Advances in the Treatment of Diabetic Foot with MSC-derived Exosomes

Qiaoya He[*]

The Second Affiliated Hospital of Harbin Medical University, Harbin Medical University, Harbin, Heilongjiang, 150000, China

Abstract. Diabetes is a chronic metabolic disease with a worldwide epidemic and its prevalence is increasing every year. Meanwhile, diabetes imposes serious economic burdens on the world. Commonly and seriously as a complication of type 2 diabetes, the diabetic foot is a multi-factorial chronic non-healing wound that not only poses serious threats to the patient's physical health but also comes along with huge financial and psychological burdens. In the long run, the risk of amputation is much higher inpatients who suffer from diabetic foot than in healthy people. [1] The prognosis for the diabetic foot is not encouraging in today's treatment paradigm, with the majority of patients having an unsatisfactory prognosis after conventional treatment, eventually leading to tissue necrosis, gangrene, and amputation. Numerous studies have suggested that mesenchymal stem cell exosomes (MSCs-Exos) have potential therapeutic value for patients with DFU and are increasingly being available in clinical practice. MSCs-Exos can accelerate DFU wound healing by promoting coagulation, inhibiting inflammatory responses, boosting cell proliferation and angiogenesis, and increasing collagen deposition. In addition, this article reviews the clinical application of MSC-derived exosomes in the treatment of diabetic foot and the advantages and limitations of MSC-derived exosomes as carriers, exploring the extraction process of exosomes. In the future, a unified and standardized guideline specification for purification and isolation techniques should also be established and enhanced, and a license for clinical application should be obtained. To verify the security and efficacy of exosomes in DFU treatment, large sample, and multi-centre clinical research should be designed.

Keywords: Diabetic foot, Mesenchymal stem cells, Exosomes, Clinical application, Extraction.

1. Introduction

1.1 Diabetes

Characterised by a relative or absolute lack of insulin secretion and chronically elevated blood glucose levels, leading to diabetes. Among these, type 2 diabetes mellitus (T2DM) is an acquired metabolic disease that increases the risk of many fatal and debilitating conditions. [2] The metabolic dysfunction of diabetes will generate a plethora of complications including retinopathy, neuropathy, heart disease, peripheral vascular disease, tissue inflammation, and ulcers [3]. Of these complications, up to 2%-3% of people with diabetes are at risk of diabetic foot ulcers [4], reducing the quality of life and survival state and bringing burdens on the healthcare system. Over the past decades, the prevalence of diabetes has increased rapidly worldwide owing to a rising aging population and changes in people's dietary habits. According to the World Health Organization, more than 420 million people worldwide were living with diabetes in 2015, and this number is expected to reach 6. 42 million by 2040.

1.2 Diabetic Foot

Diabetic foot (DFU) is a common and serious complication of diabetes, caused by ischaemic disease of the lower limbs in diabetic patients, resulting from chronic inflammation interfering with the process of tissue healing. The diabetic foot is not only a serious threat to the physical health of the patient, with symptoms such as resting pain, intermittent claudication, and ulcer infection, but also comes with a huge financial and psychological burden. The diabetic foot is characterized by high treatment costs and a long therapy cycle and is a main cause of amputation and death in diabetic patients. In a study of diabetic foot infections and amputations, the 5-year survival rate for diabetic foot patients was only about 50%, and if an extensive amputation was performed the 5-year survival rate was dramatically reduced to 8.3%. [5] Current conventional treatment of diabetic foot ulcers includes non-surgical treatment such as reduction of local pressure, infection control, improvement of blood supply, debridement and dressing changes, topical wound dressing, hyperbaric oxygen therapy, and negative pressure closed suction, and surgical treatment such as

---

[*] Corresponding author: hqy13957690944@163.com

percutaneous transluminal angioplasty. [6] The prognosis for most patients is dissatisfactory and eventually leads to tissue necrosis, gangrene, and amputation. Therefore, novel therapeutic modalities that effectively improve the prognosis of diabetic foot ulcers are a pressing technical issue for clinical application. MSCs-derived exosomes (MSCs-Exos), have demonstrated tremendous potential in promoting wound healing [7] and have been proven to have sound repair effects in the repair of various organ injuries. Because of the good stability and low immunogenicity of exosomes, MSCs-Exos has been a potential candidate for the treatment of malignant tumors, cardiovascular and respiratory diseases, endocrine metabolic diseases, osteoarthritis, endometrial injuries, etc. And exos may be safer than direct treatment with MSCs [8]

2. Biological Characteristics of Msc-Derived Exos

Mesenchymal stem cells (MSCs), a type of pluripotent stem cells with self-renewal and multidirectional differentiation potential, are a current hot issue. Their use in regenerative medicine has been reported as a promising therapeutic strategy for tissue regeneration [9]. Excessive numbers of studies have exhibited that MSCs can promote angiogenesis and cell proliferation, reduce inflammation, and produce a large number of bioactive molecules involved in the repair. Recently, a large body of evidence suggests that the positive effects of stem cell therapy may be mediated by paracrine mechanisms of MSCs, particularly through the secretion of exosomes [10], which can be functionalized to treat a variety of diseases. Exosomes are discoid vesicles [11] with a diameter of 40-160nm and consist of a lipid bilayer. The process of exosome formation involves the invagination of intracellular lysosomal particles to form intracellular multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs), which are secreted as exosomes into the extracellular matrix by cytosolic exocytosis after fusion of the outer membrane of the MVBs with the cell membrane (double invagination of the plasma membrane). Under physiological and pathological conditions, exosomes are produced and secreted by almost all cells, including immune cells (e.g. B cells, T cells, mast cells, dendritic cells), platelets, cancer cells, epithelial cells, mesenchymal cells, neurons, astrocytes, and oligodendrocytes. Exosomes are widely distributed in various body fluids, such as blood, lymph, saliva, cerebrospinal fluid, breast milk, amniotic fluid, semen, and urine. Exosomes are enriched in nucleic acids e.g. mitochondrial DNA, mRNA and non-coding RNA (miRNA, lncRNA, circRNA), proteins, lipids, etc. [12]

3. The Biological Functions of Msc-Derived Exos

Exos has specific functional, targeting, and driving mechanisms, serving as a significant mediator of cellular communication, carrying and transmitting vital signaling molecules, participating extensively in intercellular material transport and information transfer, and regulating cellular physiological activities. Exos is closely related to the occurrence and development of all kinds of diseases. Exos takes part in senescence scavenging molecule[13], antigen presentation [14], tumor progression by promoting angiogenesis and tumor cell migration during tumor cell metastasis [15-16], inhibition of the immune response [17], and disease propagation by interacting with receptor cells [18]and other physiological and pathological processes. Studies have shown that Exos accelerates the healing of chronic skin wounds mainly through mechanisms such as reducing the inflammatory response, accelerating granulation tissue and angiogenesis, repairing and replacing cells, and decreasing scar formation [19]. Related literature has revealed that exosomes may be efficient drug transport carriers on account of their natural substance transport properties, inherent long-term recycling ability, and distinguished biocompatibility. Furthermore, with the progressive research related to exosomes, they are becoming a potentially serviceable diagnostic tool in disease progression, treatment response, and predictor of overall survival.

4. Mechanisms of Exosomes Derived from Mesenchymal Stem Cells for The Treatments of Diabetic Foot

4.1 Promoting Coagulation
Studies have shown that exosomes are involved in the regulation of the hemostatic process and that they exert their procoagulant function mainly through the activation of platelets [20]. In vascular injury, tissue factor forms a complex with coagulation factor VIIa, which activates coagulation factor X, further converting prothrombin into thrombin and acting as a procoagulant [21]. Exosomes produced under tissue hypoxia will enhance their procoagulant function by further upregulating tissue factor activity, promoting prothrombin conversion and fibrin production [22]. In a rat model, EPCs-derived exosomes (EPCs-Exos) can facilitate endothelial cell repair by highly enriching miR-21-5p and specifically inhibiting the expression of the angiogenesis inhibitor platelet response protein in recipient endothelial cells [23].

4.2 Suppression of the Inflammatory Response
The healing of chronic wounds can be broadly divided into four sequential processes: the hemostatic phase, the inflammatory phase, the proliferative phase, and the remodeling phase,[24]followed by a return to homeostasis. The wounds of diabetic foot are mostly stagnant in the inflammatory phase as various exogenous and endogenous elements. The high expression of inflammatory cells and inflammatory factors leads to increased protein hydrolysis, which restrains wound healing from the inflammatory phase to the next stage, resulting in delayed wound healing [25]. Macrophages, which are inflammatory cells, play an important role in the development and resolution of inflammation. [26]

Macrophages have two phenotypes, M1 and M2, which play both pro-inflammatory and anti-inflammatory roles. Of these, M1 macrophages play a pro-inflammatory role, while M2 macrophages affectresisting inflammation. Research have indicated that exosomes perform their anti-inflammatory function by modulating immune cell activity at the site of injury and that they induce the conversion of macrophages to the M2 phenotype, which has a strong anti-inflammatory effect. At the same time, exosomes suppress B-cell maturation and T-cell proliferation, activate regulatory T-cells, degrade inflammatory factor levels and stimulate wound healing. [27]

4.3 Promoting Cell Proliferation and Angiogenesis

RNA contained within Exos is regarded as essential substance in adjusting the activity of recipient cells [28]. RNAs or proteins in MSCs-Exos can exert cell-homing effects and regulate cell proliferation and differentiation, which can limit injury, modulate immune responses, and stimulate self-repair and tissue regeneration after cell injury. [29] For example, MSCs-Exos enriched with long non-coding RNA (lncRNA) H19 can facilitate oncogene homologous phosphatase-tensin expression and observably improve fibroblast proliferation and migration, [30] inhibit p53 activity and growth differentiation factor release. There is macrophage infiltration in damaged skin to accelerate wound healing. Besides, mi RNAs released by exosomes (e.g. mi R-132 and mi R-146a) can significantly raise pro-angiogenic factor expression and increase the proliferation rate and angiogenesis of human umbilical vein endothelial cells. [31] It was demonstrated that matrix metalloproteinase-containing exosomes promote endothelial cell proliferation by facilitating cell migration and cleavage of vascular endothelial growth factor receptors, thereby accelerating trabecular neovascularization. [32] Li Luocheng et al [33] intervened with EPCs-Exos in hypoxia-treated human umbilical vein endothelial cells and discovered that the proliferation and migration ability of hypoxic endothelial cells were restored to some extent, confirming that EPCs-Exos could improve the proliferation and migration ability of hypoxic endothelial cells, reduce endothelial cell apoptosis and mitigate hypoxic injury of vascular endothelial cells. In 2004, Planat-Benard et al [34] first found that adipose-derived lineage cells and vascular endothelial cells share the same precursor cells and have the potential to differentiate into an endothelial cell phenotype and participate in angiogenesis in vivo. Subsequent studies demonstrated that ADSC in adipose tissue could participate directly in neovascularization via directional differentiation to vascular endothelial cells and smooth muscle cells. [35] Compared to bone marrow-derived stem cells, ADSC has a greater capacity to differentiate into endothelial cells. [36]

4.4 Increase Collagen Deposition and Reduce Scar Formation

Usually, diabetic foot ulcers take longer to heal and tend to cause scar formation and poor healing quality.

Fibroblasts produce varied glycoproteins and cytokines [37], which control the production and degradation of the extracellular matrix (ECM). Fibroblasts begin to synthesize and secrete large amounts of collagen fibrils and matrix components on day 4-5 of wound healing, which together with ECM components such as new capillaries, proteoglycans, hyaluronic acid, collagen, and elastin form granulation tissue. Thus can replace platelet clots, fill up the wound tissue defect and create the conditions for epidermal cell coverage[38]. During the healing process, fibroblasts can migrate to the wound surface, proliferate through mitosis and promote the capillary generation, forming a new vascular network and restoring the blood supply to the injury site. Fibroblasts,, play a very essential role in the process of wound repair. Abnormal accumulation of the extracellular matrix is one of the most important reasons for scar formation. Human adipose stem cell-derived exosomes (ADSCs-Exos) boost type I collagen, type III collagen, fibronectin, and smooth muscle actin gene expression in fibroblasts at the early stage of wound repair, while decreasing collagen expression at the late stage of healing to restrain the development of scar tissue. [33] Umbilical cord blood-derived MSC exosomes can mediate the TGF-β receptor pathway through mi R-21-5p and mir R-125b-5p to inhibit scar formation during wound healing. [39]

4.5 Promoting Wound Re-epithelialization

An important phase in the healing process is the re-epithelialization of traumatic tissue. Diabetic foot wounds are a protracted course of the disease because re-epithelialization does not take a corresponding place. In addition, plenty of immune cells such as neutrophils, monocytes, and macrophages gather on the wound, releasing hydrolytic enzymes and oxygen radicals, resulting in further tissue damage. Hence, it is important to prompt timely re-epithelialization of the wound for wound healing. Fibronectin can act as a chemokine to induce the movement of epithelial cells, fibroblasts, etc toward the wound and promote the regeneration of epithelial tissue. Zhao [26] et al. used ADSC to treat rabbit gingival wounds and found that the expression of fibronectin was upregulated in the wounds. Keratin-forming cells are the primary component of the epidermis and play a key role in epithelialization by proliferating, differentiating, and migrating to form the new epidermis. sheng et al [40] found that transplanted ADSC could promote the proliferation of epidermal cells, which in turn thickened the epidermis of skin wounds during the healing process.

5. Methods for The Extraction of Msc-Derived Exos

Methods for the extraction and identification of exosomes are the fundamental cornerstone in the study of exosomes and their practical applications. Firstly, they must be isolated and purified from biological fluids and in vitro cell cultures. The choice of exosome isolation technique significantly affects exosome yield. The selection and modification of isolation techniques should vary

according to the size, shape, density, and surface proteins of the exosomes isolated from biological fluids or cell cultures. In this section, we briefly discuss several common principles and methods of exosome isolation.[41-46] In addition, tissue sources of parental MSCs for exosome research have been isolated from various kinds of tissues, including adipose tissue, umbilical cords, bone marrow, embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs). To date, the most common strategies used to isolate pure exosomes from conditioned media are ultracentrifugation and sedimentation. [47] This is the standard method and is less costly, but is associated with potential damage to exosomes and co-precipitation of other non-exosomal contaminants (e.g. proteins)[44]. Additionally, there are membrane filtration, sucrose cushion centrifugation, HPLC gel permeation chromatography,size-exclusion chromatography, polymer precipitation, immunoaffinity capture, and microfluidics isolation. On account of the limitations of the above methods, the number of exosomes produced by MSCs is relatively small compared with other types of cells or body fluids, making it challenging to extract MSC-exos with high purity, high yields, and more functionality. Furthermore, there are problems such as homogeneity, i.e. whether exosomes from different tissue sources have the same biological efficacy has not been proven yet and there is no or comparable evidence of the effects of stem cells from different tissue sources. Therefore, further research and development of the biological properties and extraction techniques of exosomes are necessary to improve and optimize the mass production of exosome formulations for their clinical requirements. Next, the biomedical application identification for extracted exosomes is also crucial. Current methods for exosome identification include exosome size identification e.g. nanoparticle tracking analysis, exosome morphology identification e.g. scanning or transmission electron microscopy observation, exosome surface marker identification e.g. western blot analysis and flow cytometry, etc. [48,49] The content and composition of exosomes have a significant impact on disease progression, [50,51] so modification is required. i) Exogenous modification: incubation of naïve exosomes with lipophilic small molecules ii) Endogenous modification: manipulation of parental cells, either by direct loading of the therapeutic drug or transfection of parental cells with the DNA code for the therapeutic drug, with subsequent exosomes becoming carriers iii) electroporation or electropermeabilization. [52]

6. Clinical Applications of Mesenchymal Stem Cell-Derived Exosomes

MSCs-Exos are gradually drawing the interest of researchers due to their extensive availability and accessibility, showing a very promising application. Several studies have demonstrated that exosomes can be used in combination with other drugs, biomaterials, nanotechnology, etc, and improve the therapeutic effect of drugs by transporting, modulating, or slow-releasing them and so on. Exosomes are usually applied by injecting an aqueous solution of exosomes directly into the circulatory system or body cavity to enhance tissue repair. Nevertheless, direct injection of exosomes into the area of injury may not be feasible, as free exosomes in an aqueous solution are difficult to retain in the target area, leading to efflux or rapid clearance, resulting in exosomes not fully utilizing their biological functions. [53] Therefore, repair of damage in the diabetic foot requires an eligible exosome carrier that has controlled release properties of exosomes in a dose- and time-dependent manner and thus has no adverse effects on exosome internalization, and has a suitable degradation rate.

6.1 Exosomes Combined with Biomaterials

Research has demonstrated that exosomes and biomaterials can be combined to promote efficacy. Currently, the most commonly used biomaterials are biodegradable implants, tissue-derived materials, and growth factors. Moreover, various biomaterial scaffolds have been explored to deliver bioactive factors and cellular products in a controlled manner. The ideal biomaterial scaffold for interfacial tissue engineering should contain the following characteristics, (1) scaffolds capable of supporting the adhesion, proliferation, and differentiation of different types of stem cells or progenitor cell-specific phenotypes; (2) scaffolds should be degradable, with a rate of degradation compared to the rate of tissue regeneration and capable of sustaining a physiological load. [54] SUN et al. proved that exosomes can combine with multifunctional integrated nano-agents to prompt the healing of diabetic infected wounds by reducing oxidative damage and inflammatory responses in tissue wounds, facilitating angiogenesis, and inhibiting bacterial infection. [55]

6.2 Exosome Combined with Hydrogel Grafting Technique

The combination of exosomes and hydrogels is expected to be a new strategy for the alternative treatment of diabetic foot ulcer wounds. It has been exhibited that the bioactive molecules bound to the hydrogel retain their structure and function for an extended period compared to hydrogel-free administration[56]. Hydrogels are networks of polymers with high water content and are bio-derived and FDA-approved products with similar properties to the extracellular matrix, including strong biocompatibility, biodegradability, and properties that aid cell infiltration and adhesion[57]. Adipose MSC-derived exosomes incorporated into freeze-thaw-based peptide-based hydrogels were found to have healing, antibacterial, and sustained release properties of exosomes [58]. Recently, YANG et al [59] applied a combined complex of PF-127 hydrogel and human umbilical cord MSC-derived exosomes in a diabetic rat skin injury model and indicated that compared with the human umbilical cord MSC-derived exosome group alone and the PF-127 hydrogel-only control group, the combined complex group markedly shortened the wound healing time in the diabetic rat model, accelerated granulation regeneration tissue and upregulated expression of vascular endothelial growth factor, transforming growth factorβ1, CD31, and

Ki67. Chenggui Wang et al [60] developed an injectable, self-healing antibacterial peptide-based FHE hydrogel (F127/OHA-EPL) to control the discharge of AMSCs-exo and promote chronic wound healing and complete skin regeneration. A brand new method for the complete repair of chronic wounds by a multifunctional hydrogel with controlled exosome release is provided.

6.3 Exosomes Loaded with Rv (chemotactic protein), Cytokines, or Anti-inflammatory Drugs for the Treatment of Diabetic Foot

Prolonged immune cell infiltration is an established factor in the process of non-healing diabetic wounds. Pro-inflammatory cytokines including IL-1, IL-6, IL-8, and TNF-α accelerate the infiltration of inflammatory cells such as CD8 T cells, neutrophils, and macrophages into the ulcer. Continuous increasing inflammatory cell infiltration and secretion of pro-inflammatory cytokines manifest in diabetic foot ulcers. The above mechanisms suggest that targeting inflammation may offer an effective way to promote the healing of DFUs. [61] Exosomes are an underlying drug for the treatment of various inflammatory conditions and diseases. Exosomes loaded with anti-inflammatory miRNAs and cytokines can reap beneficial results and the increased stability of exosome-based anti-inflammatory mediators susceptible to endogenous degradation, [62,63] supporting the theory of using exosomes loaded with anti-inflammatory mediators for the treatment of the diabetic foot. Mechanisms applied by Rv include counteracting the inflammatory phase and initiating the regenerative phase to promote tissue healing. However, Rv is prone to oxidative instability due to its 1,4-diene structure and therefore clinical applications require the search for mediators to stabilize Rv[64]. Ways to improve Rv stability may exist in better transport systems and exosomes be stable and effective in targeting specific tissues while protecting their carriers from degradation. Thus, exosomes may have great potential in the transport of Rv[84]. Furthermore, pre-treatment or loading of exosomes with desired drugs such as pioglitazone may likewise enhance the efficacy of exosomes. [51, 65] Pioglitazone pretreated MSCs-Exos were extracted and found to promote the angiogenic capacity of HUVECs in a high glucose injury environment by activating the PI3K/AKT/e NOS pathway, thereby accelerating DFU wound healing[8, 66]. As therapeutic carriers, exosomes strengthen the therapeutic efficacy of many drugs and anti-inflammatory factors. To this end, the usage of exosomes to deliver inflammatory chemotherapeutic mediators and drugs including Rv, pro-inflammatory cytokine targeted therapy, and others, which offers the promise of promoting DFU wound healing and ending chronic inflammation with its advantages of target specificity, immune privilege, and easier handling compared to other delivery methods.

6.4 Potential of Exosomal ncRNAs (non-coding RNAs) as Novel Biomarkers and New Cell-Free Therapies in the Diagnosis, Monitoring, and Treatment of Diabetic Complications

Exosomal ncRNAs are intimately involved in the development and progression of the diabetic foot through multiple molecular mechanisms. On the one hand, exosomal ncRNAs may serve as novel biomarkers in the early prevention, diagnosis, disease monitoring, and assessment of treatment efficacy of the diabetic foot. In future studies for clinical applications, more exosomal ncRNAs are sought as novel biomarkers in human body fluids. On the other hand, loading targeted drugs such as agonists or inhibitors of ncRNAs into specific exosomes or screening exosomes naturally rich in certain ncRNAs and then introducing the exosomes into the body and transporting their molecular carriers to specific cells (e.g. human retinal endothelial cells, podocytes, fibroblasts, etc.) are the treatments of diabetes complications[12]. Stem cell-derived exosomal ncRNAs are a potential novel therapeutic agent in cell-free therapy due to their unique biological properties.

Therefore, the possibilities of secretion in the clinical use of diabetic foot need to be further exploited for the sake of better comprehension of the possible impact of exosomes on future treatments.

7. Advantages and Limitations of Msc-Derived Exosomes as Carriers

7.1 Advantages

Exosomes have been developed as drug delivery carriers for the treatment of various diseases and have the advantages of non-cytotoxicity, low immunogenicity, high circulating stability, and biocompatibility compared to cellular therapies. [67] Exosomes are a potential drug for the treatment of various inflammatory conditions and diseases, and as a therapeutic vehicle, exosomes enhance the therapeutic effects of many drugs and anti-inflammatory factors. Exosomes have the advantage of the site- or organ-specific delivery and small size and low molecular weight. [68] Exosomes have been demonstrated to be stable and effective in targeting specific tissues, and the cellular targeting function of exosomes strengthens their role as drug delivery nanoplatforms while protecting their carriers from degradation. Exosomes are equipped with therapeutically specific and sensitive and can be enhanced by fine-tuning the method of isolation of nanoparticles, as NanoPoms discussed[69]. Other types of ev and nanoparticles have limitations in clinical applications. For example, synthetic nanoparticles have been developed as a means of drug delivery, but injecting synthetic drug-laden nanoparticles into the bloodstream has two thorny problems, toxicity, and rapid phagocytic clearance. In contrast, exosomes solve the problems faced by other nanoparticle alternatives by allowing passage through the blood-brain and mucosal barriers and decreasing the incidence of drug resistance development[50,52,62,]. Compared to administration via polyethylene glycol-modified

nanoparticles, allogeneic exosomes collected from patient tissues and blood appear to have low immunogenicity, reducing the chance of toxicity and immune response while decreasing the rate of clearance of exosomes and their cargo by the mononuclear phagocyte system[52].

Moreover, exosomes are highly stable on account of their hard lipid membranes which resist rupture during freeze-thaw cycles in a hypotonic environment. [70]Table 1 summarises the mechanisms and range of applications of the different drug-loading[87]

Table 1. The mechanisms and range of applications of the different drug-loading

Drug-loading	Strategy	Structure diagram	Main drug-loading mechanism	Application range	Ref
Fabricating high drug-loading nanomedicines with porous materials as carriers	Inorganic porous materials as carriers	MSNPs/MCSNPs/MMSNPs MCNPs MMCNCs	Noncovalent electrostatic/π–π stacking/hydrogen bond	Hydrophobic drugs mostly; peptides, proteins, and gene drugs sometimes	[88-89] [90] [91]
	MOFs as carriers	MOFs	Coordinate bond/π–π stacking/hydrophobic interaction	Hydrophobic drugs mostly; gas drugs sometimes	[92]
	Protein NPs as carriers	PNPs	Covalent bond with protein scaffold/π–π stacking,hydrophobic interaction with surface of PNPs	Hydrophobic drugs mostly; peptides and gene drugs sometimes	[93]
Fabricating high drug-loading nanomedicines with drug as part of carrier	Polymer-drug conjugates	Linear polymer–drug conjugate Branched polymer–drug conjugate	Covalent bond with polymer	Hydrophobic drugs mostly; codelivery with hydrophilic drug/gene drugs sometimes	[94] [95]
	ICP I-type nanomedicines	ICP I-type	Coordinate bond	Drug with complexing ability (hydrophobic drugs and hydrophilic drugs)	[96]
Carrier-free nanomedicines	Drug nanocrystals		Crystallization	Hydrophobic drugs mostly	[97]
	Amphiphilic drug–drug conjugate		Covalent bond	Hydrophobic–hydrophilic drug pairs	[98]
	CP nanoparticles	MBioFs ICPII-type	Coordinate bond	hydrophobic drugs and hydrophilic drugs	[99] [100]

Abbreviations: MCNPs, mesoporous carbon nanoparticles; MCSNPs, mesoporous calcium silicate nanoparticles; MMCNCs, mesoporous magnetic colloidal nanocrystal clusters; MMSNPs, mesoporous magnesium silicate nanoparticles; MOFs, metal-organic frameworks; MSNPs, mesoporous silica nanoparticles; PNPs, protein nanoparticles; ICP, infinite coordination polymer; CP, coordination polymer; MBioFs, metal–biomolecule frameworks.

7.2 Limitations

Although there are many advantages to employing exosomes as drug carriers for the delivery of various mediators, some limitations remain to be considered. The loading capacity of exosomes has been identified as a potential problem for exosomal drug delivery. As exosomes naturally have proteins and nucleic acids, they may have a lower capacity compared to other nanoparticle delivery methods. Taking into account the associated drug, the method of loading the drug into the exosome, and the type of tissue from which the exosome originates, studies have observed a low range of approximately 3% to a high range of 26% for exosome volume. [52] Ev has been shown to have a limited ability to carry larger nucleic acid cargoes than siRNAs or miRNAs. [71] This limitation may also apply to exosomes. Additionally, bone marrow MSC transplantation carries the risks of possible immune rejection, poor tissue implantation, and survival rates after transplantation and prospective tumorigenicity. [72] Therefore, further development of stem cell therapy has encountered significant difficulties. Despite this, the potential of exosomes as a drug delivery method is still promising.

8. Comparison of Different Sources Mscs-Derived Exosomes for The Treatment of Diabetic Foot

Multiple types of stem cell-derived exosomes have been shown to transport various proteins and RNAs and mediate multiple cellular pathways to promote granulation tissue formation, angiogenesis, the release of various growth factors/vascular factors, modulate inflammatory and immune responses, improving the healing process of diabetic wounds in plenty of dimensions and imposing a positive impact on tissue repair. MSCs-derived exosomes of various origins as cell therapy for the treatment of DFU have attracted a lot of attention in the clinical setting. Studies have indicated that not all MSCs-derived exosomes act in the same way. Investigators performing proteomic analysis of differences in neurotrophy, axon guidance, axon growth, and neural differentiation protein secretion among MSCs of different tissue origins found that BM-SCs may be the most favorable therapeutic option for reducing oxidative stress, whereas human umbilical cord pericytes (HUCPVCs) and ADSCs are free from excitability and toxicity. On the other hand, HUCPVCs distinguished themselves in degrading abnormal targeted proteasomes [73]. When exosomes from ADSCs overexpressing Nrf2 were used to treat foot wounds in diabetic rats, a significant reduction in ulcer size was observed. Increased levels of granulation tissue formation, angiogenesis, and growth factor expression, as well as reduced levels of inflammation and oxidative stress-related proteins, were detected in the wounds. [74] Exosomes of bone marrow and umbilical cord origin have been reported to inhibit the growth and induce apoptosis of U87MG glioblastoma cells in vitro, whereas adipose-derived ones facilitate cell growth. Moreover, the placenta has drawn wide attention as an alternative source of MSCs due to its extensive availability and accessibility. The placenta is a transient fetal-maternal organ that is disposed of after delivery and does not involve invasive surgery, making it a more ethically favorable source. Recovery of large numbers of PMSCs from small pieces of tissue has also been reported. [75,76] Unlike most human tissues, placentas have lower levels of DNA methylation and they are also more compatible with the human genome. [77] As the placenta is a highly vascularised organ, it is logical that MSCs isolated from the placenta should have stronger angiogenic properties. [78,79] This hypothesis is further supported by research. In conclusion, PMSCs are readily available, and ethically popular, which can be easily isolated and have high cell yields. Their characteristics are similar to those of bone marrow MSCs, which serve as the gold standard, and are highly immunomodulatory. The mechanism by which these cells and their secretome promote angiogenesis is through the regulation of small sites of cells [80]. Table2 concludes the mechanisms of MSCs-derived exosomes from different origins.

Table 2. The mechanisms and research approachof MSCs-derived exosomes from different origins

MSCs-derived exosomes from different origins	Mechanisms	Research approach	Ref
BM-SCs	Activation of PI3K/AKT signaling pathway through miRNA-126-mediated PTEN downregulation	Subcutaneous injection	[101]
UC-MSCs	Promotes migration of human microvascular endothelial cells and activates the angiogenic response of receptor endothelial cells	By promoting streptozotocin induction and serum-free medium (MCDB131)	[102]
ADSCs	Inhibits reactive oxygen species (ROS) and inflammatory factors to enhance cellular activity, proliferation and angiogenesis of EPC	Transplantation	[103]
PMSCs	Highly vascularized, hypermethylated and more compatible with the human genome immunomodulatory	Intramuscular injection	[80]

9. Conclusion and Future Perspectives

The prevalence of diabetes mellitus is rising rapidly worldwide and has not been effectively controlled yet[81]. Treatment of diabetic foot is costly and patients have a high recurrence rate[82] and there is a lack of effective treatments. In regenerative medicine, stem cell therapy is broadly used and is an effective strategy for tissue repair and regeneration that has shown remarkable results in promoting wound healing. Stem cells from a variety of tissue sources such as bone marrow, adipose, and umbilical cord are readily available and are favored by the research community. With further studies, we have found that MSC-exos may treat DFU by promoting coagulation, modulating inflammation, and vascularisation, increasing collagen deposition, reducing scar formation, and stimulating wound re-epithelialization. Additionally, MSC-exos are enriched with a variety of nucleic acids, multiple proteins, and bioactive lipids that act primarily as intercellular communication carriers, delivering these substances between cells and thus triggering biological responses in the receptor cells. And the signal can be

transmitted across species.[85] As stem cells and exosomes continue to be intensively studied in DFU fields and bioengineering techniques continue to evolve, there are still some issues in the clinical translation of exosomes that need to be addressed. Firstly, the lack of uniform standardization of exosomes is currently a major challenge, including extraction methods of simplification, purity, variability, characterization, optimal sourcing, and immortalization, lacking uniformity and standardization of production and isolation procedures as well as optimal dosage and route of administration. Secondly, the complex composition of exosomes, which are rich in proteins, RNA, lipids, specific non-coding and miRNA-modified exosomes of epitopes and metabolic enzymes, is still unclear in terms of their function and mechanism of action, requiring longer experimental cycles to validate their safety. Thirdly, although MSCs-Exos has exhibited potential therapeutic value in DFU models, most of the relevant studies are fundamental experiments, and only a few clinical trials have reported the therapeutic effects of MSCs-Exos[86]. Therefore, it needs to overcome the obstacles between fundamental and clinical experiments. The animal model cannot fully replace the simulated human environment and thus cannot be considered clinically effective. Fourth, according to the GMP/GCP guidelines, the commercial production of exosomes on a large scale is inefficient and cannot meet the needs of clinical treatment[83]. Fifth, it needs to urgently address the issues of cell sources and types, isolation methods, doses used, transplantation methods, and amplification methods faced in exosome therapy. Therefore, larger-scale preclinical and clinical studies are needed to design and optimize the steps and methods for the clinical application of MSC-derived stem cell exosomes in the treatment of diabetic foot. Recent studies have demonstrated the potential of exosomes for the treatment of diabetic foot by combining biomaterials, combined with hydrogels and as a carrier. The potential of ncRNA (non-coding RNA) from exosomes as new biomarkers and new cell-free therapies in the diagnosis, monitoring, and treatment of diabetic complications is non-negligible. In the future, a uniform and standardized guideline specification for purification and isolation techniques should be established, with permission for clinical application obtained, designing large sample, multi-center clinical studies to validate the safety and efficacy of exosomes in DFU therapy.

References

1. Fridoni M, Kouhkheil R, Abdollhifar MA, Amini A, Ghatrehsamani M, Ghoreishi SK, Chien S, Bayat S, Bayat M. Improvement in infected wound healing in type 1 diabetic rat by the synergistic effect of photobiomodulation therapy and conditioned medium. J Cell Biochem. 2019 Jun;120(6):9906-9916. doi: 10.1002/jcb.28273. Epub 2018 Dec 16. PMID: 30556154.

2. Littig JPB, Moellmer R, Agrawal DK, Rai V. Future applications of exosomes delivering resolvins and cytokines in facilitating diabetic foot ulcer healing. World J Diabetes. 2023 Jan 15;14(1):35-47. doi: 10.4239/wjd.v14.i1.35. PMID: 36684384; PMCID: PMC9850797.

3. Prattichizzo F, De Nigris V, Spiga R, Mancuso E, La Sala L, Antonicelli R, Testa R, Procopio AD, Olivieri F, Ceriello A. Inflammageing and metaflammation: The yin and yang of type 2 diabetes. Ageing Res Rev. 2018 Jan;41:1-17. doi: 10.1016/j.arr.2017.10.003. Epub 2017 Oct 31. PMID: 29081381.

4. Everett E, Mathioudakis N. Update on management of diabetic foot ulcers. Ann N Y Acad Sci. 2018 Jan;1411(1):153-165. doi: 10.1111/nyas.13569. PMID: 29377202; PMCID: PMC5793889.

5. Vuorlaakso M, Kiiski J, Salonen T, Karppelin M, Helminen M, Kaartinen I. Major Amputation Profoundly Increases Mortality in Patients With Diabetic Foot Infection. Front Surg. 2021 Apr 30;8:655902. doi: 10.3389/fsurg.2021.655902. PMID: 33996886; PMCID: PMC8120024.

6. Huang C, Luo W, Wang Q, Ye Y, Fan J, Lin L, Shi C, Wei W, Chen H, Wu Y, Tang Y. Human mesenchymal stem cells promote ischemic repairment and angiogenesis of diabetic foot through exosome miRNA-21-5p. Stem Cell Res. 2021 Apr;52:102235. doi: 10.1016/j.scr.2021.102235. Epub 2021 Feb 11. PMID: 33601096.

7. Li C, An Y, Sun Y, Yang F, Xu Q, Wang Z. Adipose Mesenchymal Stem Cell-Derived Exosomes Promote Wound Healing Through the WNT/β-catenin Signaling Pathway in Dermal Fibroblasts. Stem Cell Rev Rep. 2022 Aug;18(6):2059-2073. doi: 10.1007/s12015-022-10378-0. Epub 2022 Apr 26. PMID: 35471485; PMCID: PMC9391246.

8. Hu Y, Tao R, Chen L, Xiong Y, Xue H, Hu L, Yan C, Xie X, Lin Z, Panayi AC, Mi B, Liu G. Exosomes derived from pioglitazone-pretreated MSCs accelerate diabetic wound healing through enhancing angiogenesis. J Nanobiotechnology. 2021 May 21;19(1):150. doi: 10.1186/s12951-021-00894-5. PMID: 34020670; PMCID: PMC8139165.

9. Lim WL, Liau LL, Ng MH, Chowdhury SR, Law JX. Current Progress in Tendon and Ligament Tissue Engineering. Tissue Eng Regen Med. 2019 Jun 26;16(6):549-571. doi: 10.1007/s13770-019-00196-w. PMID: 31824819; PMCID: PMC6879704.

10. Sun H, Pratt RE, Hodgkinson CP, Dzau VJ. Sequential paracrine mechanisms are necessary for the therapeutic benefits of stem cell therapy. Am J Physiol Cell Physiol. 2020 Dec 1;319(6):C1141-C1150. doi: 10.1152/ajpcell.00516.2019. Epub 2020 Oct 7. PMID: 33026832.

11. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science. 2020 Feb 7;367(6478):eaau6977. doi: 10.1126/science.aau6977. PMID: 32029601; PMCID: PMC7717626.

12. Xu YX, Pu SD, Li X, Yu ZW, Zhang YT, Tong XW, Shan YY, Gao XY. Exosomal ncRNAs: Novel therapeutic target and biomarker for diabetic

complications. Pharmacol Res. 2022 Apr;178:106135. doi: 10.1016/j.phrs.2022.106135. Epub 2022 Feb 19. PMID: 35192956.

13. Harding CV, Heuser JE, Stahl PD. Exosomes: looking back three decades and into the future. J Cell Biol. 2013 Feb 18;200(4):367-71. doi: 10.1083/jcb.201212113. Erratum in: J Cell Biol. 2013 Apr 29;201(3):485. PMID: 23420870; PMCID: PMC3575527.

14. Bobrie A, Colombo M, Raposo G, Théry C. Exosome secretion: molecular mechanisms and roles in immune responses. Traffic. 2011 Dec;12(12):1659-68. doi: 10.1111/j.1600-0854.2011.01225.x. Epub 2011 Jun 30. PMID: 21645191.

15. Rak J. Microparticles in cancer. Semin Thromb Hemost. 2010 Nov;36(8):888-906. doi: 10.1055/s-0030-1267043. Epub 2010 Nov 3. PMID: 21049390.

16. Hood JL, San RS, Wickline SA. Exosomes released by melanoma cells prepare sentinel lymph nodes for tumor metastasis. Cancer Res. 2011 Jun 1;71(11):3792-801. doi: 10.1158/0008-5472.CAN-10-4455. Epub 2011 Apr 8. PMID: 21478294.

17. Zhang HG, Grizzle WE. Exosomes and cancer: a newly described pathway of immune suppression. Clin Cancer Res. 2011 Mar 1;17(5):959-64. doi: 10.1158/1078-0432.CCR-10-1489. Epub 2011 Jan 11. PMID: 21224375; PMCID: PMC3155407.

18. Kujala P, Raymond CR, Romeijn M, Godsave SF, van Kasteren SI, Wille H, Prusiner SB, Mabbott NA, Peters PJ. Prion uptake in the gut: identification of the first uptake and replication sites. PLoS Pathog. 2011 Dec;7(12):e1002449. doi: 10.1371/journal.ppat.1002449. Epub 2011 Dec 22. PMID: 22216002; PMCID: PMC3245311.

19. Hyldig K, Riis S, Pennisi CP, Zachar V, Fink T. Implications of Extracellular Matrix Production by Adipose Tissue-Derived Stem Cells for Development of Wound Healing Therapies. Int J Mol Sci. 2017 May 31;18(6):1167. doi: 10.3390/ijms18061167. PMID: 28561757; PMCID: PMC5485991.

20. Zifkos K, Dubois C, Schäfer K. Extracellular Vesicles and Thrombosis: Update on the Clinical and Experimental Evidence. Int J Mol Sci. 2021 Aug 27;22(17):9317. doi: 10.3390/ijms22179317. PMID: 34502228; PMCID: PMC8431093.

21. Narauskaitė D, Vydmantaitė G, Rusteikaitė J, Sampath R, Rudaitytė A, Stašytė G, Aparicio Calvente MI, Jekabsone A. Extracellular Vesicles in Skin Wound Healing. Pharmaceuticals (Basel). 2021 Aug 18;14(8):811. doi: 10.3390/ph14080811. PMID: 34451909; PMCID: PMC8400229.

22. Whelan C, Burnley-Hall N, Morris K, Rees DA, James PE. The procoagulant effects of extracellular vesicles derived from hypoxic endothelial cells can be selectively inhibited by inorganic nitrite. Nitric Oxide. 2022 May 1;122-123:6-18. doi: 10.1016/j.niox.2022.02.002. Epub 2022 Feb 21. PMID: 35202833.

23. Hu H, Wang B, Jiang C, Li R, Zhao J. Endothelial progenitor cell-derived exosomes facilitate vascular endothelial cell repair through shuttling miR-21-5p to modulate Thrombospondin-1 expression. Clin Sci (Lond). 2019 Jul 25;133(14):1629-1644. doi: 10.1042/CS20190188. PMID: 31315970.

24. Gondaliya P, Sayyed AA, Bhat P, Mali M, Arya N, Khairnar A, Kalia K. Mesenchymal Stem Cell-Derived Exosomes Loaded with miR-155 Inhibitor Ameliorate Diabetic Wound Healing. Mol Pharm. 2022 May 2;19(5):1294-1308. doi: 10.1021/acs.molpharmaceut.1c00669. Epub 2022 Mar 16. PMID: 35294195.

25. Cooper DR, Wang C, Patel R, Trujillo A, Patel NA, Prather J, Gould LJ, Wu MH. Human Adipose-Derived Stem Cell Conditioned Media and Exosomes Containing MALAT1 Promote Human Dermal Fibroblast Migration and Ischemic Wound Healing. Adv Wound Care (New Rochelle). 2018 Sep 1;7(9):299-308. doi: 10.1089/wound.2017.0775. Epub 2018 Sep 4. PMID: 30263873; PMCID: PMC6158770.

26. Zhao H, Shang Q, Pan Z, Bai Y, Li Z, Zhang H, Zhang Q, Guo C, Zhang L, Wang Q. Exosomes From Adipose-Derived Stem Cells Attenuate Adipose Inflammation and Obesity Through Polarizing M2 Macrophages and Beiging in White Adipose Tissue. Diabetes. 2018 Feb;67(2):235-247. doi: 10.2337/db17-0356. Epub 2017 Nov 13. PMID: 29133512.

27. Vu NB, Nguyen HT, Palumbo R, Pellicano R, Fagoonee S, Pham PV. Stem cell-derived exosomes for wound healing: current status and promising directions. Minerva Med. 2021 Jun;112(3):384-400. doi: 10.23736/S0026-4806.20.07205-5. Epub 2020 Dec 2. PMID: 33263376.

28. Fu W, Liang D, Wu X, Chen H, Hong X, Wang J, Zhu T, Zeng T, Lin W, Chen S, Yan L, Ren M. Long noncoding RNA LINC01435 impedes diabetic wound healing by facilitating YY1-mediated HDAC8 expression. iScience. 2022 Mar 1;25(4):104006. doi: 10.1016/j.isci.2022.104006. Erratum in: iScience. 2022 Jun 15;25(7):104540. PMID: 35330681; PMCID: PMC8938286.

29. Qian L, Pi L, Fang BR, Meng XX. Adipose mesenchymal stem cell-derived exosomes accelerate skin wound healing via the lncRNA H19/miR-19b/SOX9 axis. Lab Invest. 2021 Sep;101(9):1254-1266. doi: 10.1038/s41374-021-00611-8. Epub 2021 May 27. PMID: 34045678.

30. Li B, Luan S, Chen J, Zhou Y, Wang T, Li Z, Fu Y, Zhai A, Bi C. The MSC-Derived Exosomal lncRNA H19 Promotes Wound Healing in Diabetic Foot Ulcers by Upregulating PTEN via MicroRNA-152-3p. Mol Ther Nucleic Acids. 2020 Mar 6;19:814-826. doi: 10.1016/j.omtn.2019.11.034. Epub 2019 Dec 14. PMID: 31958697; PMCID: PMC70054

31. Heo JS, Kim S. Human adipose mesenchymal stem cells modulate inflammation and angiogenesis through exosomes. Sci Rep. 2022 Feb 17;12(1):2776.

doi: 10.1038/s41598-022-06824-1. PMID: 35177768; PMCID: PMC8854709.

32. Han KY, Chang JH, Azar DT. MMP14-Containing Exosomes Cleave VEGFR1 and Promote VEGFA-Induced Migration and Proliferation of Vascular Endothelial Cells. Invest Ophthalmol Vis Sci. 2019 May 1;60(6):2321-2329. doi: 10.1167/iovs.18-26277. PMID: 31117124; PMCID: PMC6532701.

33. Li J, Li Z, Wang S, Bi J, Huo R. Exosomes from human adipose-derived mesenchymal stem cells inhibit production of extracellular matrix in keloid fibroblasts via downregulating transforming growth factor-β2 and Notch-1 expression. Bioengineered. 2022 Apr;13(4):8515-8525. doi: 10.1080/21655979.2022.2051838. PMID: 35333672; PMCID: PMC9161879.

34. Planat-Benard V, Silvestre JS, Cousin B, André M, Nibbelink M, Tamarat R, Clergue M, Manneville C, Saillan-Barreau C, Duriez M, Tedgui A, Levy B, Pénicaud L, Casteilla L. Plasticity of human adipose lineage cells toward endothelial cells: physiological and therapeutic perspectives. Circulation. 2004 Feb 10;109(5):656-63. doi: 10.1161/01.CIR.0000114522.38265.61. Epub 2004 Jan 20. PMID: 14734516.

35. Hutchings G, Janowicz K, Moncrieff L, Dompe C, Strauss E, Kocherova I, Nawrocki MJ, Kruszyna Ł, Wąsiatycz G, Antosik P, Shibli JA, Mozdziak P, Perek B, Krasiński Z, Kempisty B, Nowicki M. The Proliferation and Differentiation of Adipose-Derived Stem Cells in Neovascularization and Angiogenesis. Int J Mol Sci. 2020 May 27;21(11):3790. doi: 10.3390/ijms21113790. PMID: 32471255; PMCID: PMC7312564.

36. Ma T, Sun J, Zhao Z, Lei W, Chen Y, Wang X, Yang J, Shen Z. A brief review: adipose-derived stem cells and their therapeutic potential in cardiovascular diseases. Stem Cell Res Ther. 2017 Jun 5;8(1):124. doi: 10.1186/s13287-017-0585-3. PMID: 28583198; PMCID: PMC5460549.

37. Le Pillouer-Prost A. Fibroblasts: what's new in cellular biology? J Cosmet Laser Ther. 2003 Dec;5(3-4):232-8. doi: 10.1080/14764170310021869. PMID: 14741842.

38. Wang PH, Huang BS, Horng HC, Yeh CC, Chen YJ. Wound healing. J Chin Med Assoc. 2018 Feb;81(2):94-101. doi: 10.1016/j.jcma.2017.11.002. Epub 2017 Nov 21. PMID: 29169897.

39. Zhang Y, Pan Y, Liu Y, Li X, Tang L, Duan M, Li J, Zhang G. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulate regenerative wound healing via transforming growth factor-β receptor inhibition. Stem Cell Res Ther. 2021 Aug 3;12(1):434. doi: 10.1186/s13287-021-02517-0. PMID: 34344478; PMCID: PMC8336384.

40. Sheng L, Yang M, Liang Y, Li Q. Adipose tissue-derived stem cells (ADSCs) transplantation promotes regeneration of expanded skin using a tissue expansion model. Wound Repair Regen. 2013 Sep-Oct;21(5):746-54. doi: 10.1111/wrr.12080. Epub 2013 Aug 12. PMID: 23937682.

41. Alzhrani GN, Alanazi ST, Alsharif SY, Albalawi AM, Alsharif AA, Abdel-Maksoud MS, Elsherbiny N. Exosomes: Isolation, characterization, and biomedical applications. Cell Biol Int. 2021 Sep;45(9):1807-1831. doi: 10.1002/cbin.11620. Epub 2021 May 11. PMID: 33913604.

42. An Y, Zhao J, Nie F, Qin Z, Xue H, Wang G, Li D. Exosomes from Adipose-Derived Stem Cells (ADSCs) Overexpressing miR-21 Promote Vascularization of Endothelial Cells. Sci Rep. 2019 Sep 6;9(1):12861. doi: 10.1038/s41598-019-49339-y. PMID: 31492946; PMCID: PMC6731308.

43. Bu H, He D, He X, Wang K. Exosomes: Isolation, Analysis, and Applications in Cancer Detection and Therapy. Chembiochem. 2019 Feb 15;20(4):451-461. doi: 10.1002/cbic.201800470. Epub 2018 Dec 7. PMID: 30371016.

44. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in Exosome Isolation Techniques. Theranostics. 2017 Jan 26;7(3):789-804. doi: 10.7150/thno.18133. PMID: 28255367; PMCID: PMC5327650.

45. Ryu KJ, Lee JY, Park C, Cho D, Kim SJ. Isolation of Small Extracellular Vesicles From Human Serum Using a Combination of Ultracentrifugation With Polymer-Based Precipitation. Ann Lab Med. 2020 May;40(3):253-258. doi: 10.3343/alm.2020.40.3.253. PMID: 31858766; PMCID: PMC6933066.

46. Shao H, Im H, Castro CM, Breakefield X, Weissleder R, Lee H. New Technologies for Analysis of Extracellular Vesicles. Chem Rev. 2018 Feb 28;118(4):1917-1950. doi: 10.1021/acs.chemrev.7b00534. Epub 2018 Jan 31. PMID: 29384376; PMCID: PMC6029891.

47. Elahi FM, Farwell DG, Nolta JA, Anderson JD. Preclinical translation of exosomes derived from mesenchymal stem/stromal cells. Stem Cells. 2020 Jan;38(1):15-21. doi: 10.1002/stem.3061. Epub 2019 Oct 1. PMID: 31381842; PMCID: PMC7004029.

48. Lässer C, Eldh M, Lötvall J. Isolation and characterization of RNA-containing exosomes. J Vis Exp. 2012 Jan 9;(59):e3037. doi: 10.3791/3037. PMID: 22257828; PMCID: PMC3369768.

49. Zhao R, Zhao T, He Z, Cai R, Pang W. Composition, isolation, identification and function of adipose tissue-derived exosomes. Adipocyte. 2021 Dec;10(1):587-604. doi: 10.1080/21623945.2021.1983242. PMID: 34709975; PMCID: PMC8604391.

50. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011 Apr;29(4):341-5. doi: 10.1038/nbt.1807. Epub 2011 Mar 20. PMID: 21423189.

51. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, Wei J, Nie G. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for

51. targeted tumor therapy. Biomaterials. 2014 Feb;35(7):2383-90. doi: 10.1016/j.biomaterials.2013.11.083. Epub 2013 Dec 15. PMID: 24345736.

52. Batrakova EV, Kim MS. Using exosomes, naturally-equipped nanocarriers, for drug delivery. J Control Release. 2015 Dec 10;219:396-405. doi: 10.1016/j.jconrel.2015.07.030. Epub 2015 Aug 1. PMID: 26241750; PMCID: PMC4656109.

53. Imai T, Takahashi Y, Nishikawa M, Kato K, Morishita M, Yamashita T, Matsumoto A, Charoenviriyakul C, Takakura Y. Macrophage-dependent clearance of systemically administered B16BL6-derived exosomes from the blood circulation in mice. J Extracell Vesicles. 2015 Feb 9;4:26238. doi: 10.3402/jev.v4.26238. PMID: 25669322; PMCID: PMC4323410.

54. Patel S, Caldwell JM, Doty SB, Levine WN, Rodeo S, Soslowsky LJ, Thomopoulos S, Lu HH. Integrating soft and hard tissues via interface tissue engineering. J Orthop Res. 2018 Apr;36(4):1069-1077. doi: 10.1002/jor.23810. Epub 2018 Jan 5. PMID: 29149506; PMCID: PMC6467291.

55. Sun B, Wu F, Wang X, Song Q, Ye Z, Mohammadniaei M, Zhang M, Chu X, Xi S, Zhou N, Wang W, Yao C, Shen J. An Optimally Designed Engineering Exosome-Reductive COF Integrated Nanoagent for Synergistically Enhanced Diabetic Fester Wound Healing. Small. 2022 Jul;18(26):e2200895. doi: 10.1002/smll.202200895. Epub 2022 May 31. PMID: 35638464.

56. Akbari A, Jabbari N, Sharifi R, Ahmadi M, Vahhabi A, Seyedzadeh SJ, Nawaz M, Szafert S, Mahmoodi M, Jabbari E, Asghari R, Rezaie J. Free and hydrogel encapsulated exosome-based therapies in regenerative medicine. Life Sci. 2020 May 15;249:117447. doi: 10.1016/j.lfs.2020.117447. Epub 2020 Feb 19. PMID: 32087234.

57. Xiao S, Zhao T, Wang J, Wang C, Du J, Ying L, Lin J, Zhang C, Hu W, Wang L, Xu K. Gelatin Methacrylate (GelMA)-Based Hydrogels for Cell Transplantation: an Effective Strategy for Tissue Engineering. Stem Cell Rev Rep. 2019 Oct;15(5):664-679. doi: 10.1007/s12015-019-09893-4. PMID: 31154619.

58. Shen YI, Cho H, Papa AE, Burke JA, Chan XY, Duh EJ, Gerecht S. Engineered human vascularized constructs accelerate diabetic wound healing. Biomaterials. 2016 Sep;102:107-19. doi: 10.1016/j.biomaterials.2016.06.009. Epub 2016 Jun 4. PMID: 27328431.

59. Yang J, Chen Z, Pan D, Li H, Shen J. Umbilical Cord-Derived Mesenchymal Stem Cell-Derived Exosomes Combined Pluronic F127 Hydrogel Promote Chronic Diabetic Wound Healing and Complete Skin Regeneration. Int J Nanomedicine. 2020 Aug 11;15:5911-5926. doi: 10.2147/IJN.S249129. PMID: 32848396; PMCID: PMC7429232.

60. Wang C, Wang M, Xu T, Zhang X, Lin C, Gao W, Xu H, Lei B, Mao C. Engineering Bioactive Self-Healing Antibacterial Exosomes Hydrogel for Promoting Chronic Diabetic Wound Healing and Complete Skin Regeneration. Theranostics. 2019 Jan 1;9(1):65-76. doi: 10.7150/thno.29766. Erratum in: Theranostics. 2021 Nov 10;11(20):10174-10175. PMID: 30662554; PMCID: PMC6332800.

61. Rai V, Moellmer R, Agrawal DK. The role of CXCL8 in chronic nonhealing diabetic foot ulcers and phenotypic changes in fibroblasts: a molecular perspective. Mol Biol Rep. 2022 Feb;49(2):1565-1572. doi: 10.1007/s11033-022-07144-3. Epub 2022 Jan 19. PMID: 35044539.

62. Ocansey DKW, Zhang L, Wang Y, Yan Y, Qian H, Zhang X, Xu W, Mao F. Exosome-mediated effects and applications in inflammatory bowel disease. Biol Rev Camb Philos Soc. 2020 Oct;95(5):1287-1307. doi: 10.1111/brv.12608. Epub 2020 May 14. PMID: 32410383; PMCID: PMC7540363.

63. Gondaliya P, Sayyed AA, Bhat P, Mali M, Arya N, Khairnar A, Kalia K. Mesenchymal Stem Cell-Derived Exosomes Loaded with miR-155 Inhibitor Ameliorate Diabetic Wound Healing. Mol Pharm. 2022 May 2;19(5):1294-1308. doi: 10.1021/acs.molpharmaceut.1c00669. Epub 2022 Mar 16. PMID: 35294195.

64. Capó X, Martorell M, Busquets-Cortés C, Tejada S, Tur JA, Pons A, Sureda A. Resolvins as proresolving inflammatory mediators in cardiovascular disease. Eur J Med Chem. 2018 Jun 10;153:123-130. doi: 10.1016/j.ejmech.2017.07.018. Epub 2017 Jul 13. PMID: 28732558.

65. Patel N, Chin DD, Chung EJ. Exosomes in Atherosclerosis, a Double-Edged Sword: Their Role in Disease Pathogenesis and Their Potential as Novel Therapeutics. AAPS J. 2021 Jul 26;23(5):95. doi: 10.1208/s12248-021-00621-w. PMID: 34312734.

66. Prattichizzo F, De Nigris V, Spiga R, Mancuso E, La Sala L, Antonicelli R, Testa R, Procopio AD, Olivieri F, Ceriello A. Inflammageing and metaflammation: The yin and yang of type 2 diabetes. Ageing Res Rev. 2018 Jan;41:1-17. doi: 10.1016/j.arr.2017.10.003. Epub 2017 Oct 31. PMID: 29081381.

67. Xu M, Yang Q, Sun X, Wang Y. Recent Advancements in the Loading and Modification of Therapeutic Exosomes. Front Bioeng Biotechnol. 2020 Nov 11;8:586130. doi: 10.3389/fbioe.2020.586130. PMID: 33262977; PMCID: PMC7686035.

68. Zhao Z, Ukidve A, Kim J, Mitragotri S. Targeting Strategies for Tissue-Specific Drug Delivery. Cell. 2020 Apr 2;181(1):151-167. doi: 10.1016/j.cell.2020.02.001. PMID: 32243788.

69. He N, Thippabhotla S, Zhong C, Greenberg Z, Xu L, Pessetto Z, Godwin AK, Zeng Y, He M. Nano pom-poms prepared exosomes enable highly specific cancer biomarker detection. Commun Biol. 2022 Jul

4;5(1):660. doi: 10.1038/s42003-022-03598-0. PMID: 35787656; PMCID: PMC9253007.

70. Lai CP, Breakefield XO. Role of exosomes/microvesicles in the nervous system and use in emerging therapies. Front Physiol. 2012 Jun 27;3:228. doi: 10.3389/fphys.2012.00228. PMID: 22754538; PMCID: PMC3384085.

71. Familtseva A, Jeremic N, Tyagi SC. Exosomes: cell-created drug delivery systems. Mol Cell Biochem. 2019 Sep;459(1-2):1-6. doi: 10.1007/s11010-019-03545-4. Epub 2019 May 9. PMID: 31073888.

72. Lu V, Tennyson M, Zhang J, Khan W. Mesenchymal Stem Cell-Derived Extracellular Vesicles in Tendon and Ligament Repair-A Systematic Review of In Vivo Studies. Cells. 2021 Sep 27;10(10):2553. doi: 10.3390/cells10102553. PMID: 34685532; PMCID: PMC8533909.

73. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. Int J Mol Sci. 2017 Aug 25;18(9):1852. doi: 10.3390/ijms18091852. PMID: 28841158; PMCID: PMC5618501.

74. Li X, Xie X, Lian W, Shi R, Han S, Zhang H, Lu L, Li M. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. Exp Mol Med. 2018 Apr 13;50(4):1-14. doi: 10.1038/s12276-018-0058-5. PMID: 29651102; PMCID: PMC5938041.

75. Parolini O, Alviano F, Bagnara GP, Bilic G, Bühring HJ, Evangelista M, Hennerbichler S, Liu B, Magatti M, Mao N, Miki T, Marongiu F, Nakajima H, Nikaido T, Portmann-Lanz CB, Sankar V, Soncini M, Stadler G, Surbek D, Takahashi TA, Redl H, Sakuragawa N, Wolbank S, Zeisberger S, Zisch A, Strom SC. Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. Stem Cells. 2008 Feb;26(2):300-11. doi: 10.1634/stemcells.2007-0594. Epub 2007 Nov 1. PMID: 17975221.

76. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, Kanhai HH. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. Stem Cells. 2004;22(7):1338-45. doi: 10.1634/stemcells.2004-0058. PMID: 15579651.

77. Schroeder DI, Blair JD, Lott P, Yu HO, Hong D, Crary F, Ashwood P, Walker C, Korf I, Robinson WP, LaSalle JM. The human placenta methylome. Proc Natl Acad Sci U S A. 2013 Apr 9;110(15):6037-42. doi: 10.1073/pnas.1215145110. Epub 2013 Mar 25. PMID: 23530188; PMCID: PMC3625261.

78. Castrechini NM, Murthi P, Gude NM, Erwich JJ, Gronthos S, Zannettino A, Brennecke SP, Kalionis B. Mesenchymal stem cells in human placental chorionic villi reside in a vascular Niche. Placenta. 2010 Mar;31(3):203-12. doi:

10.1016/j.placenta.2009.12.006. Epub 2010 Jan 13. PMID: 20060164.

79. Chen CY, Liu SH, Chen CY, Chen PC, Chen CP. Human placenta-derived multipotent mesenchymal stromal cells involved in placental angiogenesis via the PDGF-BB and STAT3 pathways. Biol Reprod. 2015 Oct;93(4):103. doi: 10.1095/biolreprod.115.131250. Epub 2015 Sep 9. PMID: 26353894.

80. Mathew SA, Naik C, Cahill PA, Bhonde RR. Placental mesenchymal stromal cells as an alternative tool for therapeutic angiogenesis. Cell Mol Life Sci. 2020 Jan;77(2):253-265. doi: 10.1007/s00018-019-03268-1. Epub 2019 Aug 29. PMID: 31468060.

81. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. Lancet. 2017 Jun 3;389(10085):2239-2251. doi: 10.1016/S0140-6736(17)30058-2. Epub 2017 Feb 10. Erratum in: Lancet. 2017 Jun 3;389(10085):2192. PMID: 28190580.

82. Zhang P, Lu J, Jing Y, Tang S, Zhu D, Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis †. Ann Med. 2017 Mar;49(2):106-116. doi: 10.1080/07853890.2016.1231932. Epub 2016 Nov 3. PMID: 27585063.

83. Raghav A, Tripathi P, Mishra BK, Jeong GB, Banday S, Gautam KA, Mateen QN, Singh P, Singh M, Singla A, Ahmad J. Mesenchymal Stromal Cell-Derived Tailored Exosomes Treat Bacteria-Associated Diabetes Foot Ulcers: A Customized Approach From Bench to Bed. Front Microbiol. 2021 Jul 27;12:712588. doi: 10.3389/fmicb.2021.712588. PMID: 34385994; PMCID: PMC8354005.

84. Rai V, Moellmer R, Agrawal DK. Stem Cells and Angiogenesis: Implications and Limitations in Enhancing Chronic Diabetic Foot Ulcer Healing. Cells. 2022 Jul 25;11(15):2287. doi: 10.3390/cells11152287. PMID: 35892584; PMCID: PMC9330772.

85. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. Nat Rev Immunol. 2014 Mar;14(3):195-208. doi: 10.1038/nri3622. PMID: 24566916; PMCID: PMC4350779.

86. An T, Chen Y, Tu Y, Lin P. Mesenchymal Stromal Cell-Derived Extracellular Vesicles in the Treatment of Diabetic Foot Ulcers: Application and Challenges. Stem Cell Rev Rep. 2021 Apr;17(2):369-378. doi: 10.1007/s12015-020-10014-9. PMID: 32772239.

87. Shen S, Wu Y, Liu Y, Wu D. High drug-loading nanomedicines: progress, current status, and prospects. Int J Nanomedicine. 2017 May 31;12:4085-4109. doi: 10.2147/IJN.S132780. PMID: 28615938; PMCID: PMC5459982.

88. Wang B, Meng W, Bi M, Ni Y, Cai Q, Wang J. Uniform magnesium silicate hollow spheres as high drug-loading nanocarriers for cancer therapy with low systemic toxicity. Dalton Trans. 2013 Jun 28;42(24):8918-25. doi: 10.1039/c3dt50659b. Epub 2013 May 9. PMID: 23660815.

89. Wang H, Li X, Ma Z, Wang D, Wang L, Zhan J, She L, Yang F. Hydrophilic mesoporous carbon nanospheres with high drug-loading efficiency for doxorubicin delivery and cancer therapy. Int J Nanomedicine. 2016 Apr 27;11:1793-806. doi: 10.2147/IJN.S103020. PMID: 27175077; PMCID: PMC4854254.

90. Shin J, Anisur RM, Ko MK, Im GH, Lee JH, Lee IS. Hollow manganese oxide nanoparticles as multifunctional agents for magnetic resonance imaging and drug delivery. Angew Chem Int Ed Engl. 2009;48(2):321-4. doi: 10.1002/anie.200802323. PMID: 19040234.

91. Cheng K, Peng S, Xu C, Sun S. Porous hollow Fe(3)O(4) nanoparticles for targeted delivery and controlled release of cisplatin. J Am Chem Soc. 2009 Aug 5;131(30):10637-44. doi: 10.1021/ja903300f. PMID: 19722635; PMCID: PMC2739121.

92. Horcajada P, Serre C, Vallet-Regí M, Sebban M, Taulelle F, Férey G. Metal-organic frameworks as efficient materials for drug delivery. Angew Chem Int Ed Engl. 2006 Sep 11;45(36):5974-8. doi: 10.1002/anie.200601878. PMID: 16897793.

93. Ren D, Kratz F, Wang SW. Engineered drug-protein nanoparticle complexes for folate receptor targeting. Biochem Eng J. 2014 Aug 15;89:33-41. doi: 10.1016/j.bej.2013.09.008. PMID: 25018664; PMCID: PMC4090709.

94. Shen Y, Jin E, Zhang B, Murphy CJ, Sui M, Zhao J, Wang J, Tang J, Fan M, Van Kirk E, Murdoch WJ. Prodrugs forming high drug loading multifunctional nanocapsules for intracellular cancer drug delivery. J Am Chem Soc. 2010 Mar 31;132(12):4259-65. doi: 10.1021/ja909475m. PMID: 20218672.

95. Kowalczuk A, Stoyanova E, Mitova V, Shestakova P, Momekov G, Momekova D, Koseva N. Star-shaped nano-conjugates of cisplatin with high drug payload. Int J Pharm. 2011 Feb 14;404(1-2):220-30. doi: 10.1016/j.ijpharm.2010.11.004. Epub 2010 Nov 13. PMID: 21078377.

96. Tachibana M, Iwaizumi M, Tero-Kubota S. EPR studies of copper(II) and cobalt(II) complexes of adriamycin. J Inorg Biochem. 1987 Jun;30 (2):133-40. doi: 10.1016/0162-0134(87)80049-1. PMID: 3037023.

97. Zhang J, Li S, An FF, Liu J, Jin S, Zhang JC, Wang PC, Zhang X, Lee CS, Liang XJ. Self-carried curcumin nanoparticles for in vitro and in vivo cancer therapy with real-time monitoring of drug release. Nanoscale. 2015 Aug 28;7(32):13503-10. doi: 10.1039/c5nr03259h. Epub 2015 Jul 22. PMID: 26199064; PMCID: PMC4636738.

98. Huang P, Wang D, Su Y, Huang W, Zhou Y, Cui D, Zhu X, Yan D. Combination of small molecule prodrug and nanodrug delivery: amphiphilic drug-drug conjugate for cancer therapy. J Am Chem Soc. 2014 Aug 20;136(33):11748-56. doi: 10.1021/ja505212y. Epub 2014 Aug 7. PMID: 25078892.

99. Miller SR, Heurtaux D, Baati T, Horcajada P, Grenèche JM, Serre C. Biodegradable therapeutic MOFs for the delivery of bioactive molecule s. Chem Commun (Camb). 2010 Jul 7;46(25):4526-8. doi: 10.1039/c001181 a. Epub 2010 May 13. PMID: 20467672.

100. Lin W, Rieter WJ, Taylor KM. Modular synthesis of functional nanoscale coordination polymers. Angew Chem Int Ed Engl. 2009;48(4):650-8. doi: 10.1002/anie.200803387. PMID: 19065692; PMCID: PMC4040293.

101. Ding J, Wang X, Chen B, Zhang J, Xu J. Exosomes Derived from Human Bone Marrow Mesenchymal Stem Cells Stimulated by Deferoxamine Accelerate Cutaneous Wound Healing by Promoting Angiogenesis. Biomed Res Int. 2019 May 5;2019:9742765. doi: 10.1155/2019/9742765. PMID: 31192260; PMCID: PMC6525840.

102. Zhang J, Chen C, Hu B, Niu X, Liu X, Zhang G, Zhang C, Li Q, Wang Y. Exosomes Derived from Human Endothelial Progenitor Cells Accelerate Cutaneous Wound Healing by Promoting Angiogenesis Through Erk1/2 Signaling. Int J Biol Sci. 2016 Nov 25;12(12):1472-1487. doi: 10.7150/ijbs.15514. PMID: 27994512; PMCID: PMC5166489.

103. Li X, Xie X, Lian W, Shi R, Han S, Zhang H, Lu L, Li M. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutane ous wound healing by promoting vascularization in a diabetic foot ulce r rat model. Exp Mol Med. 2018 Apr 13;50(4):1-14. doi: 10.1038/s12276- 018-0058-5. PMID: 29651102; PMCID: PMC5938041.