

Biological and Bioinspired Vesicles for Wound Healing: Insights, Advances and Challenges

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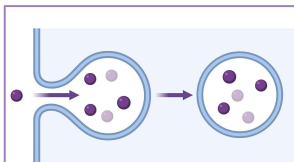
Abstract: Millions of people suffer traumatic injuries and surgical procedures each year, making effective wound management very important. Benign wound repair is essential for structural and functional recovery of damaged tissues. Natural biological vesicles have emerged as attractive candidates for accelerating wound healing due to their endogenous nature and distinctive biological properties. However, obstacles such as low separation yields, potential safety concerns, and unsatisfactory production restrict their continuous application. In recent years, bioinspired materials and technologies have played an important role in the field of remodeling wound healing. Bioinspired vesicles function by effectively transporting therapeutic substances through mimicking the chemical and biological properties in nature, and can be modified and prepared through a variety of advanced techniques such as genetic engineering, chemical modification, and physical techniques, and are good substitutes for natural vesicles. Therefore, integrating bioinspired design into vesicles to promote wound healing can improve the curative effect while maintaining good safety, which helps to accelerate the clinical transformation of vesicles. This review discusses the latest progress, barriers to clinical transformation, and future development directions of biological and bioinspired vesicles for wound healing.

Keywords: wound healing, biological vesicles, bioinspired vesicles, extracellular vesicles, preparation

Graphical Abstract

Wound treatment strategies beyond cellular vesicles

Natural source of vesicles



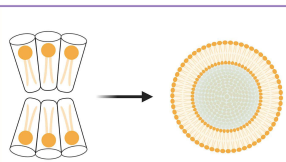
- Animal cells
- Body fluid
- Plants
- Other origins(e.g. insects)

Vesicle isolation strategies



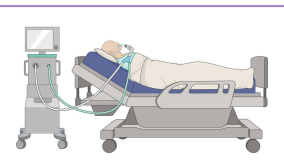
- Ultracentrifugation
- Ultrafiltration
- Immunoaffinity separation
- Volume exclusion chromatography
- Polymer precipitation method
- Microfluidic technology

Synthetic vesicle methods



- Bottom-up
- Top-down
- Biohybridisation

Clinical application difficulties



- Safety
- Biodistribution
- Degradability
- Clinical evaluation criteria

Introduction

Skin, serving as the body's largest multifunctional barrier organ, provides essential protection for internal organs against environmental threats. Numerous endogenous pathological conditions and exogenous mechanical insults that compromise cutaneous tissue integrity or architecture can result in skin wounds, posing significant life-threatening risks to patients.¹ Current epidemiological estimates indicate that dermatological pathologies affect approximately one-third of the global population, with wound management imposing substantial healthcare and socioeconomic burdens on both individuals and societies.² Beyond localized wound characteristics (including depth, dimensions, and infection status), the healing trajectory may be further complicated by systemic factors such as immunological/nutritional deficiencies, advanced age, and chronic comorbidities.³ Despite extensive research efforts to enhance cutaneous wound healing, current clinical therapeutic strategies frequently demonstrate suboptimal efficacy across diverse clinical scenarios.

Biological ingredients possess the remarkable capacity to alter the cellular milieu and impart a diverse array of stimuli to host cells. Such active ingredients can exist as biological vesicles, synthetic bioactive vesicles, or live host cells. Cell therapy entails the utilization of transplanted cells to participate in various stages of wound healing, suppress excessive local immune responses, secrete diverse growth factors, facilitate vascular regeneration and wound re-epithelialization, and ultimately expedite wound closure and enhance the quality of wound healing.⁴ Nevertheless, their sustained clinical application has been hindered by immune rejection, non-biocompatible scaffold materials, cell sources and processing techniques, maintaining cell viability throughout therapy, individual patient variations, and exorbitant costs.⁵ Biological vesicles are secreted by cells, such as extracellular vesicles, and their importance in intercellular signaling communication has been firmly established. The events mediated by biological vesicles are associated with cell proliferation, differentiation, migration, apoptosis, immunomodulation, and the ability to cross barriers, such as plasma membrane, blood-brain barrier, and gastrointestinal tract.^{6–8} Therefore, biovesicles are considered a highly promising alternative strategy for cell therapy.^{9,10} These biological vesicles have been shown to retain the efficacy of the maternal cells while avoiding problems of immune rejection, tumorigenicity, teratogenicity, and ethical perspectives. Biological vesicles are ideally suited for long-term storage due to their high stability, controllability for monitoring and assessing the healing process, precision for quantitative application, and high efficiency for enriching the wounded area.^{11,12} Furthermore, biological vesicles contain a wide range of proteins and RNAs that can stimulate cell migration, proliferation, and differentiation; they can also speed up the formation of blood vessels and extracellular matrix reconstruction by inducing inflammation in the wound; and they can function as a dual agent, promoting wound healing while preventing the formation of epileptic scars, which has good potential for regeneration and repair.¹³

Bioinspired vesicles represent a class of artificial or semi-artificial nanoscale carrier systems engineered through biomimetic principles, deriving structural and functional characteristics from natural biological vesicles (eg, extracellular vesicles, liposomes).¹⁴ Their core design paradigm involves constructing functionalized vesicles with tailored biocompatibility, targeting specificity, or environmental responsiveness by mimicking critical biological membrane features - including compositional architecture, dynamic behaviors, and signaling mechanisms of cellular/organelle membranes.¹⁵ Compared to natural counterparts, these engineered systems exhibit superior performance in structural stability, drug-loading efficiency, and functional programmability, while effectively circumventing inherent challenges of natural vesicles such as source-dependent heterogeneity and scalability limitations in manufacturing.¹⁶ Bioinspired vesicles show significant market potential for application in the field of wound healing. In terms of production cost economics, the donor is widely available and inexpensive. With the continuous optimization of the preparation process, the application of genetic engineering and chemical modification technology has increased the vesicle yield and reduced the production cost. From the point of view of market application value, bio-inspired vesicles have more significant therapeutic effects and a wider application scope. With the improvement of the quality of wound healing and the increasing demand for new therapeutic technologies, bio-inspired vesicles have a huge market potential, and their wide application will promote the development of related medical devices and pharmaceuticals and expand the market space.

The challenges such as large-scale manufacturing, purification, modification, drug loading, storage, and heterogeneity persistently impede the further transformation of biological vesicles. To circumvent the limitations of biological vesicles, synthetic nanoparticles that mimic the therapeutic properties and drug delivery capabilities of biological vesicles—such as

their nanoscale size, targeting ability, cellular internalization efficiency, and stability—have been made possible by biomimetic nanotechnology in recent years. These nanoparticles also make up for the drawbacks of biological vesicles, which include low yields, complicated isolation and purification procedures, and high costs.¹⁷ The advancement of embedded sensors and intelligent materials has driven the evolution of smart dressings for wound management, with these integrated sensors and responsive materials demonstrating the capability to detect specific biological triggers (eg, pH variations, temperature fluctuations, enzymatic activities) through precise physicochemical responses, thereby offering innovative platforms for efficient delivery of bioactive vesicles.¹⁸ On the other hand, there are still many challenges that need to be addressed before active vesicles can be used in clinical applications.¹⁹ The production process of active vesicles needs to be further optimized to increase yield and reduce cost. Also, their stability and biodistribution *in vivo* need to be studied in greater depth to ensure their safety and efficacy during treatment. In addition, the long-term storage conditions and transport requirements of active vesicles need to be clarified to ensure their feasibility and convenience in practical applications. In view of the significant advantages of bioinspired vesicles in terms of biocompatibility, therapeutic efficacy, drug delivery efficiency, production cost control, and market potential, bioinspired vesicles are becoming increasingly important in wound healing and other medical applications, and have become a preferred option that has attracted a great deal of attention.

We first systematically reviewed cutting-edge research on diverse sources and isolation techniques of biological vesicles, while highlighting the distinct characteristics of the associated strategies. Furthermore, this comprehensive analysis examines recent advancements and current limitations of bioinspired vesicle systems engineered through multiple technologies for wound healing enhancement. Finally, we synthesized data from ongoing and completed vesicles-based clinical trials, identifying multifaceted challenges in clinical translation. Our analysis suggests that enhanced mechanistic understanding of both biological and bioinspired vesicles could yield novel therapeutic candidates and precision strategies for advanced wound management.

The Origin of Biological Vesicles

Biological vesicles exhibit diverse biological origins encompassing mammalian organisms, botanical species, and specialized biological groups, each demonstrating unique therapeutic advantages in wound management due to their distinct compositional profiles and functional characteristics.^{20,21} Mammalian-derived vesicles have emerged as pivotal therapeutic agents for complex wound pathologies (eg, diabetic ulcers, burn injuries) through their superior bioactivity and precise regulatory capabilities, while plant- and insect-derived counterparts address critical gaps in conventional therapies via their distinctive components (antioxidant molecules, antimicrobial peptides) and enhanced delivery efficiency.²² This section systematically catalogs currently available vesicle sources, investigates their mechanisms of action, and elucidates their distinct therapeutic benefits in wound healing processes (Figure 1).

Animal Cells

Animal-derived biological vesicles originate from diverse cellular sources, with mesenchymal stem cells (MSCs) representing the most extensively characterized source to date.²³ MSC-derived EVs (MSC-EVs) have garnered significant scientific attention due to their demonstrated therapeutic potential in tissue regeneration processes.²⁴ Notably, cutaneous cellular components (keratinocytes and fibroblasts) and immune effector cells (macrophages) constitute additional biologically significant EV sources.²⁵ This section systematically examines the differential functional profiles and therapeutic characteristics exhibited by these cellularly derived EVs during wound healing progression.²⁶

Resident Skin Cells

Skin cell-derived biological vesicles have emerged as native therapeutic agents in wound repair, distinguished by their direct cellular origin, multifunctional efficacy, and inherent compatibility with the wound microenvironment. Unlike vesicles from non-cutaneous sources, these biological vesicles originate from peri-wound cells, demonstrating superior microenvironmental adaptation and efficient cellular uptake. Scientific consensus confirms their wound-healing enhancement through four coordinated mechanisms: promoting cellular proliferation/migration, accelerating angiogenesis/re-epithelialization, modulating immune responses, and reducing scar formation. Specifically, keratinocyte-derived exosomes mitigate pro-inflammatory cytokine release (eg, TNF- α , CD74, and inducible nitric oxide synthase [iNOS]) while

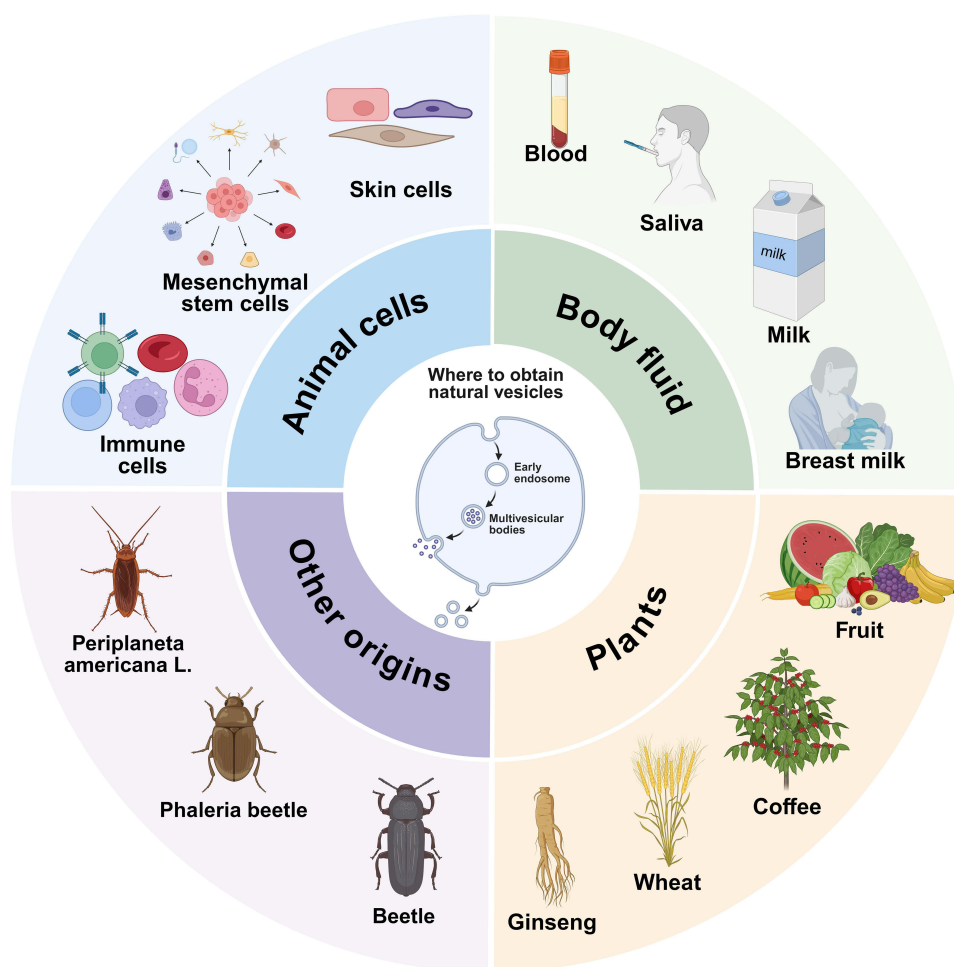


Figure 1 Natural sources of biological vesicles. The figure was created with <https://app.biorender.com/>.

enhancing anti-inflammatory mediator synthesis, thereby controlling macrophage migration/activation.²⁷ Fibroblast-derived exosomes promote aged skin wound healing through miR-125b release and fibroblast-to-myofibroblast transition induction.²⁸ During tissue remodeling, exosomes from fibroblasts and endothelial cells regulate extracellular matrix (ECM) synthesis, maturation, and reconstruction.^{29,30} Additionally, exosomes from keratinocytes and fibroblasts prevent scar formation by transferring fibroblast-developmental miRNAs to myofibroblasts and modulating ECM deposition.^{9,31} As a result, skin resident cells are considered to be one of the reliable sources of biological vesicles.

The synergistic integration of biomaterials with skin-derived biological vesicles presents a transformative strategy for wound repair, leveraging material engineering to optimize vesicle stability, targeted delivery efficiency, and functional durability, thereby maximizing therapeutic potential.³² To address the challenge of biomolecular leakage—a critical limitation in conventional delivery systems—researchers developed microneedles (MNs) encapsulating tazarotene and exosomes for localized wound application, demonstrating their dual functionality in spatiotemporally controlled release.³³ Experimental validation revealed that this MN-based platform significantly enhances cellular proliferation, migration, and angiogenesis across both in vitro and in vivo models, with murine wound studies confirming synchronized delivery of therapeutic agents and exosomes.³⁴ The elucidation of molecular mechanisms underlying skin-derived biological vesicles remains a pivotal research focus,³⁵ with metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) identified as a critical regulatory factor functioning through binding interactions with miR-124 or miR-378a-8 to exert therapeutic effects and promote tissue regeneration.³⁶ Building on this discovery, researchers engineered MALAT1-overexpressing exosomes from human keratinocytes, demonstrating that this combinatorial strategy not only activates MFGE8 via suppression of miR-1914-3p but also enhances macrophage phagocytic capacity (Figure 2), thereby establishing a dual-axis repair mechanism integrating molecular silencing with immunomodulatory

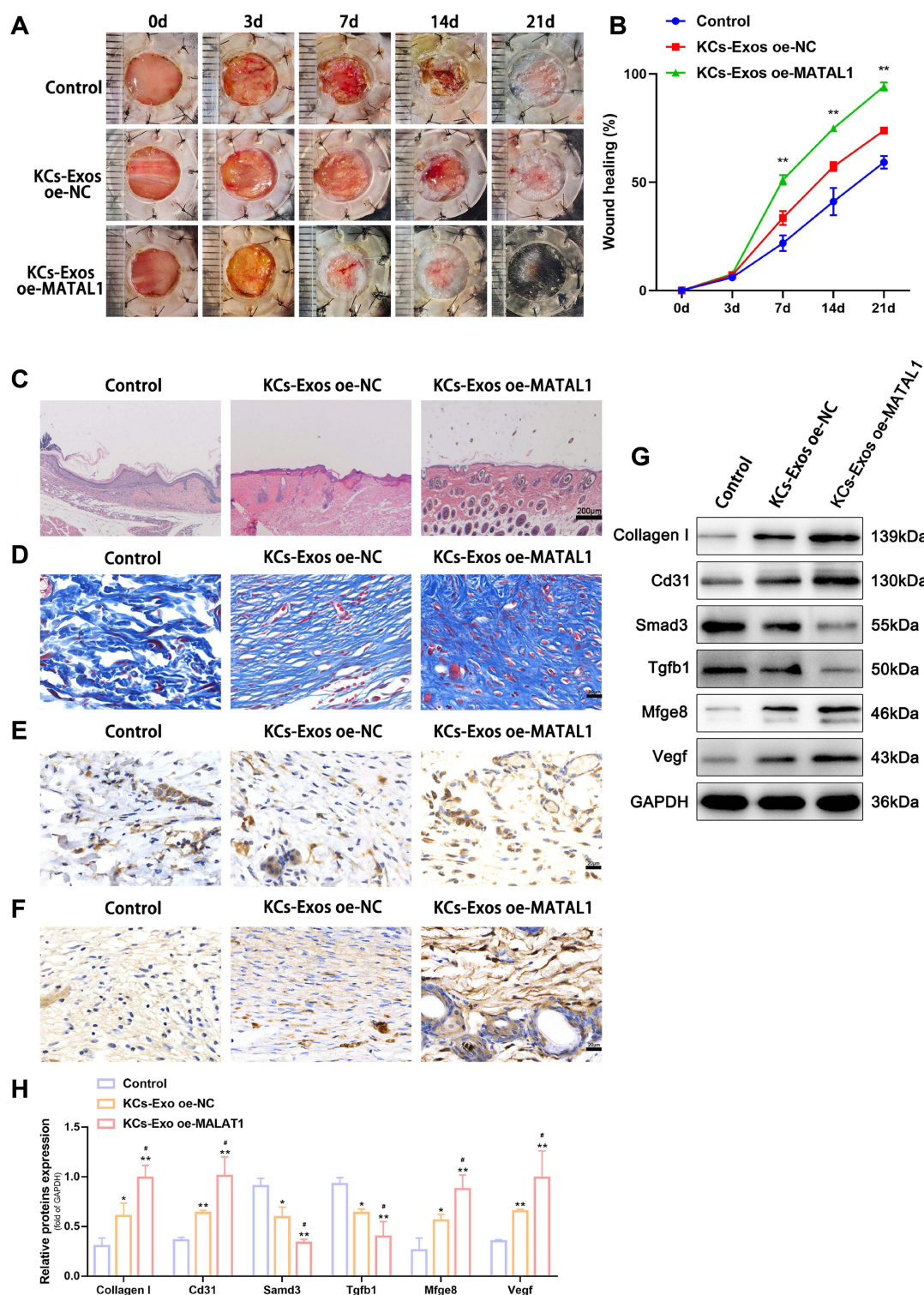


Figure 2 KCS-Exo carrying MALAT1 promoted wound healing in mice with diabetes mellitus. **(A)** Representative images of full thickness defects in mice at days 0, 3, 7, 14, and 21 days postoperatively. **(B)** Wound healing closure rates were calculated among the different groups using the ImageJ software. **(C)** H&E staining among the different groups at day 14. Scale bar: 200µm. **(D)** Masson's trichrome staining among the different groups at day 14. Scale bar: 20µm. **(E)** Representative immunohistochemical images of CD31. Scale bar: 20µm. **(F)** Representative immunohistochemical images of vascular endothelial growth factor (VEGF). Scale bar: 20µm. **(G and H)** Relative protein expression of Collagen I, Cd31, Smad3, Tgfb1, Mfge8, Vegf determined by Western blot analysis. * $p < 0.05$, ** $p < 0.01$ versus the control group; # $p < 0.05$ versus the KCS-Exo oe-NC group. Measurement data were expressed as mean \pm SD. Comparison among multiple groups was conducted using one-way ANOVA, followed by Tukey's post hoc test. **(A–H)** adapted from Kuang L, Zhang C, Li B, Deng H, Chen R, Li G. Human keratinocyte-derived exosomal MALAT1 promotes diabetic wound healing by upregulating MFGE8 via microRNA-1914-3p. *Int J Nanomed.* 2023;18:949. © 2023 The Author(s). This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at <https://www.dovepress.com/terms.php> and incorporate the Creative Commons Attribution - Non Commercial (unported, 3.0) License.³⁷

functions.³⁷ However, skin-derived biological vesicles face unique challenges in clinical translation for wound therapy. Primary skin cells demonstrate limited in vitro expansion efficiency and accelerated senescence under conventional two-dimensional (2D) culture conditions, resulting in vesicle yields significantly lower than those from mesenchymal stem cells.³⁸ Psoriasis patient-derived vesicles may exacerbate inflammatory cascades, necessitating stringent donor screening or compositional modification.³⁹ Furthermore, the absence of FDA-established quality control metrics specific to skin-derived vesicles underscores the urgent need to develop quantitative standards based on skin-specific biomarkers such as keratin 14 (KRT14) and loricrin (LOR).⁴⁰

Mesenchymal Stem Cell

Mesenchymal stem cells (MSCs) are multifunctional, self-renewing cells that can secrete growth factors or differentiate into skin cells to enhance proliferation and facilitate tissue healing.^{41,42} Macrophage polarization, myofibroblast formation, and extracellular matrix reconstitution are all influenced by a variety of cytokines secreted by MSCs, including insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), transforming growth factor- β (TGF- β), MMP-1, keratinocyte growth factor, and type I collagen.^{43,44} Because of their low immunogenicity, ease of storage, and highly efficient biological activity, MSC-derived exosomes, or MSC-exos, have drawn the attention of more researchers in light of the limited number of endogenous MSCs present during wound self-repair and the numerous adverse events that occur during MSC transplantation.⁴⁵ Adipose-derived stem cells (ADSCs), bone marrow-derived mesenchymal stem cells (BMSCs), human umbilical cord mesenchymal stem cells (hUC-MSCs), and other stem cell types serve as viable mesenchymal stem cell (MSC) sources, all demonstrating therapeutic potential in diabetic wound healing, inflammatory wound repair, and keloid formation.^{46,47}

Comparative analyses of MSC sources have revealed that human ADSCs (hADSCs) exhibit superior longevity, proliferative capacity, and resistance to senescence compared to BMSCs, therefore constituting the preferred biological vesicle source.⁴⁸ In foundational studies, the application of hADSC-derived exosomes (hADSC-Exos) encapsulated in thermosensitive PF-127 hydrogel demonstrated enhanced wound closure rates in murine models.⁴⁹ Furthermore, extensive evidence indicates that preconditioning strategies can amplify the paracrine activity of MSCs, exemplified by melatonin-pretreated MSC-derived exosomes (MT-Exo), which suppress excessive inflammation, stimulate angiogenesis and collagen deposition, and ultimately accelerate diabetic wound healing.⁵⁰ The combinatorial use of MSC-derived vesicles from multiple sources presents synergistic advantages, capitalizing on their distinct molecular profiles. Researchers developed an innovative dual-crosslinked hydrogel composed of sericin and fibroin, co-loaded with platelet-rich plasma exosomes (PRP-Exo) and MSC-derived exosomes (MSC-Exos), which accelerated diabetic wound repair through coordinated upregulation of growth factor (GF) expression while downregulating matrix metalloproteinase-9 (MMP-9), thereby promoting angiogenesis, re-epithelialization, and anti-necrotic effects (Figure 3).⁵¹

In vivo, double-cross-linked hydrogels accelerate wound healing by upregulating the expression of growth factors (GFs), downregulating the expression of matrix metalloproteinase-9, and promoting anti-NEtotic actions, angiogenesis, and re-epithelialization. Recent research attention has extended to specialized bio-derived vesicles generated under specific physiological conditions. Notably, unlike exosomes, apoptotic bodies inherently possess dual signaling capabilities - employing “find-me” signals (eg, chemokine CX3CL1) and “eat-me” signals (eg, phosphatidylserine exposure) to recruit macrophages for targeted phagocytosis.⁵² Building upon these mechanisms, researchers have successfully developed ABs@GMSs through integrated liquid-phase microfluidic technology and biosynthetic engineering, creating an injectable localized biomaterial comprising biodegradable microspheres and releasable apoptotic vesicles.⁵³ In rat models of chronic diabetic wounds, ABs@GMSs demonstrated superior efficacy in macrophage polarization and wound closure compared to conventional treatments (Figure 4).

With the advancement of research and clinical experience, clinical trials investigating MSC-Exos have demonstrated a progressive annual increase. In a recent clinical trial (NCT05475418), hADSC-Exos were isolated and applied in wound therapy research, though experimental outcomes remain pending publication. Furthermore, Exogenus Therapeutics (Portugal) is developing ExoWound, a therapeutic product combining extracellular vesicles (EVs) from human umbilical cord blood mononuclear cells (Exo-101) with sustained-release hydrogel to modulate release kinetics, achieving threefold EV yield enhancement for wound healing applications. Despite these advancements, MSC-derived

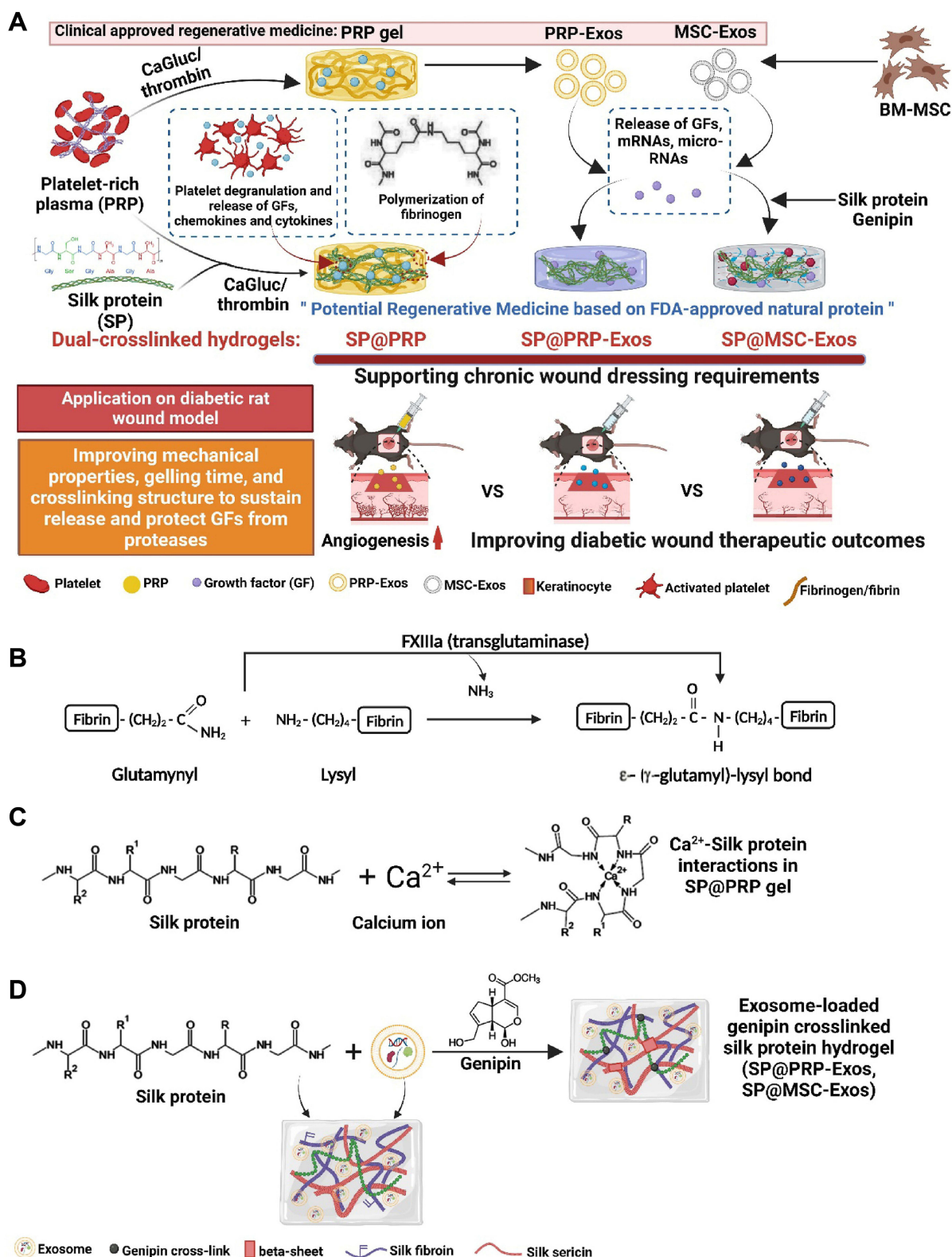


Figure 3 Schematic illustration of (A) the synthetic process for dual-crosslinked hydrogels (SP@PRP, SP@PRP, and SP@PRP) and their use as diabetic wound dressings; (B) Factor XIIIa catalyzes a chemical reaction resulting in insoluble fibrin highly covalently crosslinked through glutamine and lysine residues; (C) Ca^{2+} -silk protein interactions in SP@PRP activated with calcium gluconate, and (D) formation of exosome-loaded genipin-crosslinked silk protein hydrogels. (A–D) adapted from Bakadia BM, Ahmed AAQ, Lamboni L, et al. Engineering homologous platelet-rich plasma, platelet-rich plasma-derived exosomes, and mesenchymal stem cell-derived exosomes-based dual-crosslinked hydrogels as bioactive diabetic wound dressings. *Bioact Mater.* 2023;28:74. © 2023 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. CC BY-NC-ND 4.0.⁵¹

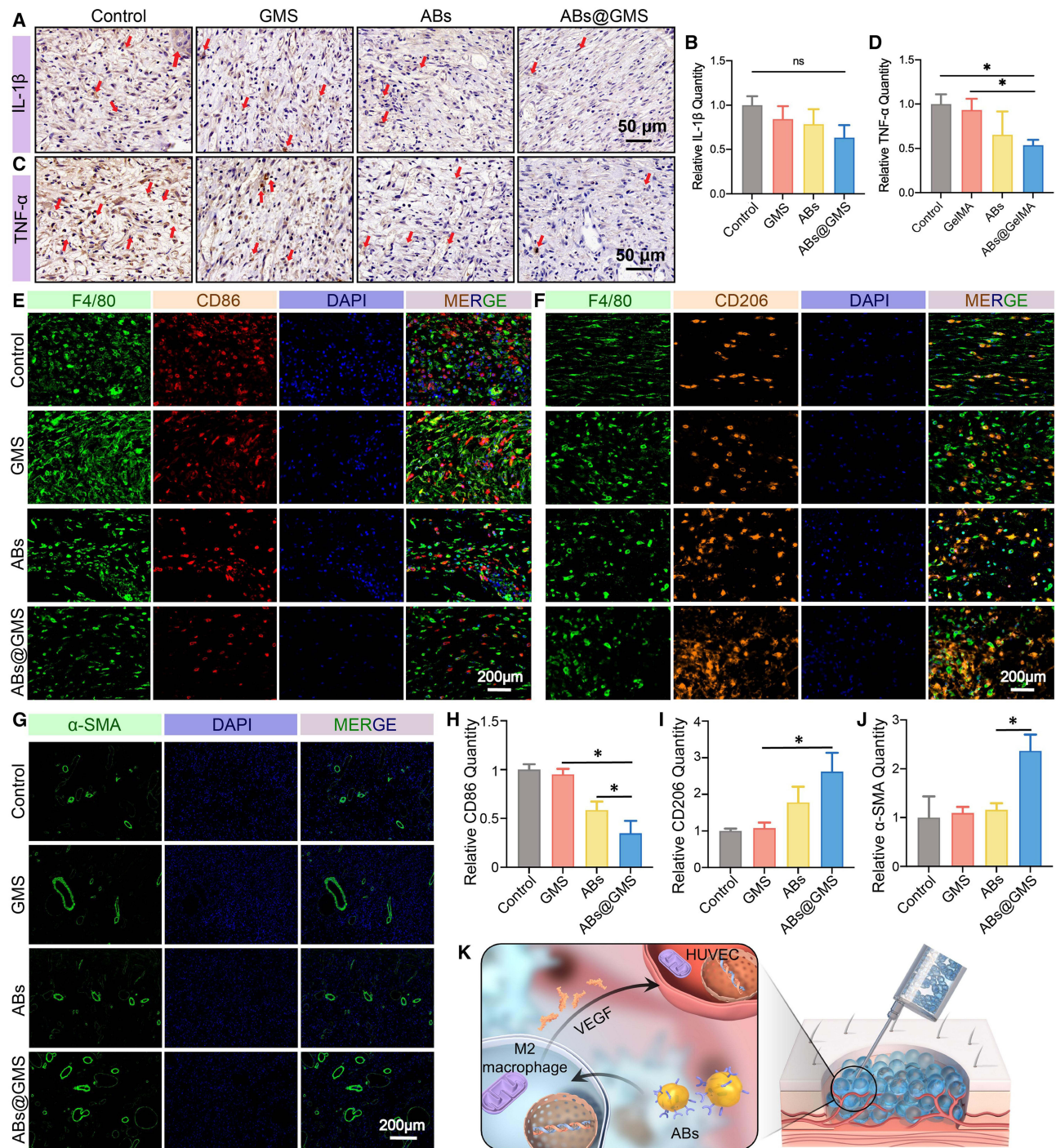


Figure 4 AB@GMSs induce the polarization of M2 macrophages and promote angiogenesis during wound healing. * $p < 0.05$. Data are expressed as mean \pm SEM. (A–K) adapted from Mao J, Qian S, Zhao Q, et al. Balancing macrophage polarization via stem cell-derived apoptotic bodies for diabetic wound healing. *Med.* 2024;5(2):148. © 2024 The Author (s). Published by Elsevier Inc. Creative Commons CC-BY-NC-ND.⁵³

biological vesicles still encounter unique clinical translation barriers in wound management, particularly the marked functional heterogeneity observed among vesicles originating from different tissue sources (bone marrow, umbilical cord, adipose). Nevertheless, MSC-derived vesicles undeniably constitute a pivotal component in future clinical strategies for cutaneous injury repair.

Immune Cells

The wound healing process typically involves infection and tissue damage triggering immune responses that recruit neutrophils, macrophages, and lymphocytes to the injury site, facilitating wound bed clearance and tissue repair.^{4,54} Immune cell-derived biological vesicles demonstrate unique advantages in wound regeneration through their inherent immunomodulatory capacities. Specifically, neutrophil-derived EVs (N-EVs) directly eradicate pathogens via myeloperoxidase while mitigating inflammatory storms through let-7 miRNA-mediated TLR4 pathway inhibition, with their spatiotemporal recruitment dynamics proving particularly advantageous for acute wound intervention.⁵⁵ Specifically, macrophage-derived EVs (M-EVs) exhibit dual functionality based on M1/M2 phenotypic polarization.⁵⁶ M1-EVs eliminate pathogens via pro-inflammatory mediators like TNF- α , while M2-EVs promote collagen deposition through TGF- β , with engineered miR-21 loading further enhancing their anti-inflammatory and pro-angiogenic effects.⁵⁷

Recent breakthroughs address diabetic wound complexities through hydrogel-based portable bioactive ink technology (PAINT), which significantly enhances macrophage M2 polarization and endothelial angiogenesis via M2-exosome (M2-Exos) delivery, achieving over 50% improvement in diabetic wound closure rates.⁵⁸ Mechanistic studies reveal monocytes' critical regulatory role:⁵⁹ monocyte deficiency induces pathological leptin overproduction causing vascular hyperdilation at infection sites, thereby delaying healing and exacerbating scarring, whereas polynuclear cells counterbalance this through ghrelin-mediated suppression of leptin-induced vascular hyperplasia while promoting collagen alignment and epithelial regeneration.⁶⁰ Immune cell precision regulation further inhibits scarring, exemplified by M2-EVs upregulating TGF- β 3 and miR-29b to reduce type I collagen deposition while maintaining skin elasticity. Innovative research on re-epithelialization demonstrates dendritic epidermal T cells (DETCs) regulate epidermal stem cell (EpSC) proliferation through exosomal communication:⁶¹ Tcr δ -knockout models show 40% EpSC population reduction and re-epithelialization delay upon DETC exosome depletion, while exogenous DETC-EV supplementation restores EpSC function via Wnt/ β -catenin pathway activation. Moreover, the integration of photothermal therapy with MN carriers expands therapeutic dimensions through localized thermal effects that suppress inflammatory cytokine storms, while enabling deep transdermal delivery of EVs via micro-needle arrays, demonstrating multimodal regenerative potential in infected wounds⁶² (Figure 5).

The clinical translation of immune cell-derived biological vesicles remains in early exploratory stages, with allogeneic compatibility issues posing unique translational challenges distinct from other cell-derived EVs. Immune cells (eg, macrophages, dendritic cells) inherently exhibit heightened immunological activity, with their secreted EVs carrying parent cell-specific antigen-presenting molecules (MHC-I/II) on surface membranes, potentially triggering recipient immune rejection. For instance, unmodified dendritic cell-derived EVs (DC-EVs) have been shown to induce CD8+ T cell activation and IgG antibody production in murine allotransplantation models, resulting in an 80% EV clearance rate within 24 hours. Further complicating this landscape, immunogenicity varies substantially across EV subtypes: activated M1 macrophage-derived EVs demonstrate elevated surface expression of CD40 and CD86 co-stimulatory molecules compared to M2-EVs, exhibiting greater propensity for host T cell activation, while neutrophil-derived EVs may trigger alternative complement pathway activation through myeloperoxidase enrichment.

Other Cells

In addressing the critical challenge of low yield in vesicle production for regenerative applications, researchers have systematically explored alternative biological sources with enhanced productivity and accessibility. Oral mucosa has emerged as an ideal candidate for cell sheet engineering due to its unique regenerative properties, demonstrating scarless healing characteristics analogous to fetal wound repair mechanisms,^{64–66} with subsequent studies utilizing in vivo wound models to validate the pro-regenerative potential of exosomes derived from oral mucosal epithelial cells (OMECs).⁶⁷ Concurrently, placental amniotic membrane serves as a prominent source of human amniotic epithelial cells (hAECs), which offer advantages including high yield extraction efficiency, low immunogenicity, and non-tumorigenic properties - researchers have successfully isolated high-purity exosomes (hAECs-Exos) from this source,⁶⁸ demonstrating their capacity to accelerate wound healing through fibroblast activation and angiogenesis stimulation via PI3K-Akt-mTOR pathway modulation.⁶⁹ Furthermore, activated platelets have been shown to enhance tissue repair through dual mechanisms of angiogenesis promotion and cellular proliferation stimulation,⁷⁰ while plant-derived resveratrol, a non-flavonoid

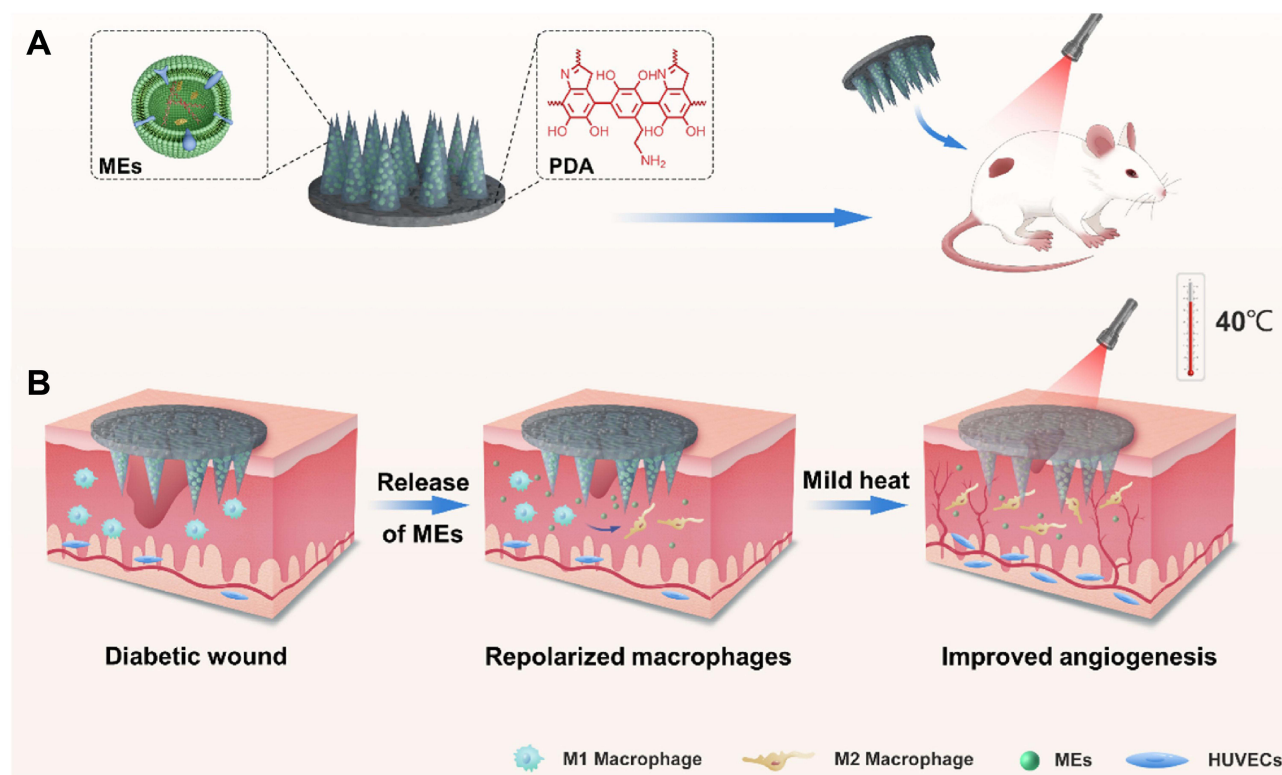


Figure 5 (A) Schematic illustration of MEs@PMN for diabetic wound healing. (B) MEs@PMN for treating diabetic wound based on anti-inflammation and angiogenesis promotion. (A and B) adapted from Zeng J, Sun Z, Zeng F, Gu C, Chen X. M2 macrophage-derived exosome-encapsulated microneedles with mild photothermal therapy for accelerated diabetic wound healing. *Mater Today Bio.* 2023;20:100649. © 2023 The Authors. Published by Elsevier Ltd. license.⁶³

polyphenolic compound from *Vitis vinifera*, exhibits complementary therapeutic effects by reducing inflammatory responses and promoting vascularization during cutaneous wound healing. This pharmacological synergy has been strategically harnessed through the development of a composite hydrogel incorporating both platelet-derived extracellular vesicles (PDEVs) and mesoporous silica nanoparticle-loaded resveratrol (MSN-Res),⁷¹ creating a multifunctional therapeutic platform that simultaneously addresses angiogenesis enhancement and inflammation regulation (Figure 6).

Vesicles of Body Fluid Origin

Biological vesicles constitute ubiquitous nanoscale communication systems within biological fluids such as bile, milk, saliva, urine, and blood, with their diagnostic and therapeutic exploitation leveraging the inherent accessibility of these liquid biopsy sources.⁷² Serum-derived exosomes (serum-Exos), the most prevalent and quantitatively dominant exosomal population in circulation, have demonstrated therapeutic efficacy in enhancing wound healing outcomes in diabetic patients. To elucidate the specific mechanistic contributions of serum-Exos to diabetic wound repair, researchers isolated substantial quantities of these nanovesicles from murine serum and administered them *in vivo*, revealing accelerated wound closure kinetics in diabetic murine models.⁷³ Post-treatment analyses confirmed that serum-Exos upregulated CD31 expression, augmented fibronectin and collagen- α synthesis, and stimulated granulation tissue formation. Further mechanistic investigations identified enhanced serine-threonine kinase Akt phosphorylation in burn-injured dermal tissues following serum-Exos administration, suggesting activation of pro-regenerative signaling pathways.⁷⁴ Emerging evidence posits that exercise-induced systemic adaptations involve the generation of “exercise factors” packaged into exosomes and released into circulation,⁷⁵ with preclinical studies achieving successful therapeutic outcomes in cutaneous wound models through exercise-modulated exosomal delivery.⁷⁶ Salivary exosomes (saliva-Exos) have garnered attention as optimal candidates for tissue regeneration due to their rich proteomic profile and growth factor content,⁷⁷ with experimental validation confirming their pro-angiogenic capacity and wound-healing acceleration.⁷⁸ Mammalian milk,

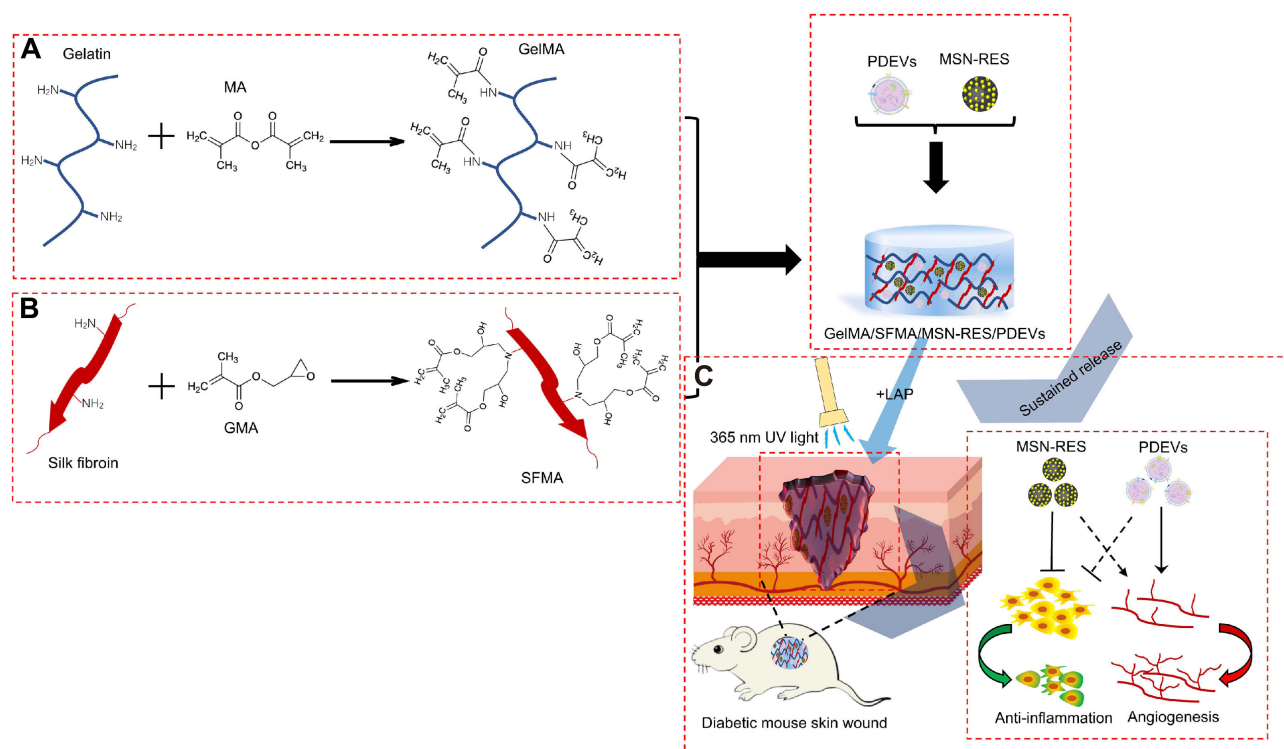


Figure 6 Schematic diagram of GelMA/SFMA/MSN-RES/PDEVs hydrogel preparation and application. **(A)** Schematic of the modification of the gelatin molecule with MA. **(B)** Schematic modification of a silk fibroin molecule with GMA. **(C)** Schematic showing the preparation of GelMA/SFMA/MSN-RES/PDEVs hydrogels used as a dressing for wounds in a diabetic mouse model. PDEVs were drawn using Figdraw (www.fgdraw.com). Figure adapted with permission Zhu W, Dong Y, Xu P, et al. A composite hydrogel containing resveratrol-laden nanoparticles and platelet-derived extracellular vesicles promotes wound healing in diabetic mice. *Acta Biomater.* 2022;154:212. © 2022 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.⁷¹

a nutritionally complex biological fluid containing developmentally critical biomolecules,⁷⁹ has been shown to promote fibroblast proliferation and collagen biosynthesis during dermal repair.^{80,81} Notably, milk-derived exosomes (mi-Exos) exhibit multifunctional regulatory properties encompassing immunomodulation, metabolic regulation, and neurodevelopmental support,⁸² establishing their potential as engineered therapeutic carriers for trauma management.⁸³ Preclinical validation studies have consistently documented mi-Exos' therapeutic efficacy in wound healing applications, further enhanced by their high yield and accessibility.^{84,85} Mechanistic evaluations revealed mi-Exos' antioxidant capacity through free radical scavenging assays and modulation of key inflammatory mediators, while their scarless healing potential was attributed to selective upregulation of TGF- β 3/TGF- β 1 ratio in regenerating tissues.⁸⁶ Fluid-derived biological vesicles face two predominant clinical translation barriers: heterogeneity control and isolation/purification technical constraints. The substantial heterogeneity of biological vesicles derived from bodily fluids manifests in dimensional variability (30–1000 nm), membrane protein compositional differences (eg, coexistence of exosomes and apoptotic bodies in plasma), and biomolecular cargo diversity, resulting in ambiguous functional definitions and inter-batch quality fluctuations (coefficient of variation >35% in critical biomarkers). Current isolation technologies are challenged by complex biological matrices containing serum protein aggregates and lipoprotein particles, with conventional ultracentrifugation methods causing structural damage to vesicle membranes while achieving suboptimal recovery rates of 5–25% as quantified by nanoparticle tracking analysis.⁸⁷

Plant-Derived Vesicles

Biomedical research has increasingly focused on plant-derived exosome-like nanovesicles (PENs) as natural intercellular communicators enriched with bioactive compounds, with systematic investigations now identifying key botanical sources including *Triticum aestivum* (wheat), *Panax ginseng*, *Aloe barbadensis*, *Citrus paradisi* (grapefruit), *Vitis vinifera* (grape), *Citrus limon* (lemon), *Brassica oleracea* (broccoli), *Daucus carota* (carrot), *Malus domestica* (apple), *Zingiber officinale*

(ginger), *Coffea arabica*, and *Citrus* species through standardized multi-omics characterization.^{88–93} Functional validation studies demonstrate species-specific therapeutic advantages: Grapefruit-derived vesicles (GEVs) enhance HaCaT keratinocyte migration rates by 2.3-fold through coordinated upregulation of wound-associated genes (COL1A1, MMP9), proteins (fibronectin, laminin-5), and cytokines (IL-6, TGF- β 1), as quantified via transcriptomic and proteomic profiling.⁹⁴ Comparative analyses reveal PENs' superior anti-inflammatory capacity and scalable production yields, positioning them as cost-effective alternatives for translational applications.⁹⁵ Current research prioritizes systematic screening of plant species using high-content wound healing assays, with *Triticum aestivum* emerging as a lead candidate through multi-parametric evaluation—wheat PENs induce 2.1-fold fibroblast proliferation, accelerate angiogenesis (1.8-fold CD31⁺ vessel density increase), and upregulate 17 core healing-related genes (FGF2, VEGF-A, TIMP1) in full-thickness wound models.⁹⁶ Parallel investigations of *Panax ginseng* demonstrate pleiotropic benefits beyond wound repair, including metabolic regulation and neurological protection,⁹⁷ while ginseng-derived nanoparticles (GDNPs) exhibit dual therapeutic action—2.4-fold collagen deposition enhancement coupled with 57% IL-1 β /IL-6 suppression in senescent skin models.⁹⁸ These findings collectively establish PENs as a pharmacologically versatile platform offering enhanced biosafety, cost efficiency, and therapeutic multifunctionality for next-generation wound management strategies.

Other Sources of Vesicles

The natural world harbors diverse biological resources with vesicle-producing potential, among which *Periplaneta americana* L. (PA)—an environmentally resilient insect with historical medicinal applications—has demonstrated therapeutic relevance.⁹⁹ PA-derived glycoproteins accelerate diabetic wound healing by modulating macrophage polarization dynamics, while exosome-like nanoparticles isolated from PA (PA-ELNs) exhibit tripartite therapeutic efficacy: enhanced re-epithelialization rates, autophagy pathway regulation, and anti-inflammatory activity, collectively contributing to improved diabetic wound closure.¹⁰⁰ These findings underscore the broad phylogenetic distribution of vesicle sources and suggest that species-specific exosomal components may harbor unique therapeutic properties warranting systematic investigation.

Isolation of Vesicles

Vesicles are nowadays heavily used for direct treatment of diseases or delivery of multiple drugs.¹⁰¹ It is crucial to understand how to produce vesicles with high purity and throughput for later clinical applications.¹⁰² Numerous methods, including size exclusion chromatography, differential ultracentrifugation, ultrafiltration, immunoaffinity capture, polymer precipitation, and microfluidics, have been used to isolate vesicles—particularly exosomes—to date (Table 1).^{72,103} To choose the best separation techniques for future uses, a thorough analysis of factors including speed, efficiency, purity, dependability, automation level, and flexibility must be made. The benefits and drawbacks of widely used separation techniques are outlined in this section (Figure 7).

Ultracentrifugation

Ultracentrifugation (including differential centrifugation and density gradient ultracentrifugation) is one of the most widely used exosome separation methods, which is called the “gold standard” for vesicles separation.¹⁰¹ Because ultracentrifugation does not need expensive or complicated sample preparation, it is commonly utilized in exocrine processing.¹⁰⁴ Regrettably, the ultracentrifugation technique yields only moderately pure samples and is time-consuming.¹⁰⁵ Furthermore, exosomes may sustain mechanical damage as a result of repeated ultracentrifugation procedures. Apoptotic fragments, protein aggregates, and other non-exosomal contaminants are often eliminated using a number of purification processes used in differential ultracentrifugation.^{105,106} The purification process includes a low-speed centrifugation phase (300 to 400 \times g) to remove cell and apoptotic debris, a high-speed centrifugation phase (2000 \times g) to eliminate larger vesicles, and an ultra-fast centrifugation phase to harvest exosomes. Exosomes with a modest yield and purity have been produced via differential ultracentrifugation, as demonstrated by numerous tests. Although the above complex centrifugation process can ensure higher purity exosomes, it is difficult to avoid the loss of exosomes during this period.

Table 1 Overview of Isolation of Vesicles

Methods for Isolating Exosome Vesicles	Characteristics	Disadvantages	Application
Ultracentrifugation	Speed: Time-consuming Purity: modest pure Yield: modest yield	Exosomes mechanical damage Loss of exosomes	Exosome separation methods
Ultrafiltration	Speed: longer; Purity: modest pure; Yield: higher throughput	Damage to the filter membrane and the separation of vesicles Only isolated small samples	Collection large size of exosomes
Polymer precipitation method	Speed: build-up; Purity: low; Yield: build-up	Some protein impurities; Limited by the supported antibodies and application scope, and the processing volume	Exosome extraction kits
Immunoaffinity separation	Speed: quick; Purity: higher; Yield: short	High cost of antibodies, exosome markers need to be screened and optimized, low yield.	Exosomes from specific sources
Volume exclusion chromatography	Speed: quick; Purity: higher; Preserving the integrity and structure of vesicles well	Not a practical way to separate exosomes from other particles of the same size	Purification
Microfluidic technology	Speed: quick; Purity: higher; Yield: boost productivity	Lengthen the time of identification and separation; Need sophisticated equipment	Separation and purification of vesicle

A gradient medium, such as sucrose, is needed for density gradient ultracentrifugation in order to enrich vesicles in density-specific layers. Density gradient technique can extract exosomes in a given size range with higher purity but lesser yield when compared to differential ultrafast centrifugation. Nevertheless, density gradient ultracentrifugation is costly and unsuitable for the large-scale separation of exosomes because of the intricate structure of gradient media.

Ultrafiltration

Using commercial membrane filters with predetermined pore sizes, the ultrafiltration technique isolates biological fluids from vesicles.¹⁰⁷ As a result, the separation is accomplished using the molecular weight and size. When compared to other methods, ultrafiltration is more straightforward and has a higher output.¹⁰⁸ However, poor vesicle production will arise from vesicle buildup and pore blockage caused by vesicle retention in the pore canal.¹⁰⁹ Damage may result from shear tension between the filter membrane and the vesicles brought on by the pressure of separation. To address these issues, techniques like membrane cleaning have been developed.

Direct current filtration (DFF) and tangential flow filtration (TFF) are the two categories into which the current vesicles ultrafiltration techniques can be separated. DFF has several drawbacks, including contaminated membranes and poor particle separation. DFF is also limited to the separation of small volume samples. The technique of tangentially moving sample fluid to filter the membrane and prevent cake formation or clogging is called TFF. TFF transfers the sample tangentially to the membrane as opposed to applying orthogonal pressure, as the aforementioned methods do. This technique prevents the buildup of particles on the membrane that causes blockage. TFF is gentler on the sample and can manage higher sample volumes than DFF. TFF will take longer to process, though, in order to obtain more pure exosomes.

Polymer Precipitation Method

Vesicles and other tiny particles are precipitated from biological fluids through polymer precipitation, which modifies the solubility or dispersity of hydrophilic polymers.¹¹⁰ One of the most often used polymers for polymer precipitation is polyethylene glycol (PEG). After centrifugation, vesicles made of hydrophilic PEG with a certain molecular weight were separated from the incubated cell culture medium or bodily fluid.¹¹¹ The researchers came up with an ideal plan by

How to separate exosomes

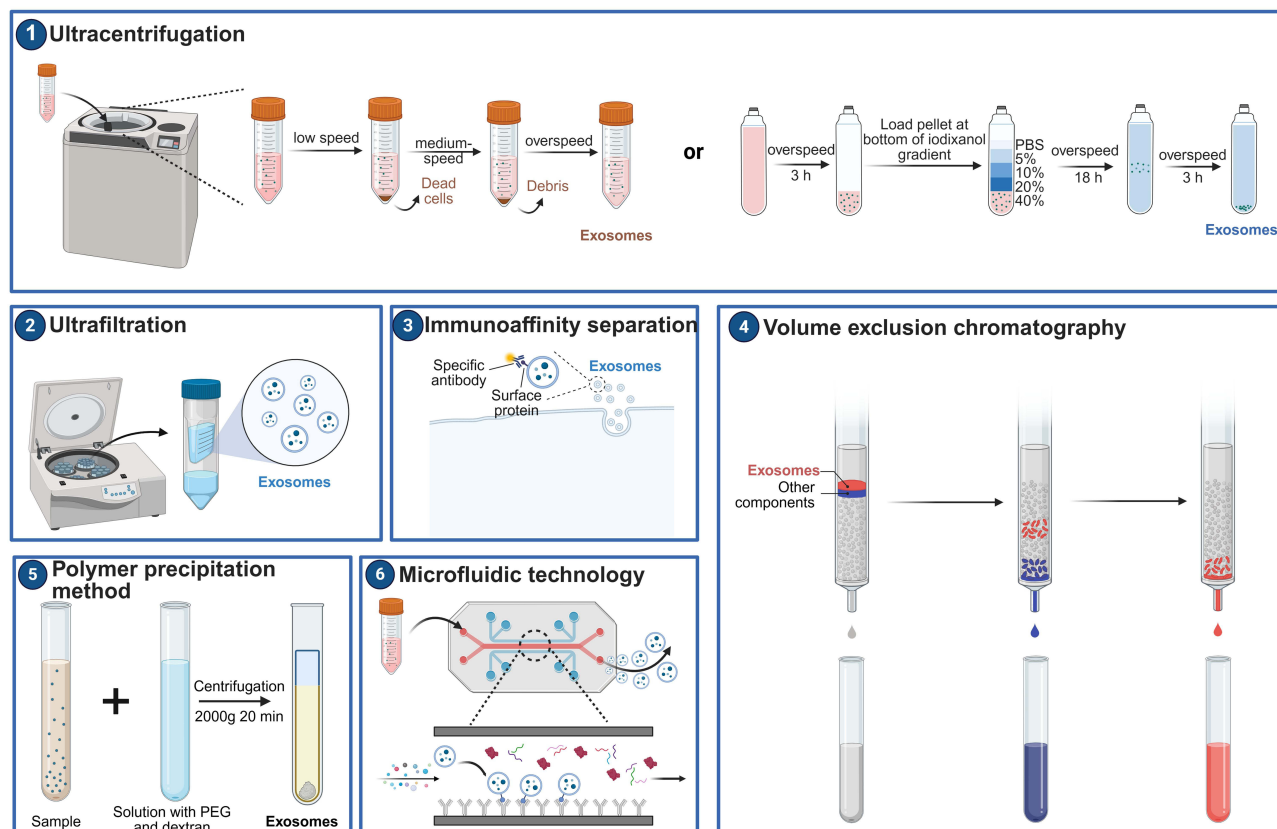


Figure 7 Methods for isolating biological vesicles (exosomes as an example). Numbers 1–6 in sequence are ultracentrifugation, ultrafiltration, immunoaffinity separation, volume exclusion chromatography, polymer precipitation, and microfluidics. The figure was created with <https://app.biorender.com/>.

modifying the precipitation, salt, and polymer levels. Certain exosome extraction kits sold commercially have also used this technique.¹¹² However, exosomes produced with this technique contain some protein impurities.¹¹³

Immunoaffinity Separation

Through the interaction of vesicles' surface proteins—such as CD9, CD63, and CD81—with particular antibodies, immunoaffinity isolation is able to collect vesicles.¹¹⁴ To facilitate separation, antibodies can be attached to a variety of support surfaces, such as magnetic beads, chromatographic substrates, plates, and microfluidic devices.¹¹⁵ To eliminate protein aggregates and other big particles, immunoaffinity capture is therefore frequently employed in conjunction with pretreatment techniques like volumetric exclusion chromatography or centrifugation. It goes without saying that immunoaffinity separation is a gentle and highly specialized technique that can keep vesicles' biological activity mostly intact after separation. However, this technique is limited by the antibodies that can be supported, the application scope is limited, and the processing volume is small.¹¹⁶ Additionally, a longer incubation period is needed for immunoaffinity isolation. For instance, when used for separation, each magnetic Dynabead from Thermo Fisher needs to be incubated for 12 hours. Longer incubation times are caused by the magnetic beads' large size (1.0–4.5 μm), limited mobility in solution, and low surface area to volume ratio. Using pH- or temperature-responsive magnetic nanoparticles can speed up this process. Because the nanoparticles have a larger surface-area-to-volume ratio and higher magneto electrophoretic mobility, after aggregation caused by changes in temperature or pH to allow for rapid magnetic separation. It is possible to shorten the periods of incubation and separation to a few minutes. Magnetism is also used in immunoaffinity techniques based on Raman scattering to separate and analyze vesicles. Molecules can be recognized by Raman

scattering due to their distinctive chemical fingerprints. High sensitivity and specificity have been achieved in the detection of breast cancer in patient samples using magnetic surface-enhanced Raman scattering. The integration of characterisation and sample processing is essential in streamlining the therapeutic and diagnostic applications of exosomes and sets them apart from alternative immunoaffinity methodologies.

Volume Exclusion Chromatography

Vesicles can be separated using a size-based technique called volumetric exclusion chromatography.¹¹⁷ This easy-to-use, low-cost vesicle separation technique prevents shear stress damage and does not require any specialized equipment. In general, this technique does a good job of preserving the integrity and structure of vesicles. It is frequently employed as a crucial stage in the purification of small EVs so that plasma impurities may be removed from them.¹¹⁸ To achieve high resolution particle size, processing parameters such as resin type, flow rate, column size, microbead loading, and system volume must be taken into account. To get a sample free of lipoprotein and protein contaminants, the material must first undergo ultracentrifugation or ultrafiltration. Size-exclusion chromatography, like other size-based separation methods, is not a practical way to separate exosomes from other particles of the same size.¹¹⁹ This issue might be resolved by combining immunoaffinity capture and separation techniques.¹²⁰

Microfluidic Technology

Microfluidic technology is a high-throughput method, which can be compatible with a variety of vesicles separation methods.¹²¹ A high-throughput technique that can work with a range of vesicles separation techniques is microfluidic technology.¹²² Microfluidic devices offer a number of benefits.¹²³ First, quicker separation times and reduced sample losses are made possible by microfluidic technology. Second, it can boost productivity and purity in a synergistic way when used in conjunction with other vesicle separation techniques. Furthermore, microfluidic devices can be expanded in accordance with actual needs and have a more compact structure than conventional separation devices. Microfluidic instruments are ideal for vesicle identification due to their small sample size and portability.

Nevertheless, there are certain issues with using microfluidic devices.¹²⁴ First, samples may obstruct the path during examination, lengthening the time needed for identification and separation. Second, the method's large-scale application is limited by the need for sophisticated equipment. In order to produce quick, effective, and high-purity vesicles separation procedures, researchers are attempting to integrate microfluidic devices with a variety of external pressures. These forces can be broadly classified into two categories: passive isolation techniques (membrane-based, column-based, and fluid dynamics) and active isolation techniques (electrical, centrifugal, and dynamical). Using acoustic dynamics as an example, it is widely accepted that larger particles in the sound field would experience stronger acoustic forces, causing them to separate from one another.¹²⁵ Researchers combined acoustic flow and droplet rotation techniques to separate and identify exosomes.¹²⁶ Experiments conducted later indicate that the gadget can handle data in less than a minute and achieve a high separation efficiency of 80–86%.

Vesicles have significantly advanced the fields of medication administration and non-invasive illness diagnostics and therapy because of their therapeutic potential and distinct biological activities. One of the main obstacles to vesicles' therapeutic evolution in the last few decades has been their isolation.¹²⁷ Currently, there are many established methods for separating vesicles that make use of cutting-edge technology like electricity, centrifugal force, microfluidics, and acoustics.¹²⁸ Ultracentrifugation (UC), the most widely utilized separation technique, exhibits recovery rates significantly influenced by sample types and operational parameters. A systematic comparative study demonstrates that UC achieves exosome recovery rates below 30% from plasma, with high centrifugal forces potentially causing partial degradation of vesicular membrane proteins such as CD63.¹¹⁸ Immunoaffinity methods (eg, antibody-conjugated magnetic bead capture) achieve high-purity isolation (>90%) but incur substantial reagent costs (antibody consumption reaching hundreds of dollars per experiment) and risk compromising vesicle integrity during elution.¹²⁹ Microfluidic platforms, despite their high-throughput processing capabilities and low shear stress characteristics, face clinical-scale implementation challenges: studies on inertial focusing chips reveal mandatory pre-filtration requirements for complex biological samples like serum to remove large particulate contaminants, accompanied by progressively increasing channel clogging rates during prolonged operation.¹³⁰ Regarding scalability, UC maintains dominance due to equipment accessibility but struggles to meet

therapeutic-grade dosage demands, while parallelized microfluidic designs (eg, multi-channel chips) theoretically enhance processing capacity yet require optimization of standardization protocols and cost-effectiveness in manufacturing.¹³¹ Hybrid strategies combining UC pre-concentration with microfluidic sorting have demonstrated improved recovery rates and purity profiles, though clinical translation necessitates rigorous GMP certification and large-scale validation.¹³²

Design and Development of Biological Vesicles

Bioinspired vesicles demonstrate optimized biomedical application potential through the integration of biomimetic principles and engineering technologies,¹³³ while preserving core functionalities of natural biological vesicles (eg, extracellular vesicles, exosomes).¹³⁴ First, enhanced stability and controllability: Whereas natural vesicles exhibit fragile membrane structures susceptible to enzymatic degradation or oxidative damage with short in vivo circulation half-lives, bioinspired counterparts achieve extended circulation durations through synthetic lipid incorporation (eg, DSPC), polymer modification (eg, polyethylene glycol), or crosslinking strategies, coupled with environment-responsive materials (eg, pH-sensitive lipids, thermosensitive polymers) for targeted drug release at lesion sites.¹³⁵ Second, breakthroughs in drug-loading efficiency and functional programmability: Natural vesicles typically exhibit drug-loading capacities below 10% due to endogenous content limitations and membrane permeability constraints, whereas bioinspired systems achieve 30%-50% loading efficiencies for small-molecule drugs, nucleic acids (eg, siRNA, mRNA), and proteins through electroporation, microfluidic manipulation, or membrane reconstitution techniques, enabling multi-drug co-loading and sequential release.¹³⁶ Third, reduced immunogenicity and allogeneic risks: While natural vesicles (particularly xenogeneic sources) may carry donor-specific antigens or pro-inflammatory factors triggering immune clearance or toxicity, bioinspired variants minimize immunogenicity through purified membrane components (eg, non-target protein removal), surface ligand engineering (eg, CD47 overexpression for immune evasion), or fully synthetic material construction, thereby improving biocompatibility.¹³⁷ Fourth, scalable manufacturing and standardization feasibility: Contrasting with natural vesicle production limited by cell culture conditions and batch-to-batch variability, bioinspired systems enable standardized large-scale manufacturing via microfluidic assembly, liposome extrusion, or membrane protein reconstitution technologies, meeting clinical-grade quality control requirements (eg, size uniformity, drug-loading consistency).¹⁵ Overall, it is anticipated that synthetic vesicles mimics would increase the effectiveness of bioactive compounds' distribution to therapeutic targets while producing scalable preparation methods and desired results.¹³⁸ The field of oncology medicines has extensively investigated this synthetic technique.¹³⁹ The blood-brain barrier's (BBB) poor drug penetration significantly reduces the effectiveness of treatment for glioblastoma multiforme (GBM). Bionic nanoparticles (NPs) embedded in GBM cell membranes have been used by researchers to successfully transport medications to GBM cells across the blood-brain barrier. In the meanwhile, a number of investigations have demonstrated that, in contrast to biological vesicles, synthetic vesicles offer larger yields, consistent efficacy, and possible therapeutic uses.¹⁴⁰ Currently, there are three types of synthetic strategies: top-down, bottom-up, and biohybrid (Figure 8). We also provided a summary of the artificial vesicles here (Table 2).

Bottom-Up

A bottom-up approach would include developing larger, more complicated structures gradually by beginning with the assembly of smaller, simpler elements.¹⁴⁸ This is comparable to the architectural construction process.¹⁴⁹ Therefore, it is evident that bottom-up approaches necessitate a thorough comprehension of the essential elements of natural exosomes. Liposomes are believed to have exceptional biocompatibility because of their ability to replicate the membrane shape of exosomes or natural cells.¹⁵⁰ These days, the focus of research is on adding lipids with certain compositions to exosome-like nanoparticles. Then, the necessary surface proteins are added to the manufactured lipid bilayers utilizing methods such as chemical biocoupling, simple incubation, and cell-free protein synthesis.^{151,152} The resultant particles, which resemble exosomes, have the benefits of controllability and clean components.¹⁵³ Bottom-up synthetic exosomes are more compliant with market rules and production standards, and they have a better acceptability rate for drugs from an industrial production perspective.¹⁵⁴ Nevertheless, there are a lot of drawbacks to using this method to make synthetic exosomes. For the present being, the loading structure and constituents of natural exosomes cannot be completely replicated using bottom-up techniques.¹⁵⁵ The active components necessary for natural exosomes to operate properly in the treatment of disease and drug delivery have not yet been identified, and a better comprehension of exosome biology is

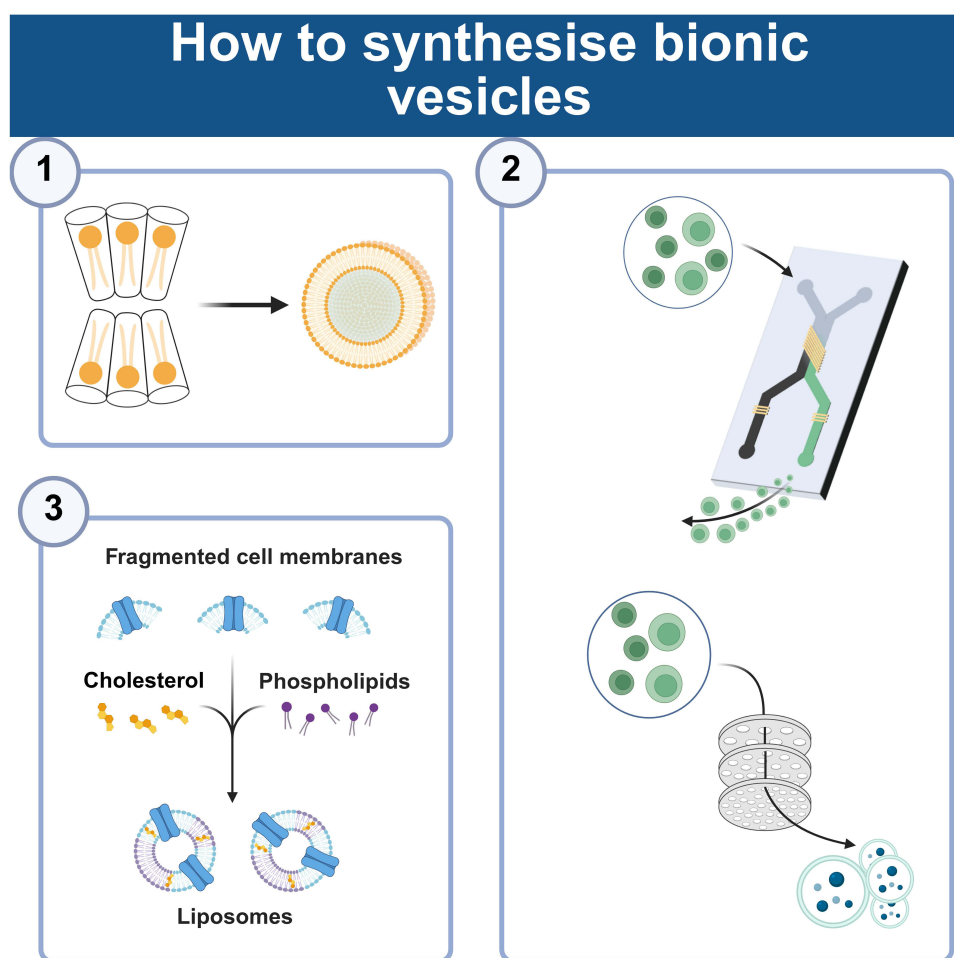


Figure 8 Strategies for artificial synthesis of bionic vesicles. Number 1–3 in sequence are bottom-up, top-down, and biohybridisation strategies. A bottom-up approach would include developing larger, more complicated structures gradually by beginning with the assembly of smaller, simpler elements. Top-down procedures begin with larger biological structures, which are subsequently broken down into smaller pieces using different techniques to create smaller vesicles. Through the process of biohybridization, natural and synthetic vesicles can be combined to create vesicles. The figure was created with <https://app.biorender.com/>.

still lacking in the clinical translation process.¹⁵⁶ It would also be challenging to adequately characterize the active components and functional mechanisms of exosomes derived from various cellular sources due to these differences.^{157,158} To guarantee the efficacy of the final manufactured exosomes, functional molecular integration of exosome-like nanoparticles even necessitates exact manufacturing and purification processes. Therefore, for bottom-up tactics to be further translated into clinical practice, several technological advances may be needed.^{151,159}

Top-Down

Typically, top-down procedures begin with larger, more complicated biological structures, which are subsequently broken down into smaller pieces using different techniques to create smaller vesicles. For instance, it is possible to produce tailored vesicles by disassembling cell membranes into tiny membrane fragments and then assembling those fragments into membrane vesicles. Top-down methods, as opposed to bottom-up ones, convert cell membranes into nanoparticles while preserving proteins or other physiologically active molecules in their native state, guaranteeing the maximum amount of biological complexity preservation.^{160–162} A top-down method of functionalizing the cell membrane guarantees that the essential characteristics of the vesicles are preserved and makes it easier to maximize their therapeutic and targeted potential. Thus, the top-down strategy helps to reduce non-specific uptake, improve delivery efficiency, isotype targeting and circulating half-life, and achieve superior therapeutic efficacy.¹⁶³ As a result, the top-down approach

Table 2 Summarization of the Synthetic Vesicles

Methods for Synthetic Vesicles	Characteristics	Exosome Source	Outcome of the Study	Application	Ref
Microfluidics	Great reproducibility High drug-carrying efficiency Consistent size, shape, rigidity, surface charge	Macrophage-derived nanomedicines	This allowed for enhanced in situ glioma targeting of drugs	Successful simplification of the bioinspired vesicle preparation and medication loading procedure	[141]
Extrusion	Excellent control and repeatability Similar biological and physical characteristics as biological vesicles	Liposome	It demonstrated their high yield and efficiency properties for effective targeted drug delivery	To produce huge quantities of bioinspired vesicles	[142]
Freeze-thawing strategy	Similar morphology and biological properties as exosomes Desirable circulating; Desirable targeting	CT26 (Mouse Colorectal Carcinoma Cells)	The hybrid NPs can evade in vivo clearance and release medications in response to temperature	Liposome production	[143]
Incubation	Gentle and successful reactions and processes	Blood exosomes	Its potential for brain-targeted delivery for the treatment of central nervous disorder	Targeted medicine delivery and cancer diagnostics	[144]
		Stem cell-derived exosomes	These exosomes were then utilized to distribute analgesics across biological barriers		[145]
Co-extrusion	Higher medication delivery yield and capacity	Bispecific CAR-T cells	The hybrid vesicles demonstrated superior anti-tumor properties in addition to their good lung accumulation capacity	Anti-tumor	[146]
	Enhanced permeability and biocompatibility	Fibroblast-derived exosomes	It has good anti-tumor properties and enable the simultaneous administration of chemodynamic and photothermal therapy		[147]

contributes to better therapeutic efficacy, isotype targeting, improved delivery efficiency, decreased non-specific absorption, and circulation half-life. Top-down strategy-based preparation techniques have been extensively developed, with extrusion and microfluidics technologies in the forefront.

Microfluidics

Microfluidics' automation, quick analysis, large throughput, and customizable detection make it a popular tool for vesicle isolation, detection, analysis, and modification.¹⁶⁴ Engineered vesicles have started to be produced on a massive scale using microfluidics in recent years.¹⁶⁵ The vesicles that are produced using microfluidic platforms exhibit great reproducibility, high drug-carrying efficiency, consistent size, shape, rigidity, and surface charge. Numerous biomimetic vesicles generated from cells and cell membranes have shown specific therapeutic efficacy, and their yield (100-fold) is substantially larger than that of natural exosomes.^{141,166,167} Potential clinical uses of microfluidics include the successful simplification of the bioinspired vesicle preparation and medication loading procedure.¹⁶⁸ In order to prepare macrophage-derived nanomedicines, a staggered herringbone micro-mixer with multiple inlets was used to precisely control the vesicle preparation process and drug loading. This allowed for enhanced in situ glioma targeting of drugs and demonstrated the great potential of microfluidics for nanomedicine preparation.¹⁴¹ Regrettably, rather than bioinspired vesicles, microfluidics has been used more in the field of wound healing to synthesize multifunctional wound healing

dressings. Diverse possibilities for cell-free wound therapy may be presented by microfluidic systems that may simultaneously synthesize therapeutic dressings and vesicles. Furthermore, even though precise and effective microfluidic chips have many advantages, their application scope and clinical prices are constrained.¹⁶⁹ All things considered, microfluidics is a promising preparation technique that may offer an effective manufacturing platform for bioinspired medication delivery and treatment systems.¹⁷⁰

Extrusion

To homogenize the size distribution of colloidal formulations (such as liposome suspensions), extrusion techniques are frequently employed. Owing to its benefits, which include excellent control and repeatability, this approach has been employed extensively in recent years to produce bioinspired vesicles. Cultured cells can be crushed and then passed through a series of progressively smaller pore-sized polycarbonate membrane filters to create vesicles that resemble biological vesicles in both their biological and physical characteristics.^{171,172} Since proteins and membrane fragments have the same physical characteristics as biological vesicles, density gradient ultracentrifugation is typically used to purify vesicles in order to increase size uniformity and sample purity.^{173,174} The yield of vesicles derived by extrusion technology is much higher than the yield of exosomes purified from the same number of cells (ie >100-fold).¹⁷¹ Furthermore, extrusion technology offers easier procedures and less equipment needed compared to other techniques. Extrusion technology is currently frequently employed to produce huge quantities of bioinspired vesicles.^{175,176} Researchers have prepared functionalized artificial vesicles by using a liposome extruder and demonstrated their high yield and efficiency properties for effective targeted drug delivery.¹⁴² To achieve evenly sized vesicles, extrusion-produced vesicles still need to go through an extra purification phase, which could add complexity and time to the entire production process.

Biohybridisation

Through the process of biohybridization, natural and synthetic vesicles can be combined to create vesicles. Biohybridisation strategies offer batch production, process control, engineered preparation, ideal stability and suitable drug loading, in addition to targeting, biological barrier penetration, recyclability, low immunogenicity and high biocompatibility.¹⁷⁷ Many investigations focused on the combination of liposomes and exosomes, with an emphasis on the biohybridization of nanomaterials and biological vesicles.^{147,178,179} Numerous techniques for biohybridization have been developed.

Freeze-Thawing Strategy

With a wealth of success, freeze-thawing-based biohybridization techniques have been used with liposome production. As a result, scientists were motivated to use it to prepare exosome-liposome hybrids and load different medications. The researchers demonstrated that the synthesized hybrids not only have similar morphology and biological properties as exosomes, but also have desirable circulating properties and targeting properties.¹⁸⁰ Moreover, by adding liposomes with various characteristics (pH-sensitive, photosensitive, and thermosensitive), hybrids can be created multipurpose. Researchers have combined heat-sensitive liposomes and gene-engineered exosomes from CT26 cells overexpressing CD47 to create hybrid nanoparticles (NPs).¹⁴³ In order to achieve targeted delivery, the hybrid NPs can evade in vivo clearance and release medications in response to temperature.

Incubation

Several biological reactions and processes can be gently and successfully induced during incubation. Researchers loaded dopamine into blood exosomes by incubation and validated its potential for brain-targeted delivery for the treatment of central nervous disorders.¹⁴⁴ In addition, the researchers constructed biologically responsive self-assembling exosomes by incubation and used them for early cancer diagnosis.¹⁸¹ It was also determined that because both liposomes and exosomes had a lipid bilayer structure, the fusion of these two types of membranes might be accomplished through incubation techniques.¹⁵⁰ Researchers created high-drug-carrying hybrid exosomes by combining liposomes and stem cell-derived exosomes through incubation. These exosomes were then utilized to distribute analgesics across biological barriers.¹⁴⁵ A promising method for targeted medicine delivery and cancer diagnostics is hybridized nanoparticles.

However, issues like particle aggregation and excessive size may result from incubation-induced spontaneous fusion. More research is required to determine how the fusion process is impacted by the incubation conditions.

Co-Extrusion

The goal of the co-extrusion technique is to physically push exosomes and liposomes to break apart and reassemble into hybrid vesicles as they go through the membrane pores that decrease gradient. When it comes to controlling yield, the co-extrusion method outperforms both freeze-thaw and incubation. Liposomes are frequently used as drug delivery vehicles because of their exceptional engineering and functional modification flexibility. However, biological vesicles are naturally advantageous for endogenous cellular processes. Exosomes and liposomes together organically have the potential to increase medication delivery yield and capacity. Additionally, it can enhance permeability and general biocompatibility.

Hybrid vesicles were created by co-extruding lung-targeted liposomes in a 1:1 ratio with exosomes from bispecific Chimeric Antigen Receptor T-cell Immunotherapy (CAR-T) cells that target mesothelin and programmed death ligand-1.¹⁴⁶ The hybrid vesicles demonstrated superior anti-tumor properties in addition to their good lung accumulation capacity. By co-extrusion of clodronate (CLD) liposomes and fibroblast-derived exosomes in a 5:1 ratio,¹⁴⁷ a hybrid drug delivery system (EL-CLD) with non-specific phagocytic suppression and fibroblast homing capabilities was created, and it was effectively used to treat pulmonary fibrosis. Bionic nanocarriers, which are produced through the co-extrusion of photo thermo sensitive liposomes and platelet exosomes, have good anti-tumor properties and enable the simultaneous administration of chemodynamic and photothermal therapy.¹⁸² Unfortunately, this strategy has not yet made its mark in the field of wound healing. When taking into account the whole strategy's economics, liposomes and exosome-like nanovesicles together might make for a more effective therapeutic approach.

Four Hurdles to Overcome: Safety, Biodistribution, Degradability, Clinical Evaluation Criteria

Over the past decade, biological vesicles have gone from being a relatively unknown topic of study to one that is gaining prominence due to the rapid advancements in nanobiotechnology. According to Grand View Research (2024), the global EV-based wound therapy market has reached 320 million (35.4780 million by 2031 at a 13.1% CAGR, primarily driven by clinical demands for refractory wounds including diabetic foot ulcers, burns, and chronic venous ulcers. We have compiled a comprehensive summary of both ongoing and completed clinical trials investigating vesicle-based therapeutic interventions for wound healing (Table 3). In a 2023 clinical investigation by Gibello et al,¹⁸³ repetitive topical administration of autologous serum-derived EVs significantly enhanced vascular density (+38%) and collagen deposition (+25%) in chronic venous ulcers, with mechanistic analyses linking these effects to the pro-angiogenic activity of platelet- and endothelial-derived vesicle subpopulations; however, the limited cohort size (n=24) and interindividual response variability necessitate expanded validation to confirm therapeutic generalizability. A 2020 double-blind trial by Kwon's team established that mesenchymal stem cell (MSC)-derived EVs accelerated epidermal regeneration post-laser therapy, reducing erythema resolution time by 4.2 days and improving scar quality (Vancouver Scar Scale score reduction: 1.8 points), though the functional contributions of specific EV subtypes (exosomes vs microvesicles) remain undefined.¹⁸⁴ Johnson et al conducted a randomized controlled trial using allogeneic platelet EVs isolated via ligand-exosome affinity purification (LEAP) chromatography,¹⁸⁵ which failed to demonstrate statistically significant differences in healing time (p=0.12) compared to controls but validated the GMP compliance of LEAP technology (purity >95%, 22% improvement in CD63 retention) and clinical safety profile (no severe adverse events), thereby providing foundational infrastructure for scalable manufacturing. Patent analyses reveal three innovative directions in wound-specific EV technologies: 1) Targeted delivery systems (integrin $\alpha 5 \beta 1$ modification in US11992527B2 enhancing inflammatory site accumulation), 2) Stability enhancement processes (EP2666459A1 extending EV storage stability at 4°C from 7 to 30 days through lipid formulation optimization), and 3) Intelligent release control (CN110563829A thermosensitive hydrogel-EV composite enabling on-demand antimicrobial peptide release during wound infection). However, there are four obstacles in the way of clinical translation that both artificial and biological vesicles must get past: sustainability, biodistribution, safety, and clinical evaluation standards (Figure 9).

Table 3 Ongoing and Completed Clinical Trials of Vesicle-Based Wound Treatment

Intervention	Conditions	Phase	ID	Status
Plasma-derived exosomes	Ulcer	Early Phase I	NCT02565264	Unknown
Adipose tissue derived exosomes	Wounds and Injuries	Not Applicable	NCT05475418	Completed
PEP (Purified Exosome Product)	Diabetic Foot Ulcer	Phase 2	NCT06319287	Recruiting
Wharton's Jelly-Derived Mesenchymal Stem Cell Exosomes	Diabetic Foot Ulcer	Phase I	NCT06812637	Completed
Purified Exosome Product	Chronic Radiation Ulcer	Phase I/2	NCT06793748	Not yet recruiting
MSC-derived Exosomes	Diabetic Foot Ulcer	Not Applicable	NCT05243368	Recruiting
Purified Exosome Product	Skin Graft	Phase I	NCT04664738	Active, not recruiting
Autologous extracellular vesicles from serum	Ulcer	Not Applicable	NCT04652531	Recruiting
Human non-autologous platelet-derived Extracellular Vesicle	Cutaneous wound healing	Phase I	ACTRN12620000944932	Completed
Adipose tissue-derived extracellular vesicles	Wound healing	Not Applicable	NCT06253975	Recruiting
Extracellular vesicles isolated from bone marrow-derived mesenchymal stem cells	Burns	Phase I	NCT05078385	Completed
Extracellular microvesicles	Diabetic Foot Ulcer	Phase I	NCT06825884	Active, not recruiting
Anti-Aging	MSC-derived exosomes	Phase I/2	NCT05813379	Unknown

Safety

This issue can be successfully resolved by natural exosomes due to their biological activity, low immunogenicity, and biodegradability. For instance, compared to artificial nanocarriers like liposomes, polymer nanoparticles, and inorganic nanoparticles, naturally occurring exosomes are more physiologically benign. Similarly, clinical results demonstrate that although natural exosomes lack sufficient efficacy in clinical trials, they have consistently demonstrated a favorable in vivo

What needs to be seriously considered

- Sustainability
- Biodistribution
- Safety
- Clinical evaluation standards

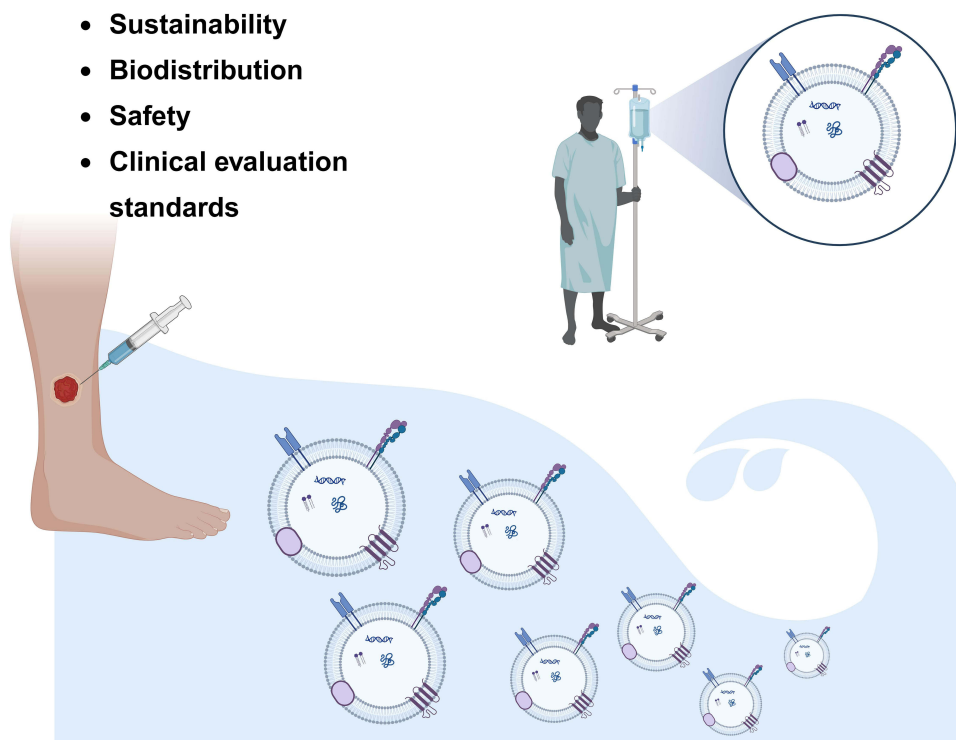


Figure 9 Four hurdles in the way of clinical translation of both artificial and biological vesicles. The figure was created with <https://app.biorender.com/>.

safety profile. Biological vesicles, on the other hand, include a number of intricate ligands and receptors that control a broad range of physiological functions. Since the underlying mechanisms are still being investigated, there may be some safety concerns. The content and function of biological vesicles may also be impacted by the kind and physiological condition of the generating cells, isolation procedures, and purification techniques, which could lead to changed therapeutic efficacy. Through regulated manufacturing procedures, artificial vesicles can alleviate the issues at hand; nevertheless, this may have unintended consequences as well, such as convoluted preparation and characterization procedures. Bioactive proteins may undergo permanent conformational changes as a result of artificial alteration of biological vesicles. Another matter of safety to take into account is immune tolerance. These problems should be resolved by cutting-edge bioengineering technologies like cell-free protein production systems. This technology can translate, fold, and incorporate a range of membrane proteins with distinct functions into liposomes in a single step, doing away with the requirement for a DNA or RNA transfection procedure. Due to advancements in cell growth and metabolic constraints, the cell-free protein synthesis system may complete the synthesis process in 3–5 hours, whereas standard mammalian cells require 3–7 days to complete the expression process. The advantages of a cell-free protein synthesis system also include flexibility, ease of operation, high throughput, and controllability across time, space, reaction conditions, and scale.

Biodistribution

The biodistribution properties of bioengineered vesicles—such as tissue distribution, blood levels, and urine clearance—determine a number of factors, including the therapeutic efficacy and possible toxicity of these extremely effective therapeutic vehicles. Following intravenous injection, it was discovered that the liver and spleen contained the greatest concentration of stem cell-derived exosomes. The distribution of tissue *in vivo* will be affected differently by various injection techniques. Researchers looked examined the impact of six different means of administration, such as intravenous, intraperitoneal, and subcutaneous injections, on tissue distribution *in vivo* to learn more about the biodistribution of exosomes. This was accomplished by combining fluorescence molecular labeling of exosomes produced from HEK293 cells with radioactive zirconium 89, along with immunofluorescence and positron emission tomography imaging.¹⁸⁶ Exosomes from HEK293 cells that overexpress PTGFRN were employed as experimental subjects, and the researchers' primary animal models included mice, rats, and non-human primates. Exosomes were primarily injected subcutaneously into the injection site or lymph nodes, intraperitoneally into the gastrointestinal tract and lymph nodes, and intravenously into the liver and spleen. Furthermore, in the context of administering cerebrospinal fluid, exosomes are dispersed via intrathecal injection along the neuraxis to the base of the skull and via intraoccipital pool and ventricular injection around the skull and cervicothoracic spine. This could raise important concerns for the long-term effectiveness and security of vesicles. Researchers have discovered that exosomes have a dense distribution that is localized to the site while treating it. This may imply that vesicle bioactivity within the wound region needs to be maintained and rapidly absorbed by researchers.

Sustainability

The primary method of biovesicle therapy for wound healing is injectable drug delivery, which is assumed to cause a quick *in vivo* clearance of vesicles, hence impacting therapeutic efficacy. As a result, more and more scientists are starting to mix bioactive carriers in an effort to improve vesicles' therapeutic effectiveness. In addition to facilitating vesicle sustained release, hydrogels act as efficient vesicle transporters by offering therapeutic advantages that vesicles cannot. Hydrogels and vesicles together have emerged as a hotspot of therapeutic research in the field of wound healing. Researchers used oxygen nanobubbles and polyvinyl alcohol/gelatin hybrid hydrogels to improve exosome delivery and reduce wound hypoxia because vesicles had a poor delivery effect in hypoxic tissues.¹⁸⁷ Furthermore, encasing exosomes in biocompatible biinks and using 3D bioprinting to create exosome-loaded 3D scaffolds can both efficiently increase the vesicles' lifespan and enhance their sustained distribution in wound tissues.¹⁸⁸ Despite producing physiochemically stable three-dimensional structures, 3D printing technology does not yield bioactive structures. To increase scaffold bioactivity, these structures ought to be enhanced with particular cell types, bioactive compounds, or exosomes.

Clinical Evaluation Criteria

With the increasing recognition of the role biological vesicles play in the development of diseases and in possible therapies, the industry sector is seeing exponential expansion. Global sales of exosome diagnostics are predicted to reach USD 321.9 million by 2026, up from USD 57.1 million in 2021, while sales of exosome therapeutics are anticipated to reach USD 169.2 million by 2026, up from USD 33.1 million in 2021.¹⁸⁹ The potential economic utility of these particles has not been completely realized, despite the noteworthy advancements in the field of vesicle biology and its applications. For clinical applications, there needs to be standardization in the production and analysis of biological vesicles. Regarding vesicle-related items intended for clinical use, there is disagreement about criteria for release and isolation techniques. The FDA has only authorized a limited number of EV products for therapeutic use thus far. Good Manufacturing Practices (GMP) are essential to guaranteeing the best possible clinical results. Biological vesicle have emerged as innovative biological agents for wound therapy, with their regulatory requirements demonstrating both differentiation and increasing stringency across major global pharmaceutical regulatory systems.¹⁹⁰ The US Food and Drug Administration (FDA) categorizes EV therapies as biological products (21 CFR 600.3), necessitating market approval through the Biologics License Application (BLA) pathway. For combination products (eg, smart dressings integrated with sensors), joint review by the Center for Drug Evaluation and Research (CDER) and Center for Devices and Radiological Health (CDRH) under 21 CFR Part 4 is required. Core regulatory mandates include: 1) Manufacturing processes must comply with Current Good Manufacturing Practice (cGMP) standards, with explicit identification methods for EV source cells (eg, mesenchymal stem cells per ISEV2018 guidelines) and purification protocols (eg, ultracentrifugation coupled with size-exclusion chromatography). Documentation must include data from ≥ 3 production batches demonstrating particle size distribution (80–200 nm), surface markers (CD9/CD63/TSG101), and exogenous contaminants (host cell protein < 50 ng/dose); 2) Preclinical validation requires standardized animal models (eg, db/db diabetic mice) demonstrating $\geq 30\%$ reduction in epithelialization time, complemented by biocompatibility (ISO 10993–5/10) and immunogenicity testing (complement activation rate $< 5\%$); 3) Clinical trials must adopt multicenter randomized controlled trial (RCT) designs with primary endpoints of complete epithelialization time and secondary endpoints encompassing infection rates, scar assessment scores, and patient-reported outcomes (PROs). The FDA mandates ≥ 1 year follow-up to evaluate long-term safety risks, particularly antibody generation against allogeneic EVs. The European Medicines Agency (EMA) regulates EV therapies under the Advanced Therapy Medicinal Products (ATMP) framework through centralized Marketing Authorization Applications (MAAs). Products targeting refractory wounds may qualify for Priority Medicines (PRIME) designation, requiring early clinical efficacy evidence (eg, Phase II trial hazard ratio $HR \geq 1.5$) and compliance with EU GMP Annex 2 specifications (cleanroom grade $\geq B$ with viral inactivation validation). EMA emphasizes orthogonal characterization methods (eg, nanoparticle tracking analysis [NTA] combined with flow cytometry) and establishment of critical quality attribute (CQA) control standards (eg, miRNA-21 content coefficient of variation $CV < 15\%$). Internationally, the ISO/TC276 draft guideline for EV characterization (2024) mandates testing of ≥ 20 parameters including particle size, zeta potential, and proteomic profiles. ICH Q5A/Q12 guidelines specify requirements for viral safety evaluation (retrovirus detection limit < 1 IU/dose) and manufacturing process change control (comparability studies for microfluidic parameter adjustments).

However, the establishment of robust GMP standards for bioinspired vesicles remains in its nascent stage, necessitating coordinated multidisciplinary efforts to address three critical challenges: (1) standardization of production processes compromised by technical complexities in synthetic biology modifications (eg, controlled insertion of artificial transmembrane proteins), (2) quality control bottlenecks arising from heterogeneous vesicle populations (typically 50–200 nm size distribution with $\pm 15\%$ batch variability), and (3) regulatory alignment for novel characterization requirements beyond conventional EV paradigms (including engineered surface topology quantification and synthetic cargo verification). Progress demands synergistic collaboration across material scientists (for functional module design), bioengineers (for scalable manufacturing development), and regulatory specialists (for QbD framework implementation), ideally through international consortia modeled after the ISO/TC276 working group on synthetic vesicles. Emerging solutions include AI-driven process optimization platforms (reducing parameter optimization time from months to weeks) and blockchain-enabled data sharing ecosystems for global GMP knowledge integration, as demonstrated by the Horizon Europe Strategic Plan establishing 23 cross-border GMP validation centers.

Conclusion and Future Direction

Chronic wound healing, particularly in refractory cases such as diabetic foot ulcers (DFUs) and venous leg ulcers (VLUs), remains a critical challenge in modern medicine. The global burden of chronic wounds has escalated due to aging populations and the rising prevalence of multidrug-resistant pathogens. These wounds are characterized by a self-perpetuating pathological triad of “sustained inflammation-oxidative stress-angiogenic dysfunction”, posing formidable challenges to conventional therapeutic approaches. A retrospective cohort study involving 889 hospitalized patients with chronic refractory cutaneous wounds revealed an all-cause mortality rate of 0.8%, with all fatalities attributed to cardiopulmonary failure secondary to systemic infections.¹⁹¹ Notably, DFU patients face disproportionately high risks of limb amputation, which independently increases mortality by 2.4-fold. Current therapeutic limitations are multifaceted: (1) Over 75% of chronic wounds develop polymicrobial biofilms, reducing antibiotic penetration by >90% and facilitating immune evasion through quorum sensing mechanisms.¹⁹² (2) Recombinant growth factor therapies (eg, platelet-derived growth factor [PDGF]) incur prohibitive costs (>\$5,000 per course), while autologous skin grafting carries risks of donor site morbidity and incomplete functional recovery.¹⁹³ (3) Existing dermal substitutes lack the capacity for dynamic microenvironment modulation, failing to synchronize vascularization with neural regeneration—a prerequisite for functional tissue restoration. These unresolved clinical demands underscore the urgent need for innovative therapeutic strategies.¹⁹⁴

Among diverse therapeutic strategies, biological vesicles exhibit remarkable therapeutic potential. As critical mediators of intercellular communication, biological vesicles carry multiple bioactive molecules such as proteins, miRNAs, and lipids, thereby regulating cellular behavior and promoting tissue repair. EVs derived from MSCs have been demonstrated to enhance wound healing in various animal models by modulating inflammatory responses, accelerating angiogenesis, and improving tissue regeneration. However, three major challenges hinder their clinical translation. First, the heterogeneity of BVs—rooted in diverse isolation methods, cellular sources, and functional characterization—remains a primary barrier. Although microfluidic-based isolation improves purity it results in a 70% loss of small-sized EVs (<50 nm).¹⁹⁵ Culture conditions significantly influence miRNA profiles in MSC-secreted EVs; for instance, hypoxic conditions (5% O₂) reduce pro-angiogenic miR-126 levels compared to normoxia.¹⁹⁶ Donor-specific variations further exacerbate functional disparities: EVs from older donors (>50 years) show a 40% slower epithelialization rate in diabetic murine wounds than those from younger donors (<30 years) (14-day healing rates: 72% vs 48%), with batch-dependent efficacy fluctuations (eg, 3.5-fold differences in pro-angiogenic activity).¹⁹⁷ Second, low production yields and limited scalability of BVs substantially increase therapeutic costs. Static MSC cultures yield only 0.1–1 mg EVs per liter of medium ($\approx 10^{10}$ – 10^{11} particles), requiring 100–1,000 liters to meet the minimum dose (10^{12} particles) for a single diabetic foot ulcer treatment.¹⁹⁸ While 3D bioreactors (eg, hollow fiber systems) elevate yields to 5–10 mg/L, the associated capital investment exceeds hundreds of thousands of dollars per unit.¹⁹⁹ Finally, the complexity of the wound microenvironment (eg, high protease activity, hypoxia, inflammation) critically compromises BV efficacy. Studies reveal that >70% of topically administered EVs are degraded by wound fluid proteases (eg, MMP-9) within 6 hours.²⁰⁰ Similarly, elevated reactive oxygen species (ROS) in diabetic wounds reduce SOD2 enzyme activity in EVs, abolishing their antioxidant capacity.²⁰¹ Bioinspired vesicles, through biomimetic design, engineered modifications, and synthetic biology technologies, systematically address the inherent limitations of natural biological vesicles (eg, exosomes, microvesicles) while demonstrating unique advantages in wound therapy. First, bioinspired strategies enhance vesicle targeting efficiency: covalent surface conjugation of metalloproteinase inhibitors (eg, TIMP-1) reduces MMP-9-mediated degradation.²⁰² Second, multifunctional preparation techniques augment pro-healing capabilities: precise loading of antimicrobial peptides (eg, LL-37) and small-molecule drugs (eg, metformin) via electroporation or microfluidic technology achieves dual therapeutic synergy.²⁰³ Engineered cells overexpressing Rab27a gene produce vesicles with 3-fold elevated VEGF payloads.²⁰⁴ Finally, bioinspired approaches enable scalable production: lipid-based self-assembly technology achieves batch yields >100 mg.²⁰⁵ Despite these theoretical advantages, their clinical translation faces critical challenges: manufacturing complexity and cost pressures, long-term safety and immunogenicity risks, and trade-offs between functional customization and inherent biological benefits. Overall, significant disparities exist between these two modalities in biological properties, stability, production costs, and clinical translation (Table 4).

In the future, the integration of multi-omics analysis, artificial intelligence algorithms, and multiscale computational models enables the establishment of a precision medicine spanning from patient-specific diagnosis to customized vesicle

Table 4 Multiple Comparisons of Biological Vesicles with Bioinspired Vesicles

Comparisons		Biological Vesicles	Bioinspired Vesicles
Healing efficiency	Bioactive Ingredients	Dependent on natural signaling molecules, moderate pro-angiogenic and immunomodulatory activity	Engineered to significantly enhance tissue repair efficacy and functional synergy of the active ingredients
	Targeting and Sustainability	Predominantly passive diffusion, short duration of action, frequent dosing required to maintain efficacy	Long-lasting action through intelligent response release system and continuous microenvironmental modulation
Stability	Physical stability	Poor storage stability, membrane components are susceptible to degradation	Materials engineering significantly improves physical stability and long-term storage performance
	Biological stability	Biomolecules are susceptible to enzymatic degradation in the body fluid environment; limited functional maintenance time	Anti-degradation design effectively extends the half-life of biologically active ingredients
Production costs	Separation and purification	Traditional separation techniques are inefficient and difficult to control purity	Novel purification strategy dramatically improves yield and batch consistency
	Scale-up potential	Individualised preparation capacity is limited, making it difficult to meet clinical demand	Standardised process supports mass production at significantly lower unit cost
Immunogenicity		Existence of endogenous risk components triggering excessive immune response	Surface modification technology effectively reduces the risk of immune recognition
Functional programmability		Natural components are fixed, with limited room for functional expansion	Modular design supports multi-functional integration and significantly improves therapeutic precision
Current status of clinical translation		Multiple mid-term trials have validated the basic efficacy, but individual differences are prominent	Front-engineered products entering late-stage trials, demonstrating breakthrough efficacy potential
Core strengths		Biological mechanism is clear, and the regulatory pathway is mature	Customisable design, controllable production costs, and superior efficacy
Key limitations		Individual differences are large, and large-scale production is difficult	Long-term biosafety to be validated

therapy. Initial characterization of critical molecular features in the wound microenvironment is achieved through whole-exome sequencing and single-cell spatial transcriptomics, particularly via detection of TGF- β signaling pathway genetic variations and macrophage polarization-associated miRNA expression profiles (eg, miR-21/-155), which identify responsive biomarkers to specific vesicle components.²⁰⁶ Building upon this foundation, Generative Adversarial Networks (GANs) trained on clinical cases generate optimal lipid composition formulations,²⁰⁷ while Graph Neural Networks (GNNs) predict molecular docking patterns between antimicrobial peptides and vesicle membranes,²⁰⁸ achieving drug loading efficiency exceeding 85%. Molecular dynamics simulations further optimize drug release kinetics by calculating coupling relationships between vesicle membrane phase transition temperatures and local blood flow rates, enabling spatiotemporal control of growth factor release.²⁰⁹ A closed-loop feedback system incorporating flexible electronic sensors continuously monitors wound pH, IL-6 concentrations, and microbiota composition, with reinforcement learning algorithms dynamically adjusting vesicle administration strategies.²¹⁰ The SMARTHeal system developed under this framework has completed Phase II clinical trials, confirming its universal applicability across chronic wounds of diverse etiologies. However, both vesicle classes must address four clinical translation barriers: manufacturing sustainability, biodistribution control, long-term safety, and standardized clinical evaluation criteria. Their complementary yet competitive development trajectories will collectively advance regenerative medicine into an era of precision and industrial scalability.

Abbreviations

ADSCs, Adipose-derived stem cells; BBB, Blood-brain barrier; BMSCs, Bone marrow-derived MSCs; CEC, Carboxyethyl chitosan; CAR-T, Chimeric Antigen Receptor T-cell Immunotherapy; DETCs, Dendritic epidermal T cells; DCMC, Dialdehyde carboxymethylcellulose; DFF, Direct current filtration; EVs, Extracellular vesicles; GBM, Glioblastoma multiforme; GFs, Growth factors; hAECs, Human amniotic epithelial cells; hUC-MSCs, Human umbilical cord MSCs; IGF-1, Insulin-like growth factor-1; IL-6, Interleukin-6; MSCs, Mesenchymal stem cells, MALAT1, Metastasis-associated lung adenocarcinoma transcript 1; MNs, Microneedles; PRP, Platelet-rich plasma; PEG, Polyethylene glycol; TFF, Tangential flow filtration; TNF- α , Tumor necrosis factor-alpha; TGF- β , Transforming growth factor- β ; VEGF, Vascular endothelial growth factor.

Data Sharing Statement

No data was used for the research described in the article.

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Disclosure

The authors declare no competing financial interests.

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