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Exosomes from foetal tissue-derived mesenchymal stem cells as a novel product for therapeutic application

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Cellular connections, secretion of soluble substances, and presence of mesenchymal stromal/stem cells (MSCs) collectively contribute to maintaining the delicate balance of tissues. In response to pathological conditions, MSCs give rise to exosomes, called MSC-derived exosomes (MSC-Exos). MSC-Exos are tiny vesicles act as soluble messengers, facilitating communication between near or distant cells, and carry essential cargo such as proteins, lipids, mRNAs, siRNAs, ribosomal RNAs, and miRNAs. The functions of MSC-Exos have been extensively compared to those of MSCs in various diseases. Notably, MSC-Exos play a supportive role in tissue healing, dampening inflammatory responses, and orchestrating immune system regulation. Owing to their remarkable regenerative potential, MSC-Exos hold immense promise in the realm of regenerative medicine. The review highlights that MSC-Exos derived from foetal tissues (such as placenta, amniotic membrane, amniotic fluid and umbilical cord) are readily available, and have higher proliferation rates and longer telomeres than those derived from adult tissues. The foetal membrane and adult tissue MSC-Exos differ in their therapeutic properties. In future, similarities and differences between the foetal membrane and adult tissue MSC-Exos should be investigated; their isolation and characterisation methods, dose and delivery mode, and long-term safety and efficacy should also be explored for promoting their clinical applications.

Keywords: Extracellular vesicles, exosomes, mesenchymal stem cells, foetal tissues, soluble mediator, cell-based therapy

INTRODUCTION

Mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) are small membrane-bound vesicles derived from MSCs and contain various bioactive molecules such as proteins, lipids, and nucleic acids. They are recognised as important constituents contributing to the therapeutic potential of MSCs (Pethe and Kale, 2021). MSCs are multi-potent cells that can differentiate into various cell types and possess regenerative, anti-inflammatory, and immuno-modulatory properties. With advances in the understanding of regenerative mechanisms, it has become evident that MSCs exert their effects through the secretion of paracrine factors, collectively called the secretome, of which exosomes form an important constituent. MSCs and their extracellular vesicles (EVs), including exosomes, have been shown to possess immense regenerative properties and therapeutic potential (Pethe and Kale, 2021).

Foetal tissue-derived mesenchymal stem cell biology

Stem cell therapy has greatly improved since the first report of the successful treatment of a mouse with a damaged heart using bone marrow cells (Zakrzewski et al. 2019). It has since gained attention as a promising cutting-edge option for treating various diseases. In humans, stem cells are non-specialised cells with a remarkable capacity for continuous regeneration. In an altered physiological environment, the ability of stem cells to constantly self-renew, multiply, and differentiate into specialised cells allows them to restore tissue to its pre-injury state (Antoniadou and David, 2016). Stem cells can be obtained from the bone marrow (BM), adipose tissue, cord blood, and umbilical cord (UC).

Stem cells are categorised as totipotent, pluri potent, multi-potent, and uni-potent based on their ability to self-renew and differentiate. The ability to mould in response to a particular circumstance is referred to as plasticity. The flexibility of stem cells refers to their capacity to differentiate into various cell types (Agrawal et al. 2009). The potency of stem cells to differentiate decreases at each step of lineage development, from early embryogenesis to mature specialised cells (Hoang et al. 2022). A classic example of a totipotent stem cell is the zygote, which is created after the fertilisation of an ovum by a sperm. The placenta, among other extra-embryonic and embryonic structures, can also be produced from it.

The blastocyst, which forms five days after fertilisation, contains the central cell mass, referred to as the embryoblast, which is surrounded by trophoblasts (Agrawal et al. 2009).

The human placenta, a non-permanent but essential organ during pregnancy, is an alternative source of stem cells. Besides playing a crucial role in selecting the best foetal growth trajectory in the uterus, embryonic stem cells (ESCs) have an advantage over adult stem cells in terms of proliferation and flexibility (Pogozhykh et al. 2018). After delivery, the placenta and UC are frequently discarded because they have historically been considered biological waste. Stem cells derived from placental and UC tissues are easily recoverable without the need for invasive procedures and are therefore not associated with the ethical dilemma associated with ESCs and those derived from the bone marrow, adipose tissue, and endometrium (Pogozhykh et al. 2018, Oliveira and Barreto-Filho, 2015). These tissues are readily accessible in large quantities. Owing to their unique characteristics, placenta-derived stem cells (PDSCs) and umbilical cord-derived stem cells (UCDSCs) provide a desirable alternative for cell therapies and regenerative medicine.

Characteristics and biological functions of foetal tissue-derived mesenchymal stem cells

Basic characteristics of human foetal tissues and development of stem cells

The zygote gives rise to the foetal membranes, which serve as auxiliary organs. The yolk sac (*saccusvitellinus*), which makes up the foetal membrane, splits into a morula (morus) and blastocyst when the early embryo travels through the fallopian tube, where fertilisation occurs. The embryo proper develops in the trophoblast and is composed of flattened cells positioned in the periphery. Tubular epithelial cells of the endometrium combine with trophoblast cells and form microvilli. The endometrial epithelium is broken down by proteolytic enzymes (zinc-containing metalloproteases) in trophoblast cells, which enables the blastocyst to enter the endometrium. The trophoblast initially consists of two layers: a *cytotrophoblastus* (inner layer) and a *syncytiotrophoblastus* (outer layer). The blastocyst is anchored in the uterus by cell fusion and can invade, whereas the inner layer is composed of highly proliferating mononuclear cells (Kulus et al. 2021). It then grows into the chorion and participates in placental development.

The amnion, which forms at approximately day seven of embryonic development, covers the embryo. Amniotic fluid, also known as liquor amnioticus, fills the cavity between the amnion and embryo. The amnion is typically not vascularised and is composed of mesenchymatous tissue and ectodermal epithelium. The amniotic epithelium is composed of large, flat, and polygonal cells with microvilli on their surfaces. It can exfoliate and enter the amniotic fluid. Amniocentesis, a procedure that collects fluid by puncturing the amniotic cavity, is used for the prenatal diagnosis of exfoliated epithelium (Nizard, 2010). Intercellular gaps, known as amniotic water vacuoles owing to their appearance, are created on the lateral sides of individual amniotic epithelial cells and are filled with microvilli and protuberances. These cells contain numerous lipid droplets and glycogen granules. Numerous reticular fibres found in the amniotic epithelial basement membrane enter the mesenchymal tissue and are abundant in fibroblasts and collagen fibres (Kulus et al. 2021). Epithelial cell creates amniotic fluid, and serous fluid also enters through the intercellular gaps from the mesenchyme. In pregnant women, the amniotic fluid is predominantly composed of water (99%); it also contains saccharides, proteins, urea, and foetal downy hair (lanugo). The amount of amniotic fluid changes over the course of pregnancy. Amniotic fluid is refreshed continuously. Both foetal and amniotic epithelial cells undergo resorption (Jain et al. 2023). The embryo benefits from the critical roles of amniotic fluid in providing a moist environment, shielding it from harm, amortisation, and metabolism.

Endo- and mesoderm-derived allantois is the final foetal membrane to develop. It originates at the back of the main intestine. Haematopoietic islands and blood vessels begin to form in the wall of the allantois at an early stage during the formation of the umbilical artery and vein. The bladder valve and the intra-embryonic portion of the allantois are united before splitting to produce the urachus and median umbilical ligament (Pogozhykh et al. 2018). The primary function of the allantois is to supply the chorion with blood arteries and establish placental circulation. The allantois is well-developed and enormous in size in some species because it also plays a role in the excretion of metabolic waste (Witkowska-Zimny and Wrobel, 2011, Spurway et al. 2012). In humans and animals, the allantois is retained in a residual form as diverticulum and caulis allantoicus.

A huge variety of placental techniques are found in mammals based on the various gestational and environmental requirements of the foetus (Spurway et al. 2012). The yolk sac and chorioallantoic placenta are the two primary types of mammalian placentas. In the first several days following implantation, often the trilaminate yolk sac placenta, which is connected to the uterine tissue, functions. Except for rats and rabbits, most mammals experience a reduction in the yolk sac placenta after the first trimester of pregnancy (Turco and Moffett, 2019). Therefore, poor structural and functional development of the yolk sac in rats is the root cause of embryonal or foetal toxicity and teratogenicity (Furukawa et al. 2019). The chorioallantoic placenta, which originates from the endometrium of the mother and the

trophectoderm of the embryo, serves as the main placenta in mammals during the medium to late stages of gestation (Turco and Moffett, 2019).

Placenta-derived mesenchymal stem cells

The origin and characteristics of human placental stem cells have not yet been thoroughly examined. Human amniotic epithelial cells (hAECs), human amniotic mesenchymal stromal cells (hAMSCs), human chorionic mesenchymal stromal cells (hCMSCs), and human chorionic trophoblastic cells (hCTCs) are the four subpopulations of placenta-derived stem cell subpopulations (Parolini et al. 2008). These cell subpopulations develop from the chorionic and amniotic membranes (AMs). According to (Parolini et al. 2008), these cell types can adhere to plastic in vitro, form fibroblast colony-forming units, advance through passages 2 to 4 in vitro, differentiate into one or more lineages, such as osteogenic, adipogenic, chondrogenic, and endothelial, and have foetal origins. In later studies, chorionic villus samples were reported to be useful for harvesting MSCs from the placenta (Roselli et al. 2013), and maternal decidua basalis (Abomaray et al. 2016). For this, small pieces of chorionic, decidua and amniotic are carefully cut and separated. The cells are then removed from the tissue by digestion with collagenase 2 and dispase II (Zhang et al. 2020, Lindenmair et al. 2012). Next, the solution is filtered and placed in culture dishes (Figure 1). hAMSCs have a fibroblast-like cell morphology, which is reportedly maintained during five iterations of in vitro cultivation. hAMSCs are three times larger than hAECs and can be easily distinguished (Parolini et al. 2008).

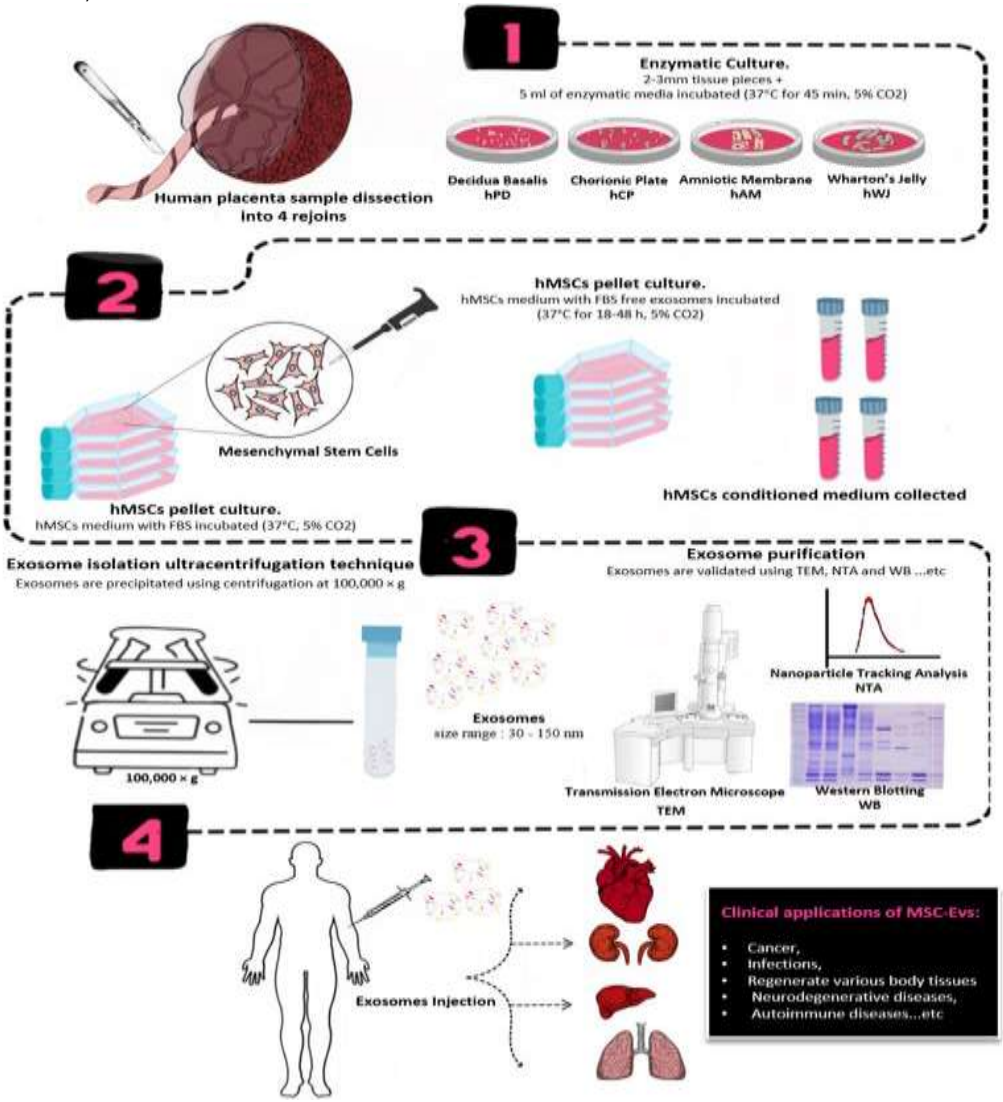

Figure 1: Technique for obtaining Exosomes from foetal tissue-derived mesenchymal stem cells stromal cellscells and purification.

No discernible changes are observed between hAMSCs isolated from various AM regions (Silini et al. 2020).

Wharton's jelly-derived mesenchymal stem cells

MSCs are multi-potent progenitor cells with the capacity to proliferate and regenerate. All adult tissues, including the bone marrow, fat, skin, placenta, and heart contain mesenchymal cells (Christodoulou et al. 2013). Upon injury, MSCs quickly move via blood vessels after the release of a variety of inflammatory cytokines and activation of inflammatory cells. With the help of their surface receptors for stroma cell-derived factor-1 (SDF1), the mesenchymal cells home into the injured area and regulate the immune system by secreting a variety of substances. The placenta, UC, amniotic fluid, and AM, which were previously considered medical waste, are rich sources of MSCs (Musiał-Wysocka et al. 2019). Stem cells from the AM, chorionic plate (CP), peritoneal, and UC tissues offer advantages over stem cells from adult tissues (Abomaray et al. 2016). Wharton's jelly (WJ), a specific mucosal connective tissue, is coated on the amniotic epithelium where two arteries and veins of the UC are implanted. UC-MSCs can differentiate into adipocytes, osteocytes, chondrocytes, neurons, and liver cells. They also have a unique capacity for self-renewal. Additionally, invader cells accumulate in inflamed or damaged tissues and hasten tissue repair by altering the immune system (Hsieh et al. 2010). The best source of MSCs is Wharton's Jelly (hWJMSCs) (Liau et al. 2020). WJMSCs are the best choice for clinical applications owing to the benefits they offer, which include increased proliferation and differentiation potential, easy and painless separation, quick access, a large supply of cells, and no ethical issues (Donders et al. 2017). Age is a significant determinant of the number of donor-isolated cells (Fan et al. 2010). Compared to older donor cells, young donor cells in culture media are more likely to multiply and age more slowly (Hoang et al. 2022). In addition, they are less vulnerable to harmful oxidative changes. WJMSCs have drawn much attention for the treatment of several diseases owing to these advantages (Hsieh et al. 2010). The conditioned medium of WJMSCs also contains several secretory factors, which mediate the immuno-modulatory activity of these cells (Kusuma et al. 2017).

Amniotic membrane-derived mesenchymal stem cells

Two types of amniotic membrane stem cells (AMSCs) originate from the amniotic mesenchymal and epithelial layers, namely, amniotic epithelial cells (AECs) and amniotic membrane mesenchymal stem cells (AM-MSCs), respectively (Cai et al. 2010). Both cell types are primarily epithelial in nature and develop during the pre-gastrulation phases of the growing embryo, prior to the separation of the three basic germ layers (Turco and Moffett, 2019). Mechanical separation of AM from the chorionic membrane and subsequent enzymatic digestion are key components in several procedures for the isolation of AECs and AM-MSCs (Oliveira and Barreto-Filho, 2015). AECs have a cobblestone epithelial appearance, whereas AM-MSCs exhibit plastic adherence and a fibroblastoid shape. AM-MSCs, and those generated from adult sources, have comparable morphology. Intriguingly, both AM-MSCs and AF-MSCs show faster rates of proliferation than MSCs generated from adult sources (Parolini et al. 2008), and have the capacity to simultaneously develop into cells from each of the three germ layers (Manuelpillai et al. 2011).

Amniotic fluid is a rich source of MSCs, and numerous methods have been developed for the isolation of these cells. MSCs can be distinguished from other cells based on their shape or other characteristics, and they typically necessitate evacuation of the amniotic fluid via amniocentesis (Bai et al. 2012). Testing for human leukocyte antigen has been used to establish the fact that MSCs originate from the foetus and not from the mother. Because the cells remain alive in the amniotic fluid and can multiply in culture, the original hypothesis that MSCs were removed from the embryo at the end of their life cycle was rejected. However, it remains unknown whether the cells come from the foetus itself, the placenta, or perhaps from the inner cell mass of the blastocyst (Turco and Moffett, 2019). MSCs obtained from amniotic fluid have a higher capacity for expansion in culture than those derived from the bone marrow. However, MSCs obtained from cultured amniotic fluid are phenotypically identical to those generated from adult bone marrow and second-trimester foetal tissue. MSCs isolated from animals and cultured in vitro have a distinct immune profile (Jain et al. 2023).

Amniotic fluid-derived mesenchymal stem cells

The largest preclinical and clinical studies of cellular treatment modules have focused on MSCs, particularly in heart repair and regeneration. Berebichez-Fridman and Montero-Olvera (2018) found that MSCs from both adult and foetal sources, such as the bone marrow, adipose tissue, UC blood, and amniotic fluid, can grow into cardiomyocytes. However, their application is constrained by low frequency (0.001–0.01%), invasive harvesting techniques, low yield, inconsistent self-renewal capacity among cell donors, and low frequency of adult-derived MSCs (Fan et al. 2010). Several MSCs produced from the foetus have also been investigated for their potential as a different source for heart regeneration. The injured myocardium was improved by MSCs produced from UC blood, placental tissue, and AMs; nonetheless, proliferation and survival were the key issues (Berebichez-Fridman and Montero-Olvera, 2018).

MSCs obtained from amniotic fluid (AF-MSCs) are an autologous cell source used to treat new-borns with

congenital heart abnormalities (CHDs). Because they can develop into cardiomyocytes and are derived from foetal tissue, AF-MSCs may represent the physiological and pathological changes in a developing foetal heart. The assessment of differences in the functional characteristics of these stem cells with the development of the foetal heart could, therefore, offer insights into the aetiology of neonatal CHDs. The ability of AF-MSCs isolated from CHD foetuses (ICHD AF-MSCs) and structurally normal foetuses (normal AF-MSCs) to proliferate and develop into cardiomyocytes was assessed (Manuelpillai et al. 2011). The immunophenotypic MSC markers expressed by ICHD AF-MSCs were comparable to those expressed by normal AF-MSCs and had similar potential for adipogenic and chondrogenic differentiation. However, they showed reduced osteogenic differentiation capacity, increased senescence, and increased expression of DNA-damaged genes. AF-MSCs serve as an example of this phenomenon. Collectively, these findings imply that AF-MSCs from ICHD foetuses exhibit abnormal patterns of proliferation and a markedly diminished capacity for cardiomyogenic differentiation. These deficiencies in ICHD AF-MSCs imply that defects in stem cells responsible for heart formation during embryogenesis may be the cause of poor heart development in foetuses with ICHD (Jain et al. 2023).

Basic functions of foetal tissue-derived mesenchymal stem cells

3.2.1. Proliferation and self-renewal potential

Various tissues contain different proportions of MSCs. For instance, the adipose tissue contains approximately 500 times more ATMSCs than the bone marrow, which has a low MSC frequency (0.001%–0.01% in the mononuclear fraction) (Oliveira and Barreto-Filho, 2015). According to (Zakrzewski et al. 2019), 0.9–1.5% of MSCs are present in the amniotic fluid. However, the success rate of Umbilical cord mesenchymal stem cells UCMSC isolation ranges from 10–63%, whereas that of isolation from the bone marrow and adipose tissue is close to 100% (Zakrzewski et al. 2019). Confluence was achieved by cultivating ATMSCs approximately on day 15 after isolation, whereas bone marrow mesenchymal stem cells BMMSCs reached confluence after approximately 22 days (Robert et al. 2020). Approximately 30 days are required to establish a primary culture of CBMSCs; BMMSCs and ATMSCs also showed higher-than-average metabolic activity (Witkowska-Zimny and Wrobel, 2011).

MSCs derived from foetal tissues grow faster than those derived from adult tissues (Choudhery et al. 2013). According to (Manuelpillai et al. 2011), the average doubling times of AF-MSCs and BMMSCs were 1.6 and 3.75 days, respectively (Manuelpillai et al. 2011). In addition, up to passage 14, the proliferation of CBMSCs increased more than two-fold, whereas that of BMMSCs decreased significantly (Hoang et al. 2022). Donor age appears to affect the rate at which MSCs proliferate as cells isolated from new-born skin expand more quickly than those isolated from adult skin (Abomaray et al. 2016). However, compared with ATMSCs isolated from 4-week-old mice, those isolated from 8-week-old mice showed considerably faster doubling times. Notably, this pattern was the opposite of that observed by (Christodoulou et al. 2013) for BMMSCs. The in vitro self-renewal ability of MSCs is limited. For example, BMMSCs can only be maintained for 12 passages before they show morphological changes indicative of ageing, whereas AF-MSCs can be maintained viable for 29 passages without observable morphological abnormalities (Agrawal et al. 2009).

Foetal tissue-derived stem cells also differ from one another. Compared with MSCs from the placenta (PMSCs), UCMSCs showed more proliferative potential, a quicker doubling time, a lower rate of apoptosis, and stronger mitotic activity. Transmission electron microscopy suggests that PMSCs are more likely than UCMSCs to adhere to surfaces (Zhu et al. 2013). According to (Bai et al. 2012), AF-MSCs and WJMSCs reached adherence at 2.7 1.6 and 6.5 1.8 days, respectively. Compared with ATMSCs, WJMSCs showed greater proliferative capability and took less time to double their population (Christodoulou et al. 2013). Atomic force microscopy has shown that UCMSCs are more capable of mass transport and cell migration than PMSCs (Zhu et al. 2013).

Gene expression profiles suggest that MSCs are at least partially specialised in accordance with their source(s). WJMSCs expressed more growth- and angiogenesis-related genes, whereas BMMSCs more closely resembled osteoblasts in terms of gene expression (Hsieh et al. 2010). These distinctions can be seen in the greater proliferative potential of WJMSCs and the superior osteogenic potential of BMMSCs (Hsieh et al. 2010). In contrast, HMSCs (derived from the heart) contained higher levels of myosin light chain-2a, miR-126, and miR-146a (Rossini et al. 2011).

Differentiation potential

The differentiation of MSCs is influenced by both transcriptional and genetic factors. Along with offering favourable conditions for growth, the microenvironment can encourage differentiation and proliferation (Kusuma et al. 2017). Because MSCs descend from the same embryonic lineage, differentiation towards osteogenesis, adipogenesis, and chondrogenesis should be straightforward. However, actual laboratory results do not match the theory. The initial cell population, origin of the starting cells, and direction of differentiation are significant determinants of differentiation capacity (Robert et al. 2020). To promote adipogenesis, the drugs dexamethasone (Dex), isobutyl-methylxanthine (IBMX), and indomethacin (IM) were combined (Figure 2).

Visualisation of lipid droplets and neutral triglycerides in cells stained with Oil red O provides evidence of a well-functioning differentiation process (Robert et al. 2020). To promote osteogenic differentiation, Dex, β-glycerophosphate (β-GP), and ascorbic acid phosphate (aP) are utilised (Hsieh et al. 2010). To verify this procedure, alkaline phosphatase activity and calcium accretion were examined. TGF-1 and TGF-2 are involved in chondrogenesis. In contrast to the mediation of ectodermal development through the neurogenic lineage by the protein kinase A (PKA) pathway, mesodermal differentiation is mediated by several other pathways, including TGF-1, PPAR-gamma, Smad3, and SOX9 (Mannino et al. 2022). Tuj-1, gamma-aminobutyric acid (GABA), MAP-2, and neurofilament 200 were used as indicators of differentiation. TGF-β, FGF, and bone morphogenetic protein (BMP) are examples of signalling pathways that are involved in the endodermal development of MSCs (Robert et al. 2020).

Research on advancing the methods used for stem cell differentiation is being carried out. Osteogenic differentiation is significantly influenced by transcription factors, such as osterix/Sp7 (Osx), runt-related transcription factor 2 (RUNX2), and Dlx5 (FarshdoustiHagh et al. 2015). Because they trigger as well as inhibit MSC differentiation, these transcription factors may act as regulators. Liang et al. (2019) recently demonstrated that utilising nanocomposites that stimulate the WNT/-catenin pathway may accelerate the differentiation of MSCs into osteogenic cells. Notably, the signalling pathways and components mentioned above that are involved in cell differentiation in a certain cell line may not be required in others, and differentiation can occur in other ways. Brady et al. (2013) used human MSCs derived from the perinatal and adult bone marrow to compare the variables that promote chondrogenesis.

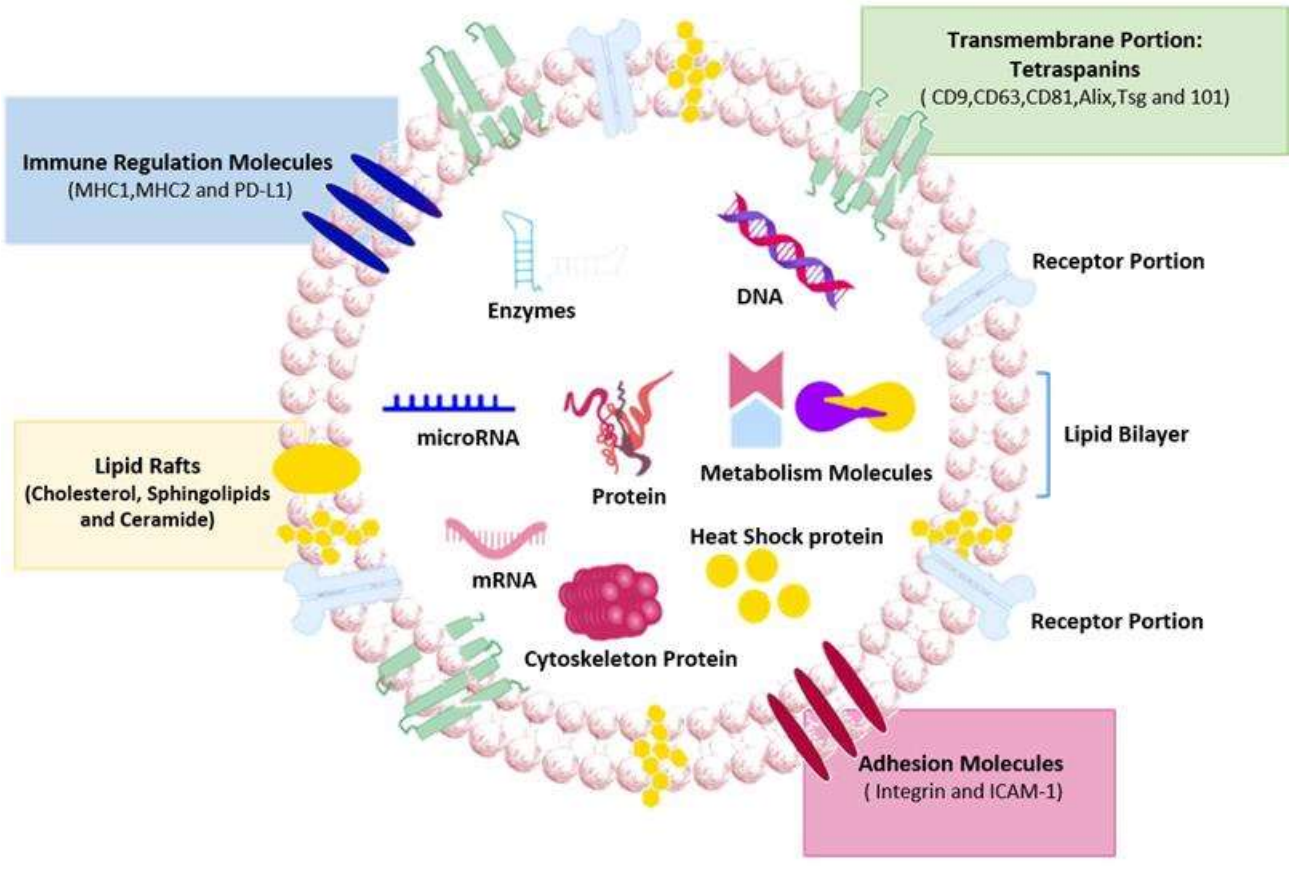

Figure 2: Typical structure of exosomes

Adult BMMSCs, but not foetal BMMSCs, trigger SMAD3 phosphorylation. Further, TGFβ3, but not BMP2, was responsible for chondrogenesis stimulation in adult BMMSCs. In contrast, BMP2, but not TGFβ3, stimulated the differentiation of foetal BMMSCs. Furthermore, when TGFβ3 and BMP-2 were applied simultaneously, chondrogenesis was induced in foetal BMMSCs. They were observed to regenerate tissues comparable to those produced by adult BMMSCs treated with TGFβ3 alone in terms of proteoglycan and type II collagen composition (Brady et al. 2013).

Tissue regeneration potential

Numerous tissue types have been found to contain MSCs; nevertheless, each of these can be categorised into one of two main types: adult and perinatal (Witkowska-Zimny and Wrobel, 2011). Tissues that can be acquired from adults are referred to as adult sources of MSCs. The milieu, growth factors, cell-to-cell interactions, external signals required for sustaining stemness, and the capacity to differentiate are provided by specialised structures known as stem cell niches, which adult MSCs typically call home(Mannino et al. 2022). While ATMSCs are acquired from stromal vascular fractions of enzymatically digested AT obtained by liposuction, lipoplasty, or lipectomy, BMMSCs are separated and cultivated in vitro from BM aspirates using the Ficoll gradient centrifugation method or red blood cell lysate buffer (Robert et al. 2020). Although BM aspiration and adipose liposuction are considered risk-free methods for BM and AT biopsies, they are invasive, uncomfortable for patients, and carry a risk of infection. This varies greatly depending on how many MSCs can be extracted from adult tissues (Rossini et al. 2011). With over 5,000 MSCs per gram of AT, AT has an MSC density at least 500 times higher than that of BM. In addition to UC, Wharton's jelly, UC blood, placental membrane, amnion, chorion membrane, and amniotic fluid are examples of placental structures derived from UC (Witkowska-Zimny and Wrobel, 2011). Because the placenta, UC, UC blood, and amnion are viewed as waste materials that are frequently discarded after birth, the collection of perinatal MSCs, such as UCMSCs, is non-invasive (Zhu et al. 2013). MSCs account for only 7–10% of the UC cells, but owing to their rapid population-doubling and -proliferation rates, they can increase in size during in vitro cultivation (Mannino et al. 2022). Compared with BMMSCs, ATMSCs exhibit a faster rate of proliferation and more colony-forming units on standardised xeno-free and serum-free culture platforms. The minimal requirements for morphology, MSC surface markers, and population-doubling time are all met by MSCs isolated from AT, BM, and UC under conventional culture conditions and xeno- and serum-free settings (Mannino et al. 2022).

The fate of these cells after administration is a major concern in MSC-based therapies, particularly when diverse delivery methods are used. To better understand the mechanisms underlying the drug action, it is crucial to understand the dispersion of these cells following injection. This information is required by authorised agencies before these cells can be used in clinical trials. Preclinical findings using several labelling methodologies provide important information, identifying MSCs that can undergo incorrect cell differentiation. Human BMMSCs and ATMSCs promptly lodge into the lungs after intravenous injection in a mouse model. After recirculation through the body, only a small number of infused cells are found, mostly in the liver after the second remobilisation (Donders et al. 2017). Intravenously injected human cells demonstrated long-term survival of up to 13 months in the bone, BM compartment, spleen, muscle, and cartilage using the technetium-99 m labelling method (Musiał-Wysocka et al. 2019, Hoang et al. 2022).

Immuno-modulatory potential

hWJMSCs appear to be a great source for cell-based reconstructive therapies and clinical trials based on preclinical and clinical investigations. WJMSCs are strong candidates for cell therapy in allogeneic transplantation because of their capacity to regulate and suppress the immune system through cell-to-cell interactions and the creation of soluble molecules. WJMSCs do not express HLA-DR and barely express class I HLA antigens. The immunosuppressant HLA-G6 is produced by WJMSCs and inhibits the cytolytic function of Natural killer (NK) cells (Abbasi et al. 2022). They lack the CD40 marker, whereas CD80 and CD86 are necessary for T-cell activation (Paladino et al. 2017). Trophoblasts that produce HLA-G6 shield the foetus from immune-based degeneration (Donders et al. 2017). Interleukin-6 (IL-6) released by UCMSCs induces tolerant phenotypes in dendritic cells (DCs) (Deng et al. 2014). MSCs produce several cytokines, including TGF-β1, interleukin 10 (IL-10), hepatocyte growth factor (HGF), prostaglandin E2 (PGE2), indolamine 2, 3-dioxygenase (IDO), and galectin-1(Fan et al. 2010). According to Paladino et al. (2017), they are crucial for immune system regulation. Studies have revealed that WJMSCs express high levels of anti-inflammatory molecules (Donders et al. 2017).

Owing to their low immunogenicity, the clinical value of WJMSCs is crucial (Varaa et al. 2019). De Girolamo et al. (2013) discovered that WJMSCs produce Major Histocompatibility Complex (MHC) class I (HLA-ABC) molecules at low to moderate levels but do not express MHC class II (HLA-DR), or co-stimulatory antigens, such as CD40, CD80, and CD86, which activate T- and B-cell-mediated responses. Their capacity to produce significant amounts of immunosuppressive cytokines, including Transforming growth factor beta (TGF-β), Interleukin 10 (IL-10) and vascular endothelial growth (VEGF), is related to their immunosuppressive potential

(de Girolamo et al. 2013). Intriguingly, T or B cells, monocytes or macrophages, DCs, neutrophils, mast cells, and natural killer cells are only a few important immune effector cells with which MSCs, specifically WJMSCs, may interact and affect their activation and activity (Abbasi et al. 2022). In WJMSC-mediated immunosuppression, soluble chemicals, and cell-to-cell communication are believed to be the major players; however, the exact mechanism of the immuno-modulatory activity remains unknown. Notably, WJMSCs and their secretomes exhibited anti-inflammatory effects in addition to their immuno-modulatory capabilities (Munoz-Perez et al. 2021). The presence of numerous secretory factors, such as prostaglandin-E2 (PGE2), is associated with the immuno-modulatory effects of the WJMSCs secretome (Donders et al. 2017). Taghavi-Farahabadi et al. (2021), demonstrated that the secretome of WJMSCs increases neutrophil function and lifespan, suggesting potential therapeutic uses for treating chronic granulomatous disease or neutropenia. Tumour necrosis factor (TNFα), G-CSF, interferon (IFN)-γ, IFN-α, IL-8, and IL-6 were among the released substances found in exosomes that were responsible for these beneficial effects (Munoz-Perez et al. 2021, Taghavi-Farahabadi et al. 2021). WJMSCs can also alter T-cell receptor-mediated T-cell activation by employing EVs enriched in programmed death-ligand 1 (PD-L1), which decreases T-cell activation in acute graft-versus-host disease(Paladino et al. 2017). By enhancing the production of lymphangiogenic factors, such as angiopoietin-2 (Ang2), prospero-homeobox protein 1 (Prox1), and phospho-Akt, WJMSC exosomes have also been shown to help treat lymphedema (Ting et al. 2021). WJMSCs and their secretomes have also been used to successfully treat morbid conditions, such as graft-versus-host disease, diabetes, and cancer, based on their immuno-modulatory effects, whether through cell-to-cell contact or soluble factors (Munoz-Perez et al. 2021, Ailuno et al. 2020, Patel et al. 2023).

Other therapeutic potential

According to Hoang et al. (2022) (Hoang et al. 2022), the multi-potency of MSCs, which allows them to replace damaged cells, facilitates tissue and organ healing. (Phinney and Pittenger, 2017) proposed that after tissue injury MSCs settle around the wound site and aid in healing by secreting trophic substances, such as growth factors, cytokines, and antioxidants. Some of these elements provide MSCs with an immuno-modulatory capacity (Musiał-Wysocka et al. 2019). In general, the biological properties of MSCs that underlie their clinical applications include (a) their capacity to localise to sites of inflammation following tissue injury when injected intravenously, (b) the secretion of numerous bioactive molecules capable of promoting the healing of injured cells and inhibiting inflammation, (c) modulation of immune functions, (d) differentiation into diverse cell types, and (e) their use as a gene therapy tool (Manuelpillai et al. 2011, Chaubey et al. 2018)

Research on the therapeutic potential of WJMSCs and their secretomes in various disease states is briefly summarised in the following sections. The secretome of WJMSCs supports various functions, including cellular homeostasis, inflammation, tissue regeneration, and immuno-modulation (Tang et al. 2021).

Long-term homing of human MSCs after injection into a range of tissues has been validated in studies on baboons (Jarrige et al. 2021). Despite the possibility that the capacity of MSCs for systemic therapeutic benefits may be diminished by retention in the lungs (Willis et al. 2017), they offer a significant benefit in the treatment of respiratory disorders. The bulk of the injected cells was found in the renal cortex after local injection of MSCs via the renal artery route, demonstrating tissue-specific homing. Numerous studies have monitored the migration of MSCs injected into humans (Berebichez-Fridman and Montero-Olvera, 2018). To cure intervertebral disc degeneration, Henriksson et al.(2019) employed MSCs labelled with iron sucrose and showed that at 8 months, but not at 28 months, chondrocytes differentiating from infused MSCs could be identified in the damaged intervertebral discs (Henriksson et al. 2019). Although information regarding the bio-distribution of infused MSCs is lacking, results from preclinical and clinical studies demonstrate a comparable set of data supporting homing, migration to the injured site, and migration to major organs where infused MSCs are located. The following in-depth and fascinating review is strongly advised (Zhuang et al. 2021).

Mechanical function of foetal tissue-derived mesenchymal stem cells

Cell-to-cell contact-mediated effects

Upon transplantation, MSCs use direct cell-to-cell contact to synergistically perform their immuno-modulatory functions at the damaged locations. Direct cell-to-cell contact between the PD-1 inhibitory molecule on T cells and its ligand PD-L1 on MSCs limits the effect of T-cell (such as IL-17-producing T cells, Th17) responses, inhibits CD3+ T-cell development, and promotes early death. The death signal is similarly transmitted, and T-cell apoptosis is triggered by interactions between TNF receptor superfamily member 6 (Fas) and FasL (Donders et al. 2017). Furthermore, found that the expression of CD54 (ICAM-1) in UCMSCs and of CD106 (VCAM-1) in PMSCs was crucial for mediating the immuno-modulatory effects on T cells (Munoz-Perez et al. 2021, Clark et al. 2019).

Paracrine and extracellular vesicle-mediated effects

Despite the low in vivo engraftment rate (0%–20%), paracrine substances, such as lipid-based mediators, growth factors, and signalling peptides, are believed to

be the cause of pleiotropic lung protection after transplantation (Yeung et al. 2019). Epithelial growth factors, including keratinocyte growth factor and pro-angiogenic factors, Lipoxin A4, and TNF-a-stimulated gene/protein 6, are secreted bioactive molecules with strong anti-inflammatory characteristics. These chemicals enable stem cells to travel to wounded tissues where they help create a non-inflammatory environment that promotes tissue regeneration, remodelling, and survival by boosting cell proliferation and preventing apoptosis (Chaubey et al. 2018). EVs are a paracrine function of MSCs that affect both tissue-resident stem cells and macrophages, resulting in a more effective reparative process by tissue-resident stem cells (Doyle and Wang, 2019). EVs are neither cytokines nor secreted soluble compounds. Exosomes and microvesicles are two types of EVs that are nanometre-sized, membrane-enclosed packets of biomolecules discharged by cells into the environment to facilitate signalling and cell-to-cell communication. These vesicular cargo macromolecules transport various physiologically active compounds, including amino acids, bioactive lipids, and nucleic acids (Veziroglu and Mias, 2020). These cell-free solutions are safer options than cell treatments. For large-scale synthesis, UCMSC exosomes and MSCs produced from adipose tissue are easier to obtain than those from BMMSCs (Choudhery et al. 2013). According to (Ragni et al. 2017), UCMSCs produce more EVs than BMMSCs.

The pleiotropic protective effects of EV-based therapies in experimental models of bronchopulmonary dysplasia(BPD) were initially identified by (Willis et al. 2017). They found that intravenous delivery of EVs derived from both BMMSCs and UCMSCs in a hypoxic mouse model reduced pulmonary hypertension and lung vascular remodelling and restricted the entry of alveolar macrophages and pro-inflammatory cytokines. Additionally, macrophage uptake resulted in a change in equilibrium towards an anti-inflammatory state (Lee et al. 2012). Mice treated with EVs showed significantly better lung function with reduced fibrosis, arteriolar muscularisation, and pulmonary hypertension. The authors concluded that macrophage immunomodulation induced by the delivery of EVs played a role in restoring lung function (Willis et al. 2017).

Exosomes

Exosomes have a flotation density of 1.10–1.21 g/mL in a sucrose gradient, which allows them to be precipitated by centrifugation at a force of 100,000 × *g* (Cai et al. 2010). Exosomes contain membrane-associated proteins, including tetraspanins proteins (CD9, CD63, CD81, Alix, Tsg and 101), MHC-I and MHC-II, heat-shock proteins (Hsp70-Hsp90), GTPases, and proteins involved in the biogenesis of multi-vesicular bodies (Xu et al. 2020). Exosomes have also been found to contain cytoskeletal proteins, such as actin, moesin, and syntenin, as well as metabolic enzymes, such as GAPDH, LDHA, PGK1, aldolase, and PKM (Figure 2) . Exosomes are a source of proteins whose levels fluctuate in response to physiological changes (Yang et al. 2015).

Exosomes are abundant in proteins, cytokines, specific lipid rafts, such as phosphoglycerides, cholesterol, ceramide, fatty acyl chains, non-coding RNAs, such as tRNAs and rRNAs, and infrequently in DNA (Yoon et al. 2014). Extensive material on exosomes is freely available online in databases such as http://exocarta.org and http://microvesicles.org (Vesiclepedia).

Exosomes are formed when multi-vesicular structures fuse with the plasma membrane. The chemical, environmental, and mechanical stressors listed by Hannafon and Ding, that cause this discharge include gamma radiation, calcium ionophores, heparanases, statins, hypoxia (low O_2), acidosis, and matrix separation (Hannafon and Ding, 2013). Additionally, cross-linked activation of TCR/CD3 in T lymphocytes, low O_2 in the placental MSC culture medium, and K^+-dependent depolarisation of neural cells encourage the induction of exosome secretion (Hannafon and Ding, 2013).

Exosomes can be isolated using microfluidic isolation, exosome precipitation, size-based isolation, immune-affinity capture, or ultracentrifugation(Figure 2) (Doyle and Wang, 2019). They are widely distributed in body fluids, such as urine, blood, saliva, and breast milk (Vlassov et al. 2012). Proteins, lipids, nucleic acids, and metabolites are among the bioactive compounds thought to be present in exosomes (Kalluri and LeBleu, 2020). To encourage cell-to-cell communication, signal transduction, immune response regulation, antigen presentation, and epigenetic reprogramming of recipient cells, these encapsulating components are selectively administered to neighbouring or distant cells (Janockova et al. 2021). The biological effects of exosomes are significantly influenced by the physiological or pathological conditions of the generated tissues or cells at the time of exosome release as well as surface receptors on recipient cells. Dental stem cell(DSC)-derived exosomes may be an ideal therapeutic tool for treating other systemic diseases as well as for tissue repair and regeneration (Mai et al. 2021).

Characteristics, components, and functions of exosomes

To ascertain the biological interactions of exosomes, it is necessary to assess their physicochemical properties. Therefore, it is crucial to accurately identify these qualities. Exosomes are the smallest EVs, have been described in several ways. These methods included flow cytometry, electron microscopy, resistive pulse sensing, nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), and atomic force

microscopy (AFM) (Phinney and Pittenger, 2017). However, each method has its limitations. Numerous biophysical, molecular, and microfluidic techniques have been used to characterise exosomes. Exosomal were characterised using biophysical methods. NTA uses optical particle tracking, a biophysical approach that can assess exosome concentration and size distribution in the 30 nm to 150 nm range in diameter. The exosomal migratory path was identified to determine particle velocity (Mai et al. 2021). Using this technique, individual nanoparticles suspended in a liquid can be monitored at the atomic level. NTA tracks each exosome through image analysis to determine its mobility. Thus, the particle size may be related to this movement (Alcayaga-Miranda et al. 2016). The size distribution, concentration, and phenotype of the particles are the outputs of this method. The benefit of employing NTA is its capacity to measure tiny particles with sizes as low as 30 nm and detect various EVs, including exosomes. The measurement takes only a few minutes, and the sample preparation is quick and simple. This technique is even more appealing because materials can be recovered in their original state when the measurements are completed (Alcayaga-Miranda et al. 2016). According to factor (Dragovic et al. 2011), using fluorescently labelled antibodies, this method may also reveal the presence of antigens on EVs. The performance of NTA is mostly dependent on sample preparation and the choice of the appropriate dilution factor (Dragovic et al. 2011).

Using DLS, sometimes referred to as photon correlation spectroscopy, exosome size can be estimated. In DLS, a monochromatic coherent laser beam passes through a suspension of particles (de Necochea-Campion et al. 2018). There are both positive and negative time-dependent fluctuations in the interference caused by the relative Brownian motion of the particles inside a sample. Although this approach is straightforward, it does not allow visualisation of the particles (de Necochea-Campion et al. 2018). One advantage of this technology is its ability to measure particles ranging in size from 1 nm to 6 m. It functions best when measuring mono-dispersed suspensions, which include only one type of suspended particle. The detection of tiny particles becomes challenging when large vesicles, even in minute amounts, are present in the suspension (Szatanek et al. 2017). The efficacy of this method was established by examining the location and quantity of EVs in red blood cells and EVs produced by ovarian cancer cells (Gercel-Taylor et al. 2012). This method can provide the range of analysed vesicle diameters; however, it cannot be used to determine the cellular origin of EVs or obtain biochemical data (Gercel-Taylor et al. 2012).

Exosomes are released by a variety of cell types, including fibroblasts, intestinal epithelial cells, neurons, adipocytes, and tumour cells. They can be detected in blood, synovial fluid, breast milk, urine, saliva, amniotic fluid, malignant effusions of ascites, and several other biological fluids. The cell of origin and the condition of the originating tissue or cell at the moment of exosome production determine the biology, function, and variety of exosomes. Exosomes can behave as cellular garbage bags that collect extra or disrupted cellular components. In addition, cell surface proteins and signalling molecules are recycled by endocytic vesicles (Gurunathan et al. 2019). Exosomes are essential components in many biological processes, including angiogenesis, antigen presentation, apoptosis, coagulation, cellular homeostasis, inflammation, and intercellular communication (Alcayaga-Miranda et al. 2016). These capabilities affect physiological and pathological processes under various conditions, including cancer, infections, neurodegenerative diseases, and autoimmune diseases. These abilities are linked to their potential for RNA, protein, enzyme, and lipid transfer (Doyle and Wang, 2019).

Exosome isolation or preparation methods

Ultracentrifugation, frequently paired with sucrose density gradients, is a fundamental and widely used technique for the isolation and purification of exosomes from cell culture supernatants and other biological fluids (Sidhom et al. 2020). Exosomes are precipitated using centrifugation at 100,000 × *g* in succession, which removes cells and larger particles (Sidhom et al. 2020). High-performance liquid chromatography (HPLC), ultrafiltration, exosome precipitation by volume exclusion of polymers, such as polyethylene glycols, and affinity purification using specific antibodies against CD9, CD63, CD81, and CD82 are other methods for separating exosomes (Nordin et al. 2015). In addition, exosome isolation kits based on effective methods offer easy separation. Low pH conditions promote the isolation of exosomes (Ban et al. 2015). AFM, scanning electron microscopy, DLS, flow cytometry, western blotting, NTA, transmission electron microscopy, and enzyme-linked immunosorbent assay (ELISA) have been used to identify exosomes (Lopez-Verrilli et al. 2016). For in vivo or in vitro applications, exosomes must be frozen after isolation and characterisation because they are unstable at 37 °C. Without the use of cryopreservatives, exosomes can be maintained at −20 °C for six months (Konala et al. 2016). The stability of exosomes was investigated by Sokolova et al. (2011), by storing them at −20, 4, and 37 °C. According to their findings, at 4 °C and 37 °C, the exosome size was reduced, and structural or degradative alterations occurred. Exosome size was unaffected by several freeze–thaw cycles (up to −20 °C) and ultracentrifugation. Therefore, storage at −20 °C or lower is safe and does not affect the size or structure of exosomes (Konala et al. 2016).

Ultracentrifugation, size exclusion chromatography (SEC), polymer precipitation, immunological affinity capture, microfluidics, and ultrafiltration (UF) are

techniques frequently employed to isolate exosomes. Exosomes can be separated from other components of biological materials using various approaches to produce pure exosome populations for further studies. Ultracentrifugation is the most popular and widely accepted technique for separating exosomes (Ailuno et al. 2020). Based on their varied sedimentation rates, proteins, vesicles, cell debris, and cells were separated into uniform suspensions via ultracentrifugation. Small-molecule protein complexes can be separated using SEC based on their sizes. Exosomes can be separated using UF, based on their size, and via immune-affinity chromatography, based on their interaction with various antigens and antibodies (Sidhom et al. 2020). Exosomes are typically precipitated from samples via polymer precipitation, which affects their solubility or dispersion in body fluids and cell cultures (Wei et al. 2021).

Translational and clinical applications of mesenchymal stem cell-derived exosomes

MSCs are promising seed cells for tissue repair and regeneration because of their distinct capacity for regeneration and the vast variety of potential applications. However, an increasing number of studies have revealed that the main biochemical mechanism by which MSCs function is through exosome secretion. Exosomes play an important role in regenerative medicine and the control of several fundamental processes. They contain RNA, proteins, lipids, and several metabolites (Clark et al. 2019). Exosomes have high drug-loading capacity, low immunogenicity, excellent biocompatibility, and few negative consequences while mimicking the functions of their parent cells (Clark et al. 2019). Six different types of dental stem cells DSCs have so far been isolated and identified: dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), dental follicle progenitor cells (DFPCs), stem cells from the apical papilla (SCAPs), and gingival mesenchymal stem cells (GMSCs). Tumour cell death, osteogenesis, angiogenesis, immunomodulation, neuronal development, and vascular expansion are dependent on DSC-derived exosomes (DSC-Exos) (Clark et al. 2019).

Recent studies have revealed that hPMSCs and their exosomes exhibit regenerative potential in animal models of various pathological conditions affecting tissues such as the liver, heart, kidney, brain, and uterus (Pethe and Kale, 2021). Additionally, their anti-inflammatory properties have been harnessed to treat conditions such as the GvHD reaction (an immunological reaction generated by mature allo-reactive donor T cells responding to allo-antigens presented on host APCs), asthma, irritable bowel disease, neurodegenerative diseases, and ovarian dysfunction. Their angiogenic properties have been used to treat chronic wounds and to repair damaged endothelial cells.

Clark et al. (2019), discussed the role of hPMSC-derived exosomesin promoting myelin regeneration in an animal model of multiple sclerosis. They found that the exosomes increased the number of oligodendrocyte precursor cells and promoted their differentiation into mature oligodendrocytes, which are responsible for the production of myelin. This study also showed that exosomes reduced inflammation and promoted re-myelination in an animal model. Amniotic fluid can also be used to obtain exosomes (hAFMSC-Exos). Similarly, AMs have been used to repair cartilage damage, nasal reconstruction, and psoriasis (Clark et al. 2019). Ridzuan et al. (2021) isolated exosomes from hUCMSCs and found that they effectively ameliorated chronic obstructive pulmonary disease (COPD)-induced inflammation in a rat model of COPD by reducing the levels of NF-κB subunit p65 in the tissue and regulating multiple pathways known to be associated with COPD .

MSCs are multi-potent progenitor cells isolated from several organs. According to a growing body of research, MSCs have self-renewal and multi-potent differentiation capabilities, which modulate immunological and inflammatory responses and reduce apoptosis and oxidative stress (Munmun and Witt-Enderby, 2021). More importantly, a large body of preclinical research has demonstrated that MSCs have the potential to treat a broad range of illnesses, including Duchenne muscular dystrophy, autoimmune diseases, cancer, liver disease, cartilage repair, heart failure, stroke, neurological disorders, diabetes mellitus, and ocular surface diseases (Luo et al. 2021). Over the past 10 years, approximately 50,000 patients have been enrolled in more than 1,000 MSC-based clinical trials, owing to the safety of autologous stem cells (Pittenger et al. 2019). Notably, accumulating research has suggested that the secretome of transplanted MSCs is primarily responsible for their therapeutic benefits (Phinney and Pittenger, 2017).

Owing to the secretome-derived bioactive substances, MSC-based therapy differs from previous forms of cell therapy in that it produces therapeutic benefits in addition to direct cell-to-cell interactions (Levy et al. 2020). MSC-EVs, including exosomes, micro vesicles, and apoptotic bodies, have recently been postulated as prospective cell-free therapeutic alternatives to MSCs (Jarrige et al. 2021). In addition to having a higher drug-loading capacity than cellular therapies, MSC-EV-based therapies have superior biocompatibility, high stability, no cytotoxicity, high specificity, low immunogenicity, competitive price, and efficient intercellular communication. Consequently, the use of exosomes in MSC-EV-based therapies makes them one of the most promising therapeutic modalities for tissue repair and regeneration (Jarrige et al. 2021).

CONCLUSIONS

Although the quality of stem cells may vary based on the

site from which they are harvested, the use of MSCs derived from foetal tissues mitigates significant ethical concerns associated with stem cells from other sources. This ethical advantage further supports the exploration and development of therapies based on MSCs such as MSC-Exos. MSC-Exos offer therapeutic benefits for various pathophysiological ailments by restoring tissues. MSC-Exos have regenerative potential and can be used to regenerate various body tissues. MSC-Exos derived from foetal tissues are considered a promising alternative to MSC-based therapies, as they offer several advantages. (1) Source and availability: Foetal membranes, such as the AM and UC, are readily available during childbirth, providing a relatively abundant source of MSCs for isolation. In contrast, isolating MSCs from adult tissues, such as bone marrow or adipose tissue, requires invasive procedures and may yield a smaller number of cells. (2) Cellular characteristics: MSCs derived from foetal membranes and adult tissues exhibit some differences in their cellular properties. Foetal membrane-derived MSCs have higher proliferation rates and longer telomeres compared to MSCs from adult tissues. These characteristics may impact the quality and quantity of exosomes derived from corresponding MSCs. (3) Exosomal composition: Exosomes derived from both foetal membrane MSCs and adult tissue MSCs contain various bioactive molecules, including proteins, lipids, and nucleic acids. However, their specific composition may differ due to the distinct cellular microenvironments and developmental stages. These differences may influence the therapeutic potential and functional properties of the exosomes. (4) Therapeutic potential: Exosomes derived from both foetal membrane and adult tissues have demonstrated therapeutic potential in regenerative medicine and other applications. While foetal membrane MSC-derived exosomes have immuno-modulatory effects, tissue repair, and anti-inflammatory properties, adult tissue MSC-Exos have applications in tissue regeneration, wound healing, and immune system modulation. The choice of exosomes may depend on the specific therapeutic goals and target tissues. Several issues limit the applicability of MSC-Exos in clinical settings and must be addressed. These include understanding the similarities and differences between exosomes derived from foetal membrane MSCs and adult tissue, standardisation of isolation and characterisation methods, optimisation of dosing and delivery, and evaluation of long-term safety and efficacy. The development of a universal and straightforward isolation strategy to effectively use MSC-Exos is crucial for promoting the use of MSCs and MSC-Exos for clinical applications.

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