.[166] *In vivo*, MSCs administration using the scaffolds of collagen hybrid improved the deposition of collagen as well as angiogenesis in diabetic wound healing.[40] Polarized macrophages are formed into two different phenotypes, M1 (proinflammatory) or M2, in response to activation cues (anti-inflammatory). Evidence suggests that macrophages, which are M2, can express mediators involved in the inflammation resolution and remodeling of tissue and hence enhance healing of the wound (Figure 6).[167]

MSCs' differentiation capacities and possible additional qualities, such as inducing the release of anti-inflammatory and pro-angiogenic mediators, emphasize their importance in wound healing and skin regeneration.

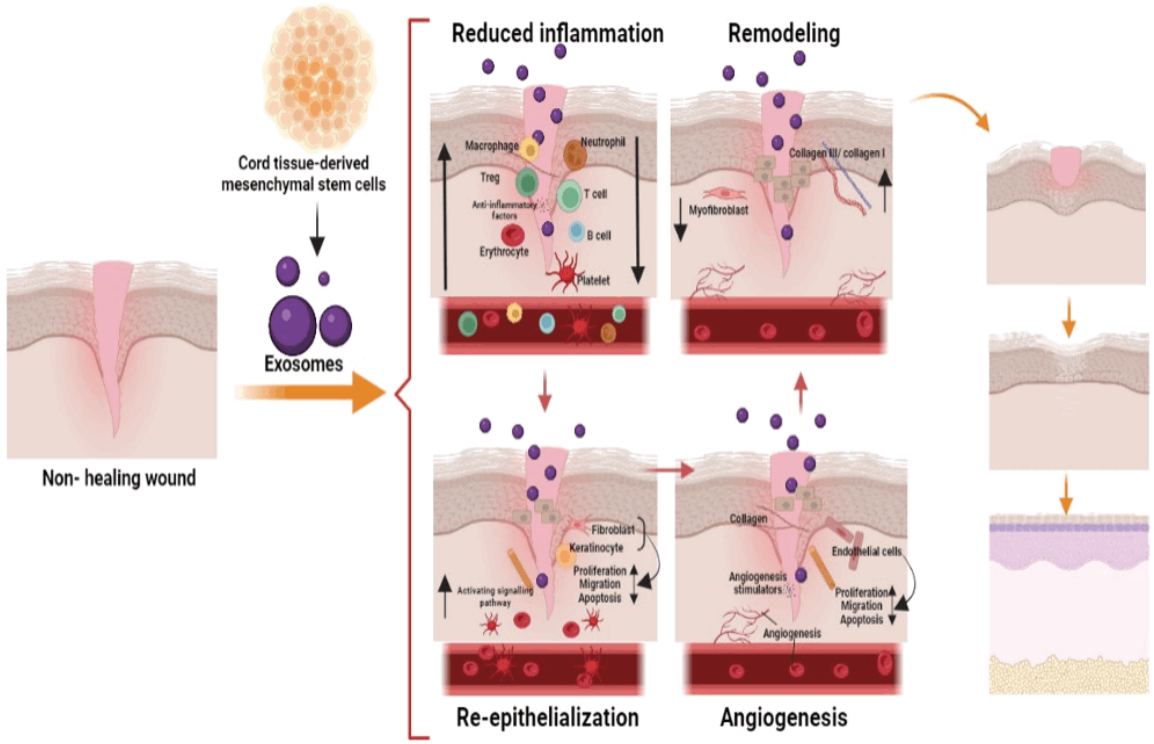

***Figure 6**. Exosomes in wound healing.*

Exosomes for the treatment of diabetes

A global metabolic disorder called diabetes mellitus (DM) is marked by a lack of insulin production, increasing blood glucose levels. Diabetes is frequently associated with several macrovascular problems, including coronary heart disease, macrovascular arteriosclerosis, microvascular disease, and hypertension. MSCs-EVs have been shown to offer outstanding healing effects when it comes to treating various organ damages as the MSCs-EVs have demonstrated sound healing effects in a variety of tissue injuries, including cardiovascular, liver, and skin wounds, which include processes of angiogenesis, cell proliferation regulation, and immunological modulation.[168]

Type 1 and type 2 are the two major types of diabetes, and both are associated with some degree of destruction of pancreatic islet cells. Diabetic patients may benefit from a potential novel medication that promotes the regeneration of cells of islets and improves the susceptibility of targeted insulin tissues.[169] Deficiency of Insulin is caused by type 1 diabetes, which destroys autoimmune tissues, particularly in the islet cells of patients. Furthermore, it has been shown that MSCs-EVs have the exclusive capacity to manage immune repair in pancreatic islet tissue to treat diabetes. MSCs-Exos can chemo-attract pancreatic tissue and activate the pancreatic and duodenal homeobox 1 pathway, further stimulating pancreatic β-cell regeneration and insulin production.[170] Many research reports have revealed that MSCs-Exos increase the regulatory T-cell levels and factors like IL-4. IL-10 and transforming growth factor (TGF). Thus, it ultimately improves diabetic mice's autoimmune response and islet regeneration. Type 2 diabetes is caused by the reduced insulin sensitivity of the peripheral tissues, which is also associated with insulin secretion defectiveness by pancreatic beta cells. The expression of muscle transporter 4 results in the reversal of peripheral insulin resistance, as well as the reduction of islet cell apoptosis and the restoration of insulin secretion function, according to studies showing the ability of MSCs-Exos to reinstate the phosphorylation protein kinase B and insulin receptor substrate 1.

As mentioned, a patient suffering from diabetes is generally associated with various other complications, one of which is diabetes ulcers, which majorly occur on the feet of patients, also called diabetic feet. MSCs-Exos improves the polarization ratio of M2/M1 (macrophage), which reduces inflammation and accelerates healing in diabetic ulcer wounds. The extent of wound vascularization impacts the wound's healing pace and remodeling. Also, as mentioned, MSCs-Exos are abundant in therapeutic noncoding RNAs and growth factors, and they may efficiently enhance the vascularization of wound skin while being more stable and safer than cell treatment. MSCs-Exos have also been shown to improve renal function, restore podocyte function, and postpone renal fibrosis.[170]

MSCs-Exos include a wide range of proteins for repair, growth cytokines, and noncoding RNAs. These have therapeutic effects that can improve organ repair done by diabetes, and their consequences aid in controlling vascularization, anti-apoptotic, and inflammation processes. MSCs-Exos may be a successful therapy method for diabetes and its associated consequences. Diabetes is frequently associated with several macrovascular problems, including hypertension, coronary heart disease, macrovascular, microvascular, and arteriosclerosis issues. MSCs-Exos have been shown to offer outstanding healing effects when it comes to treating various organ damages

as the MSCs-Exos have demonstrated good healing effects in a variety of tissue injuries, including cardiovascular, liver, and wounds on the skin, which include processes of angiogenesis, cell proliferation and regulation along with immunological modulation.

Conclusion

Therefore, stem cell therapies for various illnesses are increasingly being considered in tandem with the investigation of multiple types of stem and progenitor cells. The field of transplanting human embryonic or post-natal pluripotent stem cell-derived cells is rapidly expanding, and there have been encouraging outcomes in the grafting of dopaminergic neurons obtained from embryonic stem cells. This is because most scenarios currently cannot be imagined utilizing lineage-specific, tissue-resident natural stem cells. Does the future hold promise for MSCs as therapeutic cells? MSC treatments have been effective in maintaining patients' health. However, the safety of ES- or iPS-cell-based therapy has not yet been established. Exosome research and interest in exosome roles in disease pathology and possible treatments have led to exponential growth in the exosome field; however, inconsistent exosome collection, isolation, and analysis methods have posed a major obstacle to the field's quick progress. The International Society for Extracellular Vesicles (ISEV) has released a policy statement addressing these concerns and providing recommendations to investigators to avoid discrepancies in exosome and EV research.

Nonetheless, MSC therapies have been effective in maintaining patients' health. Examining the mechanism of exosomes in treating diseases is the primary connection to future clinical research. Exosomes are a cutting-edge therapeutic concept with unique advantages. MSC exosomes are signaling molecules that work similarly to MSCs but have a more robust membrane structure. Exosomes produced from MSCs are more immunogenic and well-tolerated than whole-cell therapy. There are more options for treating illnesses thanks to these advantages. There are several limitations to exosome manufacturing and purification, and more studies are required to determine the exact nature of their therapeutic benefits. Exosomes need to clear up a few things before they may be used in a clinical context. Detailed characterization will be required to determine the different exosome subpopulations. Exosomes have a wealth of evidence supporting their potential as novel therapeutic agents, but this needs to be clinically confirmed.

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