Review Article

An Overview About Mesenchymal Stem Cell Exosomes and their Applications in Biological Research

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ABSTRACT

Introduction: Exosomes are essential in communicating intracellularly by working as means for transport of their cargos to receiver cells.

Aim of the Work: This review aimed to shed light on the mesenchymal exosomes (MSC- exosomes) as well as their role in various biological aspects.

Material and Methods: A narrative review was conducted on the basis of publications in PubMed and Scopus databases. MSC-exosomes together with their roles in various biological aspects were discussed.

Results: In *vitro* and in *vivo* researches have shown that MSC-exosomes are related to various tissue regeneration processes (bone, dentin-pulp complex, periodontal ligaments (PDL), taste buds and paraoral tissues), treatment of different metabolic disorders (liver diseases, kidney diseases, cardiovascular diseases, diabetes types 1&2 and obesity), neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) as well as cancer. Also, they act as drug delivery mechanisms and biomarkers for multiple disorders. They also contribute in vaccines development.

Conclusions: Utilization of MSC-exosomes turns out to be of great importance in cell-free therapy. MSC-exosomes contain "cargos" which enable them to contribute in cellular functions and play pivotal role in treatment of diseases and tissue repair. MSC-exosomes play great role in tissue regeneration, including the oral and paraoral tissues. Moreover, they have therapeutic effect in different metabolic disorders and neurodegenerative diseases and they have important impact as anti-tumor agents and in reducing anticancer-therapy resistance. MSC-exosomes are beneficial biomarkers for different human diseases, both in terms of diagnosis and prognosis, and they are ideal tool for drug delivery to treat a wide range of diseases. Also, they are promising in vaccine development.

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Key Words: Biomarker; cell free therapy; mesenchymal exosomes; regeneration; vaccine.

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INTRODUCTION

Stem cells are the source of differentiated cells for tissue repair throughout development as well as for postnatal restoration of distorted or wounded tissues. Stem cells have similar general criteria:

1. They lack specialization,
2. They have an infinite capacity for rejuvenation, also can create progenies which are identical to the original cell,
3. They also show multi-lineage potential i.e. they can specialize into numerous cell kinds and proliferate indefinitely to produce more copies of the same stem cell[1,2].

Stem cells are categorized into:

1. Embryonic stem cells (ESCs),
2. Adult stem cells,
3. Perinatal stem cells present in amniotic fluid, placental tissue as well as placental blood,
4. Induced pluripotent stem cells (iPSCs) where somatic adult cells were remodeled genetically[3,4].

MESENCHYMAL STEM CELLS (MSCS)

MSCs show exclusive criteria such as: multipotency, adhesion to flexible surfaces, ability to express definite surface antigens, and capacity to develop into distinct lineages[5,6].

There are many sources of MSCs, the most common is from bone marrow (BM) as well as fatty tissues. Moreover, they are abundant in dental tissues[7,8].

Dental and oral MSCs are originated from ectomesenchyme's neural cells. They may be transformed into odontoblasts, neural progenitors, osteoblastic or chondrocytic cells and fat cells[7,9]. According to the source of the tissue, they were classified into: periodontal ligament stem cells (PDLSCs)[9], stem cells from deciduous teeth (SHED), stem cells from apical papilla (SCAP)[10], dental follicle stem cells (DFSCs)[11], salivary gland stem cells (SGSCs)[12], gingival-derived MSCs (GMSCs), dental pulp stem cells (DPSCs) available from discarded tooh after

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extraction[13], tooth germ progenitor stem cells (TGPSCs), bone marrow-MSCs (BMSCs), oral epithelial stem cells (OESCs), and periosteal-derived stem cells (PSCs)[14,15].

The common MSCs' surface markers which are also expressed by dental MSCs include; CD90 and CD105 lacking the markers CD45, and CD79a[16].

MESENCHYMAL STEM CELL EXOSOMES

MSCs are the mostly used types of therapeutic based cells for treating numerous disorders. However, injected MSCs are usually trapped in different organs such as; liver, spleen, and lungs forming emboli, so only few cells could reach the target site. Thus, concentrating on cell-free therapies turns out to be of high importance[17,18].

Extracellular vesicles (EVs), cytokines, chemokines, and growth factors are responsible for MSCs' therapeutic efficiency "which contain paracrine factors" secreted by these cells[19-21]..

The EVs are categorized on basis of their biogenesis to; (a) exosomes (40-150nm), which are free out from multivesicular bodies, (b) microvesicles (150-1000nm), which directly bud from plasma membrane, (c) apoptotic bodies (50-2000nm)[22].

Exosomes are essential mediators found in body fluids and exhibit a number of physiological and pathological relationships[23]. These nanoparticles are allowed to pass through the blood brain barrier (BBB), consequently preventing possible embolism caused by transplanted MSCs[24]. They also exhibit high drug loading potentiality and outstanding biocompatibility[25].

Biogenesis of exosomes

Endocytosis is the source of exosome biogenesis [26]. the secretory cells' membrane produces endocytic vesicles forming early endosomes, then microRNA (miRNA), messenger RNA (mRNA) and DNA are condensed within the cytoplasm producing late endosomes. These endosomes grow interiorly giving intra-luminal vesicles (ILVs) which condense to produce multivesicular bodies (MVBs) that finally bond to the cell membrane[26-29].

Properties and content of exosomes

MSC-exosomes display CD29, CD44, CD73 and CD105 surface markers and enclose large amounts of membrane and cytoplasmic proteins[30,31]. Also, they represent adhesion and signaling molecules[32]. In addition, they have growth factors and cytokines including; interleukin-6 (IL-6), interleukin-10 (IL-10), transforming growth factor beta 1 (TGFβ1) and hepatic growth factor (HGF)[33]. Meanwhile, MSC-exosomes carry mRNAs and miRNAs which are transported to receptor cells changing their manners[34-36]. All these factors enable MSC-exosomes to have a hand in certain cellular actions, such as; multiplying, bonding, transcription, immigration, or differentiation[36], and to be essential in treating some illnesses and tissue repair by inhibiting inflammation, inducing angiogenesis, preventing fibrosis, increasing neuronal survival, stimulating ECM restoration, also modulating immune cells[37].

Mechanism of action of exosomes

Typically, exosomes contain active "cargos" as proteins, lipids, and nucleic acids together with various RNA types; miRNAs, mRNAs and transfer RNAs (tRNAs) [38-40]. These cargos exhibit functional, structural, enzymatic and epigenetic activities. MSC-exosomes can engage target cells via a variety of methods to deliver their biologically active cargos to recipient cells; (1) by binding directly to membrane receptors, (2) fusion with the plasma membrane (3) crossing BBB to release these cargos directly into the cytoplasm of target cells. This aims to destabilize the target mRNAs and modulate their transcription and thus influencing various developmental and regulatory processes via activation of different signaling pathways, upregulation of different growth factors, and immunomodulation[41].

Isolation of exosomes

Isolation of exosomes can be achieved by various techniques, these are:

1- Polymer precipitation: in which mixing of the specimens with a proprietary polymer that enables fast and quantitative exosomes isolation "ExoquickTM reagent" is done to form a polymeric mesh that captures exosomes from size 60 to 180nm in diameter, then undergoes centrifugation forming a pellet. Exosomes obtained using this technique are quite homogenous yet, they may contain contaminants, such as lipoproteins[42,43].

2- Size exclusion chromatography: Exosomes travel along the filter column at various speeds regarding the size of the pore in the chromatography gel. The obtained exosomes are highly pure and of regular size. Though, the low separation volume and excessive laboratory equipment hinder its application broadly[43].

3- Differential ultracentrifugation: in this technique, isolation of the exosomes depends on their volume and physical characters. It is mostly used due to its simplicity, and efficiency. Nevertheless, the method requires much instrumentation and is time-consuming[43,44].

4- Affinity capture: this technique is based on the immunomagnetic beads that combine to specific receptor molecules present on exosomes surface such as CD9, CD63, and CD81. This method is easy, also it permits extraction of exact exosome subpopulation. However, the major limitation is the difficulty to isolate the exosomes out of the magnetic beads[43,45].

5- Density-gradient ultracentrifugation: exosomes are separated in this procedure according to their isodensity zone. Despite this technique isolates exosomes with a higher purity however, they can be crushed during the separation process[46].

6- Tangential flow filtration: clogging can be reduced in this technique as the feed stream flows parallel to a membrane under constant hydrodynamic force. Yet, the existence of nanoparticles' size equivalent to those of exosomes constitute a major obstacle in this method[42,47].

Combination of different methods can be applied to avoid mentioned disadvantages[48].

Characterization of exosomes

Various procedures were performed to verify exosomes separation, among them; nanoparticle tracking analysis, atomic force microscopy and scanning electron microscope (SEM)[49].

APPLICATIONS OF MSC-EXOSOMES IN BIOLOGICAL RESEARCH

The MSC-exosomes have become a hot research area due to their paracrine effects which activate many endogenous pathways. MSC-exosomes are significantly important in:[50-52].

1. Regeneration and repair of tissues.
2. Treatment of metabolic diseases, neurodegenerative diseases & malignant tumors.
3. Immune regulation and organism development.
4. Prognostic and diagnostic biomarkers in different diseases.
5. Vaccination and therapeutic agent delivery.

Role of MSC-exosomes in tissue regeneration

Exosomes serve as transporters to deliver their cargos to recipient cells, constituting an essential means of intercellular communication[53-55].

Bone regeneration

Concern over bone regeneration using stem cell exosomes is widespread. SCAP-exosomes are more preferable in bone regeneration than exosomes derived from BMSCs as they upregulate target genes significantly[56].

Insufficient bone volume and/or poor bone quality represent challenge in placing implants at the posterior maxilla. Several surgical techniques can be associated with biomaterials for obtaining extreme beneficial effect. Only the autogenous bone graft has the ability to manifest significant biological actions allowing for self-reproducing capacity by providing MSC-exosomes[57-59].

The osteogenic potential of GMSC-exosomes was also proved in rat model of experiment conducted by Diomede *et al*.[60] who performed 5mm cortical calvaria bone defect and applied GMSC-exosomes added to a 3D polylactic acid scaffold. The results revealed that GMSC-exosomes effectively promoted GMSCs osteogenic differentiation. This was confirmed by stimulated calcium deposition and elevated osteogenic markers' expression: Runt-related transcription factor 2 (RUNX2), osteopontin (OPN) and vascular endothelial growth factor (VEGF).

An in *vitro* study carried out by Jiang and Xu,[61] to detect the influence of GMSC-exosomes on pre-osteoblast differentiation, they cultured MC3T3-E1 pre-osteoblast cells in 20μg/mL GMSC-exosomes under osteogenic induction. After 14 days of stimulation, alkaline phosphatase (ALP) along with Alizarin Red S stain displayed that GMSC-exosomes treated group exhibited enhanced pre-osteoblast ALP activity and higher induction of mineralization in comparison to control group, (Figure1).

Other research was performed in *vitro* by Wang *et al*.[62] in which SHED-exosomes were applied for osteogenic induction in PDLSCs. The PDLSCs displayed intense Alizarin red stain, increased ALP effect together with enhanced expression of osteogenic genes: RUNX2, OPN and osteocalcin (OCN). Authors accredited their outcomes to stimulation of BMP/Smad together with Wnt/β-catenin signal pathways.

Shimizu *et al*.[63] studied the osteogenic potential of DPSC-exosomes in periodontitis of a mouse model. Periodontitis induction was performed using silk ligature around the upper second molar, after 1 week of treatment, micro-CT showed excessive loss of alveolar bone loss in the untreated group while the physiological saline (PS) group was not significantly affected, however bone loss was reduced by 50% in DPSC-exosomes treated group. The histological results confirmed that DPSC-exosomes promoted osteogenesis as significant increase in alveolar bone height was observed compared to untreated group. Researches relayed the osteogenic potential of DPSC-exosomes to their inhibitory effects on receptor activator of nuclear factor kappa beta ligand (RANKL) expression which is responsible for osteoclastogenesis plus promotion of the anti-inflammatory cytokines, (Figure 2).

Wound healing

Although similar mechanisms of tissue repair are applied in healing of skin and oral tissues, the speed of wound repair is higher in the oral cavity[64].

Liu *et al*.[65] created wound with a diameter of 20mm on the back of rats' skin, subsequently, the injured rats were separated into 2 groups. One group, was subjected to phosphate buffered saline (PBS) applied on the wound for 6min. The other group was injected with 1mg of human umbilical cord mesenchymal stem cells (hucMSCs)-derived exosomes. After 13 days' therapy, histological analysis revealed that the wound closure was noticeably faster as epidermis and auxiliary structures were visibly regenerated in the exosomes treated group, compared to PBS-infused rats which exhibited little improvement due to spontaneous healing. Immunohistochemical results for CD31, illustrated strong immunoreaction in hucMSC-exosomes-treated group compared to PBS-treated group. The authors attributed their results to the fact that hucMSC-exosomes deliver miR-21-3p and growth factors like angiopoietin 1 and 2 (Ang-1&2) to target cells and up-regulate the expression of CD31 which all lead to enhancement of angiogenesis and wound healing, (Figure3).

Hassan *et al*.[66] induced inflammatory condition in human dermal fibroblasts-adult (HDFa) cell culture using lipopolysaccharides (LPS). Then group of the cells was treated by BMSC-exosomes. After 48h of treatment, the ultra-structural examination of BMSC-exosomes treated group illustrated almost normal features. Moreover, the statistical results of cell viability% revealed that the highest cell viability% was recorded in BMSC-exosomes treated group. In addition, ELISA results of BMSC-exosomes treated group revealed significant decrease in interleukin-1β (IL-1β) and significant raise within the studied antioxidants and anti-inflammatory cytokines; Heme oxygenase (HO)-1, Nuclear factor-erythroid 2-related factor 2 (Nrf2) along with IL-10. These results are attributed to the paracrine effects of BMSC-exosomes which promote inhibitory upshot on inflammatory pathways mediated by IL-1 β and upregulate antioxidants and anti-inflammatory cytokines.

Dentin-pulp complex regeneration

Clinicians face difficulties when treating periapical or dental pulp disorders in opened apex of permanent teeth. Classical treatment methods have many restrictions. So a novel treatment modality is provided by regenerative endodontic techniques[67].

Zhuang *et al*.[68] conducted a study on 6-week-old immunodeficient mice in which, 50μg/mL SCAP-exosomes and 4×105 BMSCs with gelatin sponge were placed into tooth pieces and implanted in the subcutaneous tissue of the back of mouse for 3 months. Histological examination of SCAP-exosomes group compared to control group (BMSCs only) revealed a newly formed and uninterrupted dentin layer, polarized columnar odontoblasts, ordered arrangement and extended Tome's fibers within the dentinal tubules. Moreover, the lumen appeared more vascular and the thickness of the new dentin as well as the odontoblastic number was increased significantly in SCAP-exosomes group. These results are ascribed to the "cell homing" theory, as SCAP-exosomes achieve dentin-pulp complex restoration through modulating the passage, production, and transformation of already existing stem cells surrounding the apex of roots.

Chen *et al*.[69] demonstrated the influence of DPSC-exosomes on recruiting SCAP for pulp tissue regeneration. DPSC-exosomes and SCAP were isolated from deciduous incisor teeth of swines. Collagen gel was mixed with them and applied to pulp canal of dentin which was transplanted to the skin backs of immunodeficient mice for 8 weeks. Regeneration of pulp tissue and dentin-like mineralization was revealed histologically. The DPSC-exosomes encourage migration, proliferation and differentiation of SCAP by up-regulation of proteins related to neurogenesis and angiogenesis, (Figure 4).

Periodontal tissues regeneration

Periodontitis is a main reason for permanent damage of periodontium which form the teeth supporting periodontium. Existing treatment modalities were incapable of restoring the damaged periodontal tissues in various conditions[70-72].

Wei *et al*.[73] studied the regenerative effect of SHED-exosomes in periodontitis of a mouse model. Periodontitis induction was performed by surrounding the first molar by silk ligature. After 14 days, micro-CT showed massive alveolar bone loss buccally and lingually. After ligature removal, application of SHED-exosomes was done mesialy and distaly to the molar tooth one time a week for 6 weeks. Histological results confirmed that SHED-exosomes promoted BMSCs osteogenesis as significant increase in alveolar bone height was observed also, Masson's stained sections displayed new formation of well organized collagen fibers of periodontal ligaments (PDL) in SHED-exosomes treated group compared to non-treated one. The authors attributed the regenerative effect of SHED-exosomes on PDL to their immunomodulatory role by lowering the expression of tumor necrosis factor-α (TNF-α), in addition to their stimulatory effect on PDLSCs. Moreover, the osteogenic potential of SHED-exosomes could be ascribed to their effect in increasing the expression of osteogenic gene "RUNX2" which leads to enhanced bone formation (Figure 5).

Sun *et al*.[74] examined the role of GMSC-exosomes in the inflammatory reaction of PDLSCs produced by LPS in *vitro*. After 48h, the statistical results of ELISA and qRT-PCR assays revealed significant elevation of TNF-α level and diminished IL-10 in LPS group. This atypical alteration was upturned in GMSC-exosomes group. Data suggests that GMSC-exosomes can reduce LPS-induced inflammatory reaction in PDLSCs.

Taste perception regeneration

Tongue is considered as one of most affected anatomic structures due to aging or diseases which lead to disturbance in taste sensation that affects food intake and life's quality[75,76]. Mucosal defect in a rat tongue was performed by Zhang *et al*.[76] investigating the role of GMSC derived exosomes on taste buds regeneration. Exosomes were loaded on intestinal submucosal matrix and placed on tongue defect for 8 weeks. Immunofluorescence markers; cytokeratin 8 (CK8) and nucleoside triphosphate diphosphohydrolase-2 (NTPDase2) for types I and III bud cells were significantly expressed in GMSC-exosomes/SIS-ECM group in comparison to control group (SIS-ECM). They related this improvement to the regulating role of GMSC-exosomes on CK8 and NTPDase2 which are crucial in taste buds regeneration and reinnervation, (Figure6).

Hassan *et al*.[77] induced Alzheimer's model disease (AD) in female rats after ovariectomy, afterwards the rats received single dose of BMSC-exosomes. One month after treatment, light and transmission electron microscopic examinations demonstrated destructive changes in taste buds of circumvallate papilla and glossopharyngeal nerve fibers of AD group. Almost normal features of taste buds were detected in BMSC-exosomes group in addition

to numerously arranged glossopharyngeal nerve fibers and statistically significant increase of synaptophysin immunoreactivity area%. We related this improvement to the ability of BMSC-exosomes to inhibit lipid peroxidation and convey functional miRNAs and proteins to the neural cells which in turn promote neurogenesis, angiogenesis and synaptogenesis.

Paraoral tissues regeneration

Temporomandibular joint osteoarthritis (TMJ-OA) is a subtype of TMJ dysfunctions[78]. Zhang *et al*.[79] induced TMJ-OA in rat model using monosodium iodoacetate (MIA) and applied MSC-exosomes to study their regenerative effect. 8 weeks post treatment, the histological examination revealed efficient reestablishment of the condylar configuration with noticeable improve of cartilage thickness, synthesis of its matrix, and integrity of subchondral bone in MSC-exosomes group compared to that of untreated group. These findings imply that exosomes facilitate osteoarthritic regeneration of TMJ through reducing inflammatory process early on to reduce discomfort and tissues deterioration gathered with improved cellular production and matrix deposition.

Salivary glands damage and dysfunction changes the salivary quantity and quality. Type 2 diabetes mellitus (T2DM) is the known disease form which cause xerostomia[80]. AbuBakr *et al*.[81] performed a study evaluating BMSC-exosomes effect on submandibular salivary gland dysfunction. Diabetic rats were treated with BMSC-exosomes for 5 weeks. Light and electron microscopic results of BMSC-exosomes group revealed almost normal parenchymal structure with lessened fibrosis compared to the untreated group. These findings are credited to the anti-inflammatory, angiogenic and immunomodulatory properties of BMSC-exosomes, (Figure7).

Role of MSC-exosomes in treating metabolic disorders

The metabolic diseases are associated with impaired quality of life. The common metabolic diseases are; liver diseases, kidney diseases, cardiovascular diseases, DM types 1&2 and obesity[82].

The MSC-exosomes are unique therapeutic strategy for treatment of liver fibrosis and drug-induced liver injury[83].

In induced acute kidney injury models, MSC-exosomes improve renal inflammation, lessens apoptotic renal cells, and accelerates epithelial cells production after single administration[84].

In myocardial ischemia-reperfusion (I/R) damage, delivering MSC-exosomes preceding reperfusion markedly decreases the extent of infarct, also ameliorates the left ventricular function through anti-inflammatory effects and neovascularization[85,86].

Several researchers reported the effect of MSC-exosomes in cases of resistance to insulin. Sun *et al*.[87] revealed that application of hucMSCs-exosomes markedly improved hyperglycemia in T2DM rats. These findings suggest that hucMSCs-exosomes may ameliorate insulin sensitivity through enhancing the activity of phosphorylated insulin receptor substrate 1 (p-IRS-1), similarly by preventing release of pro-inflammatory cytokines. Also, they stimulate uptake of glucose and glycolysis in skeletal muscle by influencing glucose metabolism-related enzymes. Furthermore, hucMSCs-exosomes act through inhibition of streptozotocin (STZ)-induced β cell apoptosis[87].

The onset of insulin resistance is strongly influenced by obesity. The primary factor for insulin resistance is the ongoing inflammation in adipose tissues. Adipose-derived stem cells (ADSC) are MSCs that are obtained from abundant adipose tissue. ADSC-derived exosomes mitigate fatness caused by diet, improve glucose tolerance as well as sensitivity to insulin, and inhibit adipocyte hypertrophy[88].

Role of MSC-exosomes in treating cancer

The MSCs and their exosomes are important in tumor therapy as they can migrate from BM or other tissues to the tumor site[89].

BMSC-exosomes prevent various human and mice tumor cell lines from growing and surviving by downregulating the expression of VEGF[90,91].

An additional study showed that ADSC-exosomes application in hepatocellular carcinoma (HCC) of rat may stimulate anti-tumor reaction by promoting anti-cancer immunity, thus allowing suppression of HCC and reducing differentiation of tumor cells[83].

Because it is yet unclear how MSC-exosomes contribute to tumor formation, their use in cancer therapy must be done with caution[92].

The MSC-exosomes influence also tumor chemosensitivity. BMSC-exosomes reversed the chemoresistance of glioblastoma multiform cells by delivering anti-miR-9 which affects the level of multidrug transporter P glycoproteins[93].

Role of MSC-exosomes in treating neurodegeneration

Progressive damage of neural configuration and function induces neurodegenerative damage such as; AD and Parkinson's disease (PD) that affect variety of personal activities[94-97].

Indeed, AD is the major reason for dementia in aged individuals due to amyloid cascade progression especially amyloid beta (Aβ) peptide and tubulin associated unit (Tau) proteins. MSC-exosomes help in reducing Aβ deposition by converting them to nontoxic amyloid fibrils and by degrading them via degradation-related enzymes[98-101].

There is widespread agreement that neuroinflammation contributes significantly to the etiology of AD. MSC-exosomes exhibit an immunomodulatory function

via declining levels of major histocompatibility complex II (MHC-II) and hindering the proliferation of lymphocytes[102-104]. It has been documented that MSC-exosomes administered to mice with hippocampus injury may improve memory and learning ability by repressing microglia infiltration[105].

Role of MSC-exosomes as biomarkers

Biomarkers are mandatory for the expectation, analysis, and observation of the therapeutic achievement of different diseases. However, the conventional biomarkers are not satisfactory for a trustworthy detection[106].

The MSC-exosomes could be regarded as useful diagnostic markers in different diseases. As an example, miR-135a, miR-193b, and miR-384 derived from MSC-exosomes are possible biomarkers for diagnosis of early AD when detected in serum[107]. Also, MSC-exosome-associated miR-137 was elevated in neurons in PD[108]. Furthermore, apolipoprotein A1 (ApoA1) derived from MSC-exosomes is potential diagnostic biomarker in oral squamous cell carcinomas (OSCC)[109].

The MSC-exosomes are also useful biomarkers for monitoring the prognosis in different diseases. MSC-exosome-associated miR-133b, miR-185 and miR-205 are used for monitoring the prognosis in glioma therapy[110].

Also, MSC-exosome-associated miR-122 is used to track responsiveness of liver cancer cells to chemotherapeutic treatment[111].

Role of MSC-exosomes as a therapeutic agent delivery

MSC-exosomes, 'nature's delivery systems' have the capability to deliver drugs to target cells as they convey cargos directly into cell cytoplasm. They have the properties of an ideal drug delivery vehicle as; (1) they can be loaded with biological constituents, (2) they can penetrate the BBB, (3) they are naturally stable, (4) they are easily accommodated to membrane modifications[112,113].

Role of MSC-exosomes in vaccine development

Exosomes derived from cells have become a cutting-edge platform for the administration of vaccines with combination of immunogenicity without reactogenicity[114].

Li *et al*.[115] declared that exosomes originating from liver nonparenchymal cells may produce antiviral effect by discharging interferon-α contrary to hepatitis B virus. Vaccination against HBV is considered also as prophylactic intervention to reduce HCC incidence[116].

Clinical trials of BMSC-exosomes aerosol inhalation in severe cases hospitalized with COVID-19 demonstrated reduced lung inflammation and pathological impairment. This renders BMSC-exosomes to have important role in COVID-19 vaccines development[117].

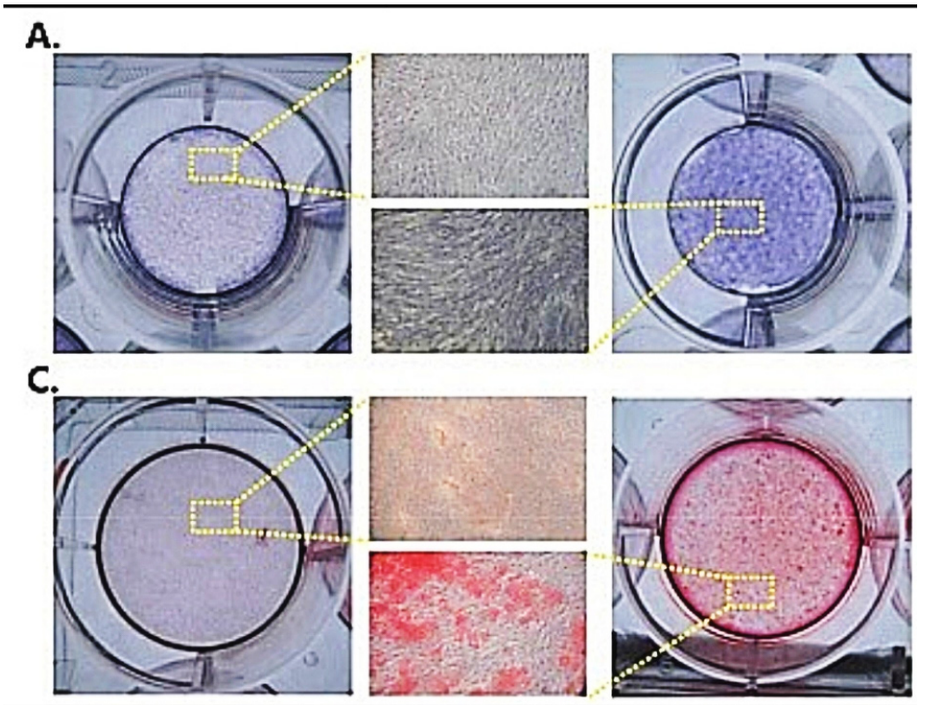

Fig. 1: Photographs showing GMSC-exosomes promote the osteogenic differentiation of pre-osteoblasts. (A)- Alkaline phosphatase staining. (C)- Alizarin Red S staining[61].

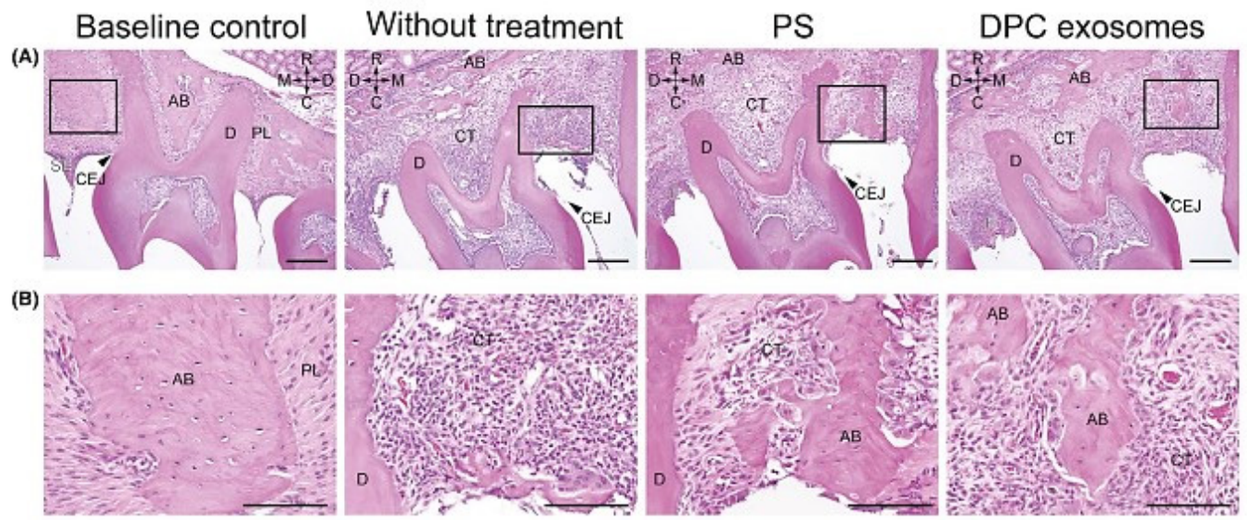

Fig. 2: Photomicrographs showing effects of DPSC-exosomes treatment on alveolar bone in a mouse model of periodontitis. (A) sagittal maxilla sections. Note that the baseline control is symmetrical in the medial-distal direction. M, mesial; D, distal; R, root; C, crown; AB, alveolar bone; D, dentin; SE, sulcular epithelium; E, epithelium; CT, connective tissue; CEJ, cement enamel junction. Black frames indicate the positions of higher-magnification images shown in B. (B) High-magnification; AB, alveolar bone; D, dentin; PL, periodontal ligament, (H&E),[63].

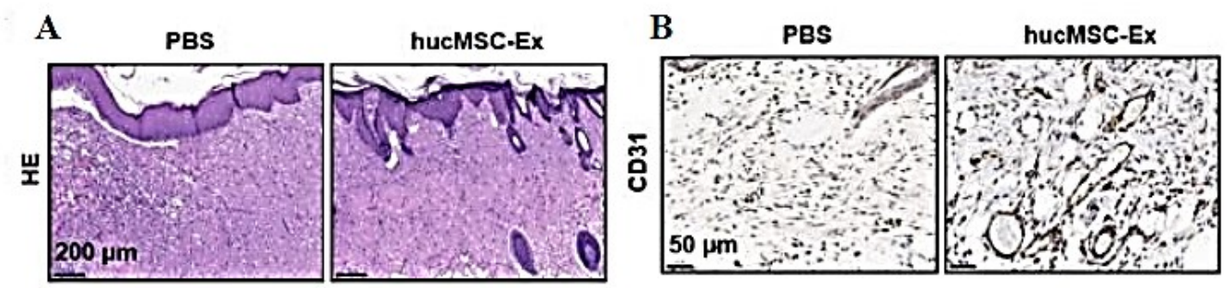

Fig. 3: Photomicrographs showing (A)- Histological structure of skin wound after treatment, (H&E). (B)- CD31 immuno-expression in skin tissues, (CD31 immunolocalization),[65].

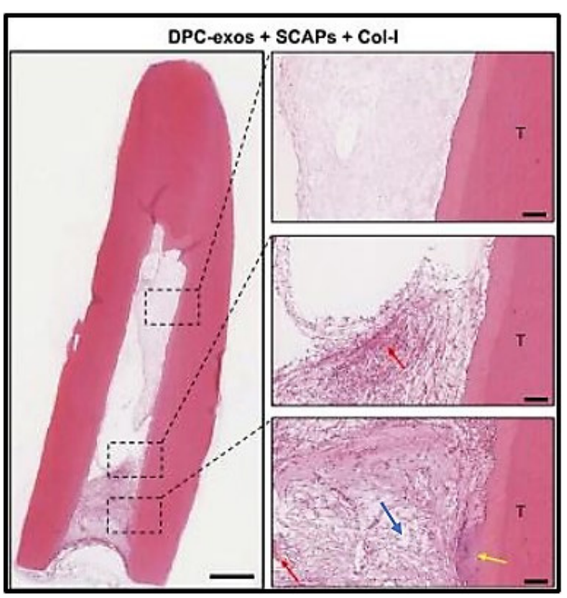

Fig. 4: Histological photomicrographs representing blood vessels (red arrows), regenerated pulp tissue (blue arrow) and regenerated dentin-like tissue (yellow arrow) in group treated by DPSC-exosomes, (H&E). Abbreviations: T, dentin tube matrix,[69].

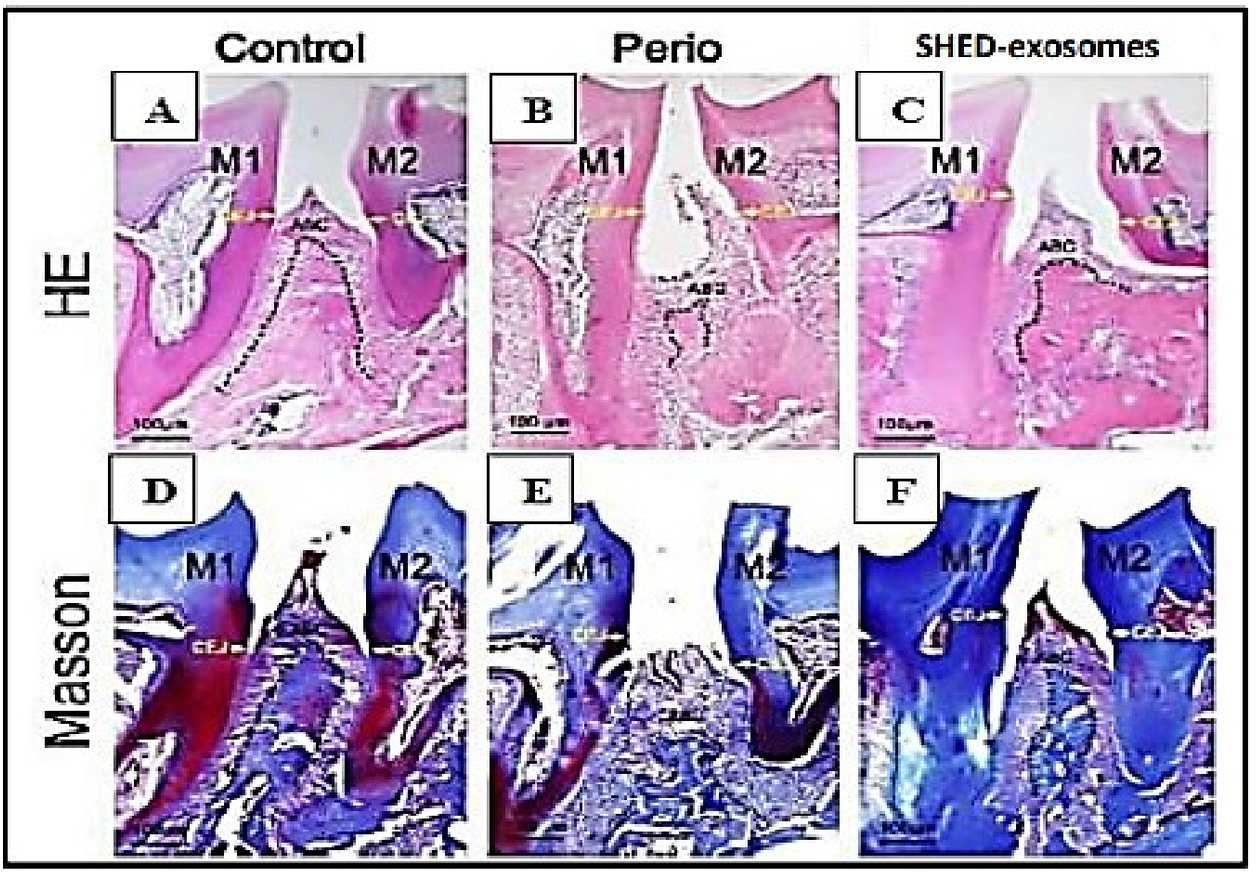

Fig. 5: Photomicrographs showing alveolar bone and PDL formation in *vivo*. A-C: representative histological sections of the alveolar bone between the first molar (M1) and the second molar (M2). The black dotted line indicates the height of the alveolar bone crest, (H&E). D–F: showing the newly formed PDL collagen fibers in different groups, (Masson's trichrome staining),[73].

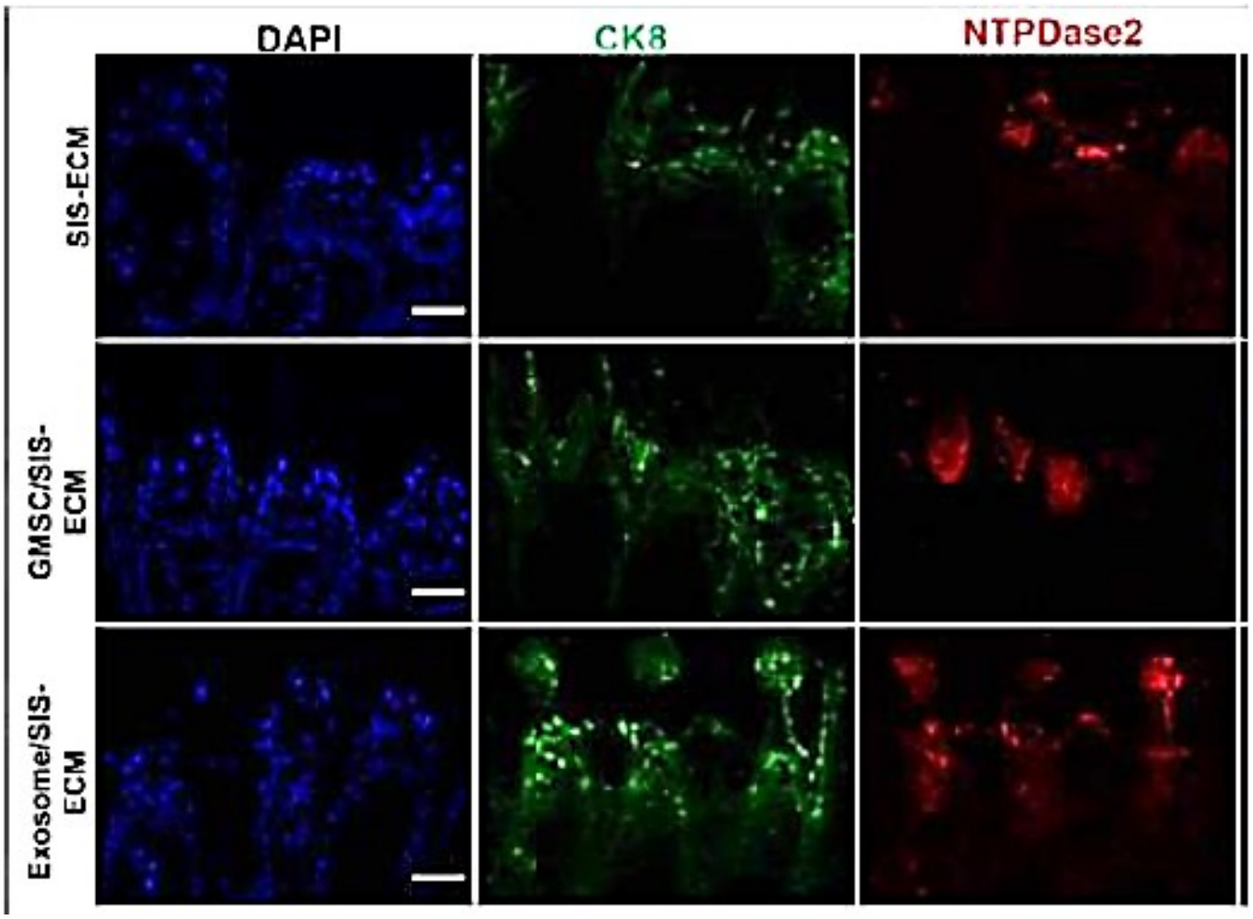

Fig. 6: Photomicrographs showing strong CK8 and NTPDase2 immuno-expression in GMSC-exosomes/SIS-ECM group, the nuclei are counterstained by 4′,6-diamidino-2-phenylindole (DAPI), (CK8 and NTPDase2 immunofluorescence labeling),[76].

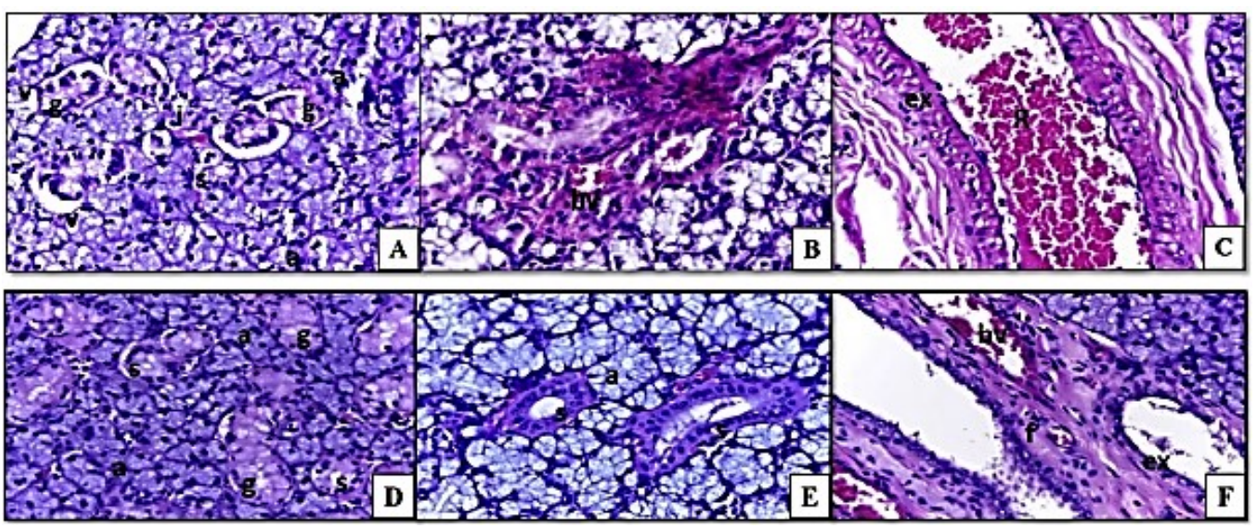

Fig. 7: Histological photomicrographs representing the diabetic submandibular salivary gland (A, B, C) and exosomes treated gland (D, E, F) showing: (A) atrophy in: acini (a), granular convoluted tubules (g), striated duct (s) & intercalated duct (i). Intracellular vacuoles (v) were noted. (B) blood vessel (bv) with thickened lining, dilatation and congestion. (C) excretory duct (ex) with intracellular vacuoles and retained secretion (R). (D) normal acinar size and architecture (a), granular convoluted tubules (g) with normal eosinophilic granular content & normal striated ducts (s). (E) striated ducts' lining with properly arranged cells (s) & mucous acinar transformation (a). (F) few dilated and congested blood vessels (bv). Less fibrosis (f) surrounding the excretory duct (ex), (H&E),[81].

CONCLUSIONS

Utilization of MSC-exosomes in therapies turns out to be of great importance to avoid all drawbacks of using transplanted stem cells.

MSC-exosomes contain "cargos" which are molecular components including lipids, nucleic acids, metabolites, and proteins which enable MSC-exosomes to contribute in cellular functions and play pivotal role in treatment of diseases and tissue repair.

MSC-exosomes play great role in tissue regeneration, including the oral and paraoral tissues, by carrying their active cargos to receiver cells to promote; osteogenesis, chondrogenesis, odontogenesis, neurogenesis, angiogenesis, wound healing and skin regeneration. Moreover, they have therapeutic effect in different metabolic disorders and neurodegenerative diseases and they have important impact as anti-tumor agents and in reducing anticancer-therapy resistance.

MSC-exosomes are beneficial biomarkers for different human diseases, both in terms of diagnosis and prognosis and they are ideal tool for drug delivery to treat wide range of diseases. Also, they are promising in vaccine development by boosting the immunogenicity without reactogenicity.

ABBREVIATIONS

BMSCs: Bone marrow mesenchymal stem cells; **MSC-exosomes:** mesenchymal exosomes; **PDLSCs:** periodontal ligament stem cells; **SCAP:** stem cells from apical papilla; **DFSCs:** dental follicle stem cells.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

نظرة عامة حول اكسوزومات الخلايا الجذعية الوسيطة وتطبيقاتها فى البحث البيولوجى

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المقدمة: تمثل الاكسوزومات طريقة مهمة للتواصل بين الخلايا من خلال العمل كموصلات لنقل المواد النشطة بيولوجيا الى الخلايا المستقبلة.

الهدف: هدفت هذه المراجعة الى القاء الضوء على اكسوزومات الخلايا الجذعية الوسيطة ودورها فى الجوانب البيولوجية المختلفة.

مواد و طرق الدراسة: تم البحث فى منشورات قاعدة بيانات PubMed و Scopus لاجراء هذه المراجعة السردية. وقد تمت مناقشة اكسوزومات الخلايا الجذعية الوسيطة ودورها فى مختلف الجوانب البيولوجية.

النتائج: أكدت الأبحاث التى اجريت داخل الجسم الحى وخارجه على مدى أهمية اكسوزومات الخلايا الجذعية الوسيطة فى تجديد الأنسجة المختلفة (العظام ، ومركب لب العاج ، وأربطة اللثة ، وبراعم التذوق والأنسجة المجاورة للفم)، وعلاج الاضطرابات الأيضية (أمراض الكبد ، وأمراض الكلى ، وأمراض القلب والأوعية الدموية ، ومرض السكري من النوعين ١ و ٢ والسمنة) والأمراض التنكسية العصبية (مرض الزهايمر) ، ومرض باركنسون ، ومرض هنتنغتون ، والتصلب المتعدد ، والتصلب الجانبي الضموري) والسرطان. كما انها تعمل كمؤشرات حيوية للأمراض المختلفة وناقلات للأدوية العلاجية، وكذلك تساهم فى تطوير اللقاحات الطبية.

الاستنتاج: تبين أن استخدام اكسوزومات الخلايا الجذعية الوسيطة فى العلاج الخالى من الخلايا له أهمية كبيرة. تحتوي الأكسوزومات على "شحنات" تمكنها من المساهمة في الوظائف الخلوية وتلعب دورًا محوريًا في علاج الأمراض وإصلاح الأنسجة. تلعب الأكسوزومات دورًا كبيرًا في تجديد الأنسجة ، بما في ذلك الأنسجة الفموية والداخلية. علاوة على ذلك ، لها تأثير علاجي في مختلف الاضطرابات الأيضية والأمراض العصبية التنكسية ولها تأثير مهم كعوامل مضادة للأورام وتقليل مقاومة العلاج المضاد للسرطان. الأكسوزومات هي مؤشرات حيوية تشخيصية وإنذارية مفيدة لمختلف الأمراض البشرية وهي أداة مثالية لتوصيل الأدوية لعلاج مجموعة واسعة من الأمراض. كما أنها واعدة في تطوير اللقاح.