Journal of Stem Cells & Regenerative Medicine
ISSN 0973 - 7154

REVIEW ARTICLE

JSRM Code: 020010300003

The Therapeutic Potential of Human Umbilical Cord Mesenchymal Stromal Cells Derived Exosomes for Wound Healing: Harnessing Exosomes as a Cell-free Therapy

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Abstract

Wound healing is a complicated process that involves many different types of cells and signaling pathways. Mesenchymal stromal cells (MSCs) have shown great potential as a treatment to improve wound healing because they can modulate inflammation, promote the growth of new blood vessels, and stimulate the regeneration of tissue. Recent evidence indicates MSCs-derived extracellular vesicles known as exosomes may mediate many of the therapeutic effects of MSCs on wound healing. Exosomes contain bioactive molecules such as proteins, lipids, and RNAs that can be transferred to recipient cells to modulate cellular responses. This article reviews current evidence on the mechanisms and therapeutic effects of human umbilical cord MSCs (hUCMSCs)-derived exosomes on wound healing. In vitro and animal studies demonstrate that hUCMSC-derived exosomes promote fibroblast proliferation/migration, angiogenesis, and re-epithelialization while reducing inflammation and scar formation. These effects are mediated by exosomal transfer of cytokines, growth factors, and regulatory microRNAs that modulate signaling pathways involved in wound healing. Challenges remain in exosome isolation methods, optimizing targeting/retention, and translation to human studies. Nevertheless, hUCMSCs-derived exosomes show promise as a novel cell-free therapeutic approach to accelerate wound closure and improve healing outcomes. Further research is warranted to fully characterize hUCMSCs-exosomal mechanisms and explore their clinical potential for wound management.

Keywords: Wound healing; hUCMSCs; Exosomes; Tissue regeneration; Extracellular vesicles

1. Introduction

Wound healing is a complex process that restores damaged tissue structure and function. The healing process can be divided into four overlapping stages: hemostasis, inflammation, proliferation, and remodeling (Figure 1). These stages are regulated via several different growth factors, cytokines, enzymes, and structural matrix proteins produced by multiple cell types, such as dermal fibroblasts, epidermal keratinocytes, and immune cells[1]. Many factors have been reported to be secreted by mesenchymal stromal cells (MSCs), including interleukin-like growth factor (IGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF), stromal cell-derived factor-1 (SDF-1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) which have a strong potential for wound healing[2]. MSCs are considered candidate cells for promoting regenerative medicine due to their ease of isolation and low immunogenicity[3]. It has been reported that MSCs can be easily isolated from various tissues, including umbilical cord blood, bone marrow, dermis, brain, teeth, menstrual blood, muscles, and placenta[4]. Among the various sources from which stem cells are harvested, the umbilical cord is an interesting source that has several advantages, including high cell numbers (per unit volume) compared to cells in bone marrow, low evidence of graft-versus-host disease (GVHD), non-invasive, no ethical consideration, ease of collection, lower risk of infectious diseases such as Epstein Barr virus (EBV) and Cytomegalovirus (CMV), high immunomodulatory activity, and painlessness in both mother and child[5]. However, using stem cells directly has risks such as tumour induction, thrombosis, and poor graft survival[6]. The human umbilical cord MSCs (hUCMSCs) have relative limitations in quantifying bioactive substances, maintaining biological activity, and in logistics delivery in clinical therapies[7]. Accordingly, finding a cell-free method with the same output and efficacy is necessary. Exosomes are extracellular vesicles (EVs) proposed as a new approach for cell-free based therapies due to their multiple biological activities and cellular communication[8]. Exosomes were first described by Harding et al. in 1983, and their existence was confirmed by Johnstone et al. in 1987[9]. A lipid bilayer membrane surrounds this smallest group of extracellular vesicles (30 and 150 nm in diameter). They originate from multivesicular bodies secreted by various cell types[10]. Exosomes reflect the state of the cell from which they originated. For instance, exosomes derived from cancer cells haul pathogenic components such as mRNA, miRNA, and proteins[11]. Exosomes extracted from various cells such as urine-derived stem cells, human induced pluripotent stem cells, human endothelial progenitor cells, and hUCMSCs. The small extracellular vesicles (sEVs) are found in body fluids, including saliva, plasma, breast milk, amniotic fluids, urine, and cell culture medium.

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doi: 10.46582/jsrm.2003003

JSRM/Vol.20 No.1, 2024; P14

Received 21sep23; Accepted 15jan24; Published online [epub ahead of regular issue]: 31May2024

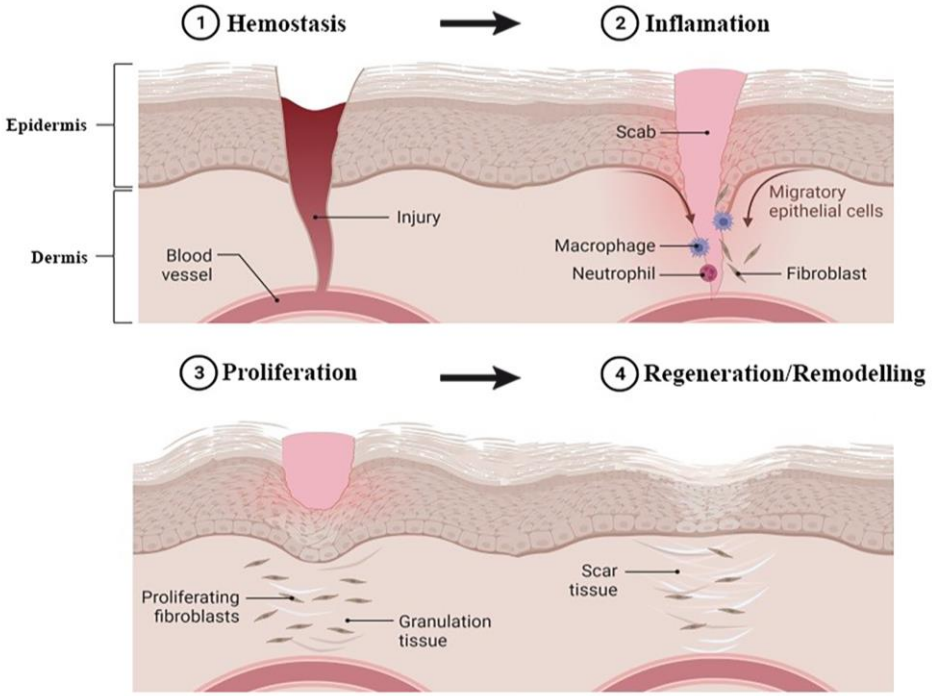

Figure 1. The image represents the successive stages of the wound healing process in the skin. The first stage is hemostasis, which is the formation of a clot to stop bleeding. It is followed by inflammation, where cells like macrophages and neutrophils clean the wound and cell migration for repair begins. The third stage is proliferation, characterized by the formation of granulation tissue and the proliferation of fibroblasts that build the extracellular matrix. The last stage is regeneration/remodeling, in which scar tissue forms and matures, and the epidermis restructures to regain its normal appearance and function as much as possible.

Table 1- Strategies to improve exosome retention time

Strategy	Examples	Description	Advantages	Challenges	Reference
Physical Protection and Sustained Release	Hydrogels	Embedding exosomes within hydrogel matrices to provide localized sustained release and protection from clearance.	Controlled release, enhanced retention, minimizes degradation.	Limited diffusion rate, potential immunogenicity.	[39]
	Microspheres	Encapsulation of exosomes within microspheres for controlled release and protection, offering extended retention.	Prolonged release, protection, controlled delivery.	Size uniformity, potential burst release, biodegradability.	[45]
	Microneedles	Incorporating exosomes into microneedles that can be inserted into the skin provides sustained release and localized concentration.	Painless administration, sustained delivery, localized concentration.	Microneedle fabrication complexity, possible skin irritation.	[47]
Surface Modification	PEGylation	Coating exosomes with polyethylene glycol (PEG) reduces immune recognition, prolongs circulation, and enhances retention.	Increased stability, prolonged half-life, reduced immune response.	Possible alteration of exosome function, variability in PEG conjugation.	[50]
	Lipid Coating	Surrounding exosomes with lipid bilayers prevents phagocytosis and enhances stability and retention.	Improved stability, prolonged circulation, and reduced immune recognition.	The complexity of the lipid coating process and potential alteration of exosome cargo.	[51]
	"Don't Eat Me" Markers	Displaying molecules like CD47 on exosome surfaces inhibits phagocytosis, leading to prolonged circulation and increased retention.	Prolonged circulation, reduced clearance, enhanced stability.	Variable efficacy, potential interference with other cellular interactions.	[52]

Table 2- Characteristics of Exosomes Derived from Different MSCs Sources

Authors	MSC Source-Exosome	Model	Main Findings	Reference
Yang et al. 2020	Umbilical Cord	Diabetic rats	hUCMSC-exos in PF-127 improved exosome ability in diabetic wound healing	[94]
Zhang et al. 2020	Umbilical Cord	Diabetic rat	Exosomes accelerated wound closure, decreased inflammation, increased collagen deposition and angiogenesis	[95]
Hu et al. 2018	Umbilical Cord	Mice	accelerate cutaneous wound healing via miR-21-3p	[60]
Zhang et al. 2015	Umbilical Cord	Rat skin burn	Enhances wound closure by activating Wnt/β-catenin in skin cells	[96]
Han et al. 2022	Bone Marrow	Diabetic mice	Exosomal lncRNA KLF3-AS1 derived from BMSCs induces angiogenesis to promote diabetic cutaneous wound healing.	[97]
Wu et al. 2020	Bone Marrow	Rat	BMSc-Exo Stimulated by Fe3O4 Nanoparticles and Static Magnetic Field Improve Wound Healing through miR-21-5p.	[61]
Jiang et al. 2020	Bone Marrow	Mice full-thickness skin wounds	Reduced inflammation and collagen deposition to prevent scarring	[98]
Ding et al. 2019	Bone Marrow	Diabetic rats	This contributed to enhanced wound healing and angiogenesis in streptozotocin-induced diabetic rats in vivo.	[99]
Shabbir et al. 2015	Bone Marrow	Diabetic mice	Topical exosomes improved wound healing through anti-inflammatory and pro-angiogenic effects (via Akt, ERK, and STAT3 signaling pathways)	[100]
Khalyfa et al. 2022	Adipose Tissue	Mice	Selenium-stimulated exosomes enhance wound healing by modulating inflammation and angiogenesis	[101]
Dong et al. 2021	Adipose Tissue	Diabetic foot ulcer rat	Can Prevent Medication-Related Osteonecrosis of the Jaw, accelerating bone remodeling, facilitating angiogenesis, and promoting wound healing.	[102]
Sheikh et al. 2020	Adipose Tissue	Rat	facilitated faster wound closure, enhanced collagen deposition, faster re-epithelialization, increased neo-vascularization,	[103]
Hu et al. 2016	Adipose Tissue	Mice	Exosomes accelerated wound closure by modulating inflammation, cell proliferation and migration	[104]
Zhang et al. 2015	Adipose Tissue	Mice full-thickness skin wounds	can promote fibroblast proliferation and migration and optimize collagen deposition via the PI3K/Akt signaling pathway	[105]
Rajendran et al. 2020	Gingival	Diabetic mice	Exosomes improved wound healing through pro-angiogenic and anti-inflammatory effects	[106]
Shi et al. 2017	Gingival	Diabetic Rat	The combination of GMSC-derived exosomes and hydrogel promote skin wound healing	[74]

The ability of skin cell proliferation, migration, angiogenesis, and skin wound closure is significantly improved after exosome injection into and around the wound site in rats[12-14]. By activating Akt, Erk, and Stat3 signalling via inducing the expression of HGF, IGF1, NGF, and SDF1, MSCs-derived exosomes from adipose, umbilical cord, and bone marrow tissues were able to promote cell migration, cell proliferation, promoted collagen synthesis. Also, increased re-epithelialization, decreased scar width, and maturation of newly formed blood vessels[15, 16]. Due to the differences in parental MSC characteristics and potentials, EVs derived from various MSC tissue origins might have different quality and therapeutic effects. In addition, changes in MSC culture conditions, cell seeding density, and passaging can also affect the secretory profile of MSCs, including exosome yields and content. Therefore, different conditions should be considered to increase the yield of MSC-exosomes and control their content[17]. Research has shown that only UCMSC-derived exosomes carry TGF-β, and the superior capacity in keratinocyte proliferation belongs to UCMSC-derived exosomes (UCMSCs-Exos)[17]. Recent studies have shown that the approximately 40-5000 nm particles released from cells are exosomes and can effectively regulate bioactive cargoes, including DNA, RNA, miRNAs, and proteins[18]. It has been reported that hUCMSCs-Exos exert a pro-angiogenic effect, promoting wound healing[13]. However, there are challenges in using exosomes for wound healing because exosomes are rapidly cleared from the application site and can only survive in the body for a short time[19]. Therefore, a combination of biomaterials and exosomes that increases the persistence of exosomes on the wound surface without affecting the biological activity of exosomes has been a novel area of research for the development of exosome-based therapies. For example, in a study, the combination of PF-127 hydrogel and hUCMSCs-Exos resulted in a remarkably accelerated wound healing, raised expression of Ki67 and CD31, and increased expression levels of VEGF and TGF-β[20].

2. Small extracellular vesicles (sEVs) are one of the most critical secreted factors released by hUCMSCs

MSCs secrete a variety of biologically active components. A large part of the bioactive factors are packaged into vesicles for export by the MSCs. While most molecules are discharged from the cells via the classical exocytosis fusion mechanism, others are transported through direct transmembrane proteins pathways. The intracellular molecular mechanisms and transmembrane process of MSCs still need to be fully understood. EVs are an exciting mechanism for MSCs to communicate with other cells. Exosomes are the smallest EVs subtypes that have been studied recently. Exosomes generally originate from endosomes because their membranes are enriched in lipid rafts involved in fusion and release cascades between intraluminal vesicles (ILVs) and multivesicular bodies (MVBs)[21]. The fusion of MVBs with the plasma membrane results in the release of exosomes. Other cells may subsequently uptake exosomes through cell-type specific membrane fusion, endocytosis, or phagocytosis[22]. Exosomes can easily pass through tissues and, thus, bypass biological barriers to transport their miRNAs, lipids, and proteins. The structure of an exosome under the transmission electron microscope (TEM) is like a “cup” or a “disk.” The exosomal surface carries specific markers such as CD81, CD9, CD63, TSG101, Alix, and HSP70[23, 24]. MSCs-Exos ameliorate experimental autoimmune pathological changes by inhibiting inflammatory cell accumulation[25]. Small extracellular vesicles (sEVs) have emerged as a new therapeutic cell-free MSC-based therapy platform. HUCMSCs-Exos have been shown to provide measurable benefits in the regeneration of tissue injury administered in various animal models. HUMSCs can inhibit the 15-LOX-1 enzyme secreted by macrophage, leading to the repair of inflammatory bowel disease (IBD) induced by dextran sulfate sodium (DSS)[26]. In a study, it has been shown that hUCMSCs-Exos can transport Wnt4 (a key factor in activating the β-catenin signalling pathway) to heal wounds and inhibit apoptosis of skin cells caused by heat stress through the activation of the AKT pathway[14]. HUCMSCs-exosomal 14-3-3ζ protein recruited p-LATS to induce Ser127 phosphorylation of YAP by forming a complex, which contributes to the regulation of skin cell proliferation by effectively coordinating the self-control of Wnt4 activity[27]. Also, in the rat second-degree burn injury model, 3,3'-diindolylmethane (DIM) increased the proliferation of MSCs by increasing the Wnt11 exosomal autocrine signalling pathway, which was related to Wnt/β-catenin activation[28]. Exosomal protein 14-3-3ζ controls YAP activities and phosphorylation, such that p-LATS binds to YAP at high cell density, and this complex limit fibroblast hyperproliferation and collagen deposition during dermal regeneration. In summary, hUCMSCs-Exos act as a signalling “brake” via modulating YAP for controlled cutaneous regeneration[27]. One of the main noncoding RNA in hUCMSCs-Exos is miR-181c, which could participate in the anti-inflammation response. miR-181c regulates attenuating burn-induced inflammation through decreasing TLR4 expression and reducing NF-κB/p65 activation[29]. HUCMSCs-Exos carriy out the specific miRNAs such as miR-23a, miR-125b, and miR-145, which are necessary for suppressing myofibroblast forma¬tion via blocking the TGF-β/Smad2 pathway[12]. The process might provide an approach to prevent scar formation during wound healing. It confirmed that hUCMSCs-Exos as a tool can significantly improve wound healing and promote collagen deposition, reducing scar formation[30].

3. Enhancing Strategies for exosome retention time

Exosomes derived from MSCs have shown promise as cell-free therapies for regenerative medicine. However, rapid clearance from circulation due to phagocytosis and filtration limits the retention time and efficacy of MSCs-derived exosomes[31]. This review discusses current and emerging strategies to improve exosome retention through physical protection, immune evasion, targeting, and combination approaches. Specific focus is given to enhancing exosomes derived from UCMSCs due to their advantages, including availability, hypo immunogenicity, and potent immunomodulatory effects[32]. A multipronged combination of physical, biological, and chemical strategies tailored to the therapeutic application and intended use can significantly improve UCMSC exosome circulation time, biodistribution, and retention at target tissues[33]. Further research is warranted to develop optimal methods that balance reduced clearance with retention of therapeutic activity. This will enable the full clinical potential of UCMSC exosomes as cell-free therapies. Strategies to improve UCMSCs exosomes retention time, including physical protection and sustained release: administration in hydrogels, microspheres, and microneedles to avoid clearance, provide sustained release, and maintains local concentration[34]. surface modification: PEGylation, lipid coating to evade phagocytosis, displaying "don't eat me" markers like CD47 (Table 1)[35]. Genetic Engineering of Parent UCMSCs: Overexpress proteins that stabilize exosomes like HSPs. Exosomes inherit properties of engineered UCMSCs[36]. targeted Biodistribution: Displaying ligands against disease-specific cells and tissues improved retention at target sites[37]. Co-administration with immunosuppressive drugs: temporary suppression of phagocytosis, and the last one is alternative administration routes: intranasal, subcutaneous etc., to avoid first pass clearance[38].

3.1. Hydrogels

Hydrogels are three-dimensional (3D) networks of hydrophilic polymers that absorb and retain large amounts of water or biological fluids. They are highly biocompatible and suitable for various biomedical applications due to their tunable characteristics, such as porosity, mechanical strength, and degradation rates[39].

Researchers have explored the interaction between exosomes and hydrogels to increase the retention time at the target site. Hydrogels can serve as exosome carriers, protecting from enzymatic degradation and mechanical clearance. They offer controlled release mechanisms, enabling sustained delivery over an extended period[40]. Researchers can tailor interactions to optimise exosome release kinetics based on the therapeutic application. Several strategies have been developed to incorporate exosomes into hydrogels effectively, such as loading exosomes into pre-formed hydrogels or using encapsulation techniques like microfluidics and electrospraying[41]. The combination of exosomes and hydrogels has shown promising results in various biomedical applications, such as tissue regeneration, wound healing, and targeted drug delivery, particularly in cancer therapy; by incorporating therapeutic cargo into hydrogels, site-specific drug release can be achieved, minimising systemic toxicity[42].

3.2. Microspheres

Microspheres, also known as microparticles or microcapsules, are tiny spherical particles made from biocompatible materials. They have been used to enhance the therapeutic potential of exosomes, offering enhanced stability and protection. Microspheres come in various forms, such as solid, porous, and hollow, and can be tailored to effectively encapsulate exosomes[43]. Encapsulation within microspheres provides a protective microenvironment, shielding exosomes from external degradation and harsh biological conditions[44]. Microspheres enable controlled release of exosomes over an extended period, providing sustained therapeutic effects. Microspheres can also be engineered to achieve targeted delivery to specific tissues or cells, thereby improving therapeutic precision. Microspheres have shown promising results in biomedical applications, including cancer therapy, regenerative medicine, and neurological disorders[45]. In cancer treatment, microspheres loaded with exosomes can directly deliver therapeutic cargo to tumor cells, promoting cell death or modulating the tumor microenvironment. In regenerative medicine, exosomes encapsulated in microspheres can facilitate tissue repair and regeneration, promoting cell growth and differentiation[46].

3.3. Microneedles

Microneedles are small, needle-like structures gaining attention for their ability to enhance drug delivery and promote localized therapeutic effects. They can facilitate controlled and targeted delivery of exosomes into the skin or underlying tissues, enhancing their penetration and retention[47]. This leads to improved exosome bioavailability and therapeutic effects. Microneedle-assisted exosome delivery enables localized therapy, focusing treatment on specific areas for better outcomes. Controlled release of exosomes over time can be achieved through various microneedles designs (solid, coated, hollow, or dissolvable microneedles) and encapsulation methods[48]. ongoing research and development aims to optimize exosome delivery and retention, including testing dissolvable microneedles and patches that release exosomes over time. Key challenges include ensuring consistent and reproducible delivery, optimizing microneedle design for specific therapeutic goals, and addressing potential immune responses or adverse reactions[49]. In summary, microneedles show potential to significantly enhance exosomes retention time at the application site, extending therapeutic effects. However, practical implementation and clinical translation of this approach require further research and development.

3.4. PEGylation

PEGylation, the process of attaching polyethylene glycol (PEG) chains to molecules, has emerged as a promising strategy to enhance the retention time of exosomes within the circulatory system. PEGylation imparts stealth-like properties to exosomes by reducing their recognition and clearance by the immune system and hepatic cells, consequently prolonging their circulation time. This improved retention enhances the potential for exosomes to reach target tissues and deliver their therapeutic cargo[50].

3.5. CD47

CD47, initially discovered as an oncogenic marker in human ovarian cancer during the 1980s, that has since been recognized in a variety of human cancers. These include acute myeloid leukemia (AML), chronic myeloid leukemia, acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), multiple myeloma (MM), bladder cancer, and several other solid malignancies. Pediatric and adult brain tumors also exhibit notable CD47 expression. CD47's elevated presence in cancer cells contributes to their evasion from phagocytosis, even with increased calreticulin levels, which typically promote phagocytosis[51,52] .

CD47, a cell surface protein, plays a pivotal role in enhancing the retention time of exosomes, small vesicles secreted by cells for intercellular communication. CD47 interacts with the integrin, thrombospondin-1 and signal regulatory protein α (SIRPα) receptor on macrophages, initiating a "don't eat me" signal that prevents phagocytosis of exosomes. This interaction inhibits the immune system's clearance mechanism and prolongs the circulation of exosomes in the bloodstream, thereby facilitating their potential therapeutic effects[35,53].

4. hUCMSC-derived exosomes and compared with exosomes derived from other MSCs sources

Several sources of MSCs, including bone marrow, embryos, umbilical cord, adipose tissue, menstrual blood, dental pulp, and gingiva, have been extensively studied for their potential therapeutic applications and have shown different translational potentials and exhibit varying properties and functions[54]. MSCs-derived extracellular vesicles (EVs) have shown translational potential in immune regulation and inflammation mediation[55-57]. hUCMSCs-Exos have a crucial role in wound healing through the Wnt4/β-catenin pathway[58]. These exosomes have angiogenic properties, promoting endothelial cell proliferation, migration, invasion, and angiogenesis. They enhance cutaneous wound healing through the activation of the Wnt4/β-catenin pathway, as well as the upregulation of phosphorylation of ERK1/2, which stimulates cell proliferation and growth. hUCMSCs have protective effects on cell viability, stimulate cell proliferation, reduce inflammation, neutrophil infiltration, and oxidative stress, and promote wound healing in various in vitro and in vivo models[59]. UCB-Exos promotes fibroblast proliferation and migration, enhancing endothelial cell angiogenic activities. MiR-21-3p, enriched in UCB-Exos, mediates regulatory effects by inhibiting PTEN and SPRY1 through its regulatory effects[60]. HBMMSCs-Exos contain anti-inflammatory miRNAs to suppress inflammation factors[61]. They promote angiogenesis, accelerate wound healing, and reduce scarring. Specific miRNAs in MSCs-derived EVs, such as miR-27b and miR-181c, regulate inflammation and inhibit myofibroblast accumulation. The administration of exosomes from hBMMSCs targeting pknox1 with miR-223 regulates macrophage polarization[62]. These exosomes contain miR-146a, a well-known anti-inflammatory miRNAs, which reduces inflammation factors such as tumour necrosis factor-α (TNF-α), Interleukin-6 (IL-6), and Interleukin-1β[63]. Low levels of miR-224-3p in bone marrow MSCs (*BMMSCs*)-Exos promote endothelial cell proliferation, migration, invasion, and angiogenesis by targeting focal adhesion kinase family interacting proteins 200 KDa (FIP200)[64]. A study by Jiang et al. demonstrated that BMMSCs exosomes suppress TGF-β1, Smad2, Smad3, and Smad4 proteins by targeting the TGF-β/Smad signalling pathway while increasing the expression of of TGF-β3

and Smad7, resulting in improved scar formation and promotion of wound healing[65]. Furthermore, miR-21-5p is overexpressed in mag-BMSCs-Exos, promoting angiogenesis in both in vivo and in vitro settings, thereby accelerating skin wound healing by targeting SPRY2 to activate the PI3K/AKT and ERK1/2 signalling pathways[61]. miR-27b and miR-181c are believed to regulate inflammation and inhibit myofibroblast accumulation[66]. Adipose-derived mesenchymal stem cells (ADMSCs)-Exos can modulate collagen production to reduce scar formation. The systemic administration of ADMSCs-Exos promotes various activities involved in wound healing, such as fibroblast function, collagen deposition, re-epithelialization, and vascularisation[67]. ADMSCs-Exos down-regulate pro-inflammatory proteins and up-regulate proteins and promote wound healing. Specific miRNAs in ADMSCs-Exos have crucial roles in fibrosis and scar formation. Additionally, fibroblasts absorb and internalise ADMSCs exosomes, resulting in increased gene expression of proteins associated with wound healing[68-70]. menstrual blood MSCs (MenSCs)-Exos can resolve inflammation by inducing polarisation of M1 to M2 macrophages, enhance neo-angiogenesis by upregulating VEGFA, accelerate re-epithelialization through the upregulation of NF-κB p65 subunit and activation of the NF-κB signalling pathway[71-73]. Gingival MSCs (GMSCs)-Exos promote re-epithelialization, angiogenesis, neuronal ingrowth, skin wound healing, and decreased cil1:col3 ratio[74]. Fetal dermal MSCs (FDMSCs)-Exos activate adult dermal fibroblasts and promote proliferation, migration, and secretion by targeting the Jagged 1 ligand in the Notch signalling pathway in wound healing. Similar effects have been observed with human-derived MSCs-Exos carrying miRNAs[75]. In summary, hUCMSC-Exos offer advantages like low immunogenicity, high yield, and a broad therapeutic spectrum. However, their widespread application faces limitations like lack of standardized protocols, inefficient delivery methods, and exosome instability. Researchers are exploring solutions like developing standardized protocols, using novel delivery methods, and improving exosome stability. Challenges include clinical translation, large-scale manufacture, and lack of definitive markers for characterizing MSCs-Exos. Solutions include optimizing culture conditions, bioengineering approaches, novel labeling techniques, and scalable purification processes. Moreover, Further research is needed to validate quality attributes and establish potency assays for clinical-grade exosome products, as referenced in some studies listed in Table 2.

5. The effects of hUCMSCs-derived exosomes on wound healing

Exosomes, which are derived from hUCMSCs, have been shown to have various beneficial effects[76, 77]. They promote angiogenesis, reduce apoptosis, and protect cells by increasing the expressions of Bcl-2 and caspase-3 while decreasing the expression of Bax, cleaved caspase-3, and cleaved PARP[78]. In vivo, hUCMSCs-Exos have been found to enhance angiogenesis under blue light exposure by upregulating miRNAs such as miR-135b-5p and miR-499a-3p in endothelial cells. Moreover, hUCMSC-derived exosomes play a crucial role in regulating inflammation by suppressing tumour necrosis factor cytokines α (TNF-α) and interleukin-1β (IL-1β) levels and upregulating IL-10 levels[79]. Over-expression of miR-181c in hUCMSCs-Exos effectively inhibits the TLR4 signalling pathway, reducing the inflammatory response in burned rats and attenuating burn-induced excessive inflammation[29].

HUCMSCs-Exos reduce scarring and myofibroblast accumulation in mouse models with skin defects. Specific miRNAs (miR-21, -23a, -125b, -145) play a key role in inhibiting myofibroblast aggregation through the factor-β2/SMAD2 pathway[12]. They promote cell proliferation and protect against oxidative stress-induced cell apoptosis in vitro by activating ERK1/2 and p38 pathways. However, UV exposure can abrogate these regulatory roles, suggesting the potential of hUCMSCs-Exos as therapeutic agents in regulating cell growth and apoptosis through exosomal shuttle of RNA, as well as their high cytokine content, including IL-6 and IL-8[14, 80]. Additionally, hUCMSCs-Exos promote angiogenesis, which is the formation of new blood vessels from pre-existing ones, by upregulating miRNAs such as miR-135b-5p and miR-499a-3p in endothelial cells[79, 81]. This process is essential for wound healing and tissue repair, as it delivers oxygen and nutrients to new tissues. Moreover, hUCMSCs-Exos have been found to reduce cell death or apoptosis by increasing the expression of anti-apoptotic proteins such as Bcl-2 and caspase-3 while decreasing the expression of pro-apoptotic proteins such as Bax, cleaved caspase-3, and cleaved PARP[82]. The findings from lab studies demonstrate that exosomes from a unique type of cell found in human umbilical cords can positively impact other cells. Moreover, the exosomes contain many cytokines, natural chemicals that can help heal. Obtaining these exosomes from the cells is a simple and efficient process, which is very promising for potential use in treating illnesses and injuries.

The mechanisms by which UCMSCs-Exos promote wound healing have yet to be fully understood. However, several potential mechanisms have been proposed. One potential mechanism is that exosomes can deliver growth factors and other signalling molecules to target cells. These molecules can then activate signalling pathways that promote cell proliferation, migration, and differentiation. For example, exosomes from UCMSCs have been shown to deliver the growth factor TGF-β, promoting fibroblasts' proliferation and differentiation[83]. Another potential mechanism is that exosomes can modulate the immune response. Exosomes can contain immunomodulatory molecules, such as cytokines and chemokines. These molecules can regulate the activity of immune cells and promote wound healing by suppressing inflammation and tissue repair. For example, exosomes from UCMSCs have been shown to suppress the production of pro-inflammatory cytokines, such as IL-1β and TNF-α[84].

6. Limitations of using exosomes and solutions to overcome the limitations

Among the major challenges of using exosomes is the short lifespan in the body, and they are quickly removed from the injection site [20]. Using biomaterial that increases the duration of exome presence on the wound without affecting their biological activity is the priority of exome therapy research. Many studies have shown that exosomes can be delivered to injury sites by different carriers[85]. Shi et al. reported that exosomes loaded on chitosan/silk hydrogel enhanced wound healing via re-epithelialization, collagen deposition, and angiogenesis[74]. Using chitosan hydrogel as a carrier of exosomes reduces their degradation rate and increases wound healing speed[86]. The combination of placenta-derived MSCs (hPMSCs) exosome and hydrogel could promote the stability of proteins and miRNAs in them[87]. Using smart hydrogel as a carrier helps increase the life span of exosomes. HUCMSCs-Exos, along with the Pluronic F-127 (PF-127) hydrogel as a carrier, promote the survival rate of the exosome, angiogenesis, and cell growth in wound healing. PF-127 is a thermosensitive hydrogel that continuously releases exosomes on the lesion's surface and accelerates skin regeneration[20]. Another challenge is to improve the extraction and purification methods of exosomes. A standard isolation and analysis technique is effective in obtaining the highest yield of exosomes[88]. In some cases, isolated exosomes overlap with other extracellular vesicles. The common method to isolate is ultra-centrifugation, which is time-consuming and expensive. It is recommended to use alternative methods that are more economical, have less time, and have higher efficiency. Recently, methods such as polymers, magnetic-activated cell sorting (MACS), immunological procedures, and microfluidics have been used [89]. In this context, the source of cell acquisition and culture conditions are important. The source of MSCs isolation impresses the cargo secreted from exosomes [88].

Improving the storage methods of exosomes is necessary to extend their application domain from the laboratory to the clinic. On the other hand, exosomes as natural vesicles are limited in clinical applications. In some cases, targeting therapeutic exosomes for special tissue/cells or loaded exosomes with modern drugs, RNA, or proteins is necessary. Recently, engineering approaches for parental cells or direct exosomes have been recommended to solve these problems. Targeting exosomes through engineering manipulation leads to chemical modification of their surface. Different techniques are used for designer exosomes, such as bio composition, electroporation, and sonication[90]. Other methods for exosome engineering are changes in DNA fragments and plasmid transfer. Also, exosome-producing cells can be enriched with the desired factor for better efficiency[91]. The studies showed that exosome modification reduces immune rejection and improves skin retention after transplantation[92]. The safety of exosome donor cells, dose and number of injections, injection site, and high charge of exosome acquisition are still challenging and under investigation. Compliance with quality control is necessary for retaining biological activity and more efficacy in manufactured products, especially in clinical applications. Storage conditions, stability of donor cells in culture, and donor cell aging should be checked constantly. There are important to assign certain standard criteria for size, purity, characteristics, and level of contamination[91].

7. Conclusions

Current evidence demonstrates that hUCMSCs-Exos exhibit therapeutic potential for accelerating wound healing. Specifically, hUCMSCs-Exos have been shown to promote several key processes involved in wound repair, including angiogenesis, cell proliferation and migration, while also reducing inflammation and apoptosis. Transfer of regulatory microRNAs by exosomes plays an important role in modulating myofibroblast accumulation and scar formation. Multiple in vivo rodent studies have confirmed that hUCMSCs-Exos accelerate wound closure and healing outcomes compared to control. However, limitations remain in the clinical translation of hUCMSCs-exosomal therapies for wound management. Rapid clearance from target tissues and variability in isolation methods present challenges. Recent research has begun investigating strategies to enhance exosome retention time and targeting, including incorporation into hydrogels, microspheres, and surface modification approaches. While significant potential exists for hUCMSCs-Exos as cell-free therapies for wound healing, further research is still needed to standardize isolation protocols, improve retention, and evaluate clinical efficacy. Optimization of exosome-based approaches through engineering and combination delivery systems will likely accelerate advancement toward regenerative medical applications. Overall, continued elucidation of mechanisms and clinical translation efforts for hUCMSCs exosomal therapeutics remain promising directions for benefiting wound treatment through cell-free strategies.

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List of Abbreviations

MSCs: Mesenchymal Stem/Stromal Cells
hUCMSCs: Human Umbilical Cord MSCs
IGF: Interleukin-like Growth Factor
PDGF: Platelet-Derived Growth Factor
TGF: Transforming Growth Factor
SDF-1: Stromal Cell-Derived Factor-1
VEGF: Vascular Endothelial Growth Factor
EGF: Epidermal Growth Factor
GVHD: Graft-Versus-Host Disease
EBV: Epstein Barr virus
CMV: Cytomegalovirus
EVs: Extracellular Vesicles
ILVs: Intraluminal Vesicles
MVBs: Multivesicular Bodies
TEM: Transmission Electron Microscope
DIM: 3,3'-Diindolylmethane
sEVs: Small Extracellular Vesicles
SIRPα: Signal Regulatory Protein α
BMMSCs: Bone Marrow MSCs
MenSCs: Menstrual Blood MSCs
ADMSCs: Adipose-derived MSCs
GMSCs: Gingival MSCs
FDMSCs: Fetal Dermal MSCs

Declarations

Conflict of interest
The authors declare that they have no conflict of interest.

Ethical approval
Not applicable.

Consent for publication
Not applicable.

Funding
Not applicable.

Data availability
Not applicable.

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