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Precision exosome engineering for enhanced wound healing and scar revision

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Abstract

The dysfunction of wound-healing processes can result in chronic non-healing wounds and pathological scar formation. Current treatment options often fall short, necessitating innovative approaches. Exosomes, extracellular vesicles secreted by various cells, have emerged as promising therapeutic agents serving as an intercellular communication system. By engineering exosomes, their cargo and surface properties can be tailored to enhance therapeutic efficacy and specificity. Engineered exosomes (eExo) are emerging as a favorable tool for treating non-healing wounds and pathological scars. In this review, we delve into the underlying mechanisms of non-healing wounds and pathological scars, outline the current state of engineering strategies, and explore the clinical potential of eExo based on preclinical and clinical studies. In addition, we address the current challenges and future research directions, including standardization, safety and efficacy assessments, and potential immune responses. In conclusion, eExo hold great promise as a novel therapeutic approach for non-healing wounds and non-healing wounds and pathological scars. Further research and clinical trials are warranted to translate preclinical findings into effective clinical treatments.

Keywords Engineered exosome, Chronic non-healing wound, Pathological scar

Introduction

Non-healing wounds, characterized by a failure to progress through the normal healing cascade, remain open for prolonged periods. Pathological scars, representing aberrant scar formation during the wound healing process, can manifest as various forms, often causing patient discomfort through pain, pruritus (itching), and erythema (redness). However, current therapeutic approaches are often limited in efficacy, and the treatment outcome remains suboptimal due to the side effects.

The past few decades have witnessed large-scale development of cytotherapy along with emerging acellular therapy. The development of engineered exosomes (eExo) with specific "4-pro" and "5-anti" effects that promote skin regeneration, becomes an active area of research. This review provides a comprehensive overview of recent research of eExo for chronic wounds and pathological scarring. The engineering strategies and the targeted biological activities of eExo are shown in Fig. 1.

Physiological wound healing

Physical wound healing is achieved through four precisely programmed and overlapping phases, and

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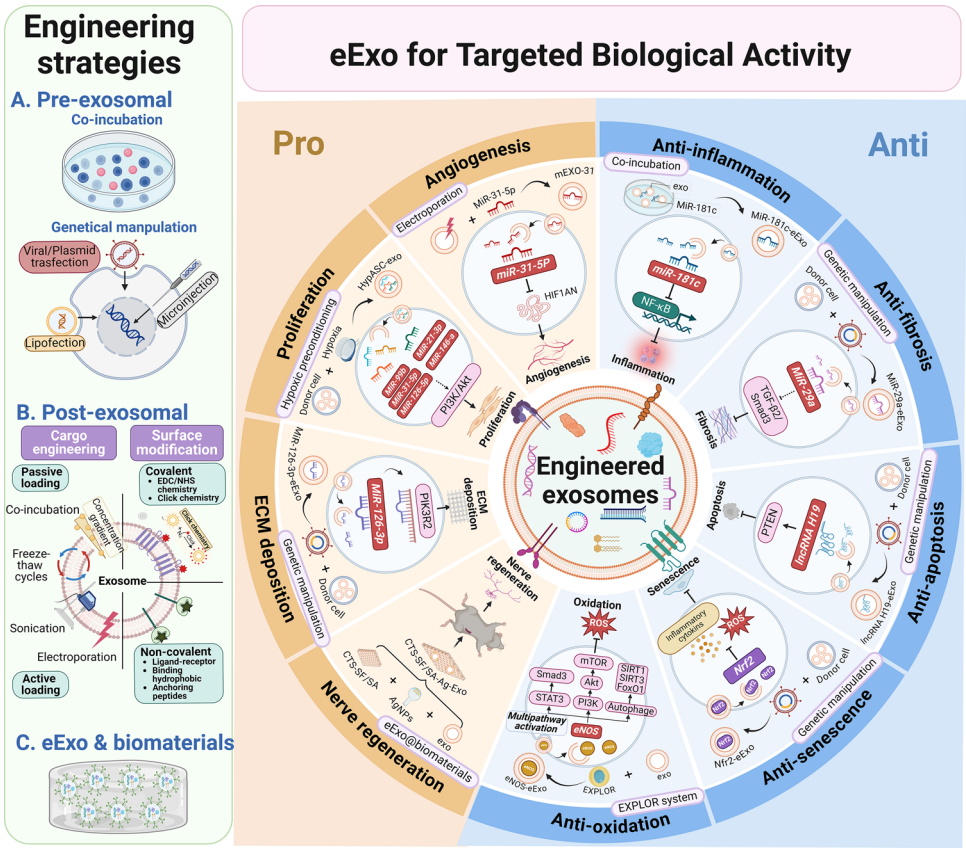

Fig. 1 A comprehensive overview of eExo for chronic wounds and pathological scarring. The engineering strategies of eExo comprise pre-exosomal, post-exosomal and biohybrid approaches. The aim of engineering strategies is to endow eExo with targeted biological activities, including "4-pro" and "5-anti" effects. "4-pro" effects refer to promoting angiogenesis, cell proliferation, ECM deposition, and nerve regeneration, while "5-anti" effects refer to anti-inflammation, anti-fibrosis, anti-apoptosis, anti-senescence, and anti-oxidation. Abbreviation: eExo, engineered exosomes

ultimately ended with normal scarring [1–3]. (1) Hemostasis phase involves blood vessel damage, hemostasis, vascular constriction, platelet activation, and fibrin clot formation. [4] (2) Inflammation phase includes neutrophil/monocyte recruitment, macrophage differentiation, chemokine/pro-inflammatory cytokine releasing, and downstream inflammatory pathway activation [5–7]. (3) Proliferation phase comprises the activation of multiple cell types (keratinocytes, fibroblasts, endothelial cells), angiogenesis, granulation tissue formation, re-epithelialization, and extracellular matrix (ECM) deposition, ultimately leading to wound closure. [4, 7–9] (4) Resolution/remodeling phase contains neovasculature regression, periodic ECM deposition, granulation tissue reconstitution, and mature scar formation (Fig. 2). [4, 10–15]

Outcomes of aberrant wound healing

When the normal healing process goes wary there are two major outcomes: an ulcerative skin defect (chronic wound) or pathological scar formation.

Chronic wounds

Chronic non-healing wounds—hereinafter referred to as chronic wounds—are defined as wounds that have "failed to proceed through a normal, orderly and timely process to establish anatomic and functional integrity" within 3 months after injury. [16, 17] Any skin lesion has the potential to become a chronic wound. According to the etiology, chronic wounds are mainly classified into ulcers of diabetic, vascular, and pressure, despite wound-specific factors, including infection, inflammation, oxygenation, radiation, and aging [18–20]. (1) Diabetic ulcers: hyperglycemia in patients with diabetes lead can lead to an increase in the production of reactive oxygen species (ROS), thereby exacerbating inflammation and oxidative stress, thus forming a vicious circle and eventually leading to difficulty healing wounds. [21] (2) Vascular ulcers: include arterial ulcers (caused by poor blood supply) and venous ulcers (mainly due to venous insufficiency). [22–24] (3) Pressure ulcers: also known as a bedsore or decubitus ulcers, are caused by sustained pressure over

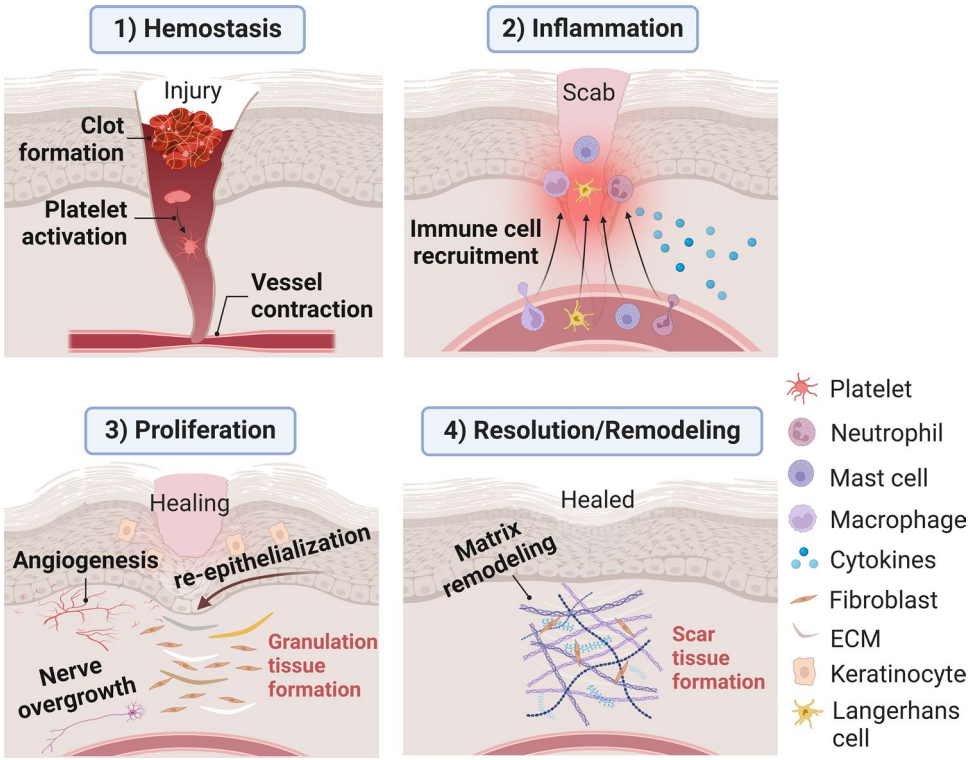

Fig. 2 Four phases of physiological wound healing. (1) Hemostasis includes vasoconstriction, clot formation, and provisional matrix formation. (2) Inflammation comprises immune cell recruitment and adaptive immune system activation. (3) Proliferation involves the cooperation of fibroblasts, keratinocytes, and endothelial cells towards wound closure. (4) Resolution/remodeling includes the reconstruction of cellular components and ECM composition

a bony prominence, ultimately leading to tissue ischemia and necrosis [25].

Different categories of chronic wounds share several common molecular and pathological characteristics. The hallmarks of chronic wounds are prolonged inflammation and inability to re-epithelialize [26–28]. Compared to acute wounds, chronic wounds are characterized by chronic inflammation, excessive ROS, impaired angiogenesis, slowed cell proliferation, delayed ECM remodeling, and elevated alkaline environment (Fig. 3). [18, 19, 21]

Pathological scarring

Another consequence of aberrant skin regeneration is pathological scarring, including keloid and hypertrophic scarring (HS). [2, 29, 30] Excessive scar tissue formation can impact life quality and lead to various complications. Pathological scars are caused by abnormal proliferation and transdifferentiation of fibroblasts, excessive ECM deposition, and chronic inflammation. [7, 31, 32] Although the exact mechanisms of pathological scar formation are not fully elucidated, the triggers are identified as immune dysregulation, mechanical stress, and hypoxia [33]. (1) Immune dysregulation: the activation of the pro-inflammatory signaling pathways of nuclear factor κB (NF-κB) and anti-inflammatory transforming growth factor β (TGF-β1) has been evidenced to increase in pathological tissues [34–37]. (2) Mechanical forces: skin sites subjected to high mechanical tension exhibit a high propensity for pathological scar formation [38]. Dohi et al. [38] and Gao et al. [39] demonstrated that keloid progression was correlated with increased mechanical strain via the Caveolin-1/Rho-associated protein kinase (ROCK) signaling pathway and YES-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) activity, respectively. (3) Hypoxia: hypoxia can promote activity and inhibit apoptosis of keloid fibroblasts [40]. Specifically, the mitochondrial function and potentiate glycolysis of keloids are attenuated under hypoxic conditions, with the upregulation of hypoxia-inducible factor-1alpha (HIF1α) and phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway [40]. The involved signaling pathways are depicted in Fig. 4.

Current therapies for chronic wounds and pathological scarring: advantages and limitations

Chronic wounds can be painful, debilitating, and even life-threatening if left untreated. As these wounds exhibit

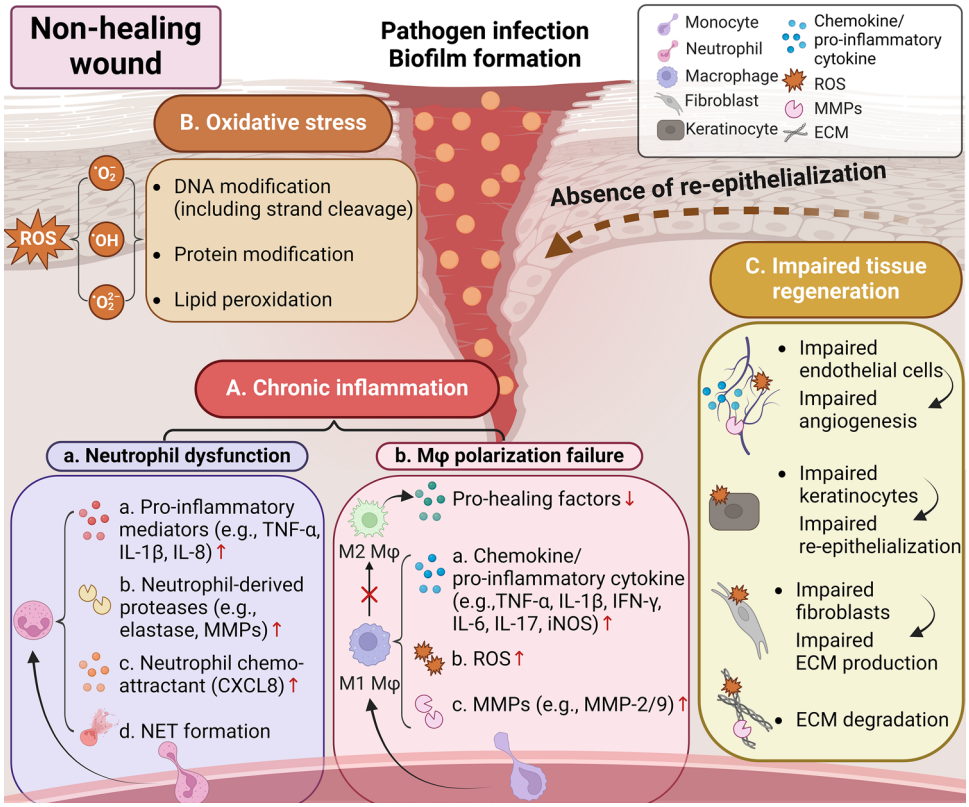

Fig. 3 Pathophysiology of non-healing wounds. Non-healing wounds are characterized by chronic inflammation, excessive oxidative stress, and impaired tissue regeneration. **A** Chronic inflammation is mainly driven by neutrophil dysfunction and macrophage (Mφ) polarization failure. Specifically, neutrophil dysfunction causes the elevation of pro-inflammatory mediators, neutrophil-driven proteases, and neutrophil chemo-attractant and neutrophil extracellular traps (NET) formation. M1/M2 macrophage alteration failure can result in elevated level of chemokines/pro-inflammatory cytokines, reactive oxygen species (ROS), and matrix metalloproteinases (MMPs), and decreased level of pro-healing factors. **B** Excessive oxidative stress can generate the modification of DNAs, proteins and lipids. **C** Impaired tissue regeneration is a result of impaired angiogenesis, re-epithelialization, and ECM production, and increased ECM degradation, caused by pro-inflammatory cytokines, ROS, and MMPs

impaired healing processes, characterized by persistent inflammation, excessive tissue breakdown, and inadequate angiogenesis, current therapeutic approaches include debridement, offloading, and topical or systemic medications to address specific underlying factors. Nevertheless, many patients still require long-term care and may undergo surgical interventions or skin grafting. However, current treatments are often limited in effectiveness despite advancements in wound care. Similarly, despite the diverse range of therapeutic options (non-surgical and surgical treatments) available for pathological scarring, the treatment outcome remains suboptimal due to the side effects and high recurrence rate. Thus, the explorations of efficient and safe novel therapeutic approaches and strategies for chronic wounds and pathological scarring constitute the focus of current research [41].

The past few decades have witnessed large-scale development of cytotherapy along with emerging acellular therapy. The development of innovative strategies, such as eExo with specific anti-inflammatory, pro-angiogenic, and anti-scarring inhibition effects that promote skin regeneration, becomes an active area of research.

Cytotherapy

The earliest attempts at cell-based therapy are attributed to Charles-Edward Brown-Séquard, the pioneer in the nineteenth century who sought to delay the senescence process in his contemporaries by injecting animal testicle extracts. [42] Afterward, Paul Niehans democratized cytotherapy in Western Switzerland in the twentieth century [43]. Cytotherapy can also be applied to acute and chronic wounds, as well as keloids and HS, potentially improving wound healing without surgical operations or donor area damage [44, 45].

A distinct advantage of stem cell-based cytotherapy is its high regenerative potential, as it can directly replace damaged skin cells. However, safety concerns, logistic

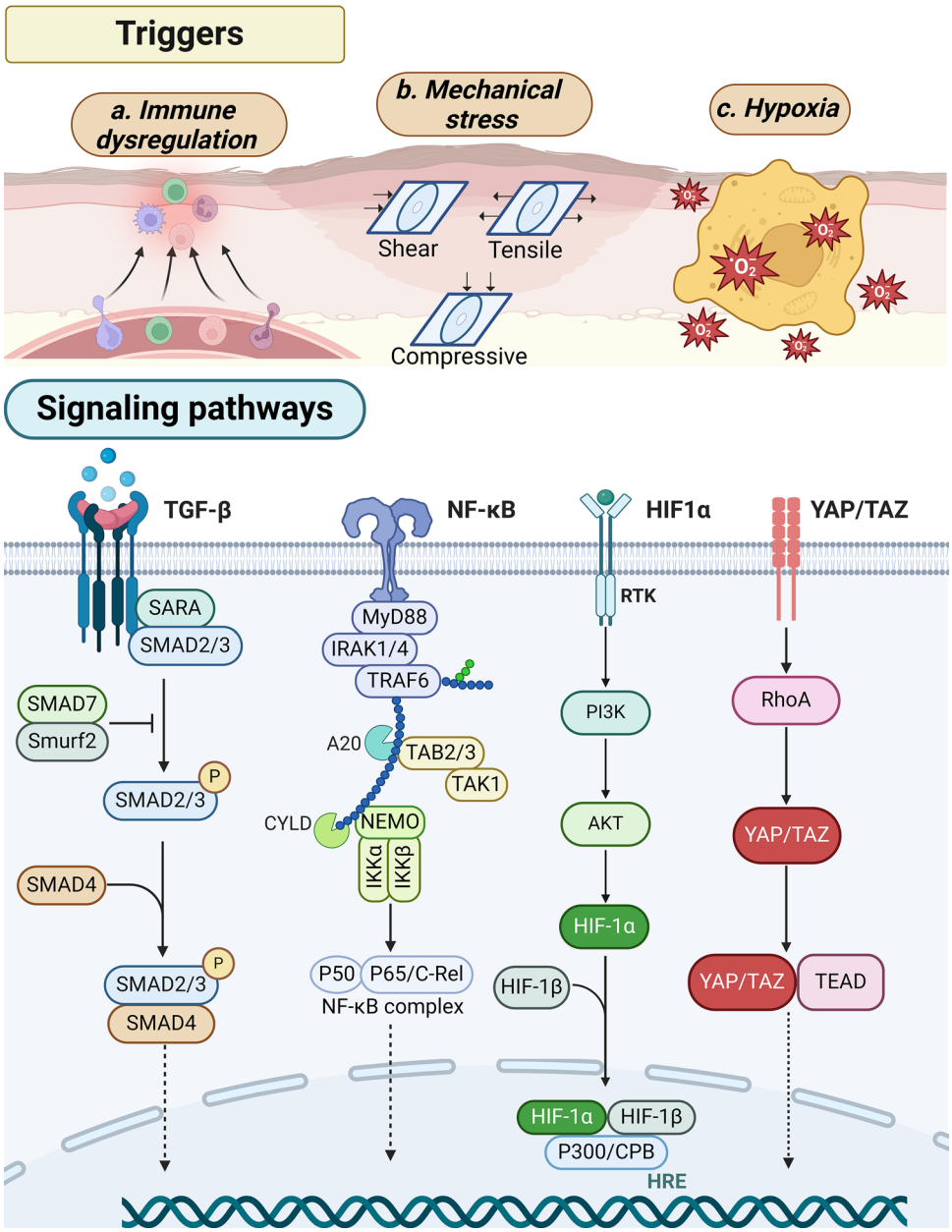

Fig. 4 Triggers and pathogenesis for pathological scar formation. Upper figure: the dominant triggers for pathological scar formation are immune dysregulation, mechanical tension, and hypoxia. Lower figure: the mainstream pathways driving pathological scar formation include the TGF-β, NF-κB, HIF1α, and YAP/TAZ signaling pathways. Abbreviations: TGF-β, transforming growth factor beta; NF-κB, nuclear factor-kappaB; HIF1α, hypoxia-inducible factor-1alpha; YAP, YES-associated protein; TAZ, transcriptional coactivator with PDZ-binding motif

challenges, and ethical considerations have hindered the widespread use of stem cell-based cytotherapies. Another consideration for cell-based therapy is donor age. Research has demonstrated that autologous cells are less likely to trigger immune rejection, making them the preferred choice for cytotherapy [46]. However, the therapeutic effect of autologous cells from older patients remains questionable, as donor age negatively affects cell function [47, 48]. As such, a safer, simpler, noninvasive, and more efficient method for wound healing and scar management is required.

Acellular therapies

Acellular therapy utilizes cellular components, such as conditioned media (CM), extracellular vesicles (EV), ECM or growth factors, without the presence of living

cells. These components can be used for skin regeneration and scar hyperplasia. [49–51]

From CM to EV Advancements in stem cell research have led to the discovery that CM from stem cells also promotes wound healing, [52, 53] even scarless wound healing [54, 55]. In addition, CM can be used for combined therapy of HS [49, 56, 57], and is a potential keloid treatment as it can prevent the proliferation and activation of keloid fibroblasts. [58] These findings indicate that the cell secretome might be an alternative for skin regeneration and scar treatment, shifting the focus from cellular to non-cellular therapies [59]. The cell secretome contains an extensive spectrum of soluble factors, including cytokines, growth factors, and microRNAs (miRNA), which are easily digested by enzymes, raising the question of whether another functional component exists in CM. [60]

The discovery of EV in CM marked a new era of acellular therapy (Fig. 5). EV, secreted by cells into the extracellular space, are lipid bilayer-delimited cargo-bearing vesicles. [61] The unique structure of EV protects the inner cargos from digestion. [62] They were first discovered by Chargaff and West in the 1940 s, who found that a "particulate fraction" sedimented at 31,000 g had a high clotting potential. [63, 64] The understanding of EV was stalled at the structural level for years until Raposo proved that EV were biologically functional in the 1990 s [65]. In 2010 s, Bruno et al. [66] reported that mesenchymal stem cells (MSC)-derived vesicles suppress acute tubular injury in mice, lifting the curtain of increased interest in EV-based therapy. Since then, studies on EV in regenerative medicine have increased exponentially, and EV-based therapeutics have been proven effective in the treatment of chronic wounds and skin scarring [67–69].

From EV to natural exosomes Exosomes are EV that originate from the endosomal system within cells and are typically between 30 and 150 nm in diameter. Exosomes have several advantages over other EV: they have a uniform size and structure, and their specific surface proteins may allow for some degree of targeting of specific tissues. The key differences of exosomes and EV are summarized in Table 1.

Compared with cytotherapy, natural exosomes possess distinct advantages, such as easy access, effortless storage, safety (non-immunogenicity, non-tumorigenicity), biocompatibility, specificity, and freedom from ethical issues (Table 2) [70].

Existing evidence suggest that exosomes play a role in intercellular communication through three mechanisms. (1) Ligand-receptor interaction: the exosomes dock on the membrane of recipient cell and bind to the surface receptors, subsequently triggering intracellular signaling. (2) Endocytosis: the internalization of exosomes can be conducted through micropinocytosis, caveolin-mediated endocytosis, lipid raft-mediated endocytosis, and phagocytosis. Afterwards, the exosomes become early endosome and undergo fast recycling (re-releasing), slow recycling (becoming MVB) or performing corresponding functions. (3) Membrane fusion: the membrane of exosomes can directly fuse with the recipient cell

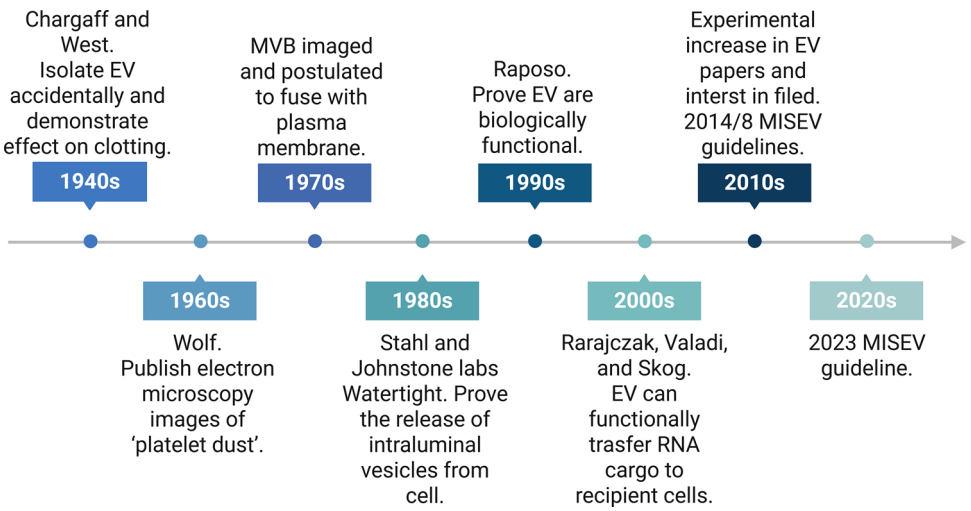

Fig. 5 Timeline for EV milestones. In 1946, Chargaff and West discovered a "particulate fraction" with high clotting potential, which marked the discovery of extracellular vesicles (EV). In the early twenty-first century, the interest in EV grew exponentially. In 2014, the International Society of Extracellular Vesicles (ISEV) proposed the Minimal Information for Studies of Extracellular Vesicles (MISEV) guideline and updated the guideline in 2018 and 2023

Table 1 Exosome *vs.* EV for therapeutic applications

Feature	Exosomes	EV
Subtypes	Specific type of EV with well-defined characteristics	Diverse population including exosomes, microvesicles, apoptotic bodies
Cargo	Well-defined and enriched in specific molecules (proteins, RNA)	More heterogeneous cargo depending on the source cell
Targeting	Can be engineered to target specific tissues (research in progress)	Targeting strategies are under development for EV in general
Production	Can be easier to produce in large quantities (especially from immortalized cells)	May be more challenging to isolate and purify specific types of EV
Standardization	More standardized isolation methods are being developed	Highly variable methods of isolation currently exist
Therapeutic potential	Promising for various applications due to well-defined cargo and potential for targeting	Variable therapeutic potential depending on the specific EV subtype
Safety	Varies depending on the source cell (adult stem cells are safest)	Varies depending on the source cell type

EV, extracellular vesicles

Table 2 Exosome therapy *vs.* stem cell therapy

Feature	Exosome Therapy	Stem Cell Therapy
Safety	Lower risk of tumor formation	Higher risk of tumor formation
Specificity	Targeted to specific cell types	Less targeted
Biocompatibility	High	Moderate
Immunogenicity	Low	High
Regenerative Potential	Moderate	High
Production and Storage	Easier	More challenging
Delivery	Non-surgical methods (topical, injection)	May require surgery
Research Stage	More advanced clinical trials	Earlier stage of research

membrane, releasing functional cargos in the cytoplasm (Fig. 6).

Natural exosome in skin regeneration and scarring

Sources of exosomes

Exosomes, derived from specific cells and tissues, have distinct components and features. Desired properties and therapeutic applications are the two dominant factors that determine the most appropriate source of exosomes (Table 3).

Immortalized cell lines

Theoretically, immortalized cell lines can be constantly divided by overcoming the Hayflick limit via genetic modifications. [76] Paprocka et al. [77] indicated that human telomerase reverse transcriptase (hTERT)-immortalized adipose-derived stem cells (ASC) secrete more pro-regenerative factors than primary ASC. A 2020 study by Hettich et al. [71] reported that exosomes derived from immortalized human keratinocytes (HaCaT cells) promoted wound healing. Despite these encouraging results, immortalized cell lines have the possibilities of oncogenicity and property changes owing to the immortalization procedure. [76] To date, no study has completely illustrated the mutations or altered properties. As exosomes share similar properties with their parental cells, these uncertainties limit large-scale studies and their use for therapeutic purposes.

Induced pluripotent stem cells, induced pluripotent stem cell-derived mesenchymal stem cells, and embryonic stem cells

Induced pluripotent stem cells (iPSC) are reprogrammed from adult cells to alleviate donor variability and scalability concerns. [72] In 2019, Lu et al. [46] indicated that exosomes released by autologous monkey iPSC accelerated wound healing. In a 2023 study by Levy et al., [72] exosomes secreted by either iPSC or iPSC-derived MSC (iMSC) contributed to inflammation resolution within the wound bed of diabetic mice and remarkably reduced the wound area. This study also demonstrated that iPSC could serve as reproducible and scalable biomanufacturing sources, which did not change with increased passages. These benefits make iPSC and iMSC favorable choices in terms of cost and reproducibility. However, their inherent tumorigenicity, immunogenicity, and heterogeneity limit their practical application.

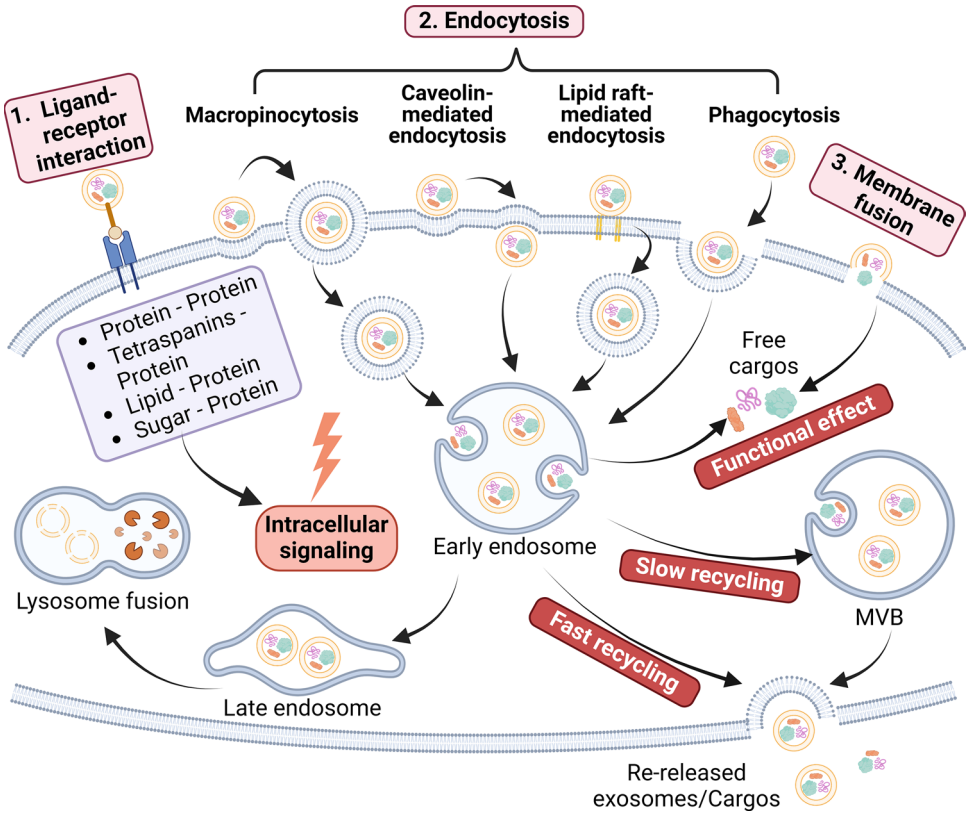

Fig. 6 Three mechanisms of exosome-mediated intercellular communication. (1) Ligand-receptor interaction: docking, binding, and triggering the intracellular signaling. (2) Endocytosis: including micropinocytosis, caveolin-mediated endocytosis, lipid raft-mediated endocytosis, and phagocytosis. The internalizing exosomes undergo fast recycling, slow recycling or performing corresponding functions. (3) Membrane fusion: directly fusing with the recipient cell membrane and releasing functional cargos in the cytoplasm

Embryonic stem cells (ESC) represent another potential option for exosome generation. Chen et al. [73] utilized ESC-exosomes (ESC-exo) to speed up wound closure of pressure ulcers in aged mice. They observed that exosomes could ameliorate endothelial senescence by activating nuclear factor-E2-related factor 2 (Nrf2) and recovering aging-related vascular endothelial dysfunction, thereby promoting wound healing [73]. Similarly, the clinical application of ESC is limited owing to immune rejection, tumorigenicity, and ethical issues. [78]

Somatic stem cells

Somatic stem cells, or adult stem cells, are undifferentiated cells in mature tissues. They are limited in their differentiation but do not pose safety and ethical concerns observed in iPSCs. [79]

General somatic stem cells The major exosome source candidates for regenerative medicine are MSC, which can be isolated from multiple tissues, including bone marrow, adipose tissue, umbilical cord, placenta, and dental pulp. [80] The prominent characteristics of MSC-exosomes (MSC-exo) for wound and scar management are anti-inflammatory, [81, 82] pro-angiogenic, [83] and the ability to decrease the pathological behavior of scar fibroblasts, [83, 84] which accelerate wound healing and prevent scar formation.

Skin-resident stem cells Skin-resident stem cells residing in niches are essential for skin regeneration. [85] The strong self-healing and regenerative ability of skin is not only attributable to its rich population of skin stem cells but also to the stem cell secretome. [86, 87] During the natural regenerative process, the basement membrane is secreted by epidermal stem cells. [87] Although no study has determined whether exosomes from skin-resident stem cells possess components specific to the niches, which might help them perform better in skin regeneration, scientists have begun to explore the roles of skin-stem cell-derived exosomes in wound healing and scar management.

Among various types of skin-resident stem cells, epidermal and hair follicle stem cells are the main topic of interest. Duan et al. [74] demonstrated that epidermal

Table 3 Differences in skin regeneration effects between exosomes from various sources

Feature	Immortalized Cell Lines	iPSC & iMSC	ESC	Somatic Stem Cells	Refs
Cell Source	Abnormal cells, may have mutations	Reprogrammed adult cells, potential for various cell types	Cells from the inner cell mass of the human blastocyst	Cells from mature tissues (bone marrow, fat, skin)	[71]
Safety	Uncertain owing to abnormal cell origin	Risk of tumor formation	Risk of tumor formation	Considered the safest, with lower tumor risk	[46, 72, 73]
Regeneration Potential	Limited—abnormal source may not promote healthy skin repair	Promising—may stimulate collagen, elastin production, and cell proliferation	Promising—anti-aging and pro-angiogenic effects	Lower than iPSC—may promote wound healing and cell migration	[73]
Skin Benefits	May promote angiogenesis (limited evidence)	May reduce inflammation, senescence, and improve collagen deposition	May improve wound healing, rejuvenate endothelial senescence	May improve wound healing and reduce inflammation and scarring	[74, 75]
Production challenge	Immortalized Cell Lines < iPSC ≈ iMSC ≈ ESC < Somatic Stem Cells				

iPSC, induced pluripotent stem cells; iMSC, iPSC-derived mesenchymal stem cells; ESC, embryonic stem cells

stem cell-derived exosomes contain specific microRNAs (miR-425-5p and miR-142-3p), playing vital roles in reducing scarring by inhibiting TGF-β1 activity. Additionally, Heras et al. [75] indicated that hair follicle stem cell-derived exosomes could promote the proliferation and migration of human dermal fibroblasts, accelerating wound healing. Nevertheless, studies on these skin-resident stem cells are still in their initial stages owing to technological limitations.

Therapeutic cargos within natural exosomes

To date, several functional therapeutic cargos within exosomes have been well defined, with proteins and nucleic acids being the two main categories that are investigated. Some examples of therapeutical biomolecules, and their functions and mechanisms in promoting wound healing and reducing skin scarring are presented in Table 4.

Drawbacks of natural exosomes

Despite the encouraging progress, the inevitable drawbacks of natural exosomes also hinder current therapeutic applications, including non-tissue-specific targeting, low-specific content for targeted tissue regeneration, low yield, and short retention time caused by rapid clearance in vivo.[98–100]

eExo: a promising new therapy

By exploiting the bioengineering strategies of exosomes, it may be possible to develop a superior approach than natural exosomes for skin wound healing and scarring management.

Superiority of eExo over natural exosome therapy

Natural exosomes carry common cargos with average specificity and exert generalized functions, limiting their efficacy. Conversely, eExo are equipped with the desired surface molecules or cargos, rendering eExo therapy a prospective approach with increased yield, specificity, efficacy, and decreased cost than natural exosome therapy (Table 5).

Surface modification endows eExo with increased specificity, whereas content modification increases the concentrations of desired cargos. Cargos residing in the cytoplasm (*e.g.,* proteins) can exist for several passages and perform desired functions. Importantly, eExo

Table 4 Therapeutic molecules of exosomes in skin regeneration and scarring

Categories	Functions	Mechanisms	Refs
Proteins			
14–3-3ζ	Control cell proliferation	Stimulate Wnt/β-catenin signaling pathway and enhance YAP Ser^{127} phosphorylation	[88]
Wnt4	Enhance proliferation and migration of skin cells	Activate Wnt/β-Catenin signaling pathway	[89]
VEGF bFGF PDGFBB CTGF	Promote angiogenesis, re-epithelialization, fibroblast proliferation, and collagen deposition	Activate PI3 K/Akt, Erk1/2, and RhoA/YAP signaling pathways	[90]
DMBT1	Promote angiogenesis	Increase VEGF-A expression, and activate PI3 K-Akt signaling pathway	[91]
Nucleic acids			
MiR-192-5p	Ameliorate collagen production	Stimulate miR-192-5p/IL-17RA/Smad axis	[92]
MiR-425-5p MiR-142-3p	Reduce scarring	Inhibit TGF-β1 activity	[74]
MiR-21 MiR-23a MiR-125b MiR-45	Suppress myofibroblast formation, and prevent excessive scarring	Inhibit TGF-β/SMAD2 signaling pathway	[93]
MiR-138-5p	Inhibit proliferation, migration, and protein expression (NF-κB, α-SMA, and TGF-β1) of fibroblasts	Downregulate SIRT1 gene	[94]
MiR-34a-5p MiR-146a-5p MiR-24-3p	Alleviate inflammation with immune modulation	Induce M2 macrophage polarization	[95]
MiR-21-3p	Accelerate angiogenesis and re-epithelialization and reduce scar widths	Activate PI3 K/Akt and ERK1/2 signaling pathway	[96]
MiR-181c	Alleviate inflammation	Suppress the TLR4 signaling pathway	[97]

YAP, YES-associated protein; Wnt4, wingless-type MMTV integration site family member 4; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; PDGF, platelet-derived growth factor; CTGF, connective tissue growth factor; DMBT1, deleted in malignant brain tumors 1; TGF-β, transforming growth factor-β; NF-κB, nuclear factor-kappa B; α-SMA, α-smooth muscle actin; SIRT1, Sirtuin 1; TLR4, toll-like receptor 4

Table 5 eExo therapy vs. natural exosome therapy

Feature	eExo therapy	Natural exosome therapy
Specificity & Targeting	Higher—engineered to target specific cell types or processes	Lower—broader range of molecules delivered
Efficacy	Potentially higher—optimized cargo for desired skin regeneration effects	Moderate—natural cargo may not be ideal for specific needs
Development Stage	Earlier stage, ongoing research	More advanced, closer to clinical applications
Yield	Improved yield	Relatively low yield
Cost	Potentially higher because of additional engineering steps	Potentially lower

eExo, engineered exosomes

also enable gene therapy through nucleic acid delivery and genetically engineered specific target presentation.[101] For example, eExo can deliver specific miRNAs, small interfering RNAs (siRNAs), and circular RNAs (circRNAs) into the recipient cells and can regulate gene expression through the following mechanisms. 1) MiRNA possesses the specific sequence of the 3′ untranslated region (3′-UTR), which enables miRNA to completely or partially complementary to its targeted mRNA, resulting in the negative regulation of the target protein expression.[102] 2) SiRNA, with a length of about 25 bp, is another category of double stranded DNA that can completely complementary to the targeted mRNA, leading to gene silencing.[103, 104] 3) CircRNA is a covalently closed RNA molecule that can regulate gene expression by acting like miRNA or protein proton sponges, interacting with U1 small nuclear ribonucleoprotein, and encoding small functional peptides (Fig. 7).[105]

Critical knowledge gaps of natural exosomes and eExo

To facilitate a more explicit comparison between natural exosomes and eExo, we critically analyzed the characteristics in terms of functional advantages, translational bottlenecks, and unresolved scientific controversies. (1) Functional advantages: eExo exhibit enhanced targeting, improved yield, and customized cargo loading, potentially leading to higher therapeutic efficacy [106]. (2) Transitional bottlenecks: natural exosomes present significant challenges related to isolation, purification, and lack of standardization [107], while eExo require a complex engineering process, pose safety concerns, and face regulatory hurdles.[108] (3) Unresolved scientific controversies: the exact mechanisms of natural exosome biogenesis and release from cells are still not fully understood.[109] Furthermore, there is no consensus on the best way to engineer exosome for therapeutic applications (Table 6).

Engineering strategies of eExo

Novel engineering strategies have been actively explored to increase exosome yield and enhance exosome therapeutic efficacy.[110, 111] Through surface or content modification, exosome engineering endows eExo with enhanced targeting abilities and increased concentrations of effector molecules. We summarize the engineering strategies into three categories: pre-exosomal, post-exosomal and biohybrid approaches (Table 7, Figs. 8, 9).

Pre-exosomal approach

Pre-exosomal modification strategies refer to the manipulation of donor cells prior to exosome isolation, which include preconditioning and genetic modification.

Preconditioning Preconditioning of parent cells via physical, chemical, biological factors or hypoxia confers exosomes with enhanced biological functions and activities, benefiting therapeutic outcomes. Ultrasonication was utilized by Wang et al.[112] to shear intact human umbilical cord MSC (hUCSC), followed by regular centrifugation and filtration, producing a higher yield of exosomes with enhanced therapeutic potential for skin rejuvenation. Chemical conjugation or membrane fusion with donor cells can generate functionalized exosomes.[113] One strategy is to utilize azide-bearing compounds, such as *N*-azidoacetylmannosamine-tetraacetylated, to functionalize exosomes with small molecules through biorthogonal chemistry.[114] Another strategy is to use membrane-fusogenic liposomes. Lee et al.[115] utilized this strategy to confer exosomes with functional lipids, drugs, fluorophores, and bio-orthogonal chemicals. Hypoxia pretreatment endows ASC-derived exosomes with enhanced pro-healing ability by activating the PI3 K/AKT signaling pathway.[116]

Genetic modification Genetic modification refers to the use of viral or non-viral vectors to load donor cells with oligonucleotides (*e.g.,* miRNA, siRNA), which results in gene overexpression or downregulation of the donor cells [117, 118]. Using this approach, exosomes secreted by donor cells are equipped with specific oligonucleotides, leading to improved targeting capabilities and therapeutic efficacy.[119, 120] For example, Huang et al.[120] demonstrated that exosomes generated from miR-31-5p-overexpressed donor cells promoted diabetic wound heal-

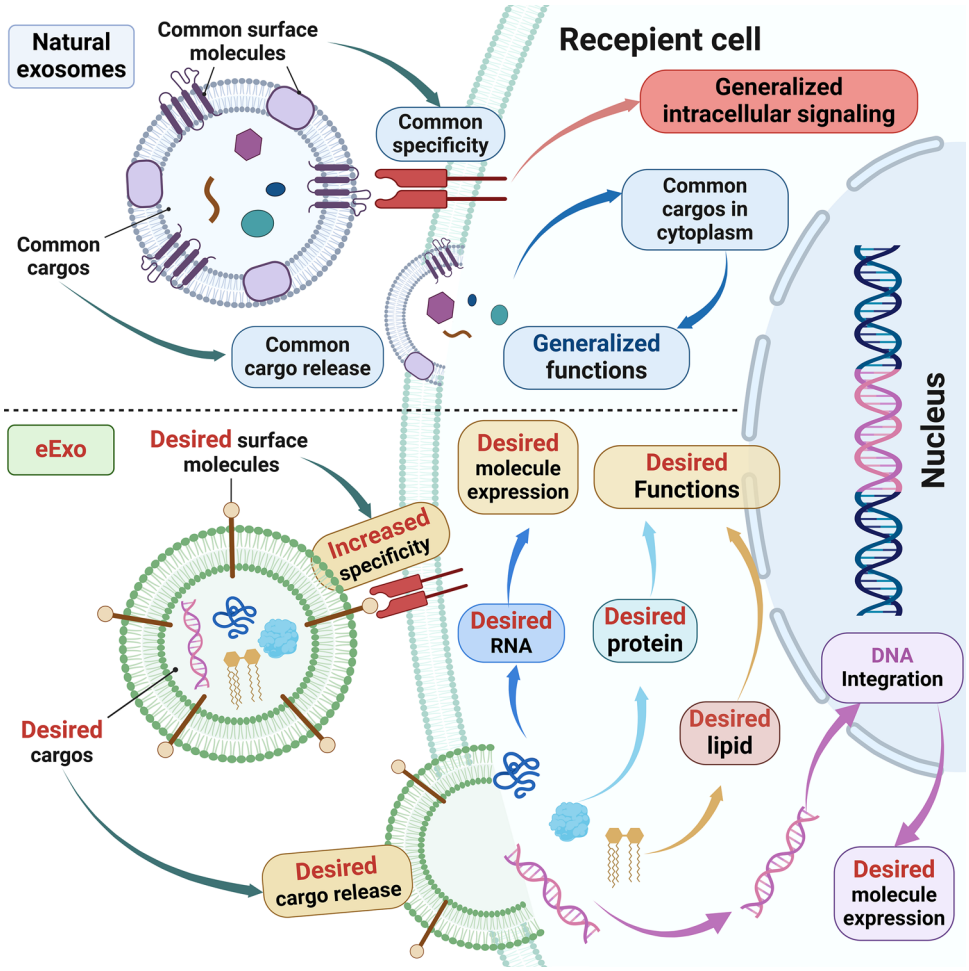

Fig. 7 Superiority of engineered exosomes (eExo). Natural exosomes carry common cargos with average specificity and exert generalized functions, limiting their efficacy. Conversely, eExo are equipped with desired cargos (*e.g.*, proteins, lipids, RNAs, and DNAs). In some cases, DNAs can enter the nucleus and integrate into the recipient cell's genome, endowing recipient cells with stable desired molecule expression. Other cargos can reside in the cytoplasm for several passages and function with the desired purpose. Abbreviation: eExo, engineered exosomes

Table 6 Critical knowledge gaps of natural exosomes and eExo

Category	Natural exosome	eExo
Functional advantages		
Targeting	Lower	Higher
Yield	Lower	Higher
Desired cargo	Lower	Higher
Therapeutic efficacy	Lower	Higher
Transitional bottlenecks		
	Isolation	Complex engineering process
	Purification	Safety concerns
	Standardization	Regulatory hurdles
Unresolved scientific controversies		
	Origin and biogenesis	Optimal engineering strategies

eExo, engineered exosomes

Table 7 Engineering strategies for exosomes

Categories	Methods	Advantages	Limitations	Examples	Refs
Pre-exosomal					
Preconditioning	Preconditioning via physical, chemical, biological factors, or hypoxia	Confer exosomes with enhanced biological functions and activities	Relative inefficiency	Chemical conjugation; Membrane fusogenic liposome	[113, 115]
Genetic manipulation	Using viral or non-viral vectors	Improve targeting capability and therapeutic efficacy	Possibility of altering donor cell gene expression and influencing the encapsulation process	RNAi therapy	[120]
Post-exosomal					
Co-incubation	Hydrophobic molecules load in exosomes along with a concentration gradient	Minimize the damage to the exosomal membrane integrity	Limited loading efficiency, difficulty in controlling loading amount, and possibility of affecting cargo properties	Drugs and RNAs	[121]
Chemical	Conjugate exosomal surface with specific targeting ligands	Rapid and selective reactions with high yield	Limited application scope	CuAAC	[124]
Sonication	Temporarily weaken exosome membranes via sonication	Facilitate the diffusion of desired agents	Might cause cargo aggregation, degradation, and asymmetry of cargo loading	Using sonication to load Erastin within exosomes	[127]
Electroporation	Utilize electrical field to induce transient permeabilization of exosome membranes	Facilitate the diffusion of desired agents	Might lead to RNA cargo aggregation	Loading Cas9 ribonucleoprotein	[129]
Extrusion	Mixed exosomes and therapeutic agents are squeezed into an extruder in the extrusion process	Induce exosomal membrane disruption and obtain cargo-loaded exosomes homogeneously	Might alter exosomal membrane structure and physicochemical properties	Dapagliflozin-loaded exosomes	[135]
Freeze–thaw	Exosomes and cargos repeat temperature changes	Facilitate cargo loading into exosomes	Limited loading efficiency and the potential of inactivating proteins, aggregating exosomes, and altering exosomal membrane stability	hCG loaded-exo	[137]
Biohybrid approach					
eExo@biomaterials	Attach eExo to biomaterials	Pro-healing and anti-scarring	n/i	SGM-miR146a-eExo from PMSC@SFP	[140]
eExo within biomaterials	Embed eExo within biomaterials	Facilitate exo delivery, enhance healing and angiogenesis, and inhibit inflammation	Might hinder wound closure if biomaterials (*e.g.,* hydrogel) fail to degrade adequately	ONB-coated ASC-exo within self-healing hydrogel dressing	[141]
eExo into nanoparticles	Load exo with nanoparticles	Exhibit synergetic functions	n/i	nSF-EPL-PDRN@Exo	[142]

CuAAC, copper-catalyzed azide alkyne cycloaddition; hCG, human chorionic gonadotropin; eExo, engineered exosomes; n/i, not investigated; SGM, silk fibroin binding peptide-Gluc-MS2; exo, exosomes; PMSC, placental mesenchymal stem cells; SFP, silk fibroin patch; ONB, oxygen nanobubbles; EPL, ε-Poly-L-Lysine; PDRN, polydeoxyribonucleotide

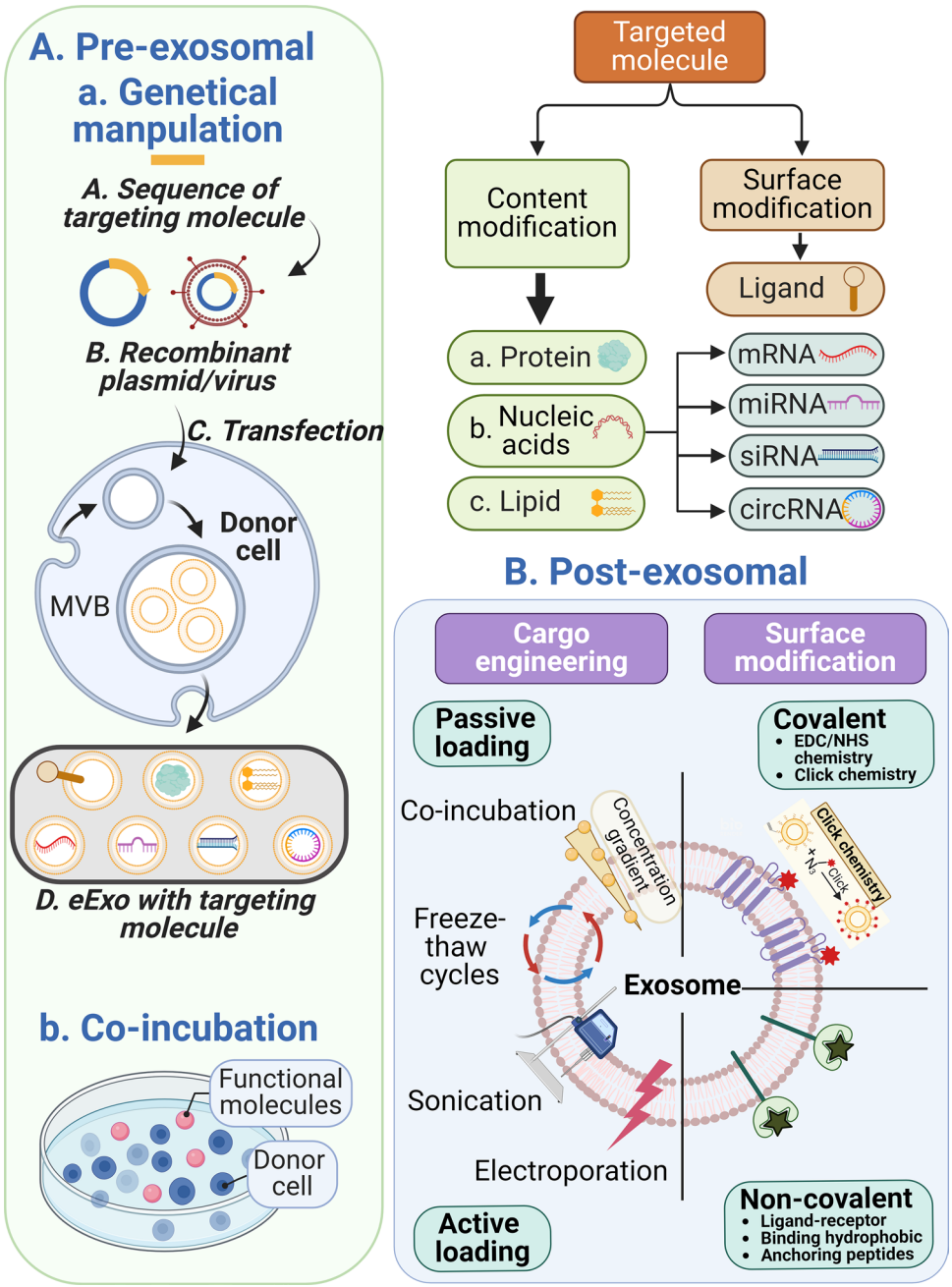

Fig. 8 Pre-exosomal and post-exosomal strategies for eExo. According to different targeted molecules, the modification of contents (proteins, lipids, and nucleic acids such as mRNA, miRNA, siRNA, and circRNA) or surfaces (ligands) can endow eExo with enhanced increased concentrations of effector molecules or targeting abilities. **A** Pre-exosomal approaches include manipulation of parental cells prior to exosome isolation, including genetic manipulation and co-incubation. **B** Post-exosomal approaches refer to the direct manipulation of exosomes after exosome isolation, comprising cargo engineering and surface modification

ing in rats. Nevertheless, several drawbacks still exist: 1) the transfected oligonucleotides may alter target gene expression in parental cells, and 2) the remaining transfection reagents may affect the encapsulation of modified exosomes.[68]

Post-exosomal approach

Post-exosomal modification strategies refer to the direct manipulation of exosomes after isolation, including co-incubation, chemical surface modification, sonication, electroporation, extrusion, and freeze–thaw cycles.

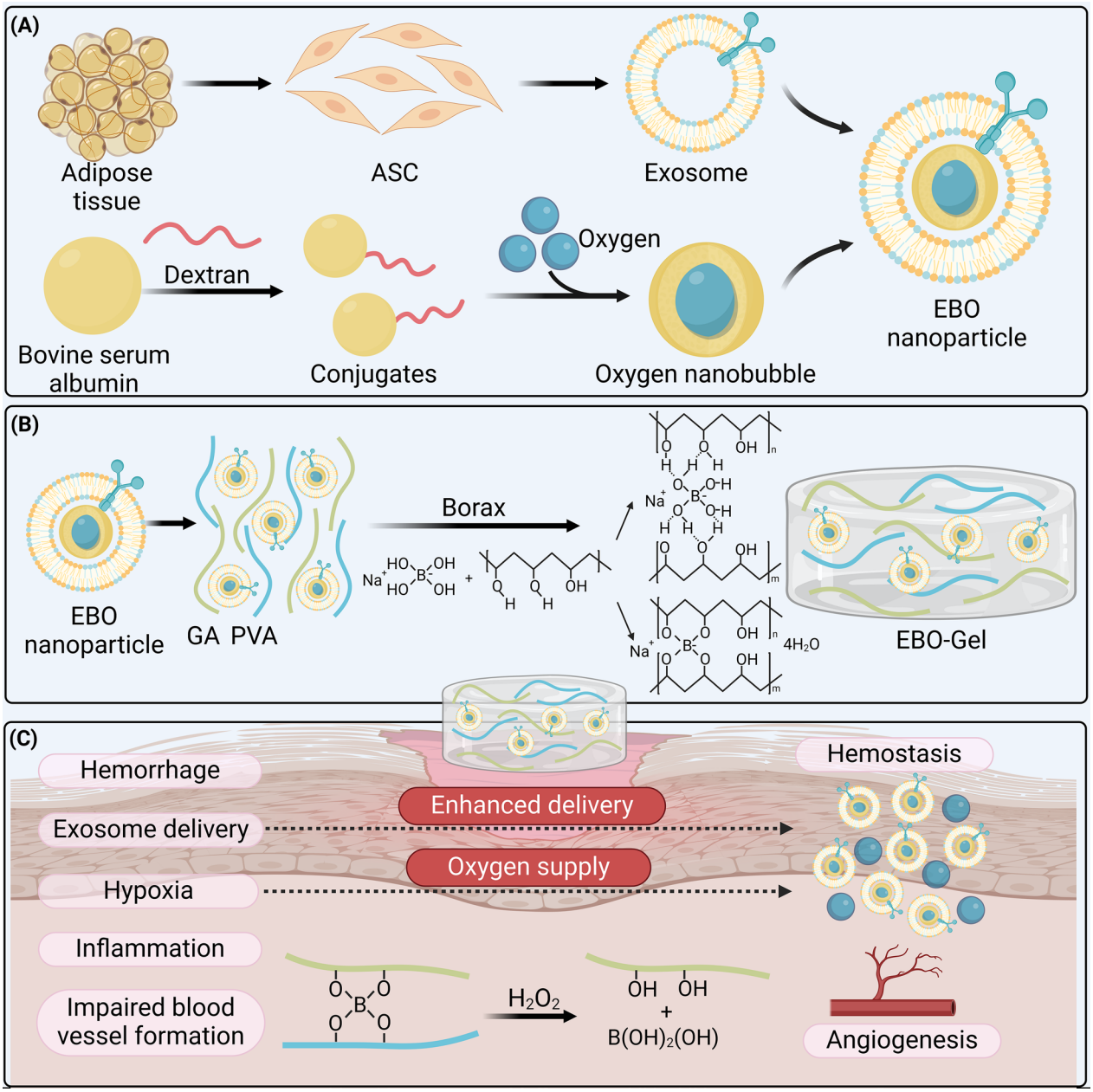

Fig. 9 Biohybrid approach for eExo. **A** Schematic of the preparation of oxygen nanobubble and EBO nanoparticles. **B** Crosslinking mechanisms and the structure of EBO-Gel. **C** EBO-Gel can enhance wound healing by hemostasis, enhanced delivery of exosomes, oxygen supply, and angiogenesis. Abbreviations: ASC, adipose-derived stem cells; EBO, exosome coated bovine serum albumin-based oxygen nanobubbles; GA, gelatin; PVA, polyvinyl alcohol.[141]

Co-incubation Along with the concentration gradient, co-incubation loads exosomes with therapeutic cargos, especially hydrophobic molecules (*e.g.,* drugs and RNAs). This procedure causes minimal damage to exosomal membrane integrity.[121] The drawbacks of co-incubation include the limited loading efficiency owing to reliance on cargo hydrophobicity and concentration gradient, difficulty in controlling the loading amount, and the use of surface permeabilizers (e.g., Triton), which may affect cargo properties and cause hemolysis in vivo.[117, 121, 122]

Chemical surface modification Chemical surface modification involves conjugating the exosomal surface with specific targeting ligands. Click chemistry is a good example of a rapid and selective reaction with high yields, mak-

ing it suitable for surface modification [123, 124]. Among the various click reactions, Cu-catalyzed azide-alkyne cycloaddition is frequently chosen for the formation of triazole bonds because it allows rapid and efficient conjugation.[124]

Sonication Sonication enables the diffusion of desired agents into the exosomal lumen by temporarily weakening the integrity of exosome membranes.[125, 126] Du et al.[127] designed exosomes by loading a ferroptosis inducer (erastin) and photosensitizer (Rose Bengal) via sonication. Fluorescence-labeled eExo were tracked to analyze their distribution in vivo. However, sonication might also cause aggregation and degradation of the delivered cargo (*e.g.,* RNAs) and asymmetry of cargo loading (outer or inner layer).[113, 122]

Electroporation Electroporation utilizes an electrical field to induce transient permeabilization of exosomal membranes for exogenous cargo loading [128]. Wan et al.[129] reported the delivery of Cas9 ribonucleoprotein into exosomes through electroporation. However, similar to sonication, electroporation can also lead to RNA cargo aggregation.[130–132] Thus, monitoring electroporation parameters and exogenous cargo concentrations is important for successful application [113, 133].

Extrusion Extrusion can induce exosomal membrane disruption and obtain cargo-loaded exosomes simultaneously. During the extrusion process, mixed exosomes and therapeutic agents were squeezed into an extruder.[134] This method can also be applied for the acquisition of exosome mimics. Zhang et al.[135] mixed dapagliflozin and iPSC-derived endothelial cells, followed by extrusion to obtain dapagliflozin-loaded exosome mimetics. These dapagliflozin-loaded exosome mimetics exhibited pro-angiogenic and pro-healing effects via the HIF-1α/VEGFA pathway in diabetic mice. However, high mechanical force may alter the exosomal membrane structure and physicochemical properties.[136]

Freeze–thaw cycles Freeze–thaw cycles utilizes the repeat temperature changes (from −80 °C to 37 °C) to load cargo into exosomes.[122] Hajipour et al.[137] reported that freeze–thaw cycles can be used for loading human chorionic gonadotropin into uterine exosomes. Nevertheless, in addition to limited loading efficiency, the shortcomings of freeze–thaw cycles include the potential to inactivate proteins, aggregate exosomes, and alter exosomal membrane stability.[113, 138]

Biohybrid approach

In biohybrid strategies, exosomes are generated by fusing exosomes with artificial materials.[98] Within the body, exosomes have a high probability of reuptake by neighboring cells. Therefore, exosomes possess a short half-life, instability, and low long-term retention after administration.[139] Hence, incorporating exosomes into biomaterials may be a powerful approach for enhancing the stability of exosomes and realizing controlled sustained release in vivo. eExo can be grafted onto biomaterial surfaces, encapsulated inside biomaterials, and integrated into nanoparticles.

eExo@biomaterials Combining eExo with biomaterials (scaffolds) is similar to the sequestration of growth factors in ECM, allowing the special attachment of eExo and biomaterials and eventually enhancing eExo stability and realizing a controlled sustained release. Considering the antimicrobial properties of silk fibroin, Li et al.[140] attached miR146a-loaded eExo to a silk fibroin patch (SFP) and investigated the pro-healing effect of the miR146a-eExo@SFP. The results showed that the miR146a-eExo@SFP group was superior to the miR146a-eExo-only and untreated groups in driving diabetic wound healing, with anti-inflammatory effects and narrowest scar widths, bringing new hope for chronic wound treatment.

eExo within biomaterials eExo within biomaterials technology is to embed eExo within biomaterials. One example is the coating of the membrane of ASC-derived exosomes with oxygen nanobubbles (ONB), allowing the reassembly of exosomes around the ONB by ultrasonication and embedding exosome-coated ONB within a self-healing hydrogel dressing.[141] This system exhibited oxygen-release properties and contributed to scarless wound healing in a rat model (Fig. 9).[141]

eExo into nanoparticles Loading exosomes with nanoparticles can lead to synergetic functions on skin regeneration. Li et al.[142] synthesized SF-ε-Poly-L-Lysine (EPL) composite nanoparticles (nSF-EPL). Further, the nSF-EPL were loaded with polydeoxyribonucleotide (PDRN) to form nSF-EPL-PDRN, which were subsequently assembled with negatively charged exosomes to generate nSF-EPL-PDRN@Exo. In vivo research results showed the composite nanoparticles exhibited excellent anti-inflammatory and pro-angiogenesis capabilities, thus accelerating the healing of chronic diabetic wounds and reducing scarring.

Comparative analysis of exosome engineering strategies

Different engineering strategies have different characteristics; therefore, a comprehensive comparative analysis of

these strategies is essential. The following matrix evaluates common engineering methods based on specificity, scalability, clinical readiness, and technical complexity (Table 8).

Reconcile the scalability of production with personalized treatment

Scalability requirements Large-scale production of eExo is essential for widespread clinical use. This involves establishing efficient and reproducible manufacturing processes, from cell culture to exosome isolation and engineering [143]. Scalable production methods need to ensure consistent quality, high yield, and cost-effectiveness.

Personalized treatment demands Personalized treatment for chronic wounds and scars depends on the individual patient characteristics. Factors such as the patient's genetic background, immune status, wound or scar severity and stage, and comorbidities can influence the optimal treatment approach [144]. In the context of exosome therapy, this means that the type of exosomes (autologous or allogeneic), their engineered modifications, and the dosing regimen may need to be tailored to the specific needs of each patient.

The choice between autologous and allogeneic exosomes Autologous exosomes, derived from the patient's own cells offer the advantage of minimal immunogenicity. Since they are genetically matched to the patient, there is a lower risk of immune rejection, making them an ideal choice for personalized treatment [145]. However, the production of autologous exosomes is highly individualized and time-consuming.

In contrast, allogeneic exosomes, sourced from healthy donors or cell lines, have the potential for scalable production [146]. A single batch of allogeneic exosomes can be used to treat multiple patients, reducing the production time and cost per patient. However, allogeneic exosomes may trigger immune responses in some patients, although the risk is generally lower compared to whole cells [147]. To mitigate this risk, careful donor selection, pre-treatment of exosomes to reduce immunogenicity, and immune monitoring during treatment are necessary.

Strategies for reconciling scalability and personalization Reconciling the scalability of eExo production with personalized treatment for chronic wounds and scars is a complex but essential task. The choice between autologous and allogeneic exosomes, combined with the development of standardized platforms [148], biomarker-guided personalization [149], and hybrid approaches [150], can help bridge this gap. Further research is needed to optimize these strategies, improve the understanding of exosome-host interactions, and ultimately translate eExo therapy into more effective and accessible treatments for patients with chronic wounds and scars.

Preserving the inherent advantages of natural exosomes during engineering processes

The prominent advantages of natural exosomes are their high biocompatibility and low immunogenicity.

Table 8 Comparative analysis of exosome engineering strategies

Categories	Specificity	Scalability	Clinical Readiness	Technical Complexity
Preconditioning	Moderate: modifies overall cellular state rather than precisely targeting specific molecules	Moderate	Moderate: more research is needed	Low
Genetic manipulation	High: enables precise control over the exosome cargo by modifying the donor cell genome	Low	Low: concerns about off-target effects, immunogenicity, and long-term safety in humans remain	High
Co-incubation	Low: lacks specificity in targeting	Moderate	Low: the stability of the loaded molecules and potential immune responses need to be addressed	Low
Chemical surface modification	Moderate: the specificity of the therapeutic effect may be limited	Moderate	Moderate: more research is required	Moderate
Sonication	Low: lacks specificity in targeting	Low	Low: concerns about potential damage to exosome functionality remain	Moderate
Electroporation	Moderate: not highly specific in targeting certain cell types or pathways	Moderate	Moderate: more research is required	Moderate
Extrusion	Low: lacks specificity in targeting	Moderate	Low: limited preclinical and clinical evidence	Moderate
Freeze–thaw	Low: lacks specificity in targeting	Low	Low: limited preclinical and clinical evidence	Low

Preserving the inherent advantages of natural exosomes during engineering processes is critical.

Engineering strategies such as physical, chemical, and genetic approaches can pose a threat to biocompatibility and immunogenicity. Preserving the high biocompatibility and low immunogenicity of natural exosomes during the engineering process is pivotal to the successful development of eExo-based therapies. This can be achieved by: (1) carefully considering the engineering methods, (2) implementing strict quality control measures, and (3) applying appropriate post-engineering treatments. These steps will help ensure that eExo retain their ability to interact harmoniously with host tissues and cells. Continued research and innovation in these areas will be crucial for maximizing the therapeutic potential of exosomes and translating them into safe and effective clinical treatments.

eExo for chronic wounds and pathological scarring–in vitro and in vivo studies

Various in vitro and in vivo studies have been conducted to investigate the functions and mechanisms of eExo in the treatment of chronic wounds and pathological scarring. Currently, inducing a full-thickness excisional wound along with an underlying disease is a prevalent approach to create chronic wound models, such as diabetic models, arterial insufficiency peripheral arterial disease models, ischemic skin flap and graft models, venous stasis ulcers, and pressure ulcers.[151] As for pathological scars, rabbit ear model is the widely used established model of HS, whereas well-accepted keloid models have not been constructed yet. Fortunately, gratifying achievements have been made.[152] Nonetheless, the functions and mechanisms underlying eExo functions have not been systematically summarized. Here, we classified eExo for chronic wounds and scarring treatment based on their major functions: "4-pro" and "5-anti" effects, and the effector molecules and pathways are also outlined (Table 9). The multiple functions of eExo applied in promoting chronic wound healing are depicted in Fig. 10.

Promoting cell proliferation

The proliferation of fibroblasts and keratinocytes is vital in wound closure.[9, 153] Engineering methodologies have been developed for promoting cell proliferation. Hypoxic preconditioning of human adipose-derived stem cells (hASC) altered the miRNA expression profiles of exosomes (HypASC-exo) [116]. HypASC-exo could promote fibroblast proliferation, migration, re-epithelialization, and ECM secretion, resulting in increased diabetic wound healing via the PI3 K/AKT signaling pathway. Electroporation was utilized in a study to overexpress miR-21-5p in ASC-exosomes (ASC-exo), which also exhibited excellent effects on promoting keratinocyte proliferation and migration through Wnt/β-catenin signaling in vitro and speeding up re-epithelialization, angiogenesis, vessel maturation, and collagen remodeling in vivo.[154] Similarly, Gondaliya et al.[155] loaded a miR-155 inhibitor into MSC exosomes via modified calcium-mediated transfection, which exhibited positive effects on keratinocyte migration, re-epithelialization, angiogenesis, and collagen deposition.

Promoting angiogenesis

Angiogenesis is essential for adequate wound healing, because new vasculature is necessary to bring nutrients, oxygen, and immune cells to the chronic healing wounds. Thus, exploring engineering strategies aiming at increasing the pro-angiogenic ability of eExo is becoming a research hotspot.

Preconditioning of parental cells could endow exosomes with enhanced pro-angiogenic ability. Yu et al.[156] reported that atorvastatin pretreatment of human bone marrow MSC (hBMSC) could enhance the pro-angiogenic capacity of hBMSC-exosomes (hBMSC-exo) in on diabetic wound through the AKT/endothelial nitric oxide synthase (eNOS) pathway. Similarly, pretreatment of BMSC with pioglitazone increased VEGF and CD31 expression in BMSC-exo and stimulated the angiogenesis of diabetic wound via the PI3 K/Akt/eNOS pathway.[157] Huang et al.[120] developed a innovative RNAi therapy by which exosomes were loaded with miR-31-5p to stimulate angiogenesis, contributing to diabetic wound healing. Yan et al.[158] directly encapsulated miR-31-5p mimics into milk-derived exosomes via electroporation, which displayed pro-angiogenic activity both in vitro and in vivo.

Promoting ECM deposition

During the early stages of wound healing, facilitating ECM synthesis and deposition is particularly beneficial. For instance, Ma et al.[159] reported exosomes from miR-126-3p-overexpressing ASC could increase collagen deposition in the wound beds in vivo,. Similarly, researches by Hu et al.,[157] Lv et al.,[154] Gondaliya et al.,[155] Ma et al.,[159] Qian et al.[160] also demonstrated eExo promote ECM deposition, which is conducive for adequate wound healing, thus preventing chronic wounds and aberrant scarring.

Promoting nerve regeneration

Nerve regeneration are important constituents for fully functional skin. Evidence indicates that traumatic impairment of peripheral innervation could lead to chronic

Table 9 Functions and mechanisms of eExo for chronic wound healing and aberrant scarring

Category	Engineering technique	Functional molecules	Source	Model	Functions	Effector molecules; pathways	Refs
4-pro							
Angiogenesis							
	Preconditioning (atorvastatin)	MiR-221-3p	hBMSC	In vitro; In vivo (rat, diabetic)	Angiogenesis↑	Activating AKT/eNOS signaling pathway	[156]
	Preconditioning (pioglitazone)	VEGF CD31	rBMSC	In vitro; In vivo (rat, diabetic)	Angiogenesis↑ Re-epithelization↑ Collagen deposition↑ ECM remodeling ↑ Wound closure rate↑	Activating PI3 K/AKT/eNOS signaling pathway	[157]
	Genetic manipulation (OE)	MiR-31-5p	HEK293	In vitro; In vivo (mouse, diabetic)	Angiogenesis↑Fibrogenesis↑ Re-epithelization↑	Inhibiting HIF-1 and EMP-1	[120]
	Electroporation	MiR-31-5p mimics	Milk	In vitro; In vivo (mouse, diabetic)	Angiogenesis↑	Activating miR-31-5p/HIF1 AN axis	[158]
Proliferation, migration, and differentiation							
	Preconditioning (hypoxia)	MiR-21-3p MiR-126-5p MiR-31-5p MiR-99b MiR-146-a	hASC	In vitro; In vivo (mouse, diabetic)	Fibroblast proliferation & migration↑ ECM secretion↑ Re-epithelialization↑ Angiogenesis↑	Activation PI3 K/Akt signaling pathway	[116]
	Electroporation	MiR-21-5p	hASC	In vitro; In vivo (rat, diabetic)	Keratinocyte proliferation& migration↑ Re-epithelialization↑ Angiogenesis↑ Vessel maturation↑ Collagen remodeling↑	Activating Wnt/β-catenin signaling pathway	[154]
	Modified calcium-mediated transfection	MiR-155 inhibitor	hMSC	In vitro; In vivo (mouse, diabetic)	Keratinocyte migration↑ Re-epithelialization↑ Angiogenesis↑ Collagen deposition↑ Inflammatory↓	Inhibiting TIMP-2, sTNF R1, RANTES, LIX, TGF-β1, IL-1β, IL-6, and TNF-α	[155]
ECM deposition							
	Genetic manipulation (OE)	MiR-126-3p	hASC	In vitro; In vivo (rat, surgical)	Collagen deposition↑ Angiogenesis↑ Wound healing rate↑	Targeting PIK3R2	[159]
Nerve regeneration							
	Asymmetric wettable dressing with a composite of exosomes and silver nanoparticles (CTS-SF/SA/Ag Exo dressing)	n/i	hUMSC	In vitro; In vivo (mouse, infected)	Nerve repair↑ Angiogenesis↑ Collagen deposition↑	n/i	[160]
5-anti							

Table 9 (continued)

Category	Engineering technique	Functional molecules	Source	Model	Functions	Effector molecules; pathways	Refs
Anti-inflammation							
	Co-incubation (miR-181c)	MiR-181c	hUCSC	In vivo (rat, burn)	Burn-induced inflammation↓	Alleviating TLR4 signaling pathway and downstream NF-κB/p65 signaling pathway	[97]
	Preconditioning (melatonin)	n/i	hBMSC	In vivo (mouse, diabetic)	Regulating macrophage M1 and M2 polarization	Activating PTEN/AKT signaling pathway	[166]
Anti-fibrosis							
	Genetic manipulation (OE)	TSG-6	hBMSC	In vivo (mouse, surgical)	Pathological scar formation↓ Collagen deposition↓	Downregulating TGF-β1, collagen I, collagen III, a-SMA, p-SMAD2^{Ser467}, and p-SMAD3$^{S423/S425}$ expression	[168]
	Genetic manipulation (OE)	MiR-29a	hASC	In vivo (mouse, thermal)	Fibrosis↓ Excessive HSFB proliferation and migration↓ Excessive scar formation↓	Inhibiting TGF-β2/Smad3 signaling pathway	[169]
Anti-apoptosis							
	Genetic manipulation (OE)	lncRNA H19	mBMSC	In vitro; In vivo (mouse, diabetic)	Apoptosis (FB)↓ Proliferation & migration (FB)↓ Inflammation↓	Regulating lncRNA H19/miR-152-3p/PTEN axis via the PI3 K/AKT signaling pathway	[172]
	Preconditioning (hypoxia)	MiR-125b	hUCSC	In vitro; In vivo (mouse, surgical)	Apoptosis (EC)↓ Proliferation & migration (EC) ↑	Activating miR-125b/TP53INP1 signaling pathway	[173]
Anti-senescence							
	Genetic manipulation (OE)	Nrf2	hASC	In vitro; In vivo (rat, diabetic)	Senescence (EPC)↓ Oxidative stress↓ Angiogenesis↑ Inflammation↓ Granulation tissue formation↑	Increased SMP30, VEGF, and p-VEGFR2; Reduced ROS, and inflammatory cytokines (*e.g.*, IL-1β, IL-6, and TNF-α)	[175]
Anti-oxidation							
	EXPLOR system (genetic engineering and optogenetic techniques)	eNOS	hUCSC	In vitro; In vivo (mouse, diabetic)	Antioxidant capacity (EC)↑ Apoptosis (EC)↓ Inflammation↓ Wound closure rate↑ Vascular neogenesis↑ Matrix remodeling↑ Skin scarring↓	SOD, T-GSH activities↑; Antioxidant-related genes (SIRT1, Nrf2)↑; Multiple phosphorylation cascades and molecular pathways, including PI3 K/Akt/mTOR and FAK/ERK1/2	[177]
	Genetic manipulation (Knockdown)	MiR-15a-3p	Peripheral blood	In vitro; In vivo (mouse, diabetic)	ROS↓ Angiogenesis↑ Wound repair↑	Activating NOX5/ROS signaling pathway	[178]

OE, overexpression; hUCSC, human umbilical cord mesenchymal stem cells; hBMSC, human bone marrow mesenchymal stem cells; hUMSC, human umbilical mesenchymal stem cells; rBMSC, rat bone marrow mesenchymal stem cells; HEK293, human embryonic kidney 293 cells; HIF-1, HIF1 AN, also named FIH; EMP-1, epithelial membrane protein-1; AKT, protein kinase B; ROS, reactive oxygen species; eNOS, endothelial nitric oxide synthase; Nrf2, nuclear factor-E2-related factor 2; hASC, human adipose-derived stem cells; n/i, not investigated; hMSC, human mesenchymal stem cells; TIMP-2, tissue inhibitor of metalloproteinase-2; sTNF R1, soluble tumor necrosis factor receptor 1; RANTES, regulated upon activation, normal T cell expressed and secreted; LIX, Lipopolysaccharide-induced CXC chemokine; TGF-β1, transforming growth factor β1; IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; TSG-6, tumor necrosis factor-stimulated gene-6; HSFB, hypertrophic scar fibroblasts; LPS, lipopolysaccharide; mBMSC, mouse umbilical cord mesenchymal stem cell; FB, fibroblasts; EPC, endothelial progenitor stem cells; EC, endothelial cells; TP53INP1, tumor protein p53 inducible nuclear protein 1; eNOS, endothelial NO synthase

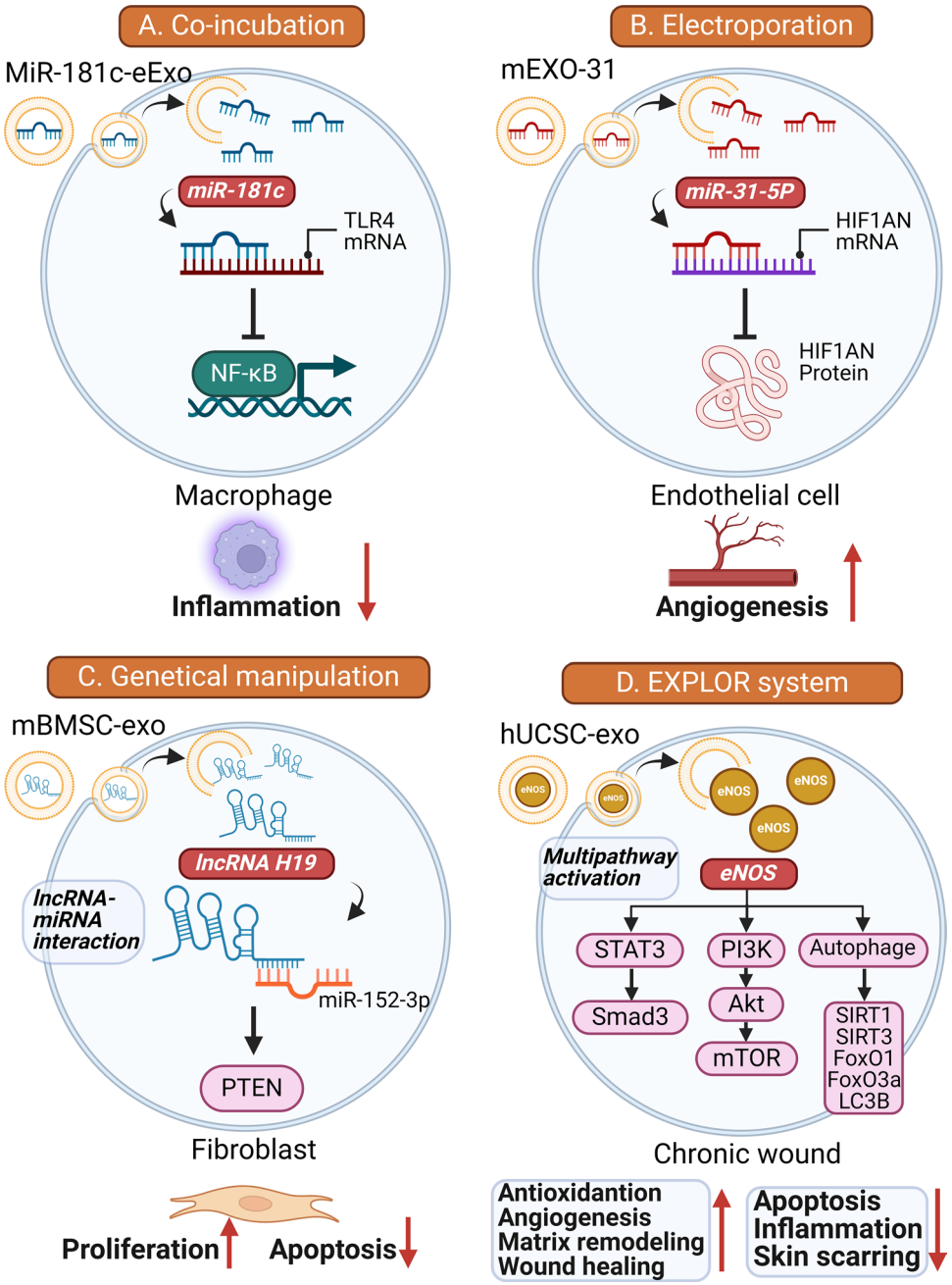

Fig. 10 Examples of eExo for promoting chronic wound healing. **A** The co-incubation of hUCSC-exo and miR-181c endows eExo with elevated level of miR-181c. MiR-181c can interact with TLR4 mRNA, alleviating TLR4 signaling pathway and downstream NF-κB signaling pathway in macrophages, subsequently reducing burn-induced inflammation.[97] **B** The utilization of electroporation encapsulates miR-31-5p mimics into milk-derived exosomes (mEXO-31). MiR-31-5p can interact with HIFIAN mRNA and decrease the expression of HIFIAN protein, displaying pro-angiogenic activity.[158] **C** eExo derived from long non-coding RNA (lncRNA) H19-transfected MSC can suppress apoptosis and inflammation and stimulate the diabetic wound healing process in vivo via the lncRNA H19/miR-152-3p/PTEN axis.[172] **D** EXPLOR system is applied to produce eNOS-enriched UCSC-derived exosomes. As results, eNOS-enriched eExo can enhance cellular antioxidant capacity, neovascularization, and matrix remodeling while alleviating apoptosis and inflammation via multipathway activation, ultimately accelerating wound closure and preventing skin scarring.[177]

wounds, which might even fail to heal. Subsequent studies indicated that denervated skin could impair normal wound healing.[161, 162] Zheng et al.[163] reported that ASC-exo encapsulated with neurotrophin-3 mRNA could restore the nerve regeneration in vivo. A previous study by Zhu et al.[164] reported that hUCSC-exosomes (hUCSC-exo) could treat cutaneous nerve damage, which further promoted skin nerve regeneration.[164] Qian et al.[160] fabricated an asymmetric wettable dressing with a composite of exosomes and silver nanoparticles to accelerate nerve repair in infected wound healing.

Anti-inflammation

Excessive and extended inflammation, independent of fibrosis, is one factor that determines pathological scarring, blocking the path to scarless healing.[29, 165] Thus, anti-inflammatory agents are the predominant target of anti-scarring management. Li et al.[97] reported that co-incubation of hUCSC-exo with miR-181c repressed the burn-induced inflammation (reduced inflammatory factor release, including IL-1β and TNF-α) by targeting toll-like receptor 4 and downstream NF-κB/p65 signaling, providing a potential target for burn therapy. Liu et al.[166] reported that exosomes derived from melatonin-pretreated hBMSC significantly suppressed the inflammatory response by regulating the ratio of M2 (anti-inflammatory) to M1 (pro-inflammatory) polarization via the phosphatase and tensin homolog (PTEN)/AKT signaling pathway, exerting strong effects on diabetic wound healing.

Anti-fibrosis

Considering that excessive matrix deposition impels fibroblasts into a state of high mechanical stress, leading to pathological scarring,[167] preventing excessive fibrosis in the wound healing process is an important aspect of anti-scarring. Jiang et al.[168] constructed tumor necrosis factor-stimulated gene-6 (TSG-6)-overexpressing and -knockdown hBMSC via lentivirus infection and collected exosomes from the two cell lines. Exosomes from TSG-6 overexpressing hBMSC exhibited excellent effects in attenuating excessive collagen and inflammatory cytokines in vivo, ultimately ameliorating pathological scar formation. Similarly, Yuan et al.[169] used a thermal mouse model to determine the anti-fibrosis and anti-scarring effect of exosomes from miR-29a-modified hASC by targeting the TGF-β2/mothers against decapentaplegic homolog 3 (Smad3) signaling pathway. Moreover, eExo combined with biomaterials exhibited pro-healing and anti-scarring effects. For instance, Li et al.[140] attached miR146a overexpression exosomes (miR146a^{OE}-eExo) to silk fibroin patches and demonstrated its superiority to the untreated group or monotherapy in promoting diabetic wound healing.

Additionally, eExo show inspiring effect on pathological scarring that already has formed. One study used eExo for scar treatment based on a New Zealand rabbit HS model. Meng et al.[170] engineered exosomes encapsulating miR-141-3p (MiR-141-3p^{OE}-eExo) and fabricated a dissolvable microneedle array (DMNAs) for sustained release. The MiR-141^{OE}-eExo@DMNAs could effectively decrease the HS thickness and improve fibroblast distribution and collagen arrangement via downregulating the TGF-β2/Smad pathway [170].

Anti-apoptosis

Inhibiting apoptosis of skin cells in the wound area also benefits wound closure.[171] Li et al.[172] demonstrated that exosomes derived from long non-coding RNA (lncRNA) H19-transfected MSC suppressed apoptosis and inflammation and stimulated the diabetic wound healing process in vivo via the lncRNA H19/miR-152-3p/PTEN axis. Similarly, Zhang et al.[173] reported that hypoxia-pretreated hUCSC induced miR-125b transcription, which was further encapsulated into hypoxic exosomes, alleviating apoptosis and promoting the proliferation of endothelial cells via the miR-125b/TP53INP1 axis.

Anti-senescence

Cell senescence usually causes the loss of cellular function and inflammation of adjacent normal cells via the senescence-associated secretory pattern.[174] Eliminating or inhibiting senescence is a potential strategy for promoting wound healing. Li et al.[175] collected exosomes from Nrf2-overexpressing hASC and revealed the pro-healing role of Nrf2 by inhibiting senescence and inflammation while promoting angiogenesis and granulation tissue formation in a diabetic rat model.

Anti-oxidation

A hostile oxidative wound microenvironment is an important obstacle in refractory wound healing [176]. Zhao et al.[177] utilized an original EXPLOR system (genetic engineering combined with optogenetic techniques) to produce eNOS-enriched UCSC-derived exosomes for treating chronic diabetic wounds. The results revealed that eNOS-enriched UCSC-exo enhanced cellular antioxidant capacity, neovascularization, and matrix remodeling while alleviating apoptosis and inflammation via multiple signaling pathways (PI3 K/Akt/mechanistic target of rapamycin [mTOR], and focal adhesion kinase/extracellular signal-regulated kinases 1 and 2 [FAK/ERK1/2]) in diabetic mice, ultimately accelerating wound closure and preventing skin scarring.

Xiong et al.[178] discovered that miR-15a-3p in circulating exosomes could induce the release of reactive oxygen species and impair wound healing. They knocked down exosomal miR-15a-3p, partially reversing its negative effects on wound healing in vitro and in vivo.

Preclinical and clinical applications of eExo for chronic wounds and pathological scars

Preclinical studies

Sousa et al.[179] reviewed the animal models used in wound healing during 2018 to 2023, reporting that rodents occupy the largest proportion of research involving exosome-based therapies (96.6%).

Despite the advantages of small animal models, there are associated limitations, including skin thickness, fast hair growth cycles, follicular patterns, eccrine glands, and wound size.[180] Moreover, rodents possess a subcutaneous panniculus carnosus muscle, which accelerates wound contraction.[181] Therefore, rodent models are commonly used in the initial stages of developing novel therapeutic approaches. More complex animal models are indispensable for further investigations because of their similarities to human species, such as rabbits, ovines, swine, dogs, and non-human primates.

However, the utilization of larger animal models is hindered by their high cost, difficult handling, and large setup requirements. Pigs, for instance, are considered the standard models for wound-healing studies because of the resemblance of their skin to that of humans. However, no studies have been conducted on this species. Likewise, no studies of eExo for chronic wound healing in rabbits, dogs, ovines, and swine have been conducted. Despite sharing many similarities with humans, non-human primates are rarely used mainly because of ethical concerns.[180]

Fortunately, the effect of eExo on pathological scars have been investigated on rabbit HS model.[170] As results, MiR-141-3p^{OE}-eExo@DMNAs could reduce ECM deposition and HS thickness. Nevertheless, more diverse and complicated animal models are necessary to bridge the gap between the current findings on functions and mechanisms and their practical application in human beings.

Clinical trials

Promotion of chronic wound healing and prevention of scar formation supplement each other. Therefore, prevention and early treatment are crucial for scar management. Although eExo exhibit a distinctive role in promoting wound healing and reducing skin scarring, the clinical application of exosomes remains fraught with manifold challenges[182]. We sourced clinical trials involving exosomes for wound healing and skin scarring from https://clinicaltrials.gov, https://www.anzctr.org.au/, and https://pubmed.ncbi.nlm.nih.gov/, and summarized the representative ongoing clinical trials in Table 10.

Phase 1 clinical trials

Most clinical trials of stem cell-CM, EV, and exosomes are in Phase I. In 2019, a phase I clinical trial (NCT04134676) of stem cell-conditioned medium was conducted to measure its therapeutic potential in chronic ulcer wounds. In 2020, the safety and therapeutic utility of clinical-grade platelet EV were also investigated by Johnson et al.[183] in a phase I clinical trial (ACTRN12620000944932). In 2020, the therapeutic value of the plasma-derived exosomes was investigated in a clinical trial (NCT02565264). A phase I clinical trial that tested the efficacy and safety of the combination treatment of exosomes derived from ASC and fractional CO_2 laser on acne scars was also conducted by KWON's team [69]. All 25 patients showed greater improvement in the appearance of acne scars than their controls over 12 weeks, exhibiting reduced postprocedural erythema and shorter downtime.[69]

Phase 2 clinical trials

In 2020, phase 1 and 2 clinical trials (NCT04326959) of UCSC-CM for keloid treatment were initiated. UCSC-CM treatment was administered to the keloids via intralesional injection, and keloid regression, including immunohistochemistry, histopathology, and imaging, was evaluated 3 months after injection. In 2024, a phase 2a multicenter prospective randomized trial (NCT06319287) to evaluate the safety and efficacy of topical (purified exosome product) PEP-TISSEEL for diabetic foot ulcers was initiated in the United States.

Phase 4 clinical trials

In 2023, a phase 4 clinical trial (NCT05887804) was conducted to compare the therapeutic potential of intralesional UCSC, UCSC-CM, and triamcinolone acetonide injections as keloid therapies. This research aimed to study keloid volume reduction and changes in the Patient and Observer Scar Assessment Scale score.

Other clinical trials

In 2022, a one-arm pilot study (NCT05475418) of human adipose tissue-derived exosomes (AT-exo) promoting wound healing was initiated by the Shanghai Ninth People's Hospital Affiliated with Shanghai Jiao Tong University. In 2024, the same team conducted a randomized, controlled, multicenter study (NCT06253975) on human adipose tissue-derived EV (AT-EV) to investigate their therapeutic efficacy for full-layer skin ulcers. Overall,

Table 10 Clinical trials of exosomes for the treatment of chronic wounds and pathological scarring

Clinical trial number/PMID	Conditions	Interventions	Phase	Outcome measures
Phase 1				
NCT04134676	Chronic Ulcer	Conditioned Media	Phase 1	Ulcer size, granulation tissue, edema, and erythema at 2 weeks
ACTRN12620000944932	Wounds	Platelet-derived EV	Phase 1	Adverse events and wound healing time
NCT02565264	Ulcer	Plasma-derived exosomes	Phase 1	Ulcer size and pain at 28 days
33,073,298	Acne scars	ASC-derived exosomes	Phase 1	Evaluation of scar improvement and side effects at 3 weeks
Phase 2				
NCT04326959	Keloid	UCSC-CM	Phase 1 & 2	Immunohistochemistry, histopathology, and imaging of keloid at 3 months
NCT06319287	Diabetic foot ulcer	PEP/TISSEEL	Phase 2	Percent area reduction of wound at 12 weeks
Phase 4				
NCT05887804	Keloid	UCSC-CM	Phase 4	Keloid volume reduction at 15 weeks, and POSAS score reduction at 17 weeks
Others				
NCT06253975	Wound heal	AT-EV	n/a	Wound healing percentage at 10 weeks
NCT05475418	Wounds and injuries	AT-exo	n/a	Wound healing percentage at 4 weeks

ASC, adipose-derived stem cells; EV, extracellular vesicles; AT-exo, adipose tissue-derived exosomes; UCSC-CM, umbilical cord mesenchymal stem cell-derived conditioned medium; PEP, purified exosome product; POSAS, Patient and Observer Scar Assessment Scale; n/a, not applicable

these studies suggest that eExo have the potential to promote wound healing and improve scar appearance in human with minimal adverse effects. However, larger and more rigorous clinical trials are required to fully evaluate the safety and efficacy of eExo-based therapies for wound healing and scar management.

Translational challenges: bridging the gap from preclinical to clinical application

eExo hold promise in the treatment of chronic wounds and scars, but there are translational challenges between preclinical findings and clinical applications. Barriers including immunogenicity, manufacturing scale-up, and regulatory considerations are listed in Table 11.

Immunogenicity

Animal models may show antibody production and cell-mediated immune responses against engineered exosomes. The source of exosomes (*for instance,* allogeneic vs. autologous) and engineering methods influence immunogenicity. High doses or certain administration routes can exacerbate the immune response [184]. Clinical trials report local inflammation, systemic immune responses, and antibody formation against exosomes in patients [185]. Patient-specific factors like immune status, age, and comorbidities also play a role in immune response. Autologous exosomes are preferred, but challenges remain in their large-scale production. Using autologous exosomes and modifying exosome surfaces with PEG or immunosuppressive ligands can reduce immunogenicity.

Manufacturing scale-up

Preclinical studies require small-scale production of eExo in laboratory settings, often with limited standardization. Methods may be labor-intensive and have low yields. Conversely, clinical trials demand large-scale production to treat a sufficient number of patients. Scaling up requires maintaining the quality, consistency, and functionality of exosomes. The lack of standardized manufacturing protocols is a major obstacle [186]. Thus, developing automated and efficient isolation and

Table 11 Barriers of preclinical vs clinical findings

Category	Preclinical studies	Clinical trails
Immunogenicity	Antibody production, and cell-mediated immune responses	Local inflammation, systemic immune responses, and antibody formation
Manufacturing scale-up	Small-scale, labor-intensive, and low yields	Large-scale, and lack of standardization
Regulatory considerations	Less complex	Complex

engineering methods, utilizing bioreactors for large-scale production, and establishing standardized manufacturing processes for quality control are of great value.

Regulatory considerations

Regulatory requirements for preclinical studies are relatively less complex but depend on proper study design and data integrity [187]. However, the regulatory landscape for eExo-based therapeutics is evolving and complex. There is a lack of clear and specific guidelines in many regions regarding product definition, quality control, and clinical trial design.

Concluding remarks and future perspectives

eExo-based therapies are experiencing"From Bench to Bedside". The preclinical milestones, clinical development, and regulatory considerations are depicted in Fig. 11**.** Despite the encouraging achievements of eExo, several challenges remain. (1) Scalability of eExo production: despite the revolution of current methods for exosome isolation and purification, more time-saving and labor-saving techniques are needed to produce large quantities of exosomes for clinical use. (2) Delivery of exosomes to target tissue: although the targeting ability of eExo has been improved in several studies, they are still rapidly cleared from circulation by the reticuloendothelial system, limiting their delivery to target tissue and thus limiting their efficacy as systemic therapeutics [188]. Unconventional delivery strategies, such as targeted exosome delivery using nanoparticles or cell-based carriers, are being developed to improve the specificity and efficacy of exosome-based therapies. (3) Safety and regulatory considerations: as with any new therapeutic approach, safety and regulatory considerations are important in the development of eExo-based therapies. Potential off-target effects, immunogenicity, and toxicity should be carefully evaluated in preclinical and clinical studies. Regulatory agencies such as the Food and Drug Agency are also developing guidelines for the use of exosome-based therapies in clinical settings.

A potential area for further exploration could is the role of advanced wound care dressings, which could provide a more complete picture of the current landscape of non-healing wound management. Such dressings often incorporate engineered exosomes and new technologies to create a favorable environment for wound healing. The dressings include hydrogel dressings, which absorb excess exudate while maintaining a moist environment; alginate dressings, derived from seaweed, which are absorbent and can help manage heavy exudate; foam dressings, which provide cushioning and absorption, making them suitable for wounds with moderate to heavy exudate; and silver-containing dressings, which can help prevent infection because silver has antimicrobial properties. Another type of advanced wound care dressing is negative pressure wound therapy (NPWT), in which gentle suction is applied to the wound, promoting drainage and reducing edema.

Despite the abovementioned challenges, the potential of eExo in wound healing and scar management remains promising. Except for conquering the challenges, future research should focus on improving engineering techniques, optimizing delivery methods, identifying specific pro-healing and anti-scarring cargo molecules, and delving into the mechanisms, to further enhance the effectiveness of eExo. Overall, eExo-based therapeutics are rapidly evolving and hold great promise for wound healing and scar prevention.

Fig. 11 From Bench to Bedside of eExo-based therapy. (A) Preclinical milestones: including in vitro studies, and animal model efficacy and safety evaluations, and lead candidate selection. (B) Clinical development: including Phase I, II, and III clinical trials. (C) Regulatory considerations: Critical aspects including IND/NDA submissions, adherence to GMP for production, and interactions with relevant regulatory agencies (*e.g.,* FDA). Abbreviations: IND, Investigational New Drug; NDA, New Drug Application; GMP, Good Manufacturing Practice; FDA, Food and Drug Agency

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Author contributions
CQL: writing- original draft preparation. CC: writing- original draft preparation. KC and ASG: preparation of the figures and tables. YYZ and QFL: writing-reviewing and editing. AA: supervision. All authors read and approved the manuscript.

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Declarations

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References

1. Monavarian M, Kader S, Moeinzadeh S, Jabbari E. Regenerative scar-free skin wound healing. Tissue Eng Part B Rev. 2019;25(4):294–311.
2. Zhang J, Zheng Y, Lee J, Hua J, Li S, Panchamukhi A, Yue J, Gou X, Xia Z, Zhu L, Wu X. A pulsatile release platform based on photo-induced imine-crosslinking hydrogel promotes scarless wound healing. Nat Commun. 2021;12(1):1670.
3. Peña OA, Martin P. Cellular and molecular mechanisms of skin wound healing. Nat Rev Mol Cell Biol. 2024;25(8):599–616.
4. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. Physiol Rev. 2019;99(1):665–706.
5. Phillipson M, Kubes P. The healing power of neutrophils. Trends Immunol. 2019;40(7):635–47.
6. Sim SL, Kumari S, Kaur S, Khosrotehrani K. Macrophages in skin wounds: functions and therapeutic potential. Biomolecules. 2022;12(11):1659.
7. Ma Y, Liu Z, Miao L, Jiang X, Ruan H, Xuan R, Xu S. Mechanisms underlying pathological scarring by fibroblasts during wound healing. Int Wound J. 2023;20(6):2190–206.
8. Moretti L, Stalfort J, Barker TH, Abebayehu D. The interplay of fibroblasts, the extracellular matrix, and inflammation in scar formation. J Biol Chem. 2022;298(2): 101530.
9. Rousselle P, Braye F, Dayan G. Re-epithelialization of adult skin wounds: cellular mechanisms and therapeutic strategies. Adv Drug Deliv Rev. 2019;146:344–65.
10. Potekaev NN, Borzykh OB, Medvedev GV, Pushkin DV, Petrova MM, Petrov AV, Dmitrenko DV, Karpova EI, Demina OM, Shnayder NA. The role of extracellular matrix in skin wound healing. J Clin Med. 2021;10(24):5947.
11. Diller RB, Tabor AJ. The role of the extracellular matrix (ECM) in wound healing: a review. Biomimetics. 2022;7(3):87.
12. Almine JF, Bax DV, Mithieux SM, Nivison-Smith L, Rnjak J, Waterhouse A, Wise SG, Weiss AS. Elastin-based materials. Chem Soc Rev. 2010;39(9):3371–9.
13. Sarangthem V, Singh TD, Dinda AK. Emerging role of elastin-like polypeptides in regenerative medicine. Adv Wound Care. 2021;10(5):257–69.
14. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. Nature. 2008;453(7193):314–21.
15. Talbott HE, Mascharak S, Griffin M, Wan DC, Longaker MT. Wound healing, fibroblast heterogeneity, and fibrosis. Cell Stem Cell. 2022;29(8):1161–80.
16. Jones RE, Foster DS, Longaker MT. Management of chronic wounds-2018. JAMA. 2018;320(14):1481–2.
17. Pignet AL, Schellnegger M, Hecker A, Kamolz LP, Kotzbeck P. Modeling wound chronicity in vivo: the translational challenge to capture the complexity of chronic wounds. J Invest Dermatol. 2024;144(7):1454–70.
18. Liu T, Lu Y, Zhan R, Qian W, Luo G. Nanomaterials and nanomaterials-based drug delivery to promote cutaneous wound healing. Adv Drug Deliv Rev. 2023;193: 114670.
19. Bowers S, Franco E. Chronic wounds: evaluation and management. Am Fam Physician. 2020;101(3):159–66.
20. Guan Y, Niu H, Liu Z, Dang Y, Shen J, Zayed M, Ma L, Guan J. Sustained oxygenation accelerates diabetic wound healing by promoting epithelialization and angiogenesis and decreasing inflammation. Sci Adv. 2021. https://doi.org/10.1126/sciadv.abj0153.
21. Yang Q, Fang D, Chen J, Hu S, Chen N, Jiang J, Zeng M, Luo M. LncRNAs associated with oxidative stress in diabetic wound healing: regulatory mechanisms and application prospects. Theranostics. 2023;13(11):3655–74.
22. C.D. Weller, C. Richards, L. Turnour, A.M. Patey, G. Russell, V. Team, Barriers and enablers to the use of venous leg ulcer clinical practice guidelines in Australian primary care: a qualitative study using the theoretical domains framework. Int J Nurs Stud, 2020. **103**: p. 103503.
23. Alam W, Hasson J, Reed M. Clinical approach to chronic wound management in older adults. J Am Geriatr Soc. 2021;69(8):2327–34.
24. Alexander JB. Lower-extremity vascular ulcers: assessment and approaches to management. Med Clin North Am. 2023;107(5):911–23.
25. Mervis JS, Phillips TJ. Pressure ulcers: pathophysiology, epidemiology, risk factors, and presentation. J Am Acad Dermatol. 2019;81(4):881–90.
26. Matoori S, Veves A, Mooney DJ. Advanced bandages for diabetic wound healing. Sci Transl Med. 2021. https://doi.org/10.1126/scitranslmed.abe4839.
27. Schilrreff P, Alexiev U. Chronic inflammation in non-healing skin wounds and promising natural bioactive compounds treatment. Int J Mol Sci. 2022;23(9):4928.
28. Li M, Hou Q, Zhong L, Zhao Y, Fu X. Macrophage related chronic inflammation in non-healing wounds. Front Immunol. 2021;12: 681710.
29. Xiaojie W, Banda J, Qi H, Chang AK, Bwalya C, Chao L, Li X. Scarless wound healing: current insights from the perspectives of TGF-β, KGF-1, and KGF-2. Cytokine Growth Factor Rev. 2022;66:26–37.
30. Berthiaume F, Hsia HC. Regenerative approaches for chronic wounds. Annu Rev Biomed Eng. 2022;24:61–83.
31. Li S, Ding H, Yang Y, Yu B, Chen M. Global research status of pathological scar reported over the period 2001–2021: a 20-year bibliometric analysis. Int Wound J. 2023;20(5):1725–38.
32. Li X, Wang X, Shen T, Xiong J, Ma Q, Guo G, Zhu F. Advances in photodynamic therapy of pathologic scar. Photodiagnosis Photodyn Ther. 2024;46: 104040.
33. Cohen AJ, Nikbakht N, Uitto J. Keloid Disorder: Genetic Basis, Gene Expression Profiles, and Immunological Modulation of the Fibrotic Processes in the Skin. Cold Spring Harb Perspect Biol. 2023;15(7):a041245.
34. Hong YK, Chang YH, Lin YC, Chen B, Guevara BEK, Hsu CK. Inflammation in Wound Healing and Pathological Scarring. Adv Wound Care. 2023;12(5):288–300.
35. Martin P, Nunan R. Cellular and molecular mechanisms of repair in acute and chronic wound healing. Br J Dermatol. 2015;173(2):370–8.
36. Wilkinson HN, Hardman MJ. Wound healing: cellular mechanisms and pathological outcomes. Open Biol. 2020;10(9): 200223.
37. Huang C, Ogawa R. Role of inflammasomes in keloids and hypertrophic scars-lessons learned from chronic diabetic wounds and skin fibrosis. Int J Mol Sci. 2022;23(12):6820.

38. Dohi T, Padmanabhan J, Akaishi S, Than PA, Terashima M, Matsumoto NN, Ogawa R, Gurtner GC. The interplay of mechanical stress, strain, and stiffness at the keloid periphery correlates with increased caveolin-1/ROCK signaling and scar progression. Plast Reconstr Surg. 2019;144(1):58e–67e.
39. Gao N, Lu L, Ma X, Liu Z, Yang S, Han G. Targeted inhibition of YAP/TAZ alters the biological behaviours of keloid fibroblasts. Exp Dermatol. 2022;31(3):320–9.
40. Wang Q, Wang P, Qin Z, Yang X, Pan B, Nie F, Bi H. Altered glucose metabolism and cell function in keloid fibroblasts under hypoxia. Redox Biol. 2021;38: 101815.
41. Chen Z, Gao J, Li L. New challenges in scar therapy: the novel scar therapy strategies based on nanotechnology. Nanomedicine. 2024;19(28):2413–32.
42. P. Abdel-Sayed, M. Michetti, C. Scaletta, M. Flahaut, N. Hirt-Burri, de A Buys Roessingh, W. Raffoul, L.A. Applegate, Cell therapies for skin regeneration: an overview of 40 years of experience in burn units. Swiss Med Wkly, 2019. 149: 20079.
43. A. Laurent, P. Abdel-Sayed, C. Scaletta, P. Laurent, E. Laurent, M. Michetti, A. de Buys Roessingh, W. Raffoul, N. Hirt-Burri, L.A. Applegate, back to the cradle of cytotherapy: integrating a century of clinical research and biotechnology-based manufacturing for modern tissue-specific cellular treatments in Switzerland. Bioengineering, 2021. 8(12).
44. Farabi B, Roster K, Hirani R, Tepper K, Atak MF, Safai B. The efficacy of stem cells in wound healing: a systematic review. Int J Mol Sci. 2024;25(5):6.
45. Chen H, Hou K, Wu Y, Liu Z. Use of adipose stem cells against hypertrophic scarring or keloid. Front Cell Dev Biol. 2021;9: 823694.
46. Lu M, Peng L, Ming X, Wang X, Cui A, Li Y, Wang X, Meng D, Sun N, Xiang M, Chen S. Enhanced wound healing promotion by immune response-free monkey autologous iPSCs and exosomes vs. their allogeneic counterparts. EBioMedicine. 2019;42:443–57.
47. Carvalho MS, Alves L, Bogalho I, Cabral JMS, da Silva CL. Impact of donor age on the osteogenic supportive capacity of mesenchymal stromal cell-derived extracellular matrix. Front Cell Dev Biol. 2021;9: 747521.
48. Horinouchi CD, Barisón MJ, Robert AW, Kuligovski C, Aguiar AM, Dallagiovanna B. Influence of donor age on the differentiation and division capacity of human adipose-derived stem cells. World J Stem Cells. 2020;12(12):1640–51.
49. Zhang C, Wang T, Zhang L, Chen P, Tang S, Chen A, Li M, Peng G, Gao H, Weng H, Zhang H, Li S, Chen J, Chen L, Chen X. Combination of lyophilized adipose-derived stem cell concentrated conditioned medium and polysaccharide hydrogel in the inhibition of hypertrophic scarring. Stem Cell Res Ther. 2021;12(1):23.
50. Yoon J, Yoon D, Lee H, Lee J, Jo S, Kym D, Yim H, Hur J, Chun W, Kim G, Cho YS. Wound healing ability of acellular fish skin and bovine collagen grafts for split-thickness donor sites in burn patients: characterization of acellular grafts and clinical application. Int J Biol Macromol. 2022;205:452–61.
51. Liu X, Cui B, Wang X, Zheng M, Bai Z, Yue O, Fei Y, Jiang H. Nature-skin-derived e-skin as versatile "wound therapy-health monitoring" bioelectronic skin-scaffolds: skin to bio-e-skin. Adv Healthc Mater. 2023;12(20): e2202971.
52. Zhou BR, Xu Y, Guo SL, Xu Y, Wang Y, Zhu F, Permatasari F, Wu D, Yin ZQ, Luo D. The effect of conditioned media of adipose-derived stem cells on wound healing after ablative fractional carbon dioxide laser resurfacing. Biomed Res Int. 2013;2013: 519126.
53. Liang X, Lin F, Ding Y, Zhang Y, Li M, Zhou X, Meng Q, Ma X, Wei L, Fan H, Liu Z. Conditioned medium from induced pluripotent stem cell-derived mesenchymal stem cells accelerates cutaneous wound healing through enhanced angiogenesis. Stem Cell Res Ther. 2021;12(1):295.
54. Li M, Luan F, Zhao Y, Hao H, Liu J, Dong L, Fu X, Han W. Mesenchymal stem cell-conditioned medium accelerates wound healing with fewer scars. Int Wound J. 2017;14(1):64–73.
55. Rong X, Chu W, Zhang H, Wang Y, Qi X, Zhang G, Wang Y, Li C. Antler stem cell-conditioned medium stimulates regenerative wound healing in rats. Stem Cell Res Ther. 2019;10(1):326.
56. Joo SY, Cho YS, Lee SY, Seo CH. Regenerative effect of combined laser and human stem cell-conditioned medium therapy on hypertrophic burn scar. Burns. 2023;49(4):870–6.
57. Hu CH, Tseng YW, Lee CW, Chiou CY, Chuang SS, Yang JY, Lee OK. Combination of mesenchymal stem cell-conditioned medium and botulinum toxin type A for treating human hypertrophic scars. J Plast Reconstr Aesthet Surg. 2020;73(3):516–27.
58. Sato C, Yamamoto Y, Funayama E, Furukawa H, Oyama A, Murao N, Hosono H, Kawakubo K, Sakamoto N, Ohnishi S. Conditioned medium obtained from amnion-derived mesenchymal stem cell culture prevents activation of keloid fibroblasts. Plast Reconstr Surg. 2018;141(2):390–8.
59. Md Fadilah NI, Mohd Abdul Kader Jailani MS, Badrul Hisham MA, Sunthar Raj N, Shamsuddin SA, Ng MH, Fauzi MB, Maarof M, *Cell secretomes for wound healing and tissue regeneration: Next generation acellular based tissue engineered products.* J Tissue Eng, 2022. 13: 204.
60. Park KS, Bandeira E, Shelke GV, Lässer C, Lötvall J. Enhancement of therapeutic potential of mesenchymal stem cell-derived extracellular vesicles. Stem Cell Res Ther. 2019;10(1):288.
61. Couch Y, Buzàs EI, Di Vizio D, Gho YS, Harrison P, Hill AF, Lötvall J, Raposo G, Stahl PD, Théry C, Witwer KW, Carter DRF. A brief history of nearly EV-erything—the rise and rise of extracellular vesicles. J Extracell Vesicles. 2021;10(14): e12144.
62. Askenase PW. exosome carrier effects; resistance to digestion in phagolysosomes may assist transfers to targeted cells; II transfers of mirnas are better analyzed via systems approach as they do not fit conventional reductionist stoichiometric concepts. Int J Mol Sci. 2022;23(11):6912.
63. Chargaff E. Cell structure and the problem of blood coagulation. J Biol Chem. 1945;160(1):351–9.
64. Chargaff E, West R. The biological significance of the thromboplastic protein of blood. J Biol Chem. 1946;166(1):189–97.
65. Raposo G, Nijman HW, Stoorvogel W, Liejendekker R, Harding CV, Melief CJ, Geuze HJ. B lymphocytes secrete antigen-presenting vesicles. J Exp Med. 1996;183(3):1161–72.
66. Bruno S, Grange C, Deregibus MC, Calogero RA, Saviozzi S, Collino F, Morando L, Busca A, Falda M, Bussolati B, Tetta C, Camussi G. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. J Am Soc Nephrol. 2009;20(5):1053–67.
67. Garima D, Sharma A, Kumar EM. Extracellular vesicle-based biovectors in chronic wound healing: biogenesis and delivery approaches. Mol Ther Nucleic Acids. 2023;32:822–40.
68. Ding JY, Chen MJ, Wu LF, Shu GF, Fang SJ, Li ZY, Chu XR, Li XK, Wang ZG, Ji JS. Mesenchymal stem cell-derived extracellular vesicles in skin wound healing: roles, opportunities and challenges. Mil Med Res. 2023;10(1):36.
69. Kwon HH, Yang SH, Lee J, Park BC, Park KY, Jung JY, Bae Y, Park GH. Combination treatment with human adipose tissue stem cell-derived exosomes and fractional CO2 laser for acne scars: a 12-week prospective, double-blind, randomized, split-face study. Acta Derm Venereol. 2020;100(18):adv00310.
70. Tan F, Li X, Wang Z, Li J, Shahzad K, Zheng J. Clinical applications of stem cell-derived exosomes. Signal Transduct Target Ther. 2024;9(1):17.
71. Hettich BF, Ben-Yehuda Greenwald M, Werner S, Leroux JC. Exosomes for wound healing: purification optimization and identification of bioactive components. Adv Sci. 2020;7(23):2002596.
72. Levy D, Abadchi SN, Shababi N, Ravari MR, Pirolli NH, Bergeron C, Obiorah A, Mokhtari-Esbuie F, Gheshlaghi S, Abraham JM, Smith IM, Powsner EH, Solomon TJ, Harmon JW, Jay SM. Induced pluripotent stem cell-derived extracellular vesicles promote wound repair in a diabetic mouse model via an anti-inflammatory immunomodulatory mechanism. Adv Healthc Mater. 2023;12(26): e2300879.
73. Chen B, Sun Y, Zhang J, Zhu Q, Yang Y, Niu X, Deng Z, Li Q, Wang Y. Human embryonic stem cell-derived exosomes promote pressure ulcer healing in aged mice by rejuvenating senescent endothelial cells. Stem Cell Res Ther. 2019;10(1):142.
74. Duan M, Zhang Y, Zhang H, Meng Y, Qian M, Zhang G. Epidermal stem cell-derived exosomes promote skin regeneration by downregulating transforming growth factor-β1 in wound healing. Stem Cell Res Ther. 2020;11(1):452.
75. Las Heras K, Royo F, Garcia-Vallicrosa C, Igartua M, Santos-Vizcaino E, Falcon-Perez JM, Hernandez RM. Extracellular vesicles from hair follicle-derived mesenchymal stromal cells: isolation, characterization and therapeutic potential for chronic wound healing. Stem Cell Res Ther. 2022;13(1):147.

76. Voloshin N, Tyurin-Kuzmin P, Karagyaur M, Akopyan Z, Kulebyakin K. Practical use of immortalized cells in medicine: current advances and future perspectives. Int J Mol Sci. 2023. https://doi.org/10.1186/s13287-022-02824-0.
77. Paprocka M, Kraskiewicz H, Bielawska-Pohl A, Krawczenko A, Masłowski L, Czyżewska-Buczyńska A, Witkiewicz W, Dus D, Czarnecka A. From primary MSC culture of adipose tissue to immortalized cell line producing cytokines for potential use in regenerative medicine therapy or immunotherapy. Int J Mol Sci. 2021;22(21):1.
78. Yu L, Wen H, Liu C, Wang C, Yu H, Zhang K, Han Q, Liu Y, Han Z, Li Z, Liu N. Embryonic stem cell-derived extracellular vesicles rejuvenate senescent cells and antagonize aging in mice. Bioact Mater. 2023;29:85–97.
79. Wang Z. Assessing tumorigenicity in stem cell-derived therapeutic products: a critical step in safeguarding regenerative medicine. Bioengineering. 2023;10(7):1.
80. Li Y, Zhu Z, Li S, Xie X, Qin L, Zhang Q, Yang Y, Wang T, Zhang Y. Exosomes: compositions, biogenesis, and mechanisms in diabetic wound healing. J Nanobiotechnol. 2024;22(1):398.
81. Liu Y, Zhang M, Liao Y, Chen H, Su D, Tao Y, Li J, Luo K, Wu L, Zhang X, Yang R. Human umbilical cord mesenchymal stem cell-derived exosomes promote murine skin wound healing by neutrophil and macrophage modulations revealed by single-cell RNA sequencing. Front Immunol. 2023;14:1142088.
82. Camões SP, Bulut O, Yazar V, Gaspar MM, Simões S, Ferreira R, Vitorino R, Santos JM, Gursel I, Miranda JP. 3D-MSCs A151 ODN-loaded exosomes are immunomodulatory and reveal a proteomic cargo that sustains wound resolution. J Adv Res. 2022;41:113–28.
83. Wang Y, Zhang Y, Li T, Shen K, Wang KJ, Tian C, Hu D. Adipose mesenchymal stem cell derived exosomes promote keratinocytes and fibroblasts embedded in collagen/platelet-rich plasma scaffold and accelerate wound healing. Adv Mater. 2023;35(40): e2303642.
84. Zhang Y, Pan Y, Liu Y, Li X, Tang L, Duan M, Li J, Zhang G. Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulate regenerative wound healing via transforming growth factor-β receptor inhibition. Stem Cell Res Ther. 2021;12(1):434.
85. Brunet A, Goodell MA, Rando TA. Ageing and rejuvenation of tissue stem cells and their niches. Nat Rev Mol Cell Biol. 2023;24(1):45–62.
86. Yang G, Waheed S, Wang C, Shekh M, Li Z, Wu J. Exosomes and their bioengineering strategies in the cutaneous wound healing and related complications: current knowledge and future perspectives. Int J Biol Sci. 2023;19(5):1430–54.
87. Fuchs E, Blau HM. Tissue stem cells: architects of their niches. Cell Stem Cell. 2020;27(4):532–56.
88. Zhang B, Shi Y, Gong A, Pan Z, Shi H, Yang H, Fu H, Yan Y, Zhang X, Wang M, Zhu W, Qian H, Xu W. HucMSC exosome-delivered 14-3-3ζ orchestrates self-control of the Wnt response via modulation of YAP during cutaneous regeneration. Stem Cells. 2016;34(10):2485–500.
89. Zhang B, Wang M, Gong A, Zhang X, Wu X, Zhu Y, Shi H, Wu L, Zhu W, Qian H, Xu W. HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. Stem Cells. 2015;33(7):2158–68.
90. Guo SC, Tao SC, Yin WJ, Qi X, Yuan T, Zhang CQ. Exosomes derived from platelet-rich plasma promote the re-epithelization of chronic cutaneous wounds via activation of YAP in a diabetic rat model. Theranostics. 2017;7(1):81–96.
91. Chen CY, Rao SS, Ren L, Hu XK, Tan YJ, Hu Y, Luo J, Liu YW, Yin H, Huang J, Cao J, Wang ZX, Liu ZZ, Liu HM, Tang SY, Xu R, Xie H. Exosomal DMBT1 from human urine-derived stem cells facilitates diabetic wound repair by promoting angiogenesis. Theranostics. 2018;8(6):1607–23.
92. Li Y, Zhang J, Shi J, Liu K, Wang X, Jia Y, He T, Shen K, Wang Y, Liu J, Zhang W, Wang H, Zheng Z, Hu D. Exosomes derived from human adipose mesenchymal stem cells attenuate hypertrophic scar fibrosis by miR-192-5p/IL-17RA/Smad axis. Stem Cell Res Ther. 2021;12(1):221.
93. Fang S, Xu C, Zhang Y, Xue C, Yang C, Bi H, Qian X, Wu M, Ji K, Zhao Y, Wang Y, Liu H, Xing X. Umbilical cord-derived mesenchymal stem cell-derived exosomal microRNAs suppress myofibroblast differentiation by inhibiting the transforming growth factor-β/SMAD2 pathway during wound healing. Stem Cells Transl Med. 2016;5(10):1425–39.
94. Zhao W, Zhang R, Zang C, Zhang L, Zhao R, Li Q, Yang Z, Feng Z, Zhang W, Cui R. Exosome derived from mesenchymal stem cells alleviates pathological scars by inhibiting the proliferation, migration and protein expression of fibroblasts via delivering miR-138-5p to target SIRT1. Int J Nanomedicine. 2022;17:4023–38.
95. Heo JS, Kim S, Yang CE, Choi Y, Song SY, Kim HO. Human adipose mesenchymal stem cell-derived exosomes: a key player in wound healing. Tissue Eng Regen Med. 2021;18(4):537–48.
96. Hu Y, Rao SS, Wang ZX, Cao J, Tan YJ, Luo J, Li HM, Zhang WS, Chen CY, Xie H. Exosomes from human umbilical cord blood accelerate cutaneous wound healing through miR-21-3p-mediated promotion of angiogenesis and fibroblast function. Theranostics. 2018;8(1):169–84.
97. Li X, Liu L, Yang J, Yu Y, Chai J, Wang L, Ma L, Yin H. Exosome derived from human umbilical cord mesenchymal stem cell mediates MiR-181c attenuating burn-induced excessive inflammation. EBioMedicine. 2016;8:72–82.
98. Li YJ, Wu JY, Liu J, Xu W, Qiu X, Huang S, Hu XB, Xiang DX. Artificial exosomes for translational nanomedicine. J Nanobiotechnol. 2021;19(1):242.
99. Deng S, Cao H, Cui X, Fan Y, Wang Q, Zhang X. Optimization of exosome-based cell-free strategies to enhance endogenous cell functions in tissue regeneration. Acta Biomater. 2023;171:68–84.
100. Shao H, Im H, Castro CM, Breakefield X, Weissleder R, Lee H. New technologies for analysis of extracellular vesicles. Chem Rev. 2018;118(4):1917–50.
101. Xu C, Jiang C, Li Z, Gao H, Xian J, Guo W, He D, Peng X, Zhou D, Li D. Exosome nanovesicles: biomarkers and new strategies for treatment of human diseases. MedComm. 2024;5(8):e660.
102. Zhang Y, Liu Q, Zhang X, Huang H, Tang S, Chai Y, Xu Z, Li M, Chen X, Liu J, Yang C. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy. J Nanobiotechnol. 2022;20(1):279.
103. Hu B, Zhong L, Weng Y, Peng L, Huang Y, Zhao Y, Liang XJ. Therapeutic siRNA: state of the art. Signal Transduct Target Ther. 2020;5(1):101.
104. Paunovska K, Loughrey D, Dahlman JE. Drug delivery systems for RNA therapeutics. Nat Rev Genet. 2022;23(5):265–80.
105. Chen LL. The expanding regulatory mechanisms and cellular functions of circular RNAs. Nat Rev Mol Cell Biol. 2020;21(8):475–90.
106. Li L, Wang F, Zhu D, Hu S, Cheng K, Li Z. Engineering exosomes and exosome-like nanovesicles for improving tissue targeting and retention. Fundam Res. 2025;5(2):851–67.
107. Zhang SH, Peng LL, Chen YF, Xu Y, Moradi V. Focusing on exosomes to overcome the existing bottlenecks of CAR-T cell therapy. Inflamm Regen. 2024;44(1):45.
108. Palakurthi SS, Shah B, Kapre S, Charbe N, Immanuel S, Pasham S, Thalla M, Jain A, Palakurthi S. A comprehensive review of challenges and advances in exosome-based drug delivery systems. Nanoscale Adv. 2024;6(23):5803–26.
109. Krylova SV, Feng D. The machinery of exosomes: biogenesis, release, and uptake. Int J Mol Sci. 2023;24(2):1337.
110. Feng ZY, Zhang QY, Tan J, Xie HQ. Techniques for increasing the yield of stem cell-derived exosomes: what factors may be involved? Sci China Life Sci. 2022;65(7):1325–41.
111. Al-Masawa ME, Alshawsh MA, Ng CY, Ng AMH, Foo JB, Vijakumaran U, Subramaniam R, Ghani NAA, Witwer KW, Law JX. Efficacy and safety of small extracellular vesicle interventions in wound healing and skin regeneration: a systematic review and meta-analysis of animal studies. Theranostics. 2022;12(15):6455–508.
112. Wang L, Abhange KK, Wen Y, Chen Y, Xue F, Wang G, Tong J, Zhu C, He X, Wan Y. Preparation of engineered extracellular vesicles derived from human umbilical cord mesenchymal stem cells with ultrasonication for skin rejuvenation. ACS Omega. 2019;4(27):22638–45.
113. Joorabloo A, Liu T. Engineering exosome-based biomimetic nanovehicles for wound healing. J Control Release. 2023;356:463–80.
114. You DG, Lim GT, Kwon S, Um W, Oh BH, Song SH, Lee J, Jo DG, Cho YW, Park JH. Metabolically engineered stem cell-derived exosomes to regulate macrophage heterogeneity in rheumatoid arthritis. Sci Adv. 2021;7(23):1.
115. Lee J, Lee H, Goh U, Kim J, Jeong M, Lee J, Park JH. Cellular engineering with membrane fusogenic liposomes to produce functionalized extracellular vesicles. ACS Appl Mater Interfaces. 2016;8(11):6790–5.
116. Wang J, Wu H, Peng Y, Zhao Y, Qin Y, Zhang Y, Xiao Z. Hypoxia adipose stem cell-derived exosomes promote high-quality healing of diabetic wound involves activation of PI3K/Akt pathways. J Nanobiotechnol. 2021;19(1):202.

117. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. Int J Nanomed. 2020;15:6917–34.
118. Raghav A, Tripathi P, Mishra BK, Jeong GB, Banday S, Gautam KA, Mateen QN, Singh P, Singh M, Singla A, Ahmad J. Mesenchymal stromal cell-derived tailored exosomes treat bacteria-associated diabetes foot ulcers: a customized approach from bench to bed. Front Microbiol. 2021;12: 712588.
119. Jafari D, Shajari S, Jafari R, Mardi N, Gomari H, Ganji F, Forouzandeh Moghadam M, Samadikuchaksaraei A. Designer exosomes: a new platform for biotechnology therapeutics. Bio Drugs. 2020;34(5):567–86.
120. Huang J, Yu M, Yin W, Liang B, Li A, Li J, Li X, Zhao S, Liu F. Development of a novel RNAi therapy: engineered miR-31 exosomes promoted the healing of diabetic wounds. Bioact Mater. 2021;6(9):2841–53.
121. Sadeghi S, Tehrani FR, Tahmasebi S, Shafiee A, Hashemi SM. Exosome engineering in cell therapy and drug delivery. Inflammopharmacology. 2023;31(1):145–69.
122. Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D. Engineering exosomes as refined biological nanoplatforms for drug delivery. Acta Pharmacol Sin. 2017;38(6):754–63.
123. Wang J, Wang M, Jiang N, Ding S, Peng Q, Zheng L. Emerging chemical engineering of exosomes as "bioscaffolds" in diagnostics and therapeutics. Genes Dis. 2023;10(4):1494–512.
124. N'Diaye ER, Orefice NS, Ghezzi C, Boumendjel A. Chemically modified extracellular vesicles and applications in radiolabeling and drug delivery. Pharmaceutics. 2022;14(3):653.
125. Lu S, Lu L, Liu Y, Li Z, Fang Y, Chen Z, Zhou J. Native and engineered extracellular vesicles for wound healing. Front Bioeng Biotechnol. 2022;10:1053217.
126. Kim MS, Haney MJ, Zhao Y, Mahajan V, Deygen I, Klyachko NL, Inskoe E, Piroyan A, Sokolsky M, Okolie O, Hingtgen SD, Kabanov AV, Batrakova EV. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. Nanomedicine. 2016;12(3):655–64.
127. Du J, Wan Z, Wang C, Lu F, Wei M, Wang D, Hao Q. Designer exosomes for targeted and efficient ferroptosis induction in cancer via chemo-photodynamic therapy. Theranostics. 2021;11(17):8185–96.
128. Xu M, Yang Q, Sun X, Wang Y. Recent advancements in the loading and modification of therapeutic exosomes. Front Bioeng Biotechnol. 2020;8: 586130.
129. Wan T, Zhong J, Pan Q, Zhou T, Ping Y, Liu X. Exosome-mediated delivery of Cas9 ribonucleoprotein complexes for tissue-specific gene therapy of liver diseases. Sci Adv. 2022;8(37):eabp9435.
130. Yang Z, Xie J, Zhu J, Kang C, Chiang C, Wang X, Wang X, Kuang T, Chen F, Chen Z, Zhang A, Yu B, Lee RJ, Teng L, Lee LJ. Functional exosome-mimic for delivery of siRNA to cancer: in vitro and in vivo evaluation. J Control Release. 2016;243:160–71.
131. Lunavat TR, Jang SC, Nilsson L, Park HT, Repiska G, Lässer C, Nilsson JA, Gho YS, Lötvall J. RNAi delivery by exosome-mimetic nanovesicles—implications for targeting c-Myc in cancer. Biomaterials. 2016;102:231–8.
132. Jeyaram A, Lamichhane TN, Wang S, Zou L, Dahal E, Kronstadt SM, Levy D, Parajuli B, Knudsen DR, Chao W, Jay SM. Enhanced loading of functional miRNA cargo via pH gradient modification of extracellular vesicles. Mol Ther. 2020;28(3):975–85.
133. Schulz-Siegmund M, Aigner A. Nucleic acid delivery with extracellular vesicles. Adv Drug Deliv Rev. 2021;173:89–111.
134. Fu S, Wang Y, Xia X, Zheng JC. Exosome engineering: current progress in cargo loading and targeted delivery. NanoImpact. 2020;20: 100261.
135. Zhang W, Wang L, Guo H, Chen L, Huang X. Dapagliflozin-loaded exosome mimetics facilitate diabetic wound healing by HIF-1α-mediated enhancement of angiogenesis. Adv Healthc Mater. 2023;12(7): e2202751.
136. Xu X, Xu L, Wen C, Xia J, Zhang Y, Liang Y. Programming assembly of biomimetic exosomes: an emerging theranostic nanomedicine platform. Mater Today Bio. 2023;22: 100760.
137. Hajipour H, Farzadi L, Roshangar L, Latifi Z, Kahroba H, Shahnazi V, Hamdi K, Ghasemzadeh A, Fattahi A, Nouri M. A human chorionic gonadotropin (hCG) delivery platform using engineered uterine exosomes to improve endometrial receptivity. Life Sci. 2021;275: 119351.
138. Zhao H, Li Z, Wang Y, Zhou K, Li H, Bi S, Wang Y, Wu W, Huang Y, Peng B, Tang J, Pan B, Wang B, Chen Z, Zhang Z. Bioengineered MSC-derived exosomes in skin wound repair and regeneration. Front Cell Dev Biol. 2023;11:1029671.
139. Kusuma GD, Barabadi M, Tan JL, Morton DAV, Frith JE, Lim R. To protect and to preserve: novel preservation strategies for extracellular vesicles. Front Pharmacol. 2018;9:1199.
140. Li Q, Hu W, Huang Q, Yang J, Li B, Ma K, Wei Q, Wang Y, Su J, Sun M, Cui S, Yang R, Li H, Fu X, Zhang C. MiR146a-loaded engineered exosomes released from silk fibroin patch promote diabetic wound healing by targeting IRAK1. Signal Transduct Target Ther. 2023;8(1):62.
141. Han X, Saengow C, Ju L, Ren W, Ewoldt RH, Irudayaraj J. Exosome-coated oxygen nanobubble-laden hydrogel augments intracellular delivery of exosomes for enhanced wound healing. Nat Commun. 2024;15(1):3435.
142. Li X, Qu S, Ouyang Q, Qin F, Guo J, Qin M, Zhang J. A multifunctional composite nanoparticle with antibacterial activities, anti-inflammatory, and angiogenesis for diabetic wound healing. Int J Biol Macromol. 2024;260(Pt 2): 129531.
143. Abbasi R, Alamdari-Mahd G, Maleki-Kakelar H, Momen-Mesgin R, Ahmadi M, Sharafkhani M, Rezaie J. Recent advances in the application of engineered exosomes from mesenchymal stem cells for regenerative medicine. Eur J Pharmacol. 2025;989: 177236.
144. Wang C, Xu M, Fan Q, Li C, Zhou X. Therapeutic potential of exosome-based personalized delivery platform in chronic inflammatory diseases. Asian J Pharm Sci. 2023;18(1): 100772.
145. Tian J, Han Z, Song D, Peng Y, Xiong M, Chen Z, Duan S, Zhang L. Engineered exosome for drug delivery: recent development and clinical applications. Int J Nanomed. 2023;18:7923–40.
146. Akhlaghpasand M, Tavanaei R, Hosseinpoor M, Yazdani KO, Soleimani A, Zoshk MY, Soleimani M, Chamanara M, Ghorbani M, Deylami M, Zali A, Heidari R, Oraee-Yazdani S. Safety and potential effects of intrathecal injection of allogeneic human umbilical cord mesenchymal stem cell-derived exosomes in complete subacute spinal cord injury: a first-in-human, single-arm, open-label, phase I clinical trial. Stem Cell Res Ther. 2024;15(1):264.
147. Samuel M, Gabrielsson S. Personalized medicine and back-allogeneic exosomes for cancer immunotherapy. J Intern Med. 2021;289(2):138–46.
148. Bahadorani M, Nasiri M, Dellinger K, Aravamudhan S, Zadegan R. Engineering exosomes for therapeutic applications: decoding biogenesis, content modification, and cargo loading strategies. Int J Nanomedicine. 2024;19:7137–64.
149. Ghosh S, Rajendran RL, Mahajan AA, Chowdhury A, Bera A, Guha S, Chakraborty K, Chowdhury R, Paul A, Jha S, Dey A, Dubey A, Gorai S, Das P, Hong CM, Krishnan A, Gangadaran P, Ahn BC. Harnessing exosomes as cancer biomarkers in clinical oncology. Cancer Cell Int. 2024;24(1):278.
150. Mondal J, Pillarisetti S, Junnuthula V, Saha M, Hwang SR, Park IK, Lee YK. Hybrid exosomes, exosome-like nanovesicles and engineered exosomes for therapeutic applications. J Control Release. 2023;353:1127–49.
151. Ghanbari M, Salkovskiy Y, Carlson MA. The rat as an animal model in chronic wound research: an update. Life Sci. 2024;351: 122783.
152. Neves LMG, Wilgus TA, Bayat A. In vitro, ex vivo, and in vivo approaches for investigation of skin scarring: human and animal models. Adv Wound Care. 2023;12(2):97–116.
153. Cañedo-Dorantes L, Cañedo-Ayala M. Skin acute wound healing: a comprehensive review. Int J Inflam. 2019;2019:3706315.
154. Lv Q, Deng J, Chen Y, Wang Y, Liu B, Liu J. Engineered human adipose stem-cell-derived exosomes loaded with miR-21-5p to promote diabetic cutaneous wound healing. Mol Pharm. 2020;17(5):1723–33.
155. Gondaliya P, Sayyed AA, Bhat P, Mali M, Arya N, Khairnar A, Kalia K. Mesenchymal stem cell-derived exosomes loaded with miR-155 inhibitor ameliorate diabetic wound healing. Mol Pharm. 2022;19(5):1294–308.
156. Yu M, Liu W, Li J, Lu J, Lu H, Jia W, Liu F. Exosomes derived from atorvastatin-pretreated MSC accelerate diabetic wound repair by enhancing angiogenesis via AKT/eNOS pathway. Stem Cell Res Ther. 2020;11(1):350.
157. Hu Y, Tao R, Chen L, Xiong Y, Xue H, Hu L, Yan C, Xie X, Lin Z, Panayi AC, Mi B, Liu G. Exosomes derived from pioglitazone-pretreated MSCs

accelerate diabetic wound healing through enhancing angiogenesis. J Nanobiotechnol. 2021;19(1):150.
158. Yan C, Chen J, Wang C, Yuan M, Kang Y, Wu Z, Li W, Zhang G, Machens HG, Rinkevich Y, Chen Z, Yang X, Xu X. Milk exosomes-mediated miR-31-5p delivery accelerates diabetic wound healing through promoting angiogenesis. Drug Deliv. 2022;29(1):214–28.
159. Ma J, Zhang Z, Wang Y, Shen H. Investigation of miR-126-3p loaded on adipose stem cell-derived exosomes for wound healing of full-thickness skin defects. Exp Dermatol. 2022;31(3):362–74.
160. Qian Z, Bai Y, Zhou J, Li L, Na J, Fan Y, Guo X, Liu H. A moisturizing chitosan-silk fibroin dressing with silver nanoparticles-adsorbed exosomes for repairing infected wounds. J Mater Chem B. 2020;8(32):7197–212.
161. Weng T, Wu P, Zhang W, Zheng Y, Li Q, Jin R, Chen H, You C, Guo S, Han C, Wang X. Regeneration of skin appendages and nerves: current status and further challenges. J Transl Med. 2020;18(1):53.
162. Chen Z, Shen G, Tan X, Qu L, Zhang C, Ma L, Luo P, Cao X, Yang F, Liu Y, Wang Y, Shi C. ID1/ID3 mediate the contribution of skin fibroblasts to local nerve regeneration through Itga6 in wound repair. Stem Cells Transl Med. 2021;10(12):1637–49.
163. Yang Z, Yang Y, Xu Y, Jiang W, Shao Y, Xing J, Chen Y, Han Y. Biomimetic nerve guidance conduit containing engineered exosomes of adipose-derived stem cells promotes peripheral nerve regeneration. Stem Cell Res Ther. 2021;12(1):442.
164. Zhu Z, Zhang X, Hao H, Xu H, Shu J, Hou Q, Wang M. Exosomes derived from umbilical cord mesenchymal stem cells treat cutaneous nerve damage and promote wound healing. Front Cell Neurosci. 2022;16: 913009.
165. Wang ZC, Zhao WY, Cao Y, Liu YQ, Sun Q, Shi P, Cai JQ, Shen XZ, Tan WQ. The roles of inflammation in keloid and hypertrophic scars. Front Immunol. 2020;11: 603187.
166. Liu W, Yu M, Xie D, Wang L, Ye C, Zhu Q, Liu F, Yang L. Melatonin-stimulated MSC-derived exosomes improve diabetic wound healing through regulating macrophage M1 and M2 polarization by targeting the PTEN/AKT pathway. Stem Cell Res Ther. 2020;11(1):259.
167. Zhang Q, Shi L, He H, Liu X, Huang Y, Xu D, Yao M, Zhang N, Guo Y, Lu Y, Li H, Zhou J, Tan J, Xing M, Luo G. Down-regulating scar formation by microneedles directly via a mechanical communication pathway. ACS Nano. 2022;16(7):10163–78.
168. Jiang L, Zhang Y, Liu T, Wang X, Wang H, Song H, Wang W. Exosomes derived from TSG-6 modified mesenchymal stromal cells attenuate scar formation during wound healing. Biochimie. 2020;177:40–9.
169. Yuan R, Dai X, Li Y, Li C, Liu L. Exosomes from miR-29a-modified adipose-derived mesenchymal stem cells reduce excessive scar formation by inhibiting TGF-β2/Smad3 signaling. Mol Med Rep. 2021;24(5):1.
170. Meng S, Wei Q, Chen S, Liu X, Cui S, Huang Q, Chu Z, Ma K, Zhang W, Hu W, Li S, Wang Z, Tian L, Zhao Z, Li H, Fu X, Zhang C. MiR-141-3p-functionalized exosomes loaded in dissolvable microneedle arrays for hypertrophic scar treatment. Small. 2024;20(8): e2305374.
171. Li JY, Ren KK, Zhang WJ, Xiao L, Wu HY, Liu QY, Ding T, Zhang XC, Nie WJ, Ke Y, Deng KY, Liu QW, Xin HB. Human amniotic mesenchymal stem cells and their paracrine factors promote wound healing by inhibiting heat stress-induced skin cell apoptosis and enhancing their proliferation through activating PI3K/AKT signaling pathway. Stem Cell Res Ther. 2019;10(1):247.
172. Li B, Luan S, Chen J, Zhou Y, Wang T, Li Z, Fu Y, Zhai A, Bi C. The MSC-derived Exosomal lncRNA H19 promotes wound healing in diabetic foot ulcers by upregulating PTEN via microRNA-152-3p. Mol Ther Nucleic Acids. 2020;19:814–26.
173. Zhang XF, Wang T, Wang ZX, Huang KP, Zhang YW, Wang GL, Zhang HJ, Chen ZH, Wang CY, Zhang JX, Wang H. Hypoxic ucMSC-secreted exosomal miR-125b promotes endothelial cell survival and migration during wound healing by targeting TP53INP1. Mol Ther Nucleic Acids. 2021;26:347–59.
174. Wang B, Pang M, Song Y, Wang H, Qi P, Bai S, Lei X, Wei S, Zong Z, Lin S, Zhang X, Cen X, Wang X, Yang Y, Li Y, Wang Y, Xu H, Huang L, Tortorella M, Cheng B, Lee Y, Qin D, Li G. Human fetal mesenchymal stem cells secretome promotes scarless diabetic wound healing through heat-shock protein family. Bioeng Transl Med. 2023;8(1): e10354.
175. Li X, Xie X, Lian W, Shi R, Han S, Zhang H, Lu L, Li M. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. Exp Mol Med. 2018;50(4):1–14.
176. Chen Y, Younis MR, He G, Zheng Z, Wang Y, Xue K, Sun J, Liu K, Huang P, Wang X. Oxidative stimuli-responsive "pollen-like" exosomes from silver nanoflowers remodeling diabetic wound microenvironment for accelerating wound healing. Adv Healthc Mater. 2023;12(23): e2300456.
177. Zhao X, Fu L, Zou H, He Y, Pan Y, Ye L, Huang Y, Fan W, Zhang J, Ma Y, Chen J, Zhu M, Zhang C, Cai Y, Mou X. Optogenetic engineered umbilical cord MSC-derived exosomes for remodeling of the immune microenvironment in diabetic wounds and the promotion of tissue repair. J Nanobiotechnol. 2023;21(1):176.
178. Xiong Y, Chen L, Yu T, Yan C, Zhou W, Cao F, You X, Zhang Y, Sun Y, Liu J, Xue H, Hu Y, Chen D, Mi B, Liu G. Inhibition of circulating exosomal microRNA-15a-3p accelerates diabetic wound repair. Aging. 2020;12(10):8968–86.
179. Sousa P, Lopes B, Sousa AC, Moreira A, Coelho A, Alvites R, Alves N, Geuna S, Maurício AC. Advancements and insights in exosome-based therapies for wound healing: a comprehensive systematic review (2018-June 2023). Biomedicines. 2023;11(8):1.
180. Grada A, Mervis J, Falanga V. Research techniques made simple: animal models of wound healing. J Invest Dermatol. 2018;138(10):2095-2105.e1.
181. Saeed S, Martins-Green M. Animal models for the study of acute cutaneous wound healing. Wound Repair Regen. 2023;31(1):6–16.
182. L Shi, D Song, C Meng, Y Cheng, B Wang, ZJG Yang, Opportunities and challenges of engineered exosomes for diabetic wound healing. 2024. 18.
183. Johnson J, Law SQK, Shojaee M, Hall AS, Bhuiyan S, Lim MBL, Silva A, Kong KJW, Schoppet M, Blyth C, Ranasinghe HN, Sejic N, Chuei MJ, Tatford OC, Cifuentes-Rius A, James PF, Tester A, Dixon I, Lichtfuss G. First-in-human clinical trial of allogeneic, platelet-derived extracellular vesicles as a potential therapeutic for delayed wound healing. J Extracell Vesicles. 2023;12(7): e12332.
184. Essola JM, Zhang M, Yang H, Li F, Xia B, Mavoungou JF, Hussain A, Huang Y. Exosome regulation of immune response mechanism: Pros and cons in immunotherapy. Bioact Mater. 2024;32:124–46.
185. Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: perspective, questions, and challenges. Cell Commun Signal. 2022;20(1):145.
186. Ahn SH, Ryu SW, Choi H, You S, Park J, Choi C. Manufacturing therapeutic exosomes: from bench to industry. Mol Cells. 2022;45(5):284–90.
187. Wang CK, Tsai TH, Lee CH. Regulation of exosomes as biologic medicines: regulatory challenges faced in exosome development and manufacturing processes. Clin Transl Sci. 2024;17(8): e13904.
188. Xu M, Feng T, Liu B, Qiu F, Xu Y, Zhao Y, Zheng Y. Engineered exosomes: desirable target-tracking characteristics for cerebrovascular and neurodegenerative disease therapies. Theranostics. 2021;11(18):8926–44.

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