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Review

Optimizing mesenchymal stem cell extracellular vesicles for chronic wound healing: Bioengineering, standardization, and safety

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ABSTRACT

Chronic wounds represent a significant global burden, afflicting millions with debilitating complications. Despite standard care, impaired healing persists due to factors like persistent inflammation and impaired tissue regeneration. Mesenchymal stem cell (MSC)-derived extracellular vesicles (EVs) offer an innovative regenerative medicine approach, delivering stem cell-derived therapeutic cargo in engineered nanoscale delivery systems. This review examines pioneering bioengineering strategies to engineer MSC-EVs into precision nanotherapeutics for chronic wounds. Emerging technologies like CRISPR gene editing, microfluidic manufacturing, and biomimetic delivery systems are highlighted for their potential to enhance MSC-EV targeting, optimize therapeutic cargo enrichment, and ensure consistent clinical-grade production. However, key hurdles remain, including batch variability, rigorous safety assessment for potential tumorigenicity, immunogenicity, and biodistribution profiling. Crucially, collaborative frameworks harmonizing regulatory science with bioengineering and patient advocacy hold the key to expediting global clinical translation. By overcoming these challenges, engineered MSC-EVs could catalyze a new era of off-the-shelf regenerative therapies, restoring hope and healing for millions afflicted by non-healing wounds.

Contents

Abbreviations: CD, cluster of differentiation; EVs, extracellular vesicles; GMP, good manufacturing practice; IL, interleukin; MMP, matrix metalloproteinase; MSCs, mesenchymal stem cells; TGF-β, transforming growth factor β; VEGF, vascular endothelial growth factor; Wnt, wingless/integrated-1.

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1. Background

Chronic nonhealing wounds, such as diabetic foot ulcers, venous leg ulcers, pressure ulcers, burn injuries, and surgical wounds, pose significant clinical challenges [1–5]. They are characterized by a high prevalence, elevated recurrence rates, and significant socio-economic burdens, with estimated costs of around $50 billion annually, particularly in countries like the United States of America [3,6]. Furthermore, up to 30% of chronic wounds do not respond to standard care involving dressings, skin grafts, and debridement after 12 weeks, underscoring the urgent need for innovative treatment options [7–9].

Extracellular vesicles (EVs) are a ubiquitous and diverse class of phospholipid-enclosed membranous structures, ranging in size from 30 nm to 5 μm, that are released by various cell types into extracellular spaces. They play a crucial role in intercellular communication by facilitating the transfer of bioactive molecular cargo between cells in both physiological and pathological conditions [10–13]. EVs are broadly categorized into three major subtypes based on their biogenesis: exosomes, microvesicles, and apoptotic bodies [10,14,15].

Exosomes, also known as small EVs, typically range from 30 to 150 nm in diameter and are produced through the endolysosomal pathway, where plasma membrane invaginations lead to the formation of multivesicular bodies containing intraluminal vesicles. Upon the fusion of multivesicular bodies with the plasma membrane, exosomes are released by the intraluminal vesicles into the extracellular environment. Conversely, microvesicles, also referred to as medium EVs, have diameters ranging from 150 nm to 1 μm and are generated *via* direct outward budding or blebbing from the plasma membrane [14,16]. This process is mediated by various signaling cascades involving proteins like RAB GTPases, ribosylation factor 6, and regulators of cytoskeletal dynamics, including Rho GTPases, which orchestrate the reorganization of the cell's structural framework [17]. Apoptotic bodies, ranging from 500 nm to 2–5 μm and falling in the category of large EVs, are released during programmed cell death and are characterized by membrane blebbing [10,18].

Despite their distinct biogenesis pathways, EVs share common features in terms of molecular composition. They are enriched in bioactive molecules such as cytokines, chemokines, growth factors, lipids, and nucleic acids including deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNAs), microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), long non-coding RNAs (LncRNAs), small nucleolar RNAs (snRNAs), small nucleolar RNAs (snoRNAs), mitochondrial RNAs (mtRNAs), and circular RNAs (CircRNAs) [16,17]. Additionally, EVs display conserved surface markers, including tetraspanins such as cluster of differentiation (CD) 63, CD81, CD9, heat shock protein 70, and posttranslationally modified surface proteins [19,20]. The cargo content of EVs is known to be sorted *via* biophysical mechanisms driven by alterations in membrane curvature and the distribution of specific components, including Bin/Amphiphysin/Rvs domain proteins and key proteins of the Endosomal Sorting Complex Required for Transport (ESCRT) machinery, such as ESCRT-I, ESCRT-II, and ESCRT-III complexes [17,21]. These intrinsic properties of EVs make them essential players in diverse intercellular communication processes, modulating various physiological and pathological functions based on the cell of origin.

EVs, with their potential to modulate tissue repair in various diseases such as cancer, neurodegeneration, and dermatological disorders (ulcers and injuries) [22,23], have garnered considerable attention as a novel therapeutic tool, particularly in wound healing. Previously, cell therapy was considered the promising alternative to conventional wound healing treatments such as wound dressings, skin grafts, and surgical debridement [24–26]. Among cell-based therapies, mesenchymal stem cells (MSCs) have attracted significant interest due to their accessibility and crucial role in wound healing. MSCs can be easily isolated from various sources, including umbilical cords, bone marrow, and adipose tissue. Additionally, they interact with various immune cells to maintain immunological homeostasis in the wound microenvironment and differentiate into

fibroblasts, chondrocytes, and osteocytes to promote tissue formation [25,26]. Nonetheless, cell transplantation encounters challenges such as interdonor variability, limited survival, immune rejection, and potential malignant transformation [27–29]. Using cell-free derivatives like MSC-EVs, these challenges could be mitigated while harnessing the regenerative capacity of their secreted paracrine factors [30–32]. Tailored *via* bioengineering techniques, engineered MSC-EVs (eMSC-EVs) are capable of actively participating in key stages of tissue healing, such as hemostasis, inflammation, proliferation, and remodeling, offering innovative biological therapies for tissue regeneration [31,32].

The intrinsic paracrine activity of MSCs can be harnessed by utilizing their secreted EVs. Importantly, the therapeutic potential of native MSC-EV strategies can be further enhanced through bioengineering approaches, including the overexpression of therapeutic factors, knockout/knockdown to eliminate harmful components, and stimulation with priming factors. For instance, eMSC-EVs can be tailored to overexpress anti-inflammatory cytokines such as interleukin (IL)-10 or tumor necrosis factor-stimulated gene-6, which are known to modulate inflammatory responses and promote tissue repair [30–32]. Knockout or knockdown strategies may involve the suppression of proinflammatory factors such as Toll-like receptor 4 to mitigate inflammatory signaling pathways within the wound microenvironment [33]. Furthermore, priming factors such as hypoxia or cytokine preconditioning can promote MSCs to release EVs enriched with angiogenic growth factors such as vascular endothelial growth factor (VEGF) and angiopoietin-1, thereby enhancing their proangiogenic properties [34,35].

MSC-EVs engineered through genetic modification, therapeutic cargo loading, and surface functionalization exhibit enhanced anti-inflammatory, pro-angiogenic, and tissue regenerative effects. In the context of chronic nonhealing wounds characterized by sustained inflammation, immune cell accumulation, senescent cell accrual, and extracellular matrix degradation, locally administered eMSC-EVs can help mitigate inflammatory responses *via* various synergistic mechanisms [31,32,36]. These mechanisms include the suppression of M1 macrophage activation, inhibition of nuclear factor kappa B signaling cascades, promotion of anti-inflammatory M2 macrophages and regulatory T cells, and downregulation of inflammatory cytokines through surface interactions and delivery of regulatory miRNA cargoes. For instance, eMSC-EVs may contain high levels of miR-146a, which can target and suppress nuclear factor kappa B signaling in immune cells, thereby attenuating inflammatory responses [37,38]. MSC-EVs engineered to carry specific pro-angiogenic miRNAs or growth factors play a crucial role in enhancing angiogenesis within the wound microenvironment by stimulating endothelial cell migration and proliferation, upregulating angiogenic growth factors, and activating downstream regeneration pathways. For example, MSC-EVs are engineered to carry miR-132, by transfecting parent MSCs with miR-132 mimics, targeting inhibitors of angiogenic signaling pathways such as Rasa1 and Spred1 [39,40]. Similarly, MSC-EVs loaded with miR-126 through electroporation have shown enhanced pro-angiogenic effects [41]. Additionally, MSCs genetically modified to overexpress fibroblast growth factor (FGF) produce EVs enriched with FGF, promoting extracellular matrix deposition and granulation tissue formation at the wound site [42,43]. Recombinant growth factors like VEGF or PDGF are also loaded into MSC-EVs to enhance their pro-angiogenic and regenerative properties [39,40].

Recent advancements have been focused on enhancing the native therapeutic efficacy of MSC-EVs through engineering strategies that focus on optimizing their molecular cargo and surface properties [44,45]. These strategies involve overexpressing angiogenic miRNAs, silencing inflammatory mRNAs, displaying tissue-targeting peptides, and incorporating drug molecules into EVs. For instance, eMSC-EVs can be designed to carry specific miRNAs, such as miR-126 or miR-210, known for their proangiogenic effects and ability to promote tissue regeneration [40,46]. In addition, modifying MSC-EV surfaces with tissue-targeting peptides, such as those containing the amino acid sequence arginine-glycine-aspartic acid that target integrin receptors on endothelial cells, can enhance their homing to injury sites [45–47]. Nevertheless, understanding the unique characteristics of MSC-EVs compared to EVs derived from other cells remains paramount.

1.1. The unique advantages of MSC-EVs for regenerative medicine

MSCs have emerged as a promising cell source for regenerative medicine due to their immunomodulatory, differentiation, and regenerative capabilities. These inherent properties are mirrored in the EVs they secrete, making MSC-EVs a potential therapeutic tool. The tissue of origin for MSCs, such as bone marrow, adipose tissue, or umbilical cord, influences the biological composition of their derived EVs. This phenomenon allows researchers to tailor treatments to specific clinical needs by selecting the most appropriate MSC source [28,29,48,49].

Beyond their source, MSC-EVs possess a unique cargo profile enriched with specific proteins, lipids, and nucleic acids. Notably, they contain miRNAs such as miR-21 and miR-146a, which play a crucial role in regulating inflammation and promoting tissue repair. Additionally, the presence of growth factors like VEGF and TGF-β in MSC-EVs further enhances their pro-angiogenic and wound healing properties, distinguishing them from EVs derived from other cell types [36,50]. Another strength of MSC-EVs lies in their cargo selectivity. These EVs can selectively package therapeutic molecules based on the physiological state and environmental cues of the MSCs. This cargo selectivity enables MSC-EVs to display specialized functions that support tissue homeostasis, immunomodulation, and regeneration more effectively compared to generic EVs [35,48,51].

Researchers are further pushing the boundaries of MSC-EV therapy by leveraging advancements in bioengineering. Techniques such as CRISPR gene editing and pre-treatment of MSCs allow for the customization of MSC-EV cargo, ultimately enhancing their therapeutic potential. This opens doors for designing MSC-EVs that carry specific cargo molecules targeting defined wound healing pathways [51–53].

Preclinical studies have demonstrated the superior therapeutic benefits of MSC-EVs in models of chronic wounds compared to EVs derived from other cell types. These studies highlight the enhanced angiogenic, anti-inflammatory, and anti-scarring properties of MSC-EVs, making them a compelling therapeutic option [54,55]. Currently, ongoing clinical trials are evaluating the safety and efficacy of MSC-EV-based therapies in humans, paving the way for their future clinical applications [29,54,55].

However, significant challenges remain before widespread clinical implementation. Scalable manufacturing of MSC-EVs with consistent quality and adherence to stringent regulatory guidelines pose significant hurdles. Variability in production processes, the need for robust quality control assays, and the evolving landscape of regulatory requirements are all aspects that require ongoing consideration for the successful implementation of engineered EV therapies.

1.2. Quality attributes and translational challenges of MSC-EV therapies

Understanding the essential quality attributes of EVs is crucial for the successful translation of their therapies into clinical applications. This involves a comprehensive analysis of their physicochemical properties, biological markers, and functional properties

specific to wound healing [29,48,52]. Establishing standardized preclinical profiling frameworks that align with global regulatory guidelines and anticipated operational infrastructure requirements is crucial for ensuring the safety, effectiveness, and quality of EV-based treatments [29,46,51].

The importance of these quality attributes can be further emphasized by considering the example of the MRG-110 clinical trial. The clinical trial of MRG-110, an anti-miR-92 drug, highlights the potential of miRNA-based therapies in regenerative medicine. The promising safety profile demonstrated in this trial is particularly relevant to the development of MSC-EV therapies, as engineered EVs often incorporate specific miRNAs to enhance their therapeutic efficacy [56–58]. However, the successful translation of miRNA-loaded EVs into clinical applications faces similar challenges as those encountered in the development of anti-miR drugs, such as scalability, standardization of potency assays, and maintenance of genomic stability.

The MRG-110 clinical trial experience underscores the importance of addressing these challenges in the context of MSC-EV therapies. Achieving scalable production of high-quality, miRNA-loaded EVs with consistent potency and genomic stability is crucial for their successful clinical translation. This requires strategic measures such as the development of standardized manufacturing processes, the establishment of robust quality control assays, and the implementation of comprehensive characterization techniques to assess EV purity, potency, and safety [46,59].

By proactively addressing these challenges and implementing appropriate strategic measures, researchers and manufacturers can ensure the timely and responsible clinical advancement of MSC-EV therapies, ultimately bringing these innovative treatments closer to patients in need.

In this review, we discuss key advancements in developing and applying EVs derived from MSCs as a cell-free therapy for chronic wound healing. We provide a critical analysis of the rapidly evolving field of MSC-EV engineering, production, and translation, with a specific focus on clinical applications in wound healing. The review delves deeply into recent advancements in modulating EV surface properties and molecular cargo to optimize their regenerative and immunomodulatory capabilities. It also highlights the critical challenges associated with achieving scalable, reproducible, current good manufacturing practice (cGMP) - compliant biomanufacturing of engineered EVs, implementing quality assurance benchmarks, and navigating regulatory requirements for novel EV therapeutics. This comprehensive analysis offers valuable insights into this promising yet intricate field, while also charting critical future directions to maximize clinical impact.

2. Genomic optimization and stability of MSC-EV-based therapies for wound healing

MSC-EVs hold significant promise for the treatment of chronic wounds. However, inherent limitations in the therapeutic efficacy and cargo capacity of these native EVs have led to recent advances in bioengineering strategies to enhance their wound-healing functions.

2.1. Engineering strategies for enhancing MSC-EV

Therapeutic Potential To further enhance the therapeutic potential of MSC-EVs, various engineering approaches have been developed. These strategies aim to optimize the molecular cargo and surface properties of MSC-EVs, enabling them to effectively target specific cell types, deliver therapeutic factors, and promote tissue regeneration. The main engineering strategies include:

2.1.1. Genetic modification of parent MSCs

One approach to enhance the therapeutic potential of MSC-EVs is to genetically modify the parent MSCs to overexpress specific therapeutic factors. For example, MSCs can be engineered to overexpress anti-inflammatory cytokines such as IL-10 or TSG-6, which are then incorporated into the secreted EVs [60]. Similarly, MSCs can be modified to overexpress growth factors like VEGF, resulting in EVs enriched with pro-angiogenic cargo [61]. These genetically engineered MSC-EVs have shown improved anti-inflammatory and pro-regenerative effects in preclinical wound healing models [60,61].

2.1.2. Loading of therapeutic cargo into EVs

Another strategy involves directly loading therapeutic molecules into MSC-EVs. This can be achieved using various techniques, such as electroporation, sonication, or incubation. For instance, MSC-EVs can be loaded with specific miRNAs, such as miR-21 or miR-146a, which have been shown to promote wound healing by regulating inflammation and tissue remodeling [62,63]. Similarly, small interfering RNAs (siRNAs) or drugs can be incorporated into MSC-EVs to target specific pathways or molecules involved in the wound healing process. These cargo-loaded MSC-EVs have demonstrated enhanced therapeutic efficacy in preclinical studies [62,64].

2.1.3. Surface modification of EVs

The surface of MSC-EVs can be engineered to display targeting ligands or peptides, enhancing their specificity and uptake by target cells or tissues. For example, MSC-EVs can be functionalized with the RGD peptide, which binds to integrin receptors expressed on endothelial cells and promotes EV uptake [40,47]. Similarly, MSC-EVs can be modified with antibodies or aptamers that recognize specific cell surface markers, allowing for targeted delivery to specific cell types involved in wound healing [40,47]. These surface-modified MSC-EVs have shown improved homing to injury sites and enhanced therapeutic effects in preclinical models [40,47].

2.1.4. Exosome surface engineering and targeting

In addition to the strategies mentioned above, direct engineering of the exosome surface is an emerging approach to enhance the targeting and therapeutic efficacy of MSC-EVs. By conjugating specific ligands, peptides, or polymers to the exosome surface, researchers can improve their binding and uptake by target cells, reduce off-target effects, and prolong their circulation time [40,47].

One common approach to exosome surface engineering is the conjugation of targeting peptides, such as the RGD motif, which binds to integrin receptors overexpressed on endothelial cells and can enhance the delivery of MSC-EVs to the wound site [40,47]. Another strategy involves the functionalization of the exosome surface with hydrophilic polymers, such as polyethylene glycol (PEG), which can increase their stability, reduce immune clearance, and prolong their circulation time [65].

Recent studies have also explored the use of click chemistry and metabolic labeling to incorporate specific functional groups onto the exosome surface, enabling the conjugation of various targeting ligands or therapeutic molecules [66,67]. However, challenges remain in developing more efficient and scalable conjugation methods, identifying novel targeting moieties, and understanding the complex interactions between engineered exosomes and the wound microenvironment.

Employing these advanced engineering strategies enables researchers to create MSC-EVs with specifically tailored therapeutic potentials, aimed at addressing distinct aspects of the wound healing process. Promising preclinical studies reveal that these

engineered MSC-EVs outperform their unmodified counterparts in reducing inflammation, promoting angiogenesis, and enhancing tissue regeneration. With ongoing advancements in this field, future research is expected to develop even more sophisticated engineering techniques to further optimize MSC-EVs, enhancing their efficacy and utility in wound healing applications.

Table 1 provides a comprehensive overview of various bioengineering strategies used to enhance the therapeutic potential of MSC-EVs. These strategies can be broadly categorized into two main approaches: direct cargo incorporation into isolated EVs and genetic modification of parent MSCs. Direct cargo loading techniques, such as electroporation, sonication, and incubation, allow for the precise modulation of EV contents by incorporating bioactive factors [62–64,68,69]. While these methods offer flexibility in tailoring therapeutic payloads, they may be associated with transient cargo loading and potential batch-to-batch variability. Genetic modification of parent MSCs using gene-editing tools, such as CRISPR/Cas9, TALENs, and viral vectors, enables the alteration of MSC genomes [70,71]. This approach results in the secretion of EVs with modified native cargo and bioactivity, ensuring the consistent generation of customized EVs. However, it is important to consider the potential impact on genomic stability, as well as the technical complexities, risk of off-target effects, and possible alterations to cell function. The table also highlights emerging strategies for surface functionalization and targeting ligand conjugation, which aim to enhance the targeting specificity, uptake, and therapeutic efficacy of MSC-EVs [47,65–67,72,73]. Ultimately, the choice between direct cargo loading and genetic engineering depends on the specific treatment context and desired outcomes, with direct EV loading allowing for maximum cargo flexibility and genetic engineering offering consistent and tailored EV production, provided genomic stability is maintained.

2.2. Advanced gene-editing technologies for MSC-EV development

Gene-editing technologies, such as CRISPR/Cas9, zinc finger endonuclease (ZFN), megaTALs, and TALENs, have revolutionized the field of regenerative medicine by enabling precise genomic modifications in MSCs. These modifications significantly enhance the therapeutic potential of MSC-derived EVs for personalized wound healing treatments [70,71,74–78]. CRISPR/Cas9, known for its precision and ease of use, has emerged as a key player in this field. However, TALENs and ZFNs offer valuable alternatives in situations where CRISPR/Cas9 may encounter limitations due to technical or ethical constraints [68,79,80].

2.2.1. Enhancing MSC-EV efficacy through gene editing

Gene-editing strategies have been pivotal in augmenting the efficacy of MSC-EVs for chronic wound treatment. Elevating the expression of hypoxia-inducible factor 1 alpha in MSCs enhances VEGF production and promotes angiogenesis in ischemic wounds [61,81]. The inhibition of antiangiogenic factors, such as thrombospondin-1, further facilitates angiogenesis [82–84], whereas the upregulation of matrix metalloproteinases (MMPs), such as MMP-1 and MMP-9, facilitates the remodeling of the extracellular matrix, which is crucial for healing complex wounds [85,86].

Additionally, the manipulation of key signaling pathways, including calmodulin-dependent protein kinase II, ephrin-A3 signaling, mitogen-activated protein kinase, wingless (Wnt)/β-catenin, phosphatidylinositol 3-kinase/protein kinase B, Notch, transforming growth factor β (TGF-β)/Smad, STAT, and Hedgehog signaling, is crucial in regulating angiogenesis, cellular proliferation, and extracellular matrix remodeling during wound healing [87–92]. For instance, activation of the Wnt/β-catenin pathway enhances dermal fibroblast migration and endothelial cell activation. This can be achieved by overexpressing Wnt ligands or β-catenin in MSCs, thereby enriching MSC-EVs with Wnt agonists [93,94]. Similarly, modulating TGF-β signaling, particularly *via* the overexpression of TGF-β1, results in EVs enriched with collagen and elastin, which are essential for matrix remodeling [82]. Introduction of the Notch intracellular domain activates transcriptional regulators that pack EVs with proangiogenic cytokines and chemokines, such as IL-6, underscoring the potential of these

Table 1
Comparison between various extracellular vesicle engineering approaches.

Approach	Method examples	Benefits	Limitations	References
Direct cargo loading	Electroporation, co-incubation, freeze-thaw, and sonication	- Precise tuning of EV cargo - Flexible incorporation of various molecules	- Transient cargo loading - Batch-to-batch variability	[62,63]
Parent cell engineering	CRISPR/Cas9, TALENs, ZFNs, and viral vectors	- Genomic integration enables stable expression - Uniform EV production	- Technical complexities - Risk of off-target effects - Cell function alteration	[68,69]
Engineering cell culture conditions	Hypoxia, inflammatory stimuli, and growth factors	- Innate EV cargo and functional modulation	- Less precise control - Limited cargo options	[70]
Bioengineered scaffolds	Microfluidics and hydrogels	- Optimized EV growth and release	- Relatively new approach - Scaffold biocompatibility issues	[69,70]
Surface Functionalization	Targeting Peptides (e.g., RGD) Hydrophilic Polymers (e.g., PEG) Click Chemistry and Metabolic Labeling	– Improved targeting and uptake by specific cell types, enhanced delivery to wound site – Increased stability, reduced immune clearance, prolonged circulation time – Versatile and specific conjugation of targeting ligands or therapeutic molecules	– Potential immunogenicity, limited stability – Potential interference with EV-cell interactions – Potential toxicity of labeling agents, need for optimization	[47,65] [66,67] [72,73]
Targeting Ligand Conjugation	Antibodies Aptamers Affinity Tags (e.g., His-tag, Strep-tag)	– High specificity and affinity for target antigens, improved EV targeting – High specificity, low immunogenicity, easy to synthesize – Facilitate EV purification and characterization, enable conjugation of targeting moieties	– Potential immunogenicity, high production costs – Limited stability, potential interference with EV-cell interactions – Potential interference with EV function, limited in vivo applicability	[40,47]

engineered EVs in wound healing applications [94–96]. The JAK-STAT signaling pathway plays a pivotal role in mediating responses to cytokines and growth factors in chronic wounds. Persistent activation of this pathway, often triggered by inflammation, can impede wound regeneration [41,97,98]. A novel approach involves engineering MSCs to overexpress the suppressor of cytokine signaling 3 (SOCS3), an intrinsic inhibitor of JAK-STAT signaling. The resulting EVs carry SOCS3 mRNA, which, upon uptake by wound-associated cells, such as macrophages, inhibits STAT binding and reduces the transcription of proinflammatory genes [99,100]. MSC-EVs engineered to carry SOCS3 have been shown to enhance the presence of factors aiding in wound resolution like insulin-like growth factor 1, demonstrating their potential in modulating cellular responses within wound environments [100].

2.3. Optimizing genomic stability of MSCs

Maintaining the genomic stability of MSCs is crucial for the production of safe and effective MSC-EVs. Genomic instability in parent MSCs can lead to the incorporation of abnormal or potentially harmful genetic material into the derived EVs, which may compromise their therapeutic efficacy and safety [101]. Therefore, it is essential to employ strategies that minimize genetic alterations during MSC culture and EV production processes.

Factors such as prolonged ex vivo expansion, suboptimal culture conditions, and exposure to stress-inducing agents can contribute to genomic instability in MSCs [102,103]. For instance, high-glucose culture conditions have been shown to increase the frequency of chromosomal aberrations in MSCs, which may be transferred to the derived EVs [104]. Similarly, the use of serum-containing media or the presence of mycoplasma contamination can induce DNA damage and genomic alterations in MSCs, potentially affecting the safety and efficacy of the resulting EV products [105].

Emerging strategies, such as optimized hypoxic culture conditions, have shown promise in minimizing stress-induced genetic alterations and preserving genomic fidelity [106]. To mitigate the risks associated with genomic instability, it is essential to implement strict quality control measures and adhere to best practices in MSC culture and EV production. This includes the use of low-passage MSCs, regular screening for mycoplasma contamination, and the implementation of standardized protocols for cell expansion and EV isolation [105]. Additionally, emerging strategies such as the use of hypoxic culture conditions or the supplementation of culture media with antioxidants have shown promise in reducing stress-induced genetic alterations and preserving genomic stability in MSCs [104,106].

Sophisticated tools, like whole-genome sequencing and comparative genomic hybridization, play a critical role in identifying and mitigating risks associated with genomic instability. Ongoing research is dedicated to exploring the effects of external factors and culture methods on genomic integrity. This research aims to understand the precise conditions and their influence on genetic alterations [101,102,110]. Developing strategies to mitigate these risks, such as optimizing culture conditions and employing precise targeted gene editing, is essential for maintaining the therapeutic potential of MSCs while minimizing unintended effects [104,105].

Regular monitoring of MSC genomic stability through advanced techniques such as whole-genome sequencing and comparative genomic hybridization is crucial for ensuring the safety and quality of MSC-EV products [101]. By comprehensively characterizing the genomic profile of parent MSCs and their derived EVs, researchers can identify potential risks and implement appropriate measures to maintain genomic stability throughout the manufacturing process, ultimately leading to the development of safer and more effective MSC-EV therapies [102,103].

2.4. Enhancing MSC-EV therapies for wound healing

2.4.1. Genetic engineering of EV cargo

MSC-derived EV therapies harness the innate regenerative potential of MSCs, which is further refined through advanced biotechnology methods. Customizing the molecular cargo of EVs emerges as a promising strategy to fully unlock their therapeutic potential in wound healing. This process involves engineering EVs to transport bioactive molecules such as growth factors, cytokines, and RNA species [40,65,107,108]. By loading EVs with angiogenic factors like VEGF and platelet-derived growth factor (PDGF), angiogenesis and tissue regeneration are stimulated at wound sites, while the inclusion of antiinflammatory molecules effectively manages chronic inflammation [43].

Utilizing gene-editing tools like CRISPR/Cas9 allows precise genetic modifications in MSCs to fine-tune their EV cargo composition. By targeting specific pathways pivotal for wound healing, such as collagen synthesis and fibroblast activation, the cargo of MSC-EVs can be optimized to enhance wound closure and tissue remodeling [53,109,110]. Moreover, downregulating proinflammatory cytokines through gene editing can mitigate scar formation and improve healing outcomes.

2.4.2. Direct drug encapsulation into EVs

Although cellular processing enables drug expression in EVs, it has inherent limitations. Therefore, direct encapsulation of therapeutic drugs into EVs using techniques such as electroporation, co-extrusion, and ultrasonication has garnered attention. Electroporation involves the use of electrical pulses to create transient pores in natural EVs, facilitating direct loading of drug molecules. However, these electrical pulses may induce EV aggregation or fusion, potentially compromising their intercellular communication efficiency. Nonetheless, one study by Johnsen et al. [111] suggests the potential of this approach in promoting wound healing. In their study, they evaluated the effects of electroporation on the structural and functional properties of adipose-derived stem cell (ASC) exosomes. Despite observing some degree of EV aggregation and fusion, they found that electroporated ASC exosomes retained their ability to promote migration and proliferation of human dermal fibroblasts in vitro, which are key processes in wound healing. These findings suggest that electroporation-mediated drug loading into EVs may be a viable strategy for enhancing their therapeutic potential in wound healing applications, although further optimization is needed to minimize potential adverse effects on EV integrity and function.

2.4.3. Integrating EVs with biomaterial scaffolds

Most EV studies entail subcutaneous administration around the wound or wound bed. However, concerns arise regarding EV diffusion away from the target site, which may diminish their therapeutic effects. An emerging solution involves immobilizing EVs on biocompatible scaffold materials to ensure sustained delivery. Several animal studies have validated the efficacy of this scaffold engineering approach. For instance, Sun et al. [112] utilized a versatile nanoagent based on 2 dimensional reductive covalent organic frameworks coated with antibacterial immuno-engineered exosomes (PCOF@E-Exo) for efficient combination therapy in diabetic wounds. Chen et al. [113] loaded adipose-derived stem cell exosomes into Ag@bovine serum albumin nanoflowers, forming a protective “pollen-flower” structure encapsulated within an injectable collagen hydrogel for concurrent oxidative stress modulation and controlled EV release. Moreover, Zhang et al. [114] developed an MSC-exosome-encapsulated adjustable Polyvinyl alcohol hydrogel for treating diabetic ulcers.

2.5. Overcoming challenges in gene editing of MSC-EVs

Integrating gene editing into MSC-EV therapies presents challenges that must be addressed to fully leverage the potential of this technology in regenerative medicine. Balancing therapeutic efficacy with patient safety is a primary concern, given the risks of off-target effects and oncogenesis associated with gene-editing tools, such as CRISPR/Cas9. Strategies to enhance targeting specificity, such as developing high-fidelity CRISPR variants and improving guide RNA design, are actively pursued. Rigorous preclinical testing and safety profiling are essential to ensure that the benefits of genetically modified MSC-EVs outweigh the potential risks [51,55,110].

Technical challenges include maintaining stable gene expression in MSCs while preserving cell functionality and viability. Developing efficient and safe delivery systems for gene-editing components is crucial [77]. Furthermore, the ethical implications of gene editing in human cells, particularly in regenerative medicine, require careful consideration. Robust ethical guidelines and regulatory frameworks are necessary to address issues, such as informed consent, long-term effects, and the potential for unintended germline modifications [115–118].

2.6. Future directions and considerations for MSC-EV therapy development

The development of effective MSC-EV therapies for wound healing requires a multifaceted approach that integrates advances in gene editing, cargo engineering, and safety profiling. Refining CRISPR strategies to enhance the precision and efficiency of gene editing in MSCs is crucial for generating EVs with optimized therapeutic properties [119,120]. For instance, a recent report by Zhang et al. [121] demonstrated that MSC-EVs engineered to overexpress miR-126 using CRISPR/Cas9 technology exhibited enhanced proangiogenic effects and accelerated wound healing in a diabetic rat model. Additionally, combining gene editing with cargo engineering techniques, such as incorporating growth factors, chemokines, and miRNAs, can further enhance the therapeutic potential of MSC-EVs [52,61]. A recent study highlighted in Zheng et al. [122] discusses the functionalization of MSC-EVs through advanced strategies such as electroporation, which allows for the incorporation of key growth factors like VEGF and bFGF. These modified MSC-EVs have demonstrated enhanced proangiogenic effects and improved wound healing capabilities in a mouse model of cutaneous injury, illustrating the potential of tailored therapeutic interventions in regenerative medicine.

To facilitate the effective integration of therapeutic cargo into MSC-EVs, the use of advanced methods such as electroporation, sonication, and microfluidic devices is essential [63]. These techniques enable the efficient loading of desired molecules into EVs while minimizing potential adverse effects on their structural and functional integrity. For example, recent advancements have utilized microfluidic devices to enhance the loading of specific miRNAs into MSC-EVs [123]. This approach has been shown to enhance angiogenesis and accelerate wound healing in models of diabetic foot ulcers. Similarly, employing advanced delivery techniques such as sonication, researchers have successfully loaded MSC-EVs with therapeutic agents, including curcumin, demonstrating enhanced wound healing outcomes in mouse models of cutaneous injury [124].

As the field of MSC-EV therapy continues to evolve, it is crucial to address ethical and regulatory considerations to ensure the responsible development and clinical translation of these therapies. This includes obtaining informed consent from cell donors and patients, assessing the risks of off-target effects associated with gene editing, and ensuring equitable access to these therapies [116,118,125]. The International Society for Cell & Gene Therapy (ISCT) has recently published a position paper [125] that provides guidance on the ethical and regulatory considerations for the clinical translation of cell and gene therapies, including MSC-EV therapies.

Future research should focus on enhancing the precision of gene editing techniques, exploring the long-term stability and consistency of gene-edited MSC-EVs, and expanding the empirical evidence base to guide clinical applications. This will require interdisciplinary collaboration among researchers, clinicians, and regulatory bodies to establish standardized protocols, share best practices, and address knowledge gaps [126–128]. Initiatives such as the International Society for Extracellular Vesicles (ISEV) [126] and the ISCT [125] play a crucial role in fostering collaborative efforts and promoting the development of standardized guidelines for the production, characterization, and clinical application of MSC-EV therapies.

3. Manufacturing systems and quality control for MSC-derived EVs

The field of regenerative medicine is rapidly advancing toward the transformative realm of MSC-EVs. This shift from traditional cell therapy manufacturing methods requires innovative solutions to overcome unique bioprocessing challenges [129,130]. Groundbreaking technologies are crucial to align with the evolving landscape of therapeutic applications and unlock the full potential of MSC-EV therapies [127,131,132].

Although two-dimensional monolayer cell cultures have been historically relied upon, cGMP standards require adaptation to effectively navigate the intricate landscape of MSC-EV production. Downstream processes such as centrifugation, filtration, chromatography, and precipitation are essential for isolating vesicle components. Therefore, advanced and efficient techniques are necessary [59,133].

Scalable bioprocessing innovations are spearheading this crucial transition. Hollow-fiber perfusion and membrane-integrated bioreactors provide promising solutions for large-scale cGMP-compliant production, ensuring consistent quality and purity. These advancements are essential for unlocking the therapeutic potential of MSC-EVs [119,134,135].

Maintaining the functional integrity of EVs throughout the manufacturing process poses a significant challenge. Shear stress in suspension bioreactors and prolonged processing can have a negative impact on EVs, potentially compromising their potency and therapeutic efficacy [135–138]. To address this challenge, innovative enclosed platforms, such as the MCube system, have been developed to facilitate scalable cGMP-compliant production while minimizing the risk of EV damage [135,139].

Ensuring stringent quality control is crucial in the development and production of MSC-EV therapies. Comprehensive profiling of omics and rigorous characterization assays are essential to confirm the identity, purity, potency, and stability of EVs [59]. However, establishing standardized benchmarks for critical quality attributes remains challenging because of the inherent variability associated with upstream processes. Initiatives such as EV-TRACK play a crucial role in promoting collaborative endeavors to enhance best practices and establish robust field standards [140]. Harmonizing global regulatory pathways and approval processes is essential to ensure that MSC-EV therapies meet diverse regulatory requirements and are accessible to patients worldwide [29,141].

The development of commercially viable cGMP-grade MSC-EV platforms presents an intricate challenge. Business viability, process control, and product quality assurance all intertwine to create a complex landscape. Integrated solutions that facilitate a collaborative effort between industry and academia are crucial for establishing a balanced and sustainable approach. These partnerships play an essential role in translating the promising clinical potential of engineered MSC-EV therapies into scalable and readily available treatment options [29,138].

cGMP standards, enforced by regulatory agencies like the U.S. Food and Drug Administration (FDA), ensure the quality, safety, and consistency of pharmaceutical products, including biologics like MSC-EVs [48,52]. These regulations punctiliously govern every facet of the manufacturing process, from initial raw material acquisition to final product release [142]. Achieving cGMP compliance for MSC-EV production necessitates careful design, installation, and consistent maintenance of manufacturing facilities and equipment, coupled with rigorous testing and qualification of raw materials. Additionally, clearly defined, thoroughly validated, and consistently executed manufacturing processes are essential, alongside robust quality control and assurance systems for continuous monitoring throughout production. Finally, comprehensive documentation of all these aspects is mandatory to ensure adherence to cGMP requirements.

Maintaining cGMP compliance is paramount for guaranteeing the quality, safety, and consistency of MSC-EV therapies. Furthermore, it paves the way for regulatory approval and successful commercialization [143,144]. Several existing MSC-EV therapies serve as exemplary models for implementing these principles. The cGMP-compliant manufacturing process employed for an MSC-EV therapy targeting graft-versus-host disease exemplifies the critical role of quality control. The consistent product quality, demonstrably successful clinical outcomes, and ultimate market approval of this therapy underscore the effectiveness and reliability of this approach [134,145].

MSC-EV therapy for myocardial infarction uses advanced bioreactor systems for scalable and controlled production, leading to consistent clinical outcomes and regulatory compliance. In the context of neurodegenerative diseases, MSC-EV therapy integrates Quality by Design principles from early development stages, ensuring process fidelity, effective scale-up, and adherence to predefined product quality standards [146]. In the context of diabetic wound healing, MSC-EV therapy employs automated and closed-system manufacturing procedures to minimize human intervention and improve product sterility and consistency. This underscores the importance of technological advancements in optimizing production efficiency [147].

These examples underscore the critical roles of meticulous process design, thorough testing, and continuous monitoring in developing high-quality and safe MSC-EV therapies. Adhering to established best practices will be crucial in overcoming the challenges of transitioning from traditional MSC production to MSC-EV manufacturing in the evolving field of MSC-EV research. Through innovative strategies and collaborative efforts, the full potential of MSC-EV therapies can be realized, offering transformative solutions for a variety of medical conditions.

4. Toxicity risk assessment and preclinical profiling of MSC-EV therapies

4.1. MSC-EV heterogeneity and complementary platforms

The inherent variability of EVs derived from different MSC sources necessitates refined characterization methods. To gain a clear understanding of how these EVs impact chronic wound healing, it is crucial to accurately identify their subclasses using advanced techniques such as high-resolution flow cytometry, which analyzes particle characteristics at a microscopic level, and multiomics computational analysis, integrating various types of biological data such as genomics, proteomics, and transcriptomics [48,52]. Collaborative efforts with wound care specialists are key to developing scalable and potent assays that enable precise predictions of the diverse bioactivities underlying the clinical potential of MSC-EV treatments [142,148].

Humanized animal models and microfluidic organ chips are transforming MSC-EV research by replicating pathologically relevant wound environments with greater precision [52,124]. Humanized models excel at evaluating immunomodulatory and tissue regenerative dynamics within complex host stromal contexts, while organ chips offer platforms for detailed, high-throughput experimental analyses [52,123]. Integrating these innovative platforms into a standardized workflow significantly improves the predictive validity of preclinical data, ultimately guiding effective translational efforts [52,123].

4.2. Refined animal models and biodistribution assessment of MSC-EVs

Developing clinically relevant animal models is crucial for bridging the gap between preclinical studies and human clinical trials in wound healing research. However, current animal models often fall short of accurately reflecting the complexities of human wound conditions, posing challenges in extrapolating findings to clinical settings [149,150]. Incorporating aspects of wound infection and chronicity into animal models enhances their clinical relevance. Chronic wounds, characterized by persistent inflammation and impaired healing, frequently involve microbial colonization or infection [150,151]. Modeling these aspects in animal studies offers valuable insights into wound pathophysiology and therapeutic responses that more closely mimic clinical scenarios [150,151].

Strategically optimizing species and strains for specific wound types, combined with advanced bioanalytical techniques, reconciles ethical considerations with the need for relevance to human trials [152]. Adhering to the principles of replacement, reduction, and refinement in animal research, as overseen by institutional animal care and use committees, ensures ethical conduct while maximizing scientific value. Employing a comprehensive panel of complementary models that encompasses a wider range of wound severities can yield more integrated and comprehensive insights [153].

Regarding in vivo evaluations of human-derived MSC-EVs, robust evidence has not yet demonstrated significant advantages associated with specific animal strains, ages, or genders. Among the available models, the nude mouse model, which involves thymectomy, might help mitigate the potential adverse effects of thymus-derived T lymphocytes on wound healing. However, this model lacks congruence with clinical conditions, as the immunodeficient state may influence wound healing dynamics. Insufficient experimental evidence supports its advantages for MSC-EV research, particularly in the context of diabetic wound healing [143]. Alternative immunodeficient models, such as SCID (Severe Combined Immunodeficiency) mice or NOD/SCID (Non-Obese Diabetic/SCID) mice, could potentially offer advantages, but further research is needed to evaluate their suitability for MSC-EV-based wound healing studies.

Table 2 summarizes key considerations for selecting appropriate animal models for preclinical MSC-EV research, including established methods for modeling type 1 and type 2 diabetes mellitus in rodents, which is crucial for evaluating the efficacy of MSC-EV

therapies in diabetic wound healing scenarios. The table delineates specific criteria to guide model selection based on wound type, research objectives, and the need for diabetes modeling [144,154,155].

The refinement of animal models enables more accurate evaluation of biodistribution and targeting of MSC-EVs [156]. Advanced imaging techniques, such as radiolabeling and fluorescence tracking, are essential for mapping the in vivo distribution of MSC-EVs. This helps predict potential accumulation in nontarget tissues and facilitates the assessment of biodistribution [157,158]. This information is crucial for understanding the systemic effects following administration and anticipating any potential off-target effects.

4.3. Comprehensive toxicological evaluation of MSC-EVs

The complex pharmacokinetic and pharmacodynamic characteristics of MSC-EVs are intricately linked to their biological nature and multifaceted modes of action. Preclinical research endeavors to unravel these profiles, guiding the development of effective therapeutic dosing strategies and administration schedules and routes for future clinical trials [159,160].

Due to the distinct characteristics of MSC-EVs, custom assays are required to assess their toxicity. These assays analyze acute, subacute, and chronic effects, taking into account the unique bioactive molecule and membrane composition of MSC-EVs [161]. This tailored approach is essential for establishing a comprehensive safety profile for MSC-EVs. Advanced in vitro models, such as 3D cell cultures and organ-on-a-chip systems, can provide more physiologically relevant platforms for toxicological evaluation of MSC-EVs [162,163].

The dose-response relationship of MSC-EVs is nonlinear and variable, influenced by factors such as EV origin, production techniques, and specific patient traits [164]. Conducting a thorough assessment of preclinical studies is essential for identifying the most effective therapeutic dosage, which can then be translated into well-defined clinical dosing regimens [165,166]. The choice of administration routes in clinical practice greatly impacts the effectiveness and safety of therapy. Preclinical studies should evaluate various administration routes, such as intravenous, subcutaneous, and topical, to determine the optimal approach for specific wound types [167].

Ensuring the stability and shelf life of MSC-EVs is critical for their clinical application. Rigorous preclinical evaluations are conducted to verify that EVs retain their therapeutic efficacy and quality across various storage conditions and durations [59]. Optimizing storage conditions, such as temperature, pH, and buffer composition, is crucial for maintaining the integrity and functionality of MSC-EVs [168].

4.4. Translational challenges and global harmonization of MSC-EV therapies

Transitioning from preclinical research to clinical applications for MSC-EV therapies poses various challenges [169]. Establishing standardized and customized evaluation protocols is essential to overcoming these obstacles. Advancing MSC-EV therapies into clinical trials with a clearly defined safety profile, ensuring both efficacy and safety in clinical settings, necessitates a deep understanding of the unique biological characteristics of EVs. Continuous research and innovation are essential for fully realizing the therapeutic potential of MSC-EVs.

Organizations like the International Society for Extracellular Vesicles and the International Society for Cell & Gene Therapy are crucial in advancing consistent and collaborative techniques for

Table 2
Summary of common animal models used in preclinical MSC-EV studies, with emphasis on diabetes modeling.

Model	Characteristics	Advantages	Disadvantages	Wound-healing applications	Diabetes Modeling Methods	Reference
Mouse	- High genetic similarity to humans - Well-characterized strains - Low cost	- Wide reagent availability - Established protocols - Ease of handling	- Small size limits treatments - Varied skin healing mechanisms	Excisional and diabetic wounds - Graft-versus-host disease - Radiation skin injury	Streptozotocin injection for T1DM modeling High-fat diet and low-dose streptozotocin for T2DM modeling Spontaneous db/db mice for T2DM	[144] [63]
Rat	- Greater wound size capacity - Established skin models	- Excisional, ischemic, and pressure ulcers - Similar healing stages to human wounds	- Limited skin grafting studies	- Burn injuries - Full-thickness excisional wounds - Chronic infected wounds	Streptozotocin injection for T1DM modeling High-fat diet and streptozotocin for T2DM modeling	[144] [154,155]
Rabbit	- Larger wound capacity - Thick dermis layer similar to that in humans	- Easy handling - Well-defined surgical protocols	- Higher cost than rodents - Behavioral constraints	- Excisional and ischemic wounds - Partial and full-thickness burns - Cutaneous radiation injury	Specific methods not well established	[154,155]
Pig	- Nearly identical skin architecture and healing - Similar subcutaneous fat	- Various debridement techniques - Split and full-thickness skin grafting - Bandaging/biopsy evaluation	- Higher housing costs - Increased staffing requirements	- Excisional, burn, diabetic, and ischemic ulcers - Venous/pressure/arterial ulcers - Topical treatment testing	Specific methods not well established	[154,155]
Dog	- Similar healing stages to humans - Large treatment areas	- Topical evaluation with bandaging - Easy infection induction	- Ethical concerns in some regions - Cost and space considerations	- Thermal/radiation injuries - Large open contaminated wounds - Antimicrobial efficacy	Specific methods not well established	[155]
Equine	- Chronic laminitis models - Similar subcutaneous adipose tissue	- Extensive skin for grafting - Evaluation of pain/infection	- Housing requirements - Staffing/procedure costs	- Hypergranulation injuries - Graft integration studies	Specific methods not well established	[155]

Diabetic, db; Diabetes Mellitus, DM; Type I diabetes, T1DM; Type II diabetes, T2DM.

cellular therapy research and development [126,128]. Establishing standardized guidelines for preclinical testing of MSC-EV therapies, including toxicity assessment, biodistribution studies, and potency assays, is essential for global harmonization. Engaging in constructive discussions with regulatory agencies is important for researchers to simplify the approval process and uphold stringent safety and ethical standards, particularly across various global regulatory environments [170,171]. Patient advocacy organizations are also vital in supporting standardized guidelines and research strategies, promoting a patient-centered perspective in the development of MSC-EV therapy [125].

By addressing the challenges associated with the heterogeneity of MSC-EVs, refining animal models, employing advanced analytical techniques, and fostering collaborative efforts for global harmonization, researchers can generate more robust and clinically relevant preclinical data to support the successful translation of MSC-EV therapies for wound healing applications.

5. Clinical translation and the regulatory landscape for engineered EV therapies

5.1. Lessons from cell therapies and advantages of EV-based approaches

The field of engineered EV therapies is evolving by leveraging insights from established cell therapy techniques, both autologous and allogeneic. Notably, autologous cell therapies like chimeric antigen receptor (CAR) T-cell treatments have shown high success rates in treating certain cancers but are constrained by significant costs and complex manufacturing processes [172,173]. These challenges underscore the need for more scalable and cost-effective alternatives such as allogeneic and acellular therapies.

Allogeneic MSC therapies, for instance, are promising but face commercialization bottlenecks exemplified by high production costs and logistical complexities seen in therapies like TEMCELL HS Inj [173,174]. These challenges underscore the need for innovative manufacturing and delivery strategies to improve patient access to such therapies.

Engineered EVs offer several advantages over traditional cell therapies, including greater stability, scalability, and reduced immunogenicity [109,175]. However, they also share some common challenges with cell therapies, such as ensuring batch-to-batch consistency and developing reliable potency tests [176]. Addressing these challenges necessitates the development of standardized manufacturing processes and robust quality control measures specifically tailored for EV therapies [177].

5.2. Navigating the global regulatory landscape and harmonization efforts

The regulatory landscape for EV therapies is complex and varies significantly across regions, impacting their development and clinical use. In the United States, the Food and Drug Administration (FDA) classifies EVs as biological products, subjecting them to stringent regulations, such as adherence to cGMP guidelines [178]. In contrast, the European Union employs a different framework, categorizing them as advanced therapy medicinal products, which necessitates distinct approaches for development and clinical trials.

Navigating these regulatory differences demands a deep understanding of each jurisdiction's specific requirements and the formulation of tailored strategies for product development and clinical trials [178]. Furthermore, global harmonization of regulatory standards spearheaded by organizations like the World Health Organization and the International Council for Harmonization is crucial. These efforts aim to standardize safety, quality, and efficacy evaluations for EV therapies worldwide, facilitating their global adoption [179].

5.3. Advancing preclinical and clinical development of EV therapies

Developing reliable potency assays and standardized immunogenicity models is essential for the successful progression of EV therapies [156]. Robust in vitro and in vivo assays that can accurately predict the therapeutic efficacy and safety of EV therapies are crucial for their clinical translation. Additionally, research using large-scale datasets and organoid systems is providing valuable insights into biodistribution and bioactivity; however, the development of scalable and representative assays remains critical for ensuring clinical reliability and safety [180].

The growing interest in EV therapies for wound healing has ignited several clinical trials, albeit in their early phases. These trials underscore the potential of EVs in therapeutic applications. Addressing challenges such as demonstrating efficacy, ensuring consistent product quality, and managing potential immune responses or toxicity arising from EV content heterogeneity is essential for advancing these therapies.

5.4. Future directions and collaborative efforts for clinical translation

Successful clinical translation of EV therapies for wound healing and other applications necessitates a multifaceted approach. This includes developing standardized, scalable, and GMP-compliant processes. Additionally, robust characterization techniques and quality control measures are essential to ensure product consistency and safety. Well-designed clinical trials are necessary to evaluate the efficacy, safety, and long-term effects of EV therapies across diverse patient populations [181,182].

Collaboration among researchers, clinicians, regulatory bodies, and industry partners is paramount to accelerate the development and commercialization of EV therapies. Engaging with patient advocacy groups and other stakeholders ensures ethical and responsible development, focusing on patient access and affordability [183].

By addressing these challenges and pursuing collaborative efforts, the field of EV therapies can progress towards providing safe, effective, and accessible treatments for patients in need. Standardized protocols, best practices, and knowledge sharing are crucial for advancing the development and commercialization of these promising therapies.

6. Conclusion and future directions

eMSC-EVs represent a significant advancement in chronic wound treatment, offering a transformative shift from passive dressings to targeted regenerative therapies [148]. This multidisciplinary field leverages the regenerative potential of stem cells within a stable, bioengineered nanocarrier platform, scrupulously designed to promote healing in non-healing wounds. As millions grapple with chronic wounds, this emerging field holds immense promise to revolutionize patient care and improve quality of life [184,185].

Realizing this potential necessitates ongoing innovation at the intersection of bioengineering, cell biology, and translational medicine. CRISPR technology allows for precise modification of MSC genomes, paving the way for next-generation therapeutic EVs selectively enriched with regenerative molecules like anti-inflammatory cytokines, proangiogenic factors, and miRNA regulators of healing pathways [186,187]. Concurrently, advancements

in microfluidic manufacturing platforms for large-scale GMP production ensure consistent quality control of these complex biologics [66,123]. Furthermore, integrating advanced analytical tools like AI-powered proteomics and high-resolution flow cytometry facilitates comprehensive molecular profiling and functional optimization of engineered EV therapies [67,188-190].

Critical to expediting clinical translation is a conscientious focus on patient safety. Rigorous preclinical assessments elucidating potential risks, including immunogenicity, tumorigenicity, off-target effects, and biodistribution profiles, are essential before human trials [191,192]. Simultaneously, innovative collaborative frameworks combining regulatory science, bioengineering innovations, regenerative medicine insights, and patient advocacy are essential for harmonizing global standards and expediting a streamlined pathway for engineered EV therapies to reach patients worldwide [169].

Exosome surface engineering and targeting represent a promising strategy to enhance the therapeutic potential of MSC-EVs for wound healing. This approach holds great promise for clinical translation by improving targeting specificity, bioavailability, and overall efficacy of MSC-EV therapies. However, continued research and development, along with interdisciplinary collaborations, are crucial to fully harness this potential.

Looking ahead, the potential of stem cell-derived EVs extends far beyond chronic wounds, potentially catalyzing a new era of tissue restoration across regenerative medicine. These biomimetic nanotherapies encapsulate the restorative capacity of stem cells in a readily available modality, offering promise in revitalizing damaged organs, alleviating neuropathic pain, and reducing human suffering globally [184]. Driven by patient-centric innovation, continuous collaboration, and profound biological insights, this nascent field holds immense promise for restoring lives with the healing power of our own cells.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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