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Regenerative potential and clinical application of mesenchymal stem cells-derived exosomes (review)

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ABSTRACT

For more than 50 years, mesenchymal stem cells have been extensively studied as a therapeutic agent in the treatment of various diseases. MSC-derived secretome including growth factors, cytokines, microvesicles and exosomes is the major driver in realizing the of beneficial effect of MSC-based therapy. Exosomes play an important role in the organism homeostasis and disease development working as a vehicle for the transfer of signaling and regulatory molecules between cells. Exosomes size, stability, and cargo content reflect the physiological state of parent cells making them an attractive new tool for regenerative medicine. Cell-free therapy or cell therapy 2.0 is being developed.

***THE PURPOSE** of this study was to analyze the literature data on the regenerative potential and clinical application of exosomes derived from mesenchymal stem cells.*

***METHODS.** An analytical review of literature data was conducted using the information analysis of Medline (PubMed), Web of Science and Scopus databases, Google Scholar and the Cochrane Central Register of Controlled Trials (CENTRAL) and other sources up to the inclusive year 2022 using the keywords: "exosomes", "mesenchymal stem cells", "cell-free therapy", "secretome", "miRNA".*

***RESULTS.** In this review, we examine the molecular profile of exosomes derived from different MSC sources and explore their biological properties, the results of clinical application of MSC-derived exosomes in the treatment of COVID-19, alopecia, skin aging and osteoarthritis. Furthermore, we analyzed the existing issues in the development and application of these new biomedical products.*

***CONCLUSION.** The study, research and development of biotechnological products based on exosomes from various stem cell types represent new stages in the development of regenerative medicine. Understanding the unique biological properties of MSCs derived from various tissue sources is one of the keys to develop effective exosome-based biotechnological products to address specific medical goals.*

***KEY WORDS:** exosomes; mesenchymal stem cells; cell-free therapy; secretome; miRNA*

Multipotent mesenchymal stromal/stem cells (MSCs) are heterogeneous population of cells presented in various tissues and organs. MSCs have unique properties, including high proliferation rate, rich secretome and the ability to differentiate into various cell types of connective tissues [1]. Their vital role is maintaining regeneration of the tissues through cell type specific differentiation, immunomodulation, secretion of trophic factors with mitogenic, anti-apoptotic, pro-angiogenic properties, secretion of extracellular matrix that organizes tissue microenvironment [1, 2]. MSCs also contribute to several pathological conditions such as cancer development or fibrotic reparative processes [3]. However, the main focus of MSCs studies during over 50 years has been their use as a key building block in the rapidly growing field of regenerative medicine. Friedenstein was the first to describe MSCs from rodent bone marrow as plastic adherent, clonogenic non-hematopoietic cells [4]. In 1992, human bone marrow MSCs were isolated and expanded in culture [5]. With increasing evidence of successful MSC culture isolation and expansion from different tissue sources (adipose tissue, skeletal muscle, umbilical cord blood, Wharton's jelly, placenta, amniotic membrane, skin, dental pulp, endometrium etc.) [6] and finding their therapeutic potential in the treatment of diverse diseases, the International Society for Cell Therapy (ISCT) has outlined minimal criteria for defining MSCs. These criteria include adherence to plastic, specific expression profile of cell surface markers and differentiation into the adipogenic, chondrogenic and osteogenic lineages *in vitro* [7]. Over 1400 MSC-based clinical trials were registered (http://www.clinicaltrial.gov) worldwide to treat different medical conditions since 1993, when the first MSC infusion in patients was con-

ducted. The first MSC therapeutic product approved in European Union was Alofice (allogenic adipose-derived MSCs) for the treatment of complex perianal fistulas in Crohn's diseases [8]. Allogeneic and autologous MSC-based products were also approved in South Korea, Canada, Japan, India [9]. MSC-based cellular therapy is the most promising regarding its effectiveness in such diseases as osteoarthritis, ischemic heart disease, graft-versus-host disease, spinal cord injury, COVID-19, and multiple sclerosis. [6].

However, the mechanisms of MSC therapeutic action are still not fully defined. There are three main ways of MSC action that mediate their beneficial effects after being transplanted into patients: (1) chemotaxis-mediated MSC migration into the injured organ or tissue and differentiation into cells of mesodermal origin; (2) reprogramming of the recipient's cells through cell-to-cell contact, the secretion of paracrine factors and extracellular vesicles resulting in tissues microenvironment reorganization, affecting immune cells, vessels etc.; (3) MSC apoptosis leading to the efferocytosis of debris and phagocyte immune cell polarization into anti-inflammatory M2-type cells (**Fig. 1**) [2]. All of them could be involved in the realization of MSC therapeutic effect after the transplantation. The balance between the three ways depends on various factors including tissue origin of MSCs, allogeneic or autologous product, systemic or local injections. Thus, mesodermal MSC differentiation is mainly realized using autologous BM-MSCs in 3D transplants for bone defect restoration but unlikely in cell suspension injection [10]. The majority of studies demonstrate a strong paracrine effect with the secretion of a huge amount of cytokines, growth factors and extracellular vesicles as the predominate pathway of MSC therapeutic action [2]. MSC-derived extracellular vesicles, especially exosomes, play a critical role in the MSC physiological function and their therapeutic effects. Exosomes composition, biological properties, mechanisms of action, higher stability compared to cell-based products make them an attractive candidate for application in regenerative medicine leading to the development of a new era in cell therapy.

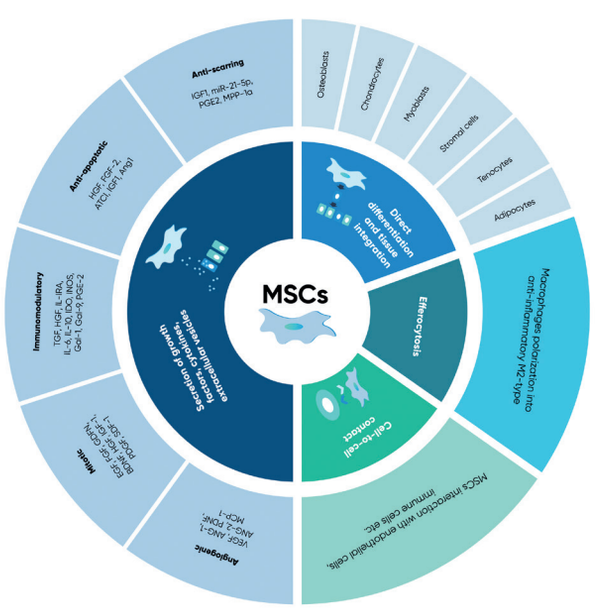

Fig. 1. The mechanism of action of MSC-based therapy.

Nanosize vesicles

Exosomes are a nanosized (30-150 nm) subset of of the endosomal origin that mediate intercellular communication by transferring functional proteins (enzymes, transcription factors, lipids, extracellular matrix proteins, receptors, growth factors, cytokines), metabolites, lipids and regulatory nucleic acids (mRNA, miRNA, lnRNA etc.) to recipient cells [11-13]. The exosomes cargo composition reflects the nature of donor cell and its physiological state.

Exosomes have essential and indispensable functions in the human organism, but surprisingly they were discovered less than 40 years ago. In the 1940s, scientists first acknowledged that there are nanoparticles with specific functions in the intercellular space. At that time, scientists considered exosomes as platelet dust and were convinced that they "carry out garbage from the body." The term "extracellular vesicle" was coined in 1971, and "exosomes" – in 1983. The first study demonstrating a direct role of exosomes in intercellular communication was published in 1996. Scientists discovered that B-lymphocytes, with the help of exosomes, can activate T-lymphocytes, thereby forming an immune response [14]. By 2006-2007 it had became clear that exosomes contain different types of RNA, including miRNAs, and their transport within the organism can change gene expression of target cells [15, 16]. From that moment on, exosome research has gained considerable popularity, accompanied by an exponential increase in publications yearly. Exosomes secreted by various cell types, isolated from blood plasma or other biological fluids, and especially produced by different types of stem cells are currently extensively investigated as diagnostic and therapeutic agents.

For years, scientists have debated what causes the therapeutic effect of MSCs. Primarily, it is achieved by releasing a number of biologically active substances, including exosomes, not by directly integrating transplanted cells into the site of damage. Initially, the therapeutic effect of MSC-derived exosomes was shown by Bruno and co-authors in 2009 [17]. The sediment fraction obtained as a result of ultracentrifugation at 100,000 ×g of the conditioned medium of MSCs was then transplanted into mice with acute kidney failure and showed a positive therapeutic effect similar in strength to the transplantation of MSCs themselves. After further examination of the obtained fraction, it was found that the impact was caused by vesicles surrounded by a membrane with an average size of 135 nm, which were exosomes.

Exosomes biogenesis is a complex multistep process. Initially, there is plasma membrane invagination that includes cell-surface proteins and soluble proteins associated with the extracellular milieu [13]. The profile of endosomal cargo depends on the nature of clustered microdomains of the plasma membrane, where the unique composition of lipids and transmembrane proteins attract specific soluble factors [18]. Next, multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs) formed through the pathway of early-sorting endosomes and late-sorting endosomes with cargo sorting and maturation in trans-Golgi network and endoplasmic reticulum. The MVBs fate could be released in two ways: degraded when fuse with lysosomes and autophagosomes or released the ILV as exosomes by fuse with plasma membrane [13]. Vesicular transport and exosomes generation are highly energetic and coordinated process with the involvement of precisely organized molecular machine. Cytoskeleton proteins, Ras-related protein GTPase Rab27a, Rab27b, Rab35 and Rab7, Sytenin-1, TSG101 (tumor susceptibility gene 101), ALIX (apoptosis-linked gene 2-interacting protein X), syndecan-1, ESCRT (endosomal sorting complexes required for transport) proteins, phospholipids, tetraspanins, ceramides, sphingomyelinases, and SNARE (soluble Nethylmaleimide-sensitive factor (NSF) attachment protein receptor) complex proteins are linked to exosomes biogenesis [12, 13].

According to available database, more than 40,000 proteins, 1000 lipids molecules and 7500 RNA are found in exosomes (http://exocarta.ludwig.edu.au, http://www.microvesicles.org). Different classes of proteins are identified in exosome membrane and cargo including tetraspanins (CD9, CD63, CD81), integrins (α4β1, αMβ2, β2, αLβ2, α3), cell surface peptidase (CD26, CD13), heat shock proteins (hsp70, hsc70, hsp90, hsp75), cytoskeletal proteins (actin, cofilin, tubulin), membrane transport and fusion (annexins I, II, IV, V, VI, RAB7, RAP1B, RABGDI), signal transduction (Gi2α, 14-3-3, Lck), metabolic enzymes (citrate synthase, malate dehydrogenase, fatty acid binding protein-3), ubiquitine/proteasome-related proteins, nuclear proteins (histone H1.1, H1.5, H1.3, H2A, H2B, H4) [12]. Lipids are the main component of exosome membrane and also

take part in their biogenesis and release. Glycosphingolipids, sphingomyelins, cholesterol, and phosphatidylserine are dominant classes of exosome lipids. Besides that, fatty acids like arachidonic acid, leukotrienes prostaglandins, phosphatidic acid, docosahexaenoic acid can be found in MSC-derived exosomes [12, 19]. Different types of RNA (miRNA, mRNA, rRNA, tRNA, lnRNA, snRNA) in exosomes cargo together with protein content underlie the exosome's function realization as an intracellular communication and a component of various signaling pathways [11, 12]. Post-translation regulation of gene expression is the main way of cell fate regulation released by miRNA.

It was found that several proteins are uniformly present in exosomes derived from different sources. The tetraspanin family (CD9, CD63 and CD81) members and proteins required in endosomal transport (Alix, TSG101) are used to clarify the extracellular vesicles subtypes and origin [11]. These markers have been defined by the International Society of Extracellular Vesicles as an extracellular vesicles hallmarks, demonstration the lipid-bilayer structure and/or- endosomal origin. Purified exosomes should be negative on expression cis-Golgi maker GM130, apoptosis related markers – calnexin, ApoA-1 and cytochrome c [20].

Exosomes can transmit information to recipient cells in several possible ways: (1) direct interaction of the exosome's transmembrane receptor with the receptor on the plasma membrane of the recipient cell, which will initiating the appropriate signaling pathway; (2) fusion of the exosome membrane with the membrane of the recipient cell and subsequent release of its contents into the cytoplasm; (3) capture (internalization) of exosomes by the cell in various ways (clathrin/caveolin-mediated endocytosis, phagocytosis, micropinocytosis) [21]. Internalization of exosomes occurs quickly and is dependent on temperature. The contents of exosomes can enter both the cytoplasm and cytoplasmic compartments (endoplasmic reticulum, Golgi complex, late endosomes, lysosomes) [11]. In addition, it was found that exosomes are able to recirculate from one cell to another, penetrating not only the surface, but also the deep layers of the tissues. Growth factors contained in exosomes are more stable and act longer than those that enter the cell without the participation of exosomes. Fusion of exosomes with lysosomes, which have an acidic environment, promotes the activation of some signaling molecules, for example, TGFβ-1, and fusion with late endosomes accelerates the release of exosome contents into the cytoplasm [12]. The exosomes miRNA, entering the cytoplasm, find their target mRNA molecule and block protein synthesis from it, thereby acting as a regulator of gene expression in the recipient cell. Thus, the ways of getting exosomes into cells are different, and the ways of implementing the informative message of exosomes are multidirectional.

Properties and molecular profile of MSC-derived exosomes

The MSCs isolated from different tissue sources are efficiently used in the treatment of different etiology wounds, musculoskeletal system disorders (osteoarthritis, degenerative disc disease), cardiovascular diseases (heart failure, ischemic heart disease, ischemic cardiomyopathy, chronic myocardial ischemia), nervous system diseases (ischemia stroke, critical limb ischemia, spinal cord injury), autoimmune diseases (multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, Sharp's syndrome), diabetes mellitus [6]. The most fully characterized MSCs used in clinical practice are MSCs isolated form umbilical cord (UC-MSCs), bone marrow (BM-MSCs), adipose tissue (ADSCs) and dental pulp (DPSCs) [22, 23]. The positive effect of MSCs application in the treatment of varying disorders is released due to their intrinsic biological properties: (1) ability to find damaged tissues or own native niches after systemic adoptive transfer by expression adhesion molecules (CD44 and integrins), chemokines receptors (CCR2, CCR7, CCR10, CXCR4, CXCR5, CXCR6), metalloproteinases (MMP-1, MMP-9) [24]; (2) trophic function – the secretion of numerous grows factors (Ang-1, EGF, FGF, GDFN, BDNF, HGF, IGF-1, PDGF, SDF-1, VEGF), cytokines and extracellular vesicles that stimulate recipient's stem cells to activation and proliferation, induce new vessel formation followed by the improvement of damaged tissue nutrition, provide anti-apoptotic and anti-oxidant effect [23]; (3) immunosuppression function – MSCs suppress the proliferation and activity of $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells, proinflammatory macrophages, neutrophils, nature killer cells and B cells. At the same time, MSCs stimulate regulatory T and B cells, anti-inflammation macrophages and immature dendritic cells. Immunomodulation properties of MSCs are released by the secretion of TGF-β, IL-6, IL-10, prostaglandin E2 (PGE2), LIF, TSG-6, inducible nitric oxide synthase (iNOS) and indoleamine 2,3-dioxygenase (IDO) etc. (**Fig. 1**), [25].

Despite the pronounced MSC therapeutic properties, the use of cell-based products has several limitations as low stability, special storage conditions, high-cost, risk of immune reaction, cannot be readily used as off-the-shelf product, cannot cross blood-brain barrier that limits MCS effectiveness and application in neurodegenerative disorders [26]. The MSC-derived exosome biological properties and characteristics as a biotechnological product could compensate the MSC disadvantages and make exosomes a new innovation tool in regenerative medicine. Exosomes secreted by MSCs lack the risk of immunogenic response, since they do not express HLA-ABC, HLA-DRDQ or co-stimulatory molecules CD80, CD86 [27, 28]. So, the use of allogenic MSCs as exosomes source in large-scale production is no limited. Exosomes lack replicating potential, they cannot change the DNA sequence of the recipient cells that minimizes the risk of malignant transformation after application [21]. Exosomes are able to penetrate the blood-brain barrier due to their nanosize that open new option for the treatment of neurological disorders [12]. Exosomes are more stable than cells and can be stored for several months at -80 °C or several weeks at +4 – +6 °C [20, 29]. This makes the transportation conditions easier and open the possibility for off-the-shelf availability.

Numerous studies have shown that MSC-derived exosomes display the key therapeutic properties of MSCs. Exosomes fusing with recipient cells transfer the proteins and miRNA resulting in the apoptosis inhibition, angiogenesis stimulation, pro-inflammatory cytokines reduction, oxidative stress attenuation, extracellular matrix remodeling, activation of niche-specific stem cells etc. [26]. MSCs derived from different tissue sources have different signaling network, transcriptome and secretome followed by unique cargo content in exosomes [26, 30, 31]. Comparative proteomic and miRNA analysis of exosomes derived from human bone marrow, adipose tissue, and umbilical cord MSCs revealed unique pattern of differentially present molecules and summarized in **Table 1** [30-32]. BM-MSC-exosomes display pronounced neuroprotective properties due to the elevated level of miR-125, 145, 18, 21, anti-apoptotic and anti-genic properties trough miR-132, 145, 125. ADSC-exosomes stronger induce phagocyte activity of neutrophils than BM-MSC-exosomes or UC-MSC-exosomes, while the angiogenesis is more specific for UC-MSC-exosomes [30]. Proteomic analysis of different types of exosomes confirmed neurogenesis features of BM-MSC-exosomes and anti-oxidant and pro-angiogenic properties of ADSC-exosomes [31]. The miRNA profiling of BM-MSC-exosomes revealed 23 prevalent miRNAs which regulate expression of more than 5000 genes related to cardiovascular system development, cell death and growth, fibrosis, vasculogenesis [32]. The anti-fibrosis effect of MSC-derived exosomes realized via miR-29- and Let-7i-mediated attenuation of TGFβ signaling that leads to the decrease of the collagen type I and fibronectin secretion [33]. The MSC-exosomes showed strong immunosuppressive effect similarly to parent cells. BM-MSC-exosomes led to the inhibition of Th1, Th17 and antigen presenting cells activation through the attenuation of TCR and TLR4 signaling followed by decreased NFAT1, NF-κB and p38 nuclear translocation. TGF-β1, PTX3, let-7b-5p, and miR-21-5p are the key molecules that underlie exosomes immunomodulatory activity [34]. Pro-angiogenic miRNA presented in MSC-exosomes induced the expression of ANGP1, ANGPT4, MAZ, NCOA1 genes that positively regulated VEGF expression. From the other side, genes involved in angiogenesis inhibition of SPINK5, ALOX5, and PPM1A depleted after exosomes treatment [32]. Cell proliferation in exosomes targeted region of damaged tissue occurs via Akt-STAT3 and ERK1/2 pathways that regulate the expression of mitogenic factors – FGF, SDF1, HGF, IL-6, IGF1 [31].

Table 1. The molecular profile and biological properties of different source-derived MSCs exosomes.

MSCs source of exosomes	Type of cargo	Content	Biological properties	Ref.
BM-MSCs	Identities markers	Positive: CD9, CD81, Alix, TSG101, flottilin-1, HSP70, β-actin, glyceraldehyde-3-phosphate dehydrogenase, CD73, CD105, CD44, CD146, CD9e, CD29. Negative: GM130		[59], [28]
	Proteins	STAT3	Wide array of cellular processes, including cellular proliferation, migration and angiogenesis by targeting the expression of many genes such as those involved in cell cycle control (c-Myc and cyclin) and encoding cytokines and growth factors (IL-6, HGF, and VEGF)	[59]
		HGF, IGF1	Cell cycle entry and proliferation, anti-apoptosis	[60]
		Notch2	Increases skeletal remodeling in osteoprogenitor cells	[31]
		ADAM9, ADAM10, CD81, CACNA2D1	Neurodevelopment, synaptic plasticity, dendritic spine morphology	
		ALDOA, APOA4, EPO, FN1, MUSK, NPTX1, PDGFRA, CSF3	Effect on the proliferation and viability of endothelial cells, fibroblasts and keratinocytes	[28]
	mRNA	CDC6, CDK8, CCNB1	Cell cycle entry and proliferation, anti-apoptosis	[60]
	miRNA	miR-23a-3p, miR-424-5p, miR-144, and miR-130a-3, miR-31-5p, miR-125a-5p, miR-126-3p, miR-221-3p, miR-132	Circulatory system development, comprised of processes related to vasculature and tube development	[33], [28]
		miR-23a-3p, miR-424-5p, miR-144-3p, miR-130-3p, miR-145-5p, miR-29b-3p, miR-29a-3p, miR-25-3p, miR-221-5p, miR-21-5p, miR-125b-5p, miR-22-3p, miR-199a-3p, and miR-191-5p	The regulation of cell growth, fibrosis, proliferation	[33]
		miR-29 family and Let-7i	Target the TGF-β pathway, collagen I synthesis	[30]
		miR-125b-5p, 181c-5p, 149-4p, 486, 143-5p	Angiogenesis, neurogenesis, anti-apoptosis	
ADSCs	Identities markers	Positive: Alix, TSG101, CD9, CD81, CD63, CD105, CD44, CD73, CD146, CD29 Negative: GM130, Calnexin, HLA-ABC and HLA-DRDPDQ, CD80, CD86		[27], [28]
	Proteins	NRF2, peroxiredoxin (PRDX) 1, 4, and 6	Reduction oxidative stress	[61, Shin, 2020 #81]
		IL-1ra, IL-6, G-CSF, Eotaxin, IP-10, and MCP-1	Chemotaxis, inflammation regulation	[62]
		ADAM9, ADAM10	Neurodevelopment, synaptic plasticity, dendritic spine morphology	[31]
		Wnt, FGF, EGF, PDGF, TGFβ, IL1R1, angiopoietin-1 (ANGPT1), WNT4, PAI-1, matrix metalloproteinase (MMP)-2 and 9	Angiogenesis, proliferation, activation of endothelial function and healing Tissue regeneration	[28], [63]
		GDNF, FGF-1, BDNF, IGF-1, NGF	Trophic support to injured neurons and promote axonal regeneration	[63]
		TGF-β, NO, IDO, HO-1, PGE2, IL-10, IL-35 and IL-1Ra	Attenuate inflammation	
	miRNA	miR-486-5p, miR-99b-5p, miR-423-5p, miR-10a-5p, miR-28-3p, miR-125b-5p	Regulate neurogenesis and brain development prevent apoptosis, facilitated viability	[64]
		miR-125b-5p, 181c-5p, 149-4p, 486, 143-5p, 146a-5p, 132-5p, and 145-5p	Th1 and Th2 activation pathways, phagocytosis,	[30]
		miR-486-5p, miR-10a-5p, miR-10b-5p, miR-191-5p, miR-222-3p and miR146a	Neurogenesis Anti-apoptotic Cell viability Inhibition of Th1 activation	[63]
		miR-21-3p miR-210, miR-378, miR-31-5p, miR-125a-5p, miR-126-3p, miR-221-3p, miR-132	Wound healing Angiogenesis	[28]
UC-MSCs	Identities markers	CD9, CD63, Rab5, CD81, TSG101		[65]
	Proteins	14-3-3ζ	Phosphorylate of YAP by transporting the 14-3-3z protein, which inhibited WNT/β-catenin signal, enhanced collagen deposition, and inhibited excess fibroblast expansion transduction, enhanced collagen deposition, and inhibited excess fibroblast expansion	[31]
		PAI-1	Plays a significant role in maintaining endothelial homeostasis and regulating fibrosis, contributes to faster wound healing by inhibiting uPA/tPA/plasminogen and plasminogen-dependent MMP activity	
		GM-CSF, IL-15, IL-6, IL-8, TNF-α, IL-1β, IL-2, and IL-10	Anti-inflammatory and immunomodulatory agent	[66]
	miRNA	miR-136	Key rejuvenation factor regulating cell senescence and survival	[67]
		miR-21, -23a, -125b, and -145	Suppressed myofibroblast formation by inhibiting the transforming growth factor-b2/SMAD2 pathway resulted in anti-scarring functions	[68]
		miRNA-146a-5p, 132-5p, 486, 145-5p, miR-132 and miR-145	Angiogenesis, Th1 and Th2 activation pathways	[30]
DPSCs	Identities markers	CD9, CD63, and CD81		[69]
	miRNA	miR-27a and miR-22, miR-130a-3p, miR-513b-5p, miR-30b-5p, miR-34a- 5p, miR-324-5p, and miR-378f	Osteogenesis	[69]
		let-7 and miR-29(a,b)-3	Angiogenesis	[69]

Compared to other types of MSC-exosomes, DPSC-exosomes may possess better therapeutic potential in dental diseases, neurological disorders, ophthalmological diseases and wounds that is associated with the neural-crest origin of DPSCs. These cells demonstrate more pronounced ability to neuronal and glial differentiation than UC-MSCs, ADSCs or BM-MSCs which determines the inherited therapeutic cargos in their secreted exosomes [35]. Despite the limited studies, DPSC-derived exosomes show a great promise in the treatment of disorders with neurological component.

Considering all the above, the exosomes therapeutic potential can vary depending on the tissue origin of MSCs and their biological properties (**Fig. 2**). Based on analyzed exosomes and parent cells molecular profile we hypothesized that BM-MSC-derived exosomes may be more effective in the treatment of musculoskeletal disorder since one of BM-MSC physiological roles is maintaining osteoblast/osteoclast functions. ADSC-derived exosomes similar to the cells derived from metabolic active adipose tissue display strong immunomodulation properties and could be applicable in the treatment of autoimmune diseases or chronic inflammation ablation. UC-MSCs are the youngest MSCs among adult stem cells with a huge amount of bioactive molecules that are transported via exosomes inducing proliferation, angiogenesis, extracellular matrix remodeling, and anti-apoptosis. Thus, UC-MSC-exosomes could be more effective in the treatment of dermatological, pulmonary diseases and anti-aging. DPSC-derived exosomes may have predominant neuroprotective properties due to the neural crest origin of parent cells. Comprehensive studies of biological properties and *in vivo* therapeutic effect of exosomes derived from MSCs of different origin should be conducted to choose the most effective treatment option.

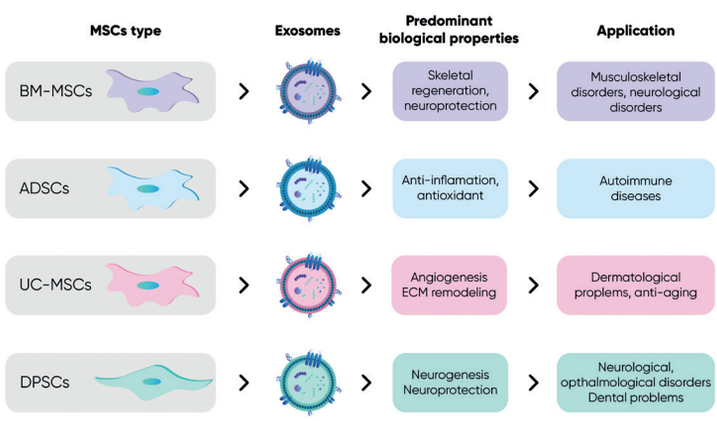

Fig. 2. The proposed hypothesis of different exosomes types application in therapy based on their MSCs source and predominant biological properties.

Exosomes biodistribution

Although biotechnological products do not belong to classic pharmaceuticals but to Advanced Therapies Medicinal Products (ATMPs) by Regulation 1394/2007/EC, the European Medicines Agency (EMA), the principles of pharmacokinetics are important for understanding of their effects on the human body. A few preclinical studies on animal models have shown that in the case of intravenous administration, the largest concentration of exosomes (70-95 %) leaves the bloodstream within the first 30 minutes [36, 37]. After one hour of systemic administration, exosomes are not detected in the bloodstream. Redistribution of exosomes in the body occurs in at least three phases: fast (less than an hour), middle (from 2 to 12 hours), and late (more than 24 hours). The liver, spleen, lungs, and kidneys are the organs that exosomes reach primarily. When administered intravenously, the highest concentration of exosomes is detected in the liver, and remains permanently high for 24 hours [38]. A lower concentration of exosomes is detected in the following organs: heart, brain, bones, gastrointestinal tract in the middle phase of redistribution. This redistribution of exosomes after systemic administration can be partly explained by the peculiarities of the cellular and molecular composition of both exosomes and target organs. For example, the accumulation of exosomes in the liver is possible due to the presence of Kupffer cells – specialized macrophages that absorb exosomes. In the lungs, exosomes are adsorbed by fibroblasts and epithelial cells. Adsorption rate depends on the presence of special molecules – α6β1 integrins – on the surface of exosomes. Exosomes are removed from the body through the kidneys and liver [36]. The use of exosomes by local injection predominates in clinical studies, with a limited number of clinical results published with the intravenous route of administration. However, it was shown that intravenous administration of high concentrations of exosomes (from 10 billion per mL) was easily tolerated by patients and effective in overcoming the severe cases of COVID-19 [39].

Clinical translation of MSC-derived exosomes

The search was conducted using PubMed and www.clinicaltrials.gov. The keywords used were "exosomes" and "mesenchymal stem cells" with the filter "clinical trials". By October 2022, we have found 104 papers and 293 clinical trials in which exosome-based formulations are applied. The articles were selected in two stages. Firstly, the abstracts identified were downloaded, and the list was narrowed using the inclusion or exclusion criteria. Then, the full texts of this list were retrieved and evaluated for eligibility. The reference lists of the identified publications were hand-searched for additional relevant studies, and these were subject to the same two-stage selection. All the studies conducted on human subjects with published results were included.

Out of the 293 trials and 104 articles, 47 met the inclusion criteria. The registered clinical trial list that concerns MSC-derived exosomes is presented in **Table 2**. The most frequently MSC exosomes safety and effectiveness are evaluated for pulmonary disease (n = 13), dominant COVID-19 pneumonia, dermatological problems (n = 9) (hair loss, psoriasis, wound, ulcer, dystrophic epidermolysis bullosa), ophthalmological diseases (n = 4) (dry eyes, retinitis pigmentosa, macular halls) and orthopedic disorders (n = 6). The ADSCs, UC-MSCs, PI-MSCs, BM-MSCs and amniotic fluid are the most popular sources of exosomes in clinical trials (**Tables 2, 3**). The doses of exosomes applied are significantly varied from several million to more than 100 billion. Most of these trials are in phase I or II and are under extensive research. The published results of MSC-exosome-based clinical trials or case report are listed in **Table 3**.

Table 2. List of registered clinical trials of MSC-derived exosomes.

N	Clinical Trial Identifier	Study title	Condition	Source of exosomes	Administrated dosage	Number of patients	Clinical trial phase	Location	Status
1	NCT05658094	Exosome Effect on Prevention of Hair loss	Androgenic Alopecia	PI-MSCs	4 injection with an interval of 14 days during two months, 10×10^{11} exosomes	20	A Phase 1/2, Open-label Study	Iran	Recruiting
2	NCT05523011	Safety and Tolerability Study of MSC Exosome Ointment	Adult healthy subjects/ Psoriasis	MSCs	from Day 1 to 20, the study product will be applied with a gap of 4 hours, 100 µg MSC exosomes/g ointment	10	A Phase 1, Open-label Study	Singapore	Completed
3	NCT05499156	Safety of Injection of Placental Mesenchymal Stem Cell Derived Exosomes for Treatment of Resistant Perianal Fistula in Crohn's Patients	Perianal Fistula in Patients With Crohn's Disease	PI-MSCs	n/d	n/d	A Phase 1/2, Open-label Study	Tehran, Iran	Active, not recruiting
4	NCT03384433	Allogenic Mesenchymal Stem Cell Derived Exosome in Patients With Acute Ischemic Stroke	Cerebrovascular Disorders	n/d	MSC-generated exosomes transfected by miR-124, one month after attack, via Stereotaxis/Intraparanchymal	n/d	A Phase 1/2, Open-label Study	Iran	Unknown
5	NCT04276987	A Pilot Clinical Study on Inhalation of Mesenchymal Stem Cells Exosomes Treating Severe Novel Coronavirus Pneumonia	Coronavirus	Allogeneic AD-MSCs	5 times aerosol inhalation of MSC-derived exosomes (2.0×10^8 nanovesicles/3 mL at Day 1, Day 2, Day 3, Day 4, Day 5	24	Phase 1	Wuhan, China	Completed
6	NCT05490173	The Pilot Experimental Study of the Neuroprotective Effects of Exosomes in Extremely Low Birth Weight Infants	Premature Birth Extreme Prematurity Preterm Intraventricular Hemorrhage Hypoxia-Ischemia, Cerebral Neurodevelopmental Disorders	n/d	Will be administrated intranasal in ELBW infants	n/d	Not Applicable	Russian Federation	Not yet recruiting
7	NCT04270006	Evaluation of Adipose Derived Stem Cells Exo.in Treatment of Periodontitis (exosomes)	Periodontitis	AD-MSCs	n/d	n/d	Early Phase 1	Egypt	Unknown
8	NCT04798716	The Use of Exosomes for the Treatment of Acute Respiratory Distress Syndrome or Novel Coronavirus Pneumonia Caused by COVID-19 (ARDOXSO)	Covid19 Novel Coronavirus Pneumonia Acute Respiratory Distress Syndrome	n/d	For all: period of 5 days, with a minimum of 24 hours between doses recorded. First Cohort: intravenously every other day on an escalating dose: ($2:4:8\times10^9$/mL). Dose escalation will begin at 2×10^9 exosomes. Second Cohort: IV every other day on an escalating dose ($8:4:8\times10^9$/mL). Dose escalation will begin at 4×10^9 exosomes. Third Cohort: IV every other day ($8:8:8\times10^9$/mL) Fourth Cohort: IV every other day ($8:8:8\times10^9$/mL); placebo (~25 %)	n/d	Phase 1 Phase 2	United States	Not yet recruiting
9	NCT02565264	Effect of Plasma Derived Exosomes on Cutaneous Wound Healing	Ulcer	Plasma-derived exosomes	The plasma-derived exosomes will be applied to the participants' ulcers daily for 28 days	n/d	Early Phase 1	Kumamoto, Japan	Unknown
10	NCT04202783	The Use of Exosomes In Craniofacial Neuralgia	Craniofacial Neuralgia	Neonatal stem cell products (NSCP)	All patients will receive the same amount (5 mL concentrated) of exosomes delivered via ultrasound-guided, regional epineural injection and the same amount (5 mL unconcentrated) delivered via IV. Patients will be given 3 mL IV (45mg of the exosome product containing $15\text{-}21\times10^6$ NSCP), and 3 mL of the exosome hyperconcentrate product delivered epineurally using ultrasound guidance (15 mg of the exosome product carrying $5\text{-}7\times10^6$ NSCP).	n/d	Not Applicable	California, United States,	Suspended
11	NCT04202770	Focused Ultrasound and Exosomes to Treat Depression, Anxiety, and Dementias	Refractory Depression Anxiety Disorders Neurodegenerative Diseases	Amniotic fluid	15 cc of unconcentrated solution allogenic exosomes (equivalent to 21 million stem cells, Kimera Corporation) IV in 200 ccs of normal saline dripped over thirty minutes to one hour.	n/d	Not Applicable	California, United States	Suspended
12	NCT05216562	Efficacy and Safety of EXOSOME-MSC Therapy to Reduce Hyper-inflammation In Moderate COVID-19 Patients (EXOMSC-COV19)	SARS-CoV2 Infection	n/d	Patients: IV injection of Exosome-MSC Placebo	60	Phase 2/3	Indonesia	Recruiting
13	NCT05060107	Intra-articular Injection of MSC-derived Exosomes in Knee Osteoarthritis (ExoOA-1) (ExoOA-1)	Osteoarthritis, Knee	Allogeneic mesenchymal stromal cells	Single intra-articular injection of exosomes: $3\text{-}5\times10^{11}$ particles/dose	n/d	Phase 1	Chile	Not yet recruiting

N	Clinical Trial Identifier	Study title	Condition	Source of exosomes	Administrated dosage	Number of patients	Clinical trial phase	Location	Status
14	NCT04356300	Exosome of Mesenchymal Stem Cells for Multiple Organ Dysfunction Syndrome After Surgical Repair of Acute Type A Aortic Dissection	Multiple Organ Failure	n/d	Exosome of MSC at a dose of 150 mg will be given intravenously to patients once a day for 14 times.	n/d	Not Applicable	China	Not yet recruiting
15	NCT04544215	A Clinical Study of Mesenchymal Progenitor Cell Exosomes Nebulizer for the Treatment of Pulmonary Infection	Carbapenem-resistant Gram-negative Bacilli-induced Pulmonary Infection	ADSCs (haMPC-Exos)	Low-dose group: 7 times aerosol inhalation (8.0×10^8 nanovesicles/3 mL each day); high-dose group: 7 times aerosol inhalation (16.0×10^8 nanovesicles/3 mL Day 1-7); placebo	60	Phase 1/2	Shanghai, China	Recruiting
16	NCT05191381	Immune Modulation by Exosomes in COVID-19 (IMECOV19)	COVID-19 Critical Illness Hypercytokinemia Lung Fibrosis	n/d	Application of exosomes in a whole blood assay	40		Ulm, Germany	Recruiting
17	NCT05738629	Safety and Efficacy of Pluripotent Stem Cell-derived Mesenchymal Stem Cell Exosome (PSC-MSC-Exo) Eye Drops Treatment for Dry Eye Diseases Post Refractive Surgery and Associated With Blepharospasm	Dry Eye Disease	Pluripotent Stem Cell-derived Mesenchymal Stem Cell Exosome (PSC-MSC-Exo)	Participants will receive artificial tears for 2 weeks to get the normalized baseline, followed by PSC-MSC-Exo eye drop intervention for 12 weeks.	n/d	Phase 1/2	China	Not yet recruiting
18	NCT05261360	Clinical Efficacy of Exosome in Degenerative Meniscal Injury (KNEEXO)	Degenerative Meniscal Injury	SF-MSC-EX (synovial fluid-derived mesenchymal stem cells-derived exosomes	Experimental 1: The left knee will receive 1 mil. cells/kg SF-MSC-EX by intra-articular injection. Experimental 2: The right knee will receive 1 mil. cells/kg SF-MSC by intra-articular injection. Placebo	30	Phase 2	Eskisehir, Turkey	Recruiting
19	NCT05413148	The Effect of Wharton Jelly-derived Mesenchymal Stem Cells and Stem Cell Exosomes on Visual Functions in Patients With Retinitis Pigmentosa	Retinitis Pigmentosa	Wharton Jelly	2 experimental groups: Single subtenon injection for single eye; Placebo	135	Phase 2/3	Kayseri, Turkey	Recruiting
20	NCT02138331	Effect of Microvesicles and Exosomes Therapy on β-cell Mass in Type I Diabetes Mellitus (T1DM)	Diabetes Mellitus Type 1	n/d	n/d	n/d	Phase 2/3	Egypt	Unknown
21	NCT04213248	Effect of UMSCs Derived Exosomes on Dry Eye in Patients With cGVHD	Dry eye symptoms in patients with chronic Graft Versus Host Diseases (cGVHD).	Umbilical Cord	Participants will receive artificial tears for 2 weeks to get the normalized baseline, followed by UMSC-exo 10 μg/drop, four times a day for 14 days.	27	Phase 1/2	China	Recruiting
22	NCT05475418	Pilot Study of Human Adipose Tissue Derived Exosomes Promoting Wound Healing	Wounds and Injuries	ADSCs		n/d	Not Applicable	China	Not yet recruiting
23	NCT04602442	Safety and Efficiency of Method of Exosome Inhalation in COVID-19 Associated Pneumonia (COVID-19EXO2)	Covid19	n/d	Experimental 1: Twice a day during 10 days inhalation of 3 mL special solution contained $0.5\text{-}2\times10^{10}$ of nanoparticles (exosomes) of the first type; Experimental 2: Twice a day during 10 days inhalation of 3 mL special solution contained $0.5\text{-}2\times10^{10}$ of nanoparticles (exosomes) of the second type. Placebo: Twice a day during 10 days inhalation of 3 mL special solution free of nanoparticles (exosomes).	30	Phase 1/2	Russian Federation	Completed
	NCT04491240	Evaluation of Safety and Efficiency of Method of Exosome Inhalation in SARS-CoV-2 Associated Pneumonia. (COVID-19EXO)							
24	NCT04313647	Tolerance Clinical Study on Aerosol Inhalation of Mesenchymal Stem Cells Exosomes In Healthy Volunteers	Healthy	n/d	Once aerosol inhalation of MSC-derived exosomes. Experimental 1: 2.0×10^8 nanovesicles/3 mL); Exp. 2: 4.0×10^8/3 mL; Exp.3: 8.0×10^8/3 mL; Exp.4: 12.0×10^8/3 mL; Exp.5: 16.0×10^8/3 mL	24	Phase 1	Shanghai, China	Completed
25	NCT03437759	MSC-Exos Promote Healing of MHs (MSCs)	Macular Holes	n/d	Experimental: After air-liquid exchange, 50 μg or 20 μg MSC-Exo in 10 μl PBS was dripped into vitreous cavity around MH, leaving 20 % SF6 or air as tamponade. Control: only pars plana vitrectomy (PPV) and ILM peeling.	44	Early phase 1	China	Active, not recruiting

N	Clinical Trial Identifier	Study title	Condition	Source of exosomes	Administrated dosage	Number of patients	Clinical trial phase	Location	Status
26	NCT05387278	Safety and Effectiveness of Placental Derived Exosomes and Umbilical Cord Mesenchymal Stem Cells in Moderate to Severe Acute Respiratory Distress Syndrome (ARDS) Associated With the Novel Corona Virus Infection (COVID-19)	COVID-19	Umbilical Cord	Experimental: The treatment consists of administration of WJ-Pure™ and EV-Pure™ plus standard care. Placebo: Cryopreservation media plus standard care	20	Phase 1	Missouri, The United States	Recruiting
27	NCT04388982	the Safety and the Efficacy Evaluation of Allogenic Adipose MSC-Exos in Patients With Alzheimer's Disease	Alzheimer Disease	Allogeneic ADSCs	Low-dosage: 5μg MSCs-Exos, Total volume: 1 mL Frequency: Twice a week Duration: 12 weeks; Mid-Dosage: 10 μg MSCs-Exos, Total volume: 1 mL Frequency: Twice a week Duration: 12 weeks; High-dosage: 20 μg MSCs-Exos, Total volume: 1 mL. Frequency: Twice a week Duration: 12 weeks	n/d	Phase 1/2	Shanghai, China	Unknown
28	NCT04173650	MSC EVs in Dystrophic Epidermolysis Bullosa	Dystrophic Epidermolysis Bullosa	AGLE-102		n/d	Phase 1/2	Aegle Therapeutics	Not yet recruiting
29	NCT04134676	Therapeutic Potential of Stem Cell Conditioned Medium on Chronic Ulcer Wounds	Chronic Ulcer	Wharton's Jelly	The Conditioned Medium gel was applied for 2 weeks to the wound and closed by transparent dressing.	38	Phase 1	Jakarta, Indonesia	Completed
30	NCT04657406	Expanded Access to Zofin for Patients With COVID-19	COVID 19	Human amniotic fluid (HAF)	Standard care + 1 mL of Zofin on day 0, day 4 and day 8, containing $1\text{-}5\times10^{11}$ particles/mL	n/d		Organicell Regenerative Medicine	Available
31	NCT04384445	Zofin (Organicell Flow) for Patients With COVID-19	COVID 19	Human amniotic fluid (HAF	Experimental: 1 mL, containing $2\text{-}5\times10^{11}$ particles/mL + Standard Care. The Zofin dose will be diluted in 100 mL of sterile saline at subject's bedside. Placebo: saline IV with 1 mL + Standard Care. The Placebo dose will be diluted in 100 mL of sterile saline at subject's bedside	20	Phase 1/2	Organicell Regenerative Medicine	Active, not recruiting
32	NCT04493242	Extracellular Vesicle Infusion Treatment for COVID-19 Associated ARDS (EXIT-COVID19)	COVID-19	BM-MSCs	Placebo: Normal saline 100 mL; Experimental 1: Normal saline 90 mL and ExoFlo 10 mL, which is 800 Billion EV; Exp. 2: Normal saline 85 mL and ExoFlo 15 mL, which is 1.2 Trillion EV	102	Phase 2	The United States	Completed
33	NCT05228899	Zofin to Treat COVID-19 Long Haulers	COVID-19	Human amniotic fluid (HAF)	Exp 1: 1 mL of Zofin IV, containing $2\text{-}5\times10^{11}$ particles/mL. Zofin will be diluted in 100 mL of sterile saline. Placebo: 1mL of Placebo (normal saline) will be diluted in 100 mL of sterile saline.	30	Phase 1/2	Organicell Regenerative Medicine	Recruiting

Table 3. Summary of published clinical application of MSC-exosomes.

Target Health Condition	Paper title	Patients Trial Phase (if applicable)	Stem Cells' Source	Administrated Dosage	Clinical Trial Identifier (if applicable)	Reference
Skin aging	The Utilization of Human Placental Mesenchymal Stem Cell Derived Exosomes in Aging Skin: An Investigational Pilot Study	n = 40	Human PI-MSCs	5.0 mL of 5×10^{8} exosomes suspended in saline Facial micro needling	-	[52]
Acne scars	Combination Treatment with Human Adipose Tissue Stem Cell-derived Exosomes and Fractional CO_2 Laser for Acne Scars: A12-week Prospective, Double-blind, Randomized, Split-face Study	n = 25	Allogeneic ADSCs-conditioned medium (ASCE)	9.78×10^{10} particles/mL (for the day of FCL treatment) or 1.63×10^{10} particles/mL (for days subsequent to FCL treatment) in a gel solution containing 30 % ASCE.	-	[53]
Alopecia	Hair Regeneration Treatment Using Adipose-Derived Stem Cell Conditioned Medium: Follow-up With Trichogram	n = 22	Allogeneic ADSCs-conditioned medium (AAPE; Prostemics)	3-4 mL, 6 sessions of injections every 3 to 5 weeks. Nappage and papule injections	-	[48]
	The Latest Advance in Hair Regeneration Therapy Using Proteins Secreted by Adipose-Derived Stem Cells	n = 25	Allogeneic ADSCs-conditioned medium (AAPE; Prostemics)	3-4 mL 4 sessions of injections every 3 to 5 weeks. Nappage and papule injections	-	[49]
	Clinical use of conditioned media of adipose tissue-derived stem cells in female pattern hair loss: a retrospective case series study	n = 27	Allogeneic ADSCs-conditioned medium (AAPE; Prostemics)	4 mL of 5×10^{8} cells, 12 injections, 1 every week. Micro-needle roller	-	[50]

Target Health Condition	Paper title	Patients Trial Phase (if applicable)	Stem Cells' Source	Administrated Dosage	Clinical Trial Identifier (if applicable)	Reference
COVID 19	Nebulized exosomes derived from allogenic adipose tissue mesenchymal stromal cells in patients with severe COVID-19: a pilot study	n = 7 Phase I	Allogeneic ADSCs	2.0×10^8 nanovesicles/3 mL on days 1, 2, 3, 4, 5. Aerosol inhalation	NCT04276987	[42]
	Treatment of a COVID-19 long hauler with an amniotic fluid-derived extracellular vesicle biologic	n = 1	Zofin is derived from human amniotic fluid.	3.26×10^{11}/mL 1 mL of Zofin on Day 0, 4, 8 IV	-	[45]
	Exosome derived from bone marrow mesenchymal stem cells as treatment for severe COVID 19	n = 24 Phase II	Allogeneic BM-MSCs (ExoFlo)	15 mL of ExoFlo was added to 100 mL of normal saline. 1.2×10^{12} particles IV	NCT04493242	[44]
	Nebulization Therapy with Umbilical Cord Mesenchymal Stem Cell Derived Exosomes for COVID-19 Pneumonia	n = 7	Umbilical Cord	1×10^6 cells/kg predicted body weight. Aerosol inhalation	ChiCTR2000030261	[43]
COPD	Molecular and Cellular Mechanisms Responsible for Beneficial Effects of Mesenchymal Stem Cell-Derived Product "Exo-d-MAPPS" in Attenuation of Chronic Airway Inflammation	n = 30	Placental MSCs	0.5 mL/once per week for three weeks	-	[70]
Idiopathic pulmonary fibrosis	Treatment of idiopathic pulmonary fibrosis with an extracellular vesicle isolate product	n = 1	Allogeneic BM-MSCs (ExoFlo)	3 doses of ExoFlo: on August 2019 – 2 cc of nebulized ExoFlo; On May 2020 – 10 cc IV; on August, 2020 – 5 cc IV	Case report	[71]
Elbow Arthritis	Treatment Of Elbow Arthritis With A Bone Marrow Derived Mesenchymal Stem Cell Extracellular Vesicle Isolate Product	n = 1	Allogeneic BM-MSCs (ExoFlo)	2cc of $>10\times10^8$ exo/cc. Intra-articular	Case report	[55]
Osteoarthritis	IRB Approved Pilot Safety Study of an Extracellular Vesicle Isolate Product Evaluating the Treatment of Osteoarthritis in Combat-Related Injuries	n = 33 OA of the knee (n = 58), shoulder (n = 32), elbow (n = 16), hip (n = 12), ankle (n = 8), wrist (n = 6)"	Allogeneic BM-MSCs (ExoFlo)	2 mL of 30×10^8 exo/mL. Every patient had four joints injected (n = 132). Intra-articular	-	[55]
	Intra-Articular Injection of an Extracellular Vesicle Isolate Product to Treat Shoulder Osteoarthritis in an Athlete	n = 1	Allogeneic BM-MSCs (ExoFlo)	2 mL of 30×10^8 exo/mL. Intra-articular	-	[56]
Hip labral tears	Intra-Articular Injection of an Extracellular Vesicle Isolate Product to Treat Hip Labral Tears	n = 1	Allogeneic BM-MSCs (ExoFlo)	2 mL of 30×10^8 exo/mL. Intra-articular	-	[57]
Graft-versus-host disease	MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease	n = 1	Allogeneic BM-MSCs	4×10^7 MSCs = 1 unit. 1 dose- 1/10 MSCs Exo unit. As within 2 days no side effects were observed, unit amounts were gradually increased and administered every 2–3 days until a four-time dosage (4 units) was reached.	-	[72]
Chronic kidney disease	Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases	n = 20 (patients) n = 20 (placebo)	UC-MSCs	1 dose – $(1\times10^{10}$ p/g) IV 2 dose – 100 µg/kg/dose	-	[73]

COVID-19

Exosomes derived from MSCs similarly to parent cells demonstrated strong immunomodulatory properties. Exosomes-mediated immunomodulation released by the delivery of various biological factors including cytokines (interleukin- (IL-) 2, IL-6, IL-1β, IL-10, tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), interferon (IFN)-γ, etc.), and chemokines (C-C motif ligand (CCL)-2, CCL-3, CCL-7, C-X-C motif chemokine (CXC)-12, CXC-14)) (**Table 1**) and by direct interaction with cells of immune system [34, 40]. MSCs exosomes suppress the activation of T cells and antigen-presenting cells (APCs), inhibit the development of T helper 1 (Th1) and Th17 cells, while induce regulatory T cell (Treg) generation and M2-type macrophage polarization [34]. Thus, similarly to MSCs, MSC-exosomes can be effective in disease treatment where immune hyperactivation is present including autoimmune diseases, graft-versus-host disease, solid organ transplantation or local inflammatory reaction.

Coronavirus infection leading to a severe form of pneumonia is associated with "cytokine storm" – an extreme inflammatory response, in which inflammatory cytokines are rapidly secreted in a massive amount [41]. Both MSC therapy and MSC exosome therapy are applied in clinical trials for the treatment of severe and mild forms of COVID-19. Due to their nanosize, exosomes could be administered to the lungs directly via aerosol inhalation, while MSCs under the high nebulizer pressure are destructed and should be administrated only systemically. Inhalation of exosomes compared to intravenous MSC administration could potentially increase treatment efficacy through more targeted delivery. To explore the safety and efficiency of aerosol inhalation of exosomes derived from allogenic adipose-derived MSCs, phase II single-arm, open-labelled, interventional trial was completed for the treatment of patients hospitalized with novel coronavirus severe pneumonia [42]. Scientists reported that no adverse events, immediate clinical instability, or dose-relevant toxicity were observed at any of the doses tested. Also, the inflammation biomarker rate decreased significantly, including C-reactive protein (CRP) and lactate dehydrogenase (6/7 patients), interleukin-6 (IL-6) (5/7 patients). Another trial using nebulized exosomes derived from umbilical cord MSCs [43] was performed. The efficacy was measured regarding changes in the level of CRP and oxygen saturation. For patients with mild cases, there was a significant difference in the duration of hospitalization between the group who received exosome treatment from the beginning and those who received no exosome treatment or only received exosome treatment once at very late stage. Also, the saturation level increased up to 98-100 % in patients with mild cases of Covid-19. The results clearly show that nebulization treatment of MSC-derived exosomes can promote the absorption of pulmonary lesions and reduce the duration of hospitalization for mild cases of COVID-19 pneumonia. The subsequent study of Exo-Flo, bone marrow derived exosomes, has the most noticeable results among others, with the mean reduction of CRP being 77 % and the average increase of PaO_2/FiO_2 ratio up to 191 % comparing the baseline with 14 days after treatment [44]. Due to its ability to restore oxygenation, downregulate

cytokine storm and reconstitute immunity, ExoFlo is considered a promising therapeutic candidate for severe COVID-19. In this case [45], one patient was enrolled, and a chest X-ray was performed to evaluate the efficiency of the treatment. On day 4, a decrease in small consolidations in both lungs was reported. There were no new pulmonary abnormalities, pneumothorax or pleural effusion. Also, oxygen saturation increased by 98 % and continued to be measured at normal levels up to day 60 (when the study was stopped). By day 21, the patient reported feeling as if he had made a full recovery that continued to improve by day 60.

The mentioned clinical studies demonstrated MSCs exosomes efficiency and safety in the treatment of coronavirus related pneumonia. The accumulation of evidences allow to assume that MSC derived exosomes are able to restore damaged lung tissue through the reduction of inflammation by polarization of M1 macrophages into immunosuppressive M2-type, stimulation of immunosuppressive IL-10 production that inhibit cytotoxic T-lymphocytes and neutrophils, reducing their tissue infiltration, stimulation of epithelial cells regeneration by trophic factors HGF, VEGF, KGF delivery, stimulation of angiogenesis, suppression of collagen deposition and accordingly fibrosis. Exosomes stability, accessibility, strong biological effect and possibility of inhalation make this biomedical product a more perspective candidate in lung diseases treatment than MSC-based therapy. Moreover, positive efficiency in COVID-19 treatment opens new direction of exosomes application in pulmonary disorders such as the treatment of chronic obstructive pulmonary disease, asthma, pulmonary fibrosis, post-COVID-19.

Androgenic alopecia

Androgenic alopecia (AGA) is the most common form of hair loss characterized by a receding frontal hairline in men and diffuse hair thinning in women, with frontal hairline retention, and can impact an individual's quality of life [46]. MSC exosomes show promise in hair restoration as they contain potent cytokines and growth factors that promote hair growth [47]. In the study by Fukuoka H. et al., hair numbers were significantly increased after treatment in both male and female patients [48]. The mean increase was 29 ± 4.1 in male patients and 15.6 ± 4.2 in female patients. Also, in the half-side comparison study, the increase in hair numbers was significantly higher on the treatment side than on the placebo side. The next two studies reported the use of an allogeneic ADSC-conditioned medium (AAPE; Prostemics), which confirmed the efficiency of exosome-based products [49, 50]. ASDC-CM promoted hair growth at the frontal region of the head in patients with androgenic alopecia. The medium time for hair regrowth was 1,5 years for men younger than 50 and 2,5 years for women younger than 60. Even more important is that the medium VAC score was 4.2 (where 1 – worse and 5 – excellent improvement), meaning that after the treatment, patients felt happier and more satisfied with their looks. The minoxidil (2 %) and the ADSC-CM product were compared, with the last one being more effective within a shorter period in terms of the percentage improvement [50]. Moreover, mean hair density increased by 16.4 % (from 105.4 to 122.7 hairs/cm^2) over the 12 weeks of treatment, and mean hair thickness increased by 11.3 % from 57.5 μm to 64.0 μm. None of the studies reported any adverse effects. No patient experienced irritation or itching.

Exosomes have no serious side effects and show encouraging results in androgenic alopecia treatment. The mechanisms that underlie such positive effect of MSCs secretome application on hair growth may be explained by the exosome involvement in the activation of dermal papilla stem cells, which prolong anagen phase of hair cycle, the stimulation to hair stem cells proliferation – new hairs growth, angiogenesis stimulation – tissue nutrition improvement. Exosome based therapy has a great potential for the treatment of different types of non-scarring alopecia including alopecia areata, post-COVID-19 and stress-mediated ones. MSC exosomes as biomedical product demonstrated several advantages compared to PRP treatment or Rigenera technology that are widely used in hair loss treatment. Both PRP and Rigenera technology are invasive procedures for patients, the composition of active substances in final product is unknown exactly compared to exosomes that are fully characterized on molecular profile and quantity indicators. Moreover, the amount and profile of growth factors in PRP drastically decrease with the age and depend on patient's lifestyle and general health. MSC-derived exosome product despite the allogenic origin does not cause immune response and can be standardized as a product.

Dermatologic applications

MSC-exosomal therapies have been proposed as a possible solution to the current lack of effective therapeutic interventions for skin regeneration and rejuvenation. Extracellular vesicles (especially exosomes), which transfer cocktails of functional cargo (such as proteins, lipids, miRNAs, other RNAs, and DNA) horizontally between cells, making them multipotent stimulants of endogenous tissue repair [51]. The study by Chernoff G. et al. demonstrated that applying human PI-MSC-derived exosomes improved the tone, quality, and clarity of their skin compared to the Control Group, with a reduction in wrinkles, pores, pigment, oiliness, and improvement in evenness of skin and vascularity for women with the skin aging consequences [52]. Also, there were no adverse, allergic or hypersensitivity reactions reported. There was a constant progression of satisfaction with the results in the Treatment Group from 30 to 120 days, compared with a high degree of dissatisfaction with the results in the Control Group. In the study by Kwon H. et al., they evaluated exosomes' clinical efficacy and safety of adipose-derived MSCs as adjuvant therapy after applying fractional CO_2 laser for acne scars [53]. Three treatment sections with fractional laser were made, and after that ASCE product in the gel form was applied. Treatment with ASCE afforded more favorable responses and a shorter recovery time combined with FCL for acne scarring.

The MSC-derived exosomes showed anti-fibrotic properties, induced proliferation of fibroblasts and keratinocytes, inhibited autoreactive lymphocytes, mediated extracellular matrix remodeling that make exosomes a promising cell-free biotechnological product in the treatment of various dermatological problems like wound, burns, atopic dermatitis, psoriasis and anti-aging therapy [51]. Exosome-based therapy for dermatological/cosmetological purpose can be formulated as solution for injection or in the gel form combined with amino acids, polynucleotides, hyaluronic acids, collagen etc. In the treatment course, exosomes may be applied as monotherapy or in combination with the injection of dermal fibroblasts and also as additional post-procedural care after laser therapy or aggressive peeling procedure.

Musculoskeletal disorders

Knee osteoarthritis and limb arthritis are the leading causes of functional musculoskeletal disabilities in adults. The goals of managing these diseases are directed toward symptomatic pain relief and the attainment of a functional quality of life. The treatment strategy ranges from conservative to surgical management with reparative and restorative techniques. MSC-derived exosomes are the alternative to conservative and non-effective techniques [54]. They act as a directed therapy to halt the disease progression and provide a pain-free range of movements with increased cartilage quality on regeneration. Among all the available MSCs, BM–derived MSCs and their exosomes have been proven to have higher cartilage regenerating potential. A study on ExoFlo, exosomes derived from BM-MSCs, showed that they could be used to significantly contribute to functional and pain improvements in osteoarthritis (OA) at six months [55]. The 33 Navy SEAL veterans with the OA of the knee (n = 58), shoulder (n = 32), elbow, hip (n = 12), ankle (n = 8), and wrist (n = 6) participated in this study and six months post ExoFlo injection improved 77 % in BPI, 80 % in ODI, 76 % in LEFS, 51 % in UEFS, and 77 % in QD. Most improvements (95 %) occurred within the first six weeks post-treatment. Thus, they felt these pain reliefs very quickly and there were no complications or adverse events, minor or major. No patient was observed to have accelerated OA progression from the ExoFlo injection. Another case of ExoFlo administration is the intra-articular injection of an athlete's shoulder [56]. The patient's self-rated overall shoulder improvement was

75 % within two weeks after the injection. The QuickDASH, The Upper Extremity Functional Index, and the Brief Pain Inventory were all improved within two weeks, and that improvement was maintained up to 12 weeks. Considering the elbow OA, there is a vast void between non-operative and operative approaches. The use of acellular products is very perspective regarding painlessness and long-term results. Bender J, Dordevic M. reported a case of a young, very active chiropractor with OA of the dominant right elbow [57]. She was treated with a single intra-articular injection of ExoFlo containing active Growth Factors (over 800) and extracellular vehicles over 10 billion per 2 cc. The results were encouraging 18 months following the injection, patient's elbow is 90 % improved. Furthermore, the elbow range of motion has returned to equal to the opposite elbow following the injection. Thus, the regenerative therapies with MSCs are being seen to hold a future in the management of OA, and exosome-based technologies hold the key to unlocking the potential for the preservation and regeneration of the limbs.

MSC-derived exosomes are a new step in the development of cell therapy that give rise to the era of cell-free therapy or cell therapy 2.0 in regenerative medicine. Exosomes as one of the active components of MSC therapy could be isolated, purified, concentrated in high amount and thoroughly characterized with the aim of therapeutic application and large-scale production. The emergence of a new biomedical product as exosomes is associated with understanding the mechanism of MSCs action, searching the ways to overcome challenges of cell therapy such as the risk of malignant transformation, immune response and low percentage of cells viability after transplantation. Indeed, compared to MSCs, exosomes are characterized by a number of advantages, such as higher stability, relatively simple storage and transport conditions, availability, the ability to pass through the blood-brain barrier, the possibility of being used outside the hospital as nasal spray or aerosol for inhalation. No risk of oncotransformation or immune response that is why allogenic MSCs could be used as an exosomes source. From the other side, exosomes in contrast to MSCs cannot change their "behavior" after interaction with tissue microenvironment and are not able to perform effective homing that calls into question the effectiveness of intravenous exosomes administration. However, similarly to parent cell exosomes demonstrate huge regenerative potential via the ability to prevent cell apoptosis, induce tissue resident stem/progenitor cells proliferation, induce angiogenesis and immunomodulation. Besides the direct therapeutic application, exosomes are considered as the effective instrument of disease diagnostics, prognostics and prediction, target therapy and vaccine development. Since 2010, the quantity of scientific publication with the key word "exosomes" has drastically increased from 5-10/year to more than 5000/year. Early clinical trials and case reports showed that exosomes application could be considered as safe and effective. Nevertheless, many challenges exist around MSC-derived exosome application in medical practice. The development of manufacturing solutions following the GMP standards and assessing the safety and efficacy of EV-based therapies in clinical trial protocols should be in focus of scientific community. It is clear that exosomes quantity and quality characteristics reflect the physiological state of parent cells. Published data are often controversial in understanding the molecular profile of different types of exosomes that could be associated with MSC culture condition, seeding density, passage, donor features etc. This is the reason why MSC-exosomes that underlie commercialized biomedical product should be fully characterized according to the ISEV 2018 recommendation or their future update. At the same time, will the choice of MSCs from different tissue sources as cell source of exosomes affect treatment effectiveness? (**Fig. 2**). Hoang D. et al. discuss the interconnection between MSC tissue origin and MSC effectiveness in downstream therapeutic applications [23]. BM-MSCs are more effective in neurological disorders treatment, ADSCs – in metabolic disorder treatment, wound healing and reproductive system regeneration, while UC-MSCs showed higher efficiency in pulmonary disease treatment. Considering that exosomes reflect parent cells biology and have differentially presented signaling molecules depending on MSC source, the comparative studies of MSC-derived exosome effectiveness *in vitro* and *in vivo* should be performed. This will justify the choice of a cellular source of exosomes for the particular medical purposes. How to calculate the exosomes doses for treatment is the next open question. If gold standard for MSC treatment is the 1 million per kg body mass, what about exosomes? 1 billion per kg body mass, cm^2? How to standardize exosome-based therapy between different hospitals, countries? The International Expert Consensus on the Cell Therapy assumed formula "DOSES" that should be applied in communication between clinicians about MSC therapy. This tool is based on the reporting of 5 core items: donor, origin of tissue, separation from other cell types/preparation method, exhibited cell characteristics associated with behavior, and the site of delivery [58]. The similar instrument should be also development for understanding the effectiveness of MSC-derived exosome therapy.

CONCLUSION

The study, research and development of biotechnological products based on exosomes from different cell types represent a new frontier in regenerative medicine. Based on the significant success of exosome therapy for neurodegenerative disorders, autoimmune, cardiovascular, orthopedic diseases, hair loss, respiratory diseases and dermatological problems, as well as for cancer diagnosis, it is clear that the potential for its application is wide and, therefore, raises considerable interest. There is an obvious need for the further research on the safety and efficacy of exosome-based products. Complex molecular profiling of the exosomes cargo, as well as in vitro and in vivo studies, will allow to determine the optimal algorithm for selecting the composition of exosomes obtained from MSCs of various tissue sources (umbilical cord, placenta, adipose tissue, bone marrow or dental pulp). This is the basis for biotechnological products design to address specific medical goals and contribute to the development of regenerative medicine as a whole. Exosomes are expected to be key agents in understanding the treatment of unsolved aspects of many conditions for which conventional treatments or diagnostics are not available yet.

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