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Paving New Roads with Mesenchymal Stem Cell Derived Exosomes: A Promising Approach in Tissue Regeneration-An Update

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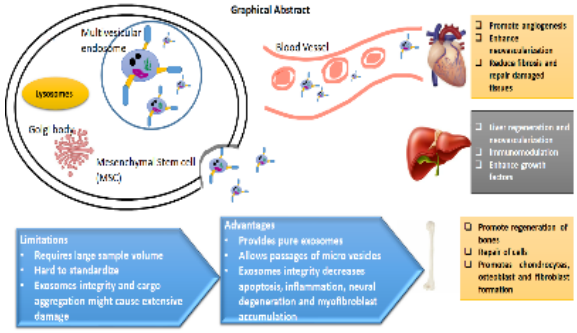

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KEYWORDS
Mesenchymal Stem cells (MSCs) exosomes, Regenerative medicine, Immunomodulation, tissue repair, cell differentiation

ABSTRACT:

Immunomodulation is possible with the existence of mesenchymal stem cells and its regenerative potentiality. The interdisciplinary field of life sciences and engineering is renamed as Regenerative medicine. Several tissues and organs are lost during injury or disease, in such cases regenerative medicine plays a key role to restore the lost parts of organs and tissues as well as aid in the healing of wounds. The repair in tissues is possible with the introduction of Mesenchymal Stem cells (MSCs) exosomes which have triggered remodelling reactions. The multivesicular body is surrounded by plasma membrane referred to as exosomes which are extracellular vesicles. These vesicles aid in intracellular communication, cell differentiation, immune signalling, angiogenesis and stress response. The productive biological properties of these exosomes include stability, less toxicity, biocompatibility with abundant complex molecules of enzymes, proteins, nucleic acids, transcription factors, cytokines and cell surface receptors. Exosomes are primes candidates for proficient exchange of cargos which promotes engineering of tissues and expected to solve main medical problems. Therefore, our article prime focus is to highlight on mechanism of actions of MSCs-exosomes and its clinical transformation in field of therapeutics. Further, have provided insights on its application for wide variety of diseases and limitations on using in relation with realistic instances.

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1. Introduction

From decades study on exosomes in response to human health is in acceleration. The traditional method for In-vitro evolution of living organisms along with the power of regulating expressions of genes is termed Exosomes (Exos). These exos act as biologically active molecules which can diffuse locally or pass-through blood vessels to remote organs/tissues to perform significant functions in the healing of human tissues (1-2). The pluripotent (MSCs) have the ability to differentiate into different lineages which further progress from mesoderm. Their in-vitro capability of differentiation is widely accepted entreaty in cell-based therapy since it has the potential to regenerate tissues (3). Contemporarily, the greater demands for the development of exosomes with relation to human health.

Nonetheless, exosomes are naturally small sized particles in nano scale has several benefits in contrast to engineered nanoparticles (4-5). The heterogenous exosomes are extracellular vehicles (EVs) are naturally appearing nano to micro-sized membrane vesicles discharged from fundamentally various cell types. These, Extracellular vesicles are encircled by a lipid bilayer membrane and vary in size from 30 to 10,000 nm in diameter. Nevertheless, exosomes are materialized as a unique and significant trouper in intracellular & intercellular communication specifically for their capability in transferring their biological content, entailing of lipids, proteins, and nucleic acids to target cells (6-8). It is predominantly apparent that EVs display main role in the physiological regulation processes along with repair of damaged tissues (9-10) and sustenance of stem cell (11). In various pathological circumstances EVs played a key role in treatment of maladies like viral infections (12-13) cancer (14-16), and neurodegenerative disease (17).(figure-1).

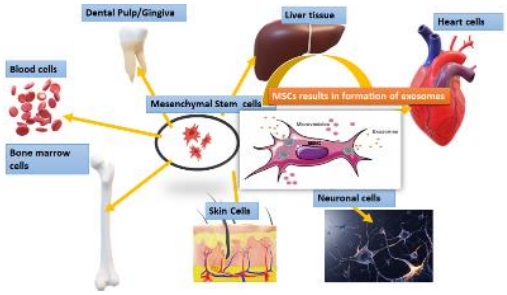

Figure-1: Mesenchymal Stem Cells (MSCs) are used to treat a wide range of disorders & can be isolated from many organs, tissues, and cells.

In early 19th century Pan & Johnstone discovered exosomes, while Johnstone during examining the maturation mechanisms in sheep reticulocytes discovered exosomes (18–20). However, researchers investigated the bleb formation from endocytosis which fuses with lysosomes and results in formation of a micro-vesicle, later termed as exosomes which contains proteins, lipids, and enzymes (21). (Shown in figure-2). Earlier it was assumed that exosomes are apoptotic bodies for disposal of cellular debris (22). There has been a lot of work done to better understand the functional biology of exosomes and to use them in clinical settings (23). The miraculous potential of exosomes which are obtained from donor cell and its sustenance in outer environment and growth In-vitro condition has proved to be promising therapeutic approach for various maladies such as cancer with tumor specific biomarkers (24), liposome mediated drug delivery in cardiovascular problems (25-28), MSCs in Orthopaedics (29-30), dentistry (31) and currently in treatment of COVID-19 (32-34). SARS-COV2 malady outbreak created global pandemic situation from late 2019 and still today facing the critical situation.

Our article provides broad insights on available exosomes and its applications in various diseases including COVID-19 and its treatment with Mesenchymal stem cells (MSCs) as an option since it possesses regenerative, immunomodulatory, antitumour, anti-inflammatory, along with antiviral actions Although, several advantages of these exosomes-based therapy but also have limitations which is been revealed in our article. Multidisciplinary research needs to carried out to exploit standard methods for isolation, storage, and increased secretion of exosomes in both donor as well as recipient for large scale valorization. With reference to current advancements and challenges, our review provides concise role of exosomes in different maladies and its treatment potentiality along with overview on role of exosomes in relation to their origin and functions are distinctively elaborated in our updated review.

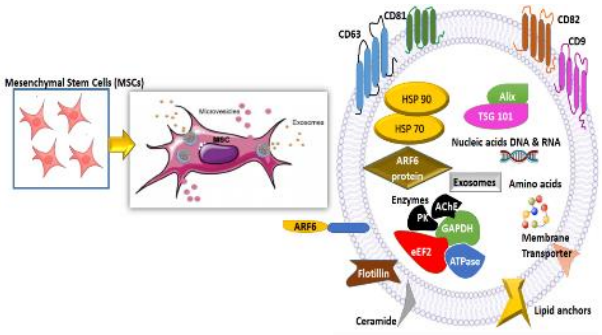

Figure-2: The internal contents of exosomes and outer membrane receptors

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2. Exosomes biogenesis

Exosomes are created by the plasma membrane invaginating inward, which creates endosomal membrane. The plasma membrane, however, is where microvesicles first appear. (35). Early endosomes (EEs), which are formed by the fusion of endocytic vesicles, are the first stage of biogenesis (36). Later, formed EEs either return the cargo proteins outwards or packed into late endosomes (LEs) (37). Thus, the Endosomal-sorting complex required for transport (ESCRT) is dependent on the packing of proteins into multivesicular bodies and intraluminal vesicles (ILVs). Commonly 4 ESCRT complexes of protein such as ESCRT0, ESCRTI, ESCRTII, ESCRTIII along with AAA ATPase Vps4 accessory protein complex which depends on ubiquitin protein for further mechanism or undergoes ubiquitin independent mechanism of action for selection of required cargo and formation of target exosomes (38-42).

ESCRT-0, the complex's initial component, is a heterodimer comprised of STAM1/2 and HRS, both of which can detect cargo that has been ubiquitylated (43, 44). The cytoplasmic protein HRS, the central RR/KHHCR, the N-terminal WxxD, and early endosome antigen 1 (EEA1), which aids in the formation of endosomes, are all found in the FYVE domain. The phosphatidylinositol 3-phosphate (PtdIns-3) that is created by these components (45–46) binds to the phosphatidylinositol 3-phosphate (PtdIns3P), a lipid that is present in large amounts on the surface of pre-MVB endosomes (47). Then, HRS recruits clathrin (48), aiding in the corralling and clustering of ubiquitylated cargo (49–50). Together with ESCRT-I and ESCRT-II, which also contain ubiquitin-interaction domains, ESCRT-0 works at the site of ILV formation to create a sorting domain with a high affinity for ubiquitylated cargo (51–52). ESCRT-III is simultaneously recruited, which encourages membrane deformation and tightens the neck of the ensuing invagination (53-57). The ESCRT complex is deconstructed by the ATPase VPS4 and its co-factor VTA1 (59) after ubiquitin has been removed from the cargo by de-ubiquitylating enzymes (DUBs) (58), allowing the recycling of its component parts. Theoretically, blocking any of these elements should stop the formation of exosomes that are dependent on ESCRT, but not without interfering with additional steps such as lysosomal targeting (60).(Shown in figure-3)

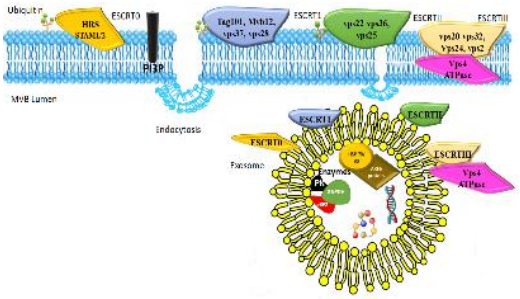

Figure-3: Ubiquitin dependent sorting of ESCRT complex proteins machinery results in formation of Exosomes

3. MSCs derived Exosomes formation and its potential function

In accordance to the exosome formation which mainly depends on the size, shape, markers, origin and based on it extracellularlly are classified into 1) microvesicles, 2) Apoptotic bodies 3) Membrane fragments and 4) Exosomes. The extracellular vesicles are also termed as microvesicles which approximately about 20 to 1000nm and mainly involved in budding of plasma membrane. Whereas apoptotic bodies about 1000-5000nm in diameter and release the engulfed materials as apoptotic cells to exterior. However, membrane fragments formed from epithelial cell membrane are grossly 50-80nm with CD-133 marker. Moreover, fusion of multivesicular bodies results in formation of exosomes which are approximately 40-100nm in size and mainly produced through endosomal alleyway. (61-69)

Numerous disorders, including cancer, cardiovascular disease, and neurological disease, have been related to the growth of exosomes. Exosomes are involved in a number of physiological processes, such as the presentation of antigens, RNA transfer, and tissue healing. (70–75). According to data from past studies, exosomes have specialised roles and significant involvement in coagulation, intercellular communication, and waste product control. Their roles include regulating the immune system, stimulating vascular regeneration, mediating cell proliferation, migration, apoptosis, and differentiation. They aid in sustaining the physiological state of the organism, and contribute to disease advancements (76).

4. Tissue engineering & regenerative therapy

The Food and Drug Administration (FDA) has approved biologics products depicted in Table-1. The tissue engineering & regenerative therapy has developed in industry from two decades ago and further clearance or approval. For therapy either autologous /allogeneic or differentiated cells with significant proliferative potential

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is used (77-78). The first biologic in orthopaedic medicine to receive FDA approval for the treatment of localised articular cartilage abnormalities is Carticel. However, autologous chondrocytes from articular cartilage were extracted, grown ex vivo, and then implanted at the damage site, which led to a strong repossession. (79). Several components used in current regenerative medicine which mimics the native tissues of extracellular matix (ECM) and signals the growth cells for altered behaviour as well as differentiation (80). (figure-4)

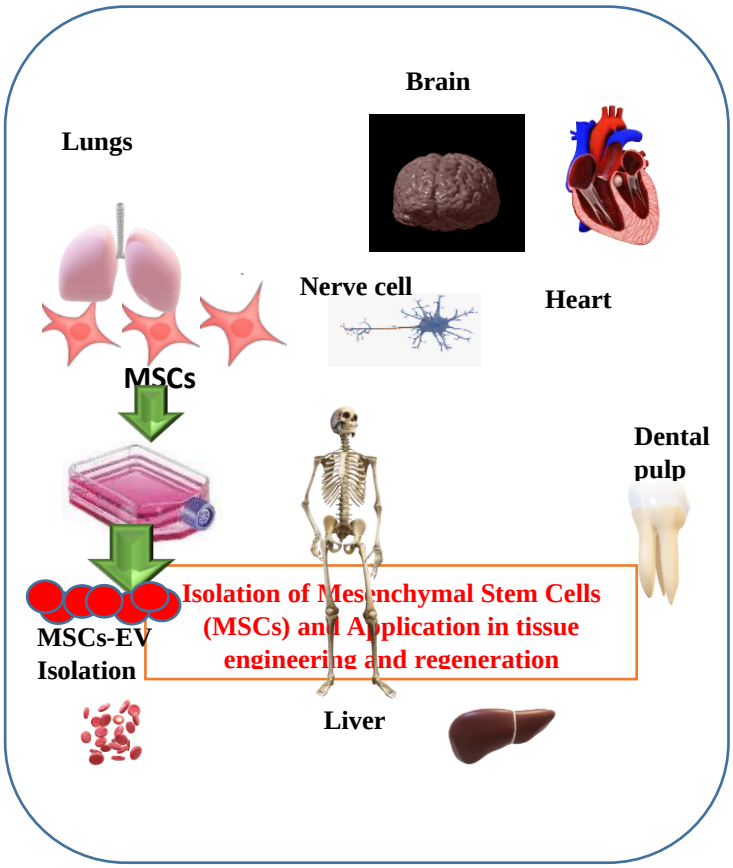

Figure-4: Isolation of Mesenchymal Stem Cells (MSCs-EV) for Application in Regenerative Medicine and Tissue engineering.

Extracellular vesicles and microvesicles are both heterogeneous lipid bilayers that are surrounded by vesicles released by different cell types, including MSCs. Additionally, MSCs can serve as intermediaries for cellular communication. The significant involvement of EVs includes physiological and pathological, as well as different biological processes participating in modulating immune responses, coagulation, maintaining homeostasis, angiogenesis, tumour growth, and inflammation (81–84). But small EVs, medium EVs, and large EVs are categorised in accordance with their size, shape, and place of origin (85–86). The release of extracellular vehicles (EVs), as opposed to cellular engraftment and subsequent reaction on the damaged site, provides evidence that MSCs have beneficial effects that are attributed to their paracrine function. (87–90). In addition, MSCs mainly involves in procedures such as isolation, purification of MSC-EVs (**91**). Additionally, the released MSCs -EV used for regenerative therapy has indicated potential biological effects. This is because MSCs frequently need to be cultivated in vitro and the final product is released after purification (92–93).

Table-1: FDA Approved Biologics for Tissue regenerative medicine

Name	Category	Biologic activity	FDA Approved
GINTUIT, Apligraf	Biologics	fibroblasts in bovine collagen and Allogenic cultured keratinocytes	Diabetic foot ulcers, leg, topical mucogingival condition
laViv		Autologous fibroblasts	refining nasolabial crinkle appearance
Carticel		Autologous chondrocytes	Defects in Cartilage arising from acute/repetitive damage
Cord blood		Progenitor cells & Hematopoietic stem cells	Immunological & Hematopoietic rebuilding after myeloablative therapy
Osteogenic protein-1	Biopharmaceuticals	BMP-7	Tibia non-union
Infuse bone graft,		BMP-2	lower spine fusion, Tibia fracture & non-union

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Regranex	Cell-based medical devices	PDGF-BB	Diabetic ulcers-lower extremity
GEM 125		tricalcium phosphate, PDGF-BB	Defects in Periodontal
Dermagraft		Allogenic fibroblasts	Diabetic foot ulcer
Celution		Cell extraction	Transfer of autologous adipose stem cells

5. Preclinical and clinical stages of Investigation

Wide range of approaches at both the preclinical and clinical stages of examination are at present reconnoitred. The studies on regenerative medicine are broadly divided into three subsections (i) reiterating the structure of organ and tissue through scaffold fabrication (ii) 3D bioprinting and self-assembly (iii) Association of graft with the host through vascularization and excitation (iv) modifying the host environment to instigate regenerative potential specifically through altering immune response. Table-2 depicts the methods employed currently for recognition and identification from different cell sources for regenerative therapy.

Table-2: Depicts the different cell sources and secreted factors revealing therapeutic effect

Source	Species	Secreted factor	Disease/Lesion	Effect	References
Adipose tissue	Equine	Tissue vascularization	Tendinopathy	enhanced tendon healing, Instigate tendon fiber organization, dwindled inflammatory infiltration, enhanced type I collagen formation	(94-95)
Adipose tissue	Canine	orientation of the myofibers	Semi-tendinous muscle lesion	Enhanced lameness	(96)
Adipose tissue		Insulin growth factor-I (IGF-I) Fragments of bone and cartilage	degenerative joint disease (DJD)/ Osteoarthritis	steroidal or non-steroidal anti-inflammatory drugs (NSAID) Platelet-rich plasma, or stem cells, are able to mend damaged tissue and reduce inflammation.	(97)
Bone Marrow	Murine	MIG, VEGF, MIP-1α, MCP-1	Ischemia	Decreased caspase-3 activity, Angiogenesis Promotion	(98)
Bone Marrow		SDF-1	Bronchopulmonary dysplasia	Angiogenesis, increased alveolarization, macrophage Infiltration, diminished alveolar space	(99)

6. New cell sources for regenerative medicine

In regenerative medicine the main stratagem particularly used is sample cell source. Nonetheless, it is difficult to recognize and derive adequate numbers of therapeutic cells. Stem cells, progenitor cells can be derived from both adult as well as embryonic tissues. However, earlier studies have broadly explored regenerative medicine and used adult tissue-derived cells clinically due to their ready availability along with safety. Most of the adult tissue derived cells are utilized clinically and are FDA-approved in regenerative medicine therapies till date.

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7. Delivery methods for exosomes

The various cutting-edge methods for getting exosomes to their sites of action, as well as their drawbacks and difficulties, were examined and presented. Despite the fact that this approach has a rapid liver and renal clearance, intravenous administration (IV) is the most popular (100). This approach was frequently employed for ailments including cardiovascular, neoplastic, and orthopaedic diseases (101). The subcutaneous (SC) route has been employed mostly in aesthetic and cosmetic reasons, while intramuscular (IM) has been used primarily in neuromuscular and musculoskeletal problems. In neurological diseases such Alzheimer's, Parkinson's, and Creutzfeldt-Jakob disease, intrathecal administration is preferable (102-103). In order to treat wounds and ulcers, local aerosol sprays are used (104). This is the recommended path for hair development and regeneration in age-related therapies (105-106). Exosomal therapy has been studied in interesting detail as a potential therapeutic option for the coronavirus disease-2019 (COVID-19) pandemic (107).

8. Advantages and challenges of MSCs derived Exosomes

- Several investigations have shown that embryonic stem cell-derived EVs perform the same tasks as parent cells.
- The immune rejection in cell transplantation, stress reactions caused by necrosis or abnormal differentiation are avoided by Exosome-based therapies
- The chief advantages of exosomes is that they are mediators of stem cell paracrine activity and transmit information to neighbouring cells for treatment of disease.
- The exosomes can be amalgamated with existing/newly developed approaches with specific ingredients.
- Tissue engineering and regenerative exosomes can be loaded into drug carriers to treat lesion tissue.
- Today, MSCs-derived exosomes are considered to be effective in comparison to cell therapy.

9. Challenges/Disadvantages

- Before use, it is important to thoroughly research their origins and how they interact with nearby cells.
- The variability of MSC-exosomes is the most challenging issue.
- Further, it is difficult to purify EV population.
- • The International Society for Extracellular Vesicles (ISEV) advises having a thorough understanding of the biogenesis pathway.
- In addition, basic information on biochemical composition, size and cell origin should be provided for In vitro and In vivo functional assays.

10. Conclusive Remarks

The mesenchymal stem cells derived exosomes are paving new roads for tissue engineering and future regenerative medicine which robustly evolving to become a reality for therapeutic use in patients. The wide composition of exosomes includes growth factors, miRNA and several proteins to fight against diseases. Further, clinical trials need to be carried out for stem cell derived exosomes release for clinical application. Although, various literature reports have revealed the exosomal usage in healing of wide variety of maladies such as regeneration of skin, wound healing but the exact mode of action and role of exosomes need to be explored more. Nonetheless, for better comprehension on exosomes need to explore on area of mechanism of action, trafficking of exosomes and cellular uptake. Furthermore, challenge's part on exosomes must be concentrated for enhancing its future therapeutic use. Thus, it is significant that isolation, optimization, purification and quality control are highly promising in tissue engineering and regenerative medicine before clinical use.

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Contributions

KRP and KRD contributed to writing, and drawing figures and tables in this review article. KRP solely drafted this review article all authors reviewed, corrected, suggested, and finally approved the article.

Compliance with Ethics Requirements

This article does not contain any studies with animals performed by any of the authors.

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

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