

Extracellular vesicles and their mimetics: clinical application prospects in medical aesthetics

Zixuan Sun^{1,2,†}, Jiali Li^{2,†}, Zhijing Wu², Jiabin Zhang², Tangrong Wang², Yuzhou Zheng², XueZhong Xu¹, Wei Ding¹, Hui Qian^{2,*}, Yulin Tan^{1,*}

¹Department of General Surgery, Wujin Hospital Affiliated with Jiangsu University, No. 2 Yongning North Road, Jiangsu, Changzhou 213017, China

²Jiangsu Key Laboratory of Medical Science and Laboratory Medicine, Department of Laboratory Medicine, School of Medicine, Jiangsu University, No. 301 Xuefu Road, Jiangsu, Zhenjiang 212013, China

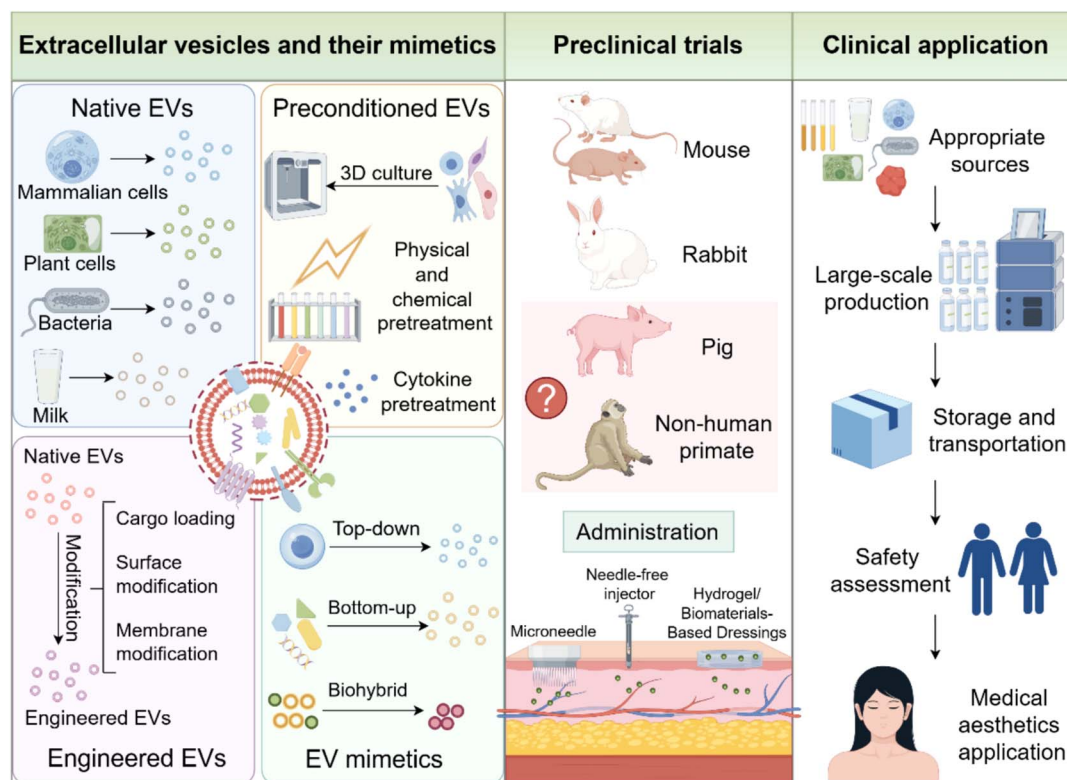
*Corresponding author. Yulin Tan, E-mail: tanyulin@wjrmmy.cn; Hui Qian, E-mail: 100007341@ujs.edu.cn

†Zixuan Sun and Jiali Li have contributed equally to this work.

Abstract

The persistent desire for beauty has fueled the rapid development of medical aesthetics. Multiple approaches, including cosmetics, drugs, and cell therapies, have been developed to improve skin conditions. However, none of these methods achieves the intended outcomes and may produce adverse repercussions. Consequently, it is crucial to develop effective and safe therapies to promote skin repair and regeneration. Extracellular vesicles (EVs) are cell-derived, lipid bilayer-encapsulated nanoparticles containing multiple active components. There is now strong evidence that EV therapies can improve skin barrier function, reduce scarring, inhibit aging and pigmentation, and suppress hair loss, highlighting the potential for cosmeceutical development based on EVs. This review summarizes the latest applications of natural EVs, preconditioned and engineered EVs, and EV mimetics in medical aesthetics. Additionally, the article discusses advanced delivery strategies for EVs and the commercial prospects of EVs and provides insights into present security concerns, regulations, and the field's future directions.

Graphical Abstract



Keywords: Extracellular vesicles; Extracellular vesicle mimetics; Medical aesthetics; Cosmeceutical; Commercial landscape

Received: November 27, 2024. Revised: April 27, 2025. Accepted: May 12, 2025

© The Author(s) 2025. Published by Oxford University Press.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site-for further information please contact journals.permissions@oup.com.

Highlights

- This article reviews the application of EVs and their mimetics in regenerative dermatology.
- EVs using adjunctive procedures (microneedles, needleless syringes, and hydrogels) could improve the therapeutic effect.
- Some companies are selling EV products for topical skin applications, but issues still exist regarding their purity, dosage, and penetration level.

Background

Beauty is an ever-lasting pursuit of humanity. Medical aesthetics refers to the use of medical means, including drugs, instruments, and surgery, to change the external shape and color, and hence, augment the body's external beauty [1, 2]. Improving skin barrier function, reducing scars, combating aging, preventing pigmentation, and inhibiting hair loss are the main goals of medical aesthetics. Currently, vitamin C [3], hyaluronic acid [4, 5], and botulinum toxin [6] are often used in medical aesthetics to diminish pigmentation and eliminate wrinkles, thereby promoting facial rejuvenation. However, controlling the injection dosage is difficult, and inappropriate injections can lead to serious complications. Additionally, although fat grafting has become common in medical aesthetics, it can result in localized contour shape abnormalities and increased foreign body sensation [7]. Driven by growing demand, recent research has concentrated on the production of novel ingredients exhibiting superior functions that combine beauty and medicinal attributes.

Nanoparticles (NPs) have been used as carriers of active ingredients to enhance their bioavailability and efficacy owing to their good skin penetration and adjustable release properties. The predominant nanocarriers used in the cosmeceutical domain include liposomes, nanocapsules, micelles, and metal NPs [8]. However, the immunogenicity and potential toxicity of these NPs are the major limitations to their development. In contrast to the aforementioned NPs, cell-derived NPs, known as extracellular vesicles (EVs), have low immunogenicity and high biocompatibility and have garnered significant interest in the cosmeceutical domain. EVs can be classified into several subtypes, which researchers previously typically classified as exosomes (30–150 nm) and microvesicles (MVs, 50–1000 nm) based on biogenesis [9] (Figure 1). However, the use of terms associated with difficult-to-identify biogenesis pathways is no longer recommended in the MISEV2023 guidelines of the International Society for Extracellular Vesicles (ISEV) [10]. Consequently, in this article, the term EVs predominantly denotes all NPs, except when a particular designation is applied according to the study's context.

EVs can be released by almost all cells and can be isolated from cell supernatants, a wide range of tissues, body fluids, biofluids (such as milk), and plants. Compared with cell therapy, EVs offer enhanced safety owing to their ability to pass through filters, lack of proliferation or differentiation capabilities, and the presence of clear and stable biological cargo. EVs play crucial roles in tissue repair and immune regulation by transporting proteins, nucleic acids, lipids, and metabolites [11]. The immunomodulatory effects of EVs further enhance their potential as biomarkers for inflammatory skin diseases, allowing for noninvasive prediction of the onset of these conditions [12, 13]. In addition, the expression of surface proteins, such as CD47, can reduce the clearance of EVs by the mononuclear phagocytic system [14]. Increasing evidence indicates that EVs of different origins possess similar therapeutic significance for

improving skin conditions [15–18]. For instance, Zhang *et al.* [19] discovered that EVs derived from adipose and umbilical cord mesenchymal stem cells (MSCs) can effectively alleviate skin aging by reducing UVB-induced Notch activation through the key protein component TIMP1 in their cargo. In a clinical study, the local application of adipose-derived MSCs (ADSCs)-EVs formulations showed skin-brightening effects in volunteers with hyperpigmentation [20]. In another clinical study, ADSC-EVs were applied to atrophic acne scars treated with a fractional carbon dioxide laser (FCL) to reduce the adverse effects of FCL by accelerating tissue regeneration and wound healing [21]. A growing number of clinical trials on EVs from different sources aimed at improving skin barrier function (NCT05969717), delaying skin aging (NCT05813379), inhibiting hair loss (NCT06539273, NCT05658094, NCT06571799, NCT06239207, NCT06482541, NCT06697080), and treating melasma (NCT06221787, NCT06677931) have also significantly enhanced the commercial potential of EVs in medical aesthetics. In addition to natural EVs, there is considerable research potential to enhance the therapeutic effects of EVs by altering the culture environment of parent cells through methods such as hypoxic culture and the addition of cytokines [22, 23] or by using EVs as drug carriers to improve cellular targeting and uptake. To achieve low cost and large-scale production, EV mimetics have emerged as promising alternatives to EVs. Compared with human induced pluripotent stem cell (iPSC)-derived EVs, EV mimetics derived from human iPSCs have significantly increased production and demonstrated therapeutic potential in alleviating replicative senescence of human dermal fibroblasts (HDFs) [24]. EVs delivered with adjuvant therapies (e.g. microneedling) offer the potential to further augment transdermal administration and regulate their release kinetics to improve clinical outcomes [15]. These advantages render EVs attractive candidates for use in cosmeceuticals.

In this review, we summarize recent findings on natural EVs, preconditioned and engineered EVs, and EV mimics in medical aesthetics. We also discuss advanced delivery strategies for EVs, the clinical trial enrollment of EVs related to skin improvement, and the commercial outlook for EVs. Finally, we provide insights into the future directions required to develop reproducible and clinically effective EV therapeutics.

Review

Application of native EVs in medical aesthetics

Repairing the skin barrier, reducing scarring, combating aging, preventing pigmentation, and inhibiting hair loss remain the most common sought-after effects of medical aesthetics. EVs have garnered significant attention owing to their nano-size, low immunogenicity, intercellular communication capabilities, and the fact that they carry well-defined and stabilized biological cargoes that are crucial in aesthetics. The emerging role of EVs in medical aesthetics highlights their potential for the development of cosmetics based on EVs (Figure 2).

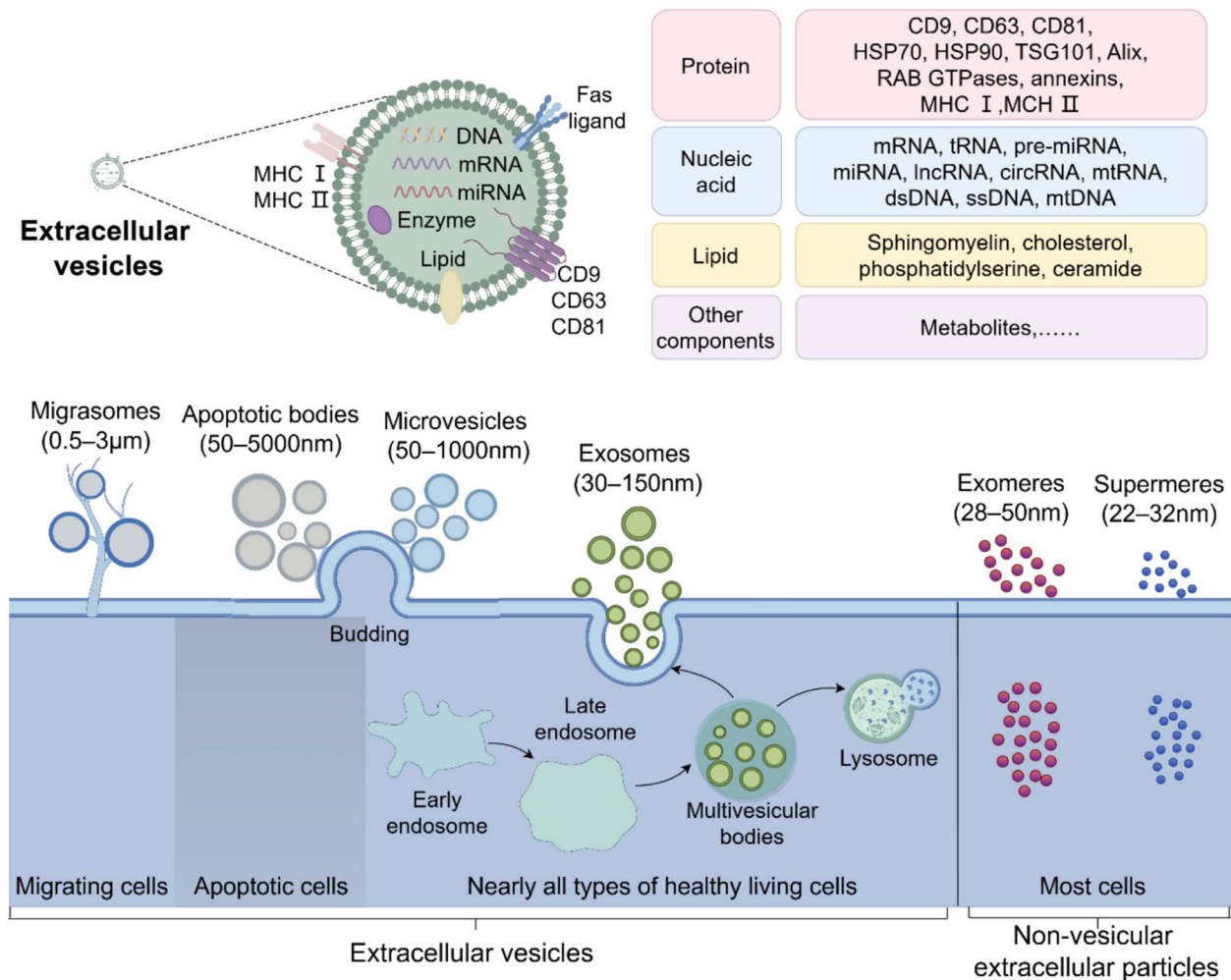


Figure 1. Classification, biogenesis, and composition of EVs (by Figdraw). Migrasomes are membranous vesicles generated by migrating cells at the near ends. Apoptotic bodies are generated through the blebbing of the plasma membrane after cell apoptosis. Microvesicles originate from the direct budding of the plasma membrane, whereas exosomes are generated through the endosomal pathway. These EVs have a lipid bilayer, encapsulating many components. In addition, exomeres and supermeres are newly discovered non-vesicular extracellular nanoparticles, and their roles need further investigation. MHC: major histocompatibility complex; mRNA: messenger RNA; miRNA: microRNA; HSP70: heat shock protein 70; HSP90: heat shock protein 90; TSG101: tumor susceptibility 101; Alix: ALG-2 interacting protein-X; tRNA: transfer RNA; pre-miRNA: precursor microRNA; lncRNA: long non-coding RNA; circRNA: circular RNA; mtRNA: mitochondrial RNA; dsDNA: double-stranded DNA; ssDNA: single-stranded DNA; mtDNA: mitochondrial DNA

Mammalian cell-derived EVs

Mesenchymal stem cell-derived EVs. MSCs have been extracted from adipose tissue [25, 26], umbilical cord [27], bone marrow [28], placenta [29], and hair follicles [30, 31] and have demonstrated significant promise as therapies for scarring, hair loss, skin barrier [32–34], aging, and pigmentation [35] through paracrine mechanisms (e.g. secretion of EV). Since the liposuction procedure is considered secure and free of ethical issues and a large number of ADSCs can be obtained, ADSC-EVs show credible and reliable clinical applications. ADSC-EVs have been proven to enhance hair regrowth through modulating the Wnt/ β -catenin signaling pathway, TNF- α signaling pathway, and growth regulators [36–38]. In skin photoaging model, ADSC-EVs resisted skin aging by reducing reactive oxygen species (ROS) production and inflammatory responses [18]. In addition, ADSC-EVs regulated the Smad signaling pathway in scar formation by targeting IL-17RA through miR-192-5p, thereby reducing hypertrophic scar fibrosis [39]. Several studies have highlighted the regulatory and temporal effects

of ADSC-EVs on collagen synthesis during wound repair, which may be important in attenuating fibrotic proliferation and scar formation [40–42]. The umbilical cord is another important source of MSCs, and EVs derived from human umbilical cord MSCs (hucMSCs) may also regulate the activity of hair follicle keratinocytes and induce hair follicle regeneration by secreting growth factors such as FGF-7, BMP-4, and versican [43]. Although EVs originate from different sources, they have shown similar therapeutic effects in terms of medical aesthetics (Table 1).

Mature somatic cell-derived EVs. Fibroblasts are more closely associated with the skin than stem cells. They are widely distributed, easy to obtain, more easily survive *in vitro*, and proliferate rapidly. Therefore, some studies have investigated the application of fibroblast-derived EVs in skin aesthetics. Oh *et al.* [44] found that fibroblast-derived EVs promoted gene expression related to scar-free wound healing in fibroblasts and accelerated wound healing in mice. In addition, fibroblast-derived EVs rich in Wnt3a may activate the

Table 1. The roles of native EVs in medical aesthetics

Origins	Cargos in EVs	Models	Mechanisms	Functions	Ref.
ADSCs	Undefined	Hypertrophic scar rabbits	Undefined	Inhibit collagen deposition and myofibroblast aggregation, prevent the formation of hypertrophic scars	[25]
ADSCs	Proteins and miRNAs	UVB-irradiated HDFs	↑TIMP-1, ↑TGF-β1	Promote dermal matrix synthesis	[26]
ADSCs	Undefined	AD mice	Undefined	Inhibit inflammation, improve skin barrier function	[32]
ADSCs	Undefined	AD mice	↓JAK/STAT signaling pathway	Reduce skin inflammation, promote skin barrier repair	[33]
ADSCs	Undefined	AGA mice	↑Wnt/β-catenin signaling pathway	Antagonize the inhibitory effect of DHT on hair follicle growth	[36]
ADSCs	Undefined	Hair-depilated mice	↓miR-22, ↓TNF-α signaling pathway, ↓Wnt/β-catenin signaling pathway	Promote hair growth	[37]
ADSCs	Undefined	Nude mice	↑PDGF, ↑VEGF, ↓TGF-β1	Promote hair regeneration	[38]
ADSCs	Undefined	Photoaging mice	↓NF-κB1 signaling pathway, ↓ROS	Reduce wrinkles, reduce ROS production	[18]
ADSCs	miR-192-5p	Excisional wound mice	↓IL-17RA	Attenuate the deposition of collagen and the formation of hypertrophic scar	[39]
HucMSCs	GPX-1	UVB-irradiated fibroblasts	Undefined	Prevent cell cycle arrest, reduce ROS	[27]
HucMSCs	Undefined	Subjects (female, aged 18–55)	↓Neurovascular hyperreactivity	Repair skin barrier, improve clinical symptoms in patients with sensitive skin	[34]
HucMSCs	FGF-7, BMP-4, and versican	AA mice	Undefined	Promote hair regrowth	[43]
BMSCs	Undefined	Hair-depilated mice	↑Akt/ERK pathway, ↑VEGF, ↑IGF-1, ↑Wnt signaling pathway, ↑Versican	Promote hair growth	[28]
P-MSCs	miR-145-5p	Skin wound mice	↓CDKN1A, ↑Erk/Akt signaling pathway	Accelerate wound closure, reduce scar width	[29]
hASCs	miR-181a-5p and miR-199a	Pigmentation mice	↓MITF, ↑autophagy	Inhibit melanin synthesis	[35]
DSCCs	Undefined	Senescent cells	↑Wnt/β-catenin signaling pathway	Restore the biological functions and improve its hair follicle induction ability of aging DPCs	[30]
DPCs	miR-140-5p	Hair-depilated mice	↓BMP2 signaling pathway, ↑Wnt signaling pathway, ↑Shh signaling pathway	Accelerate hair follicle elongation and cell proliferation activation	[31]
Fibroblasts	Undefined	Skin wound mice	Undefined	Promote gene expression related to scar-free wound healing and accelerate wound healing	[44]
Fibroblasts	Wnt3a	DPCs and ORSCs	↑Wnt/β-catenin signaling pathway	Promote elongation of the hair shaft	[45]
HDFs	Undefined	Pigmentation mice	↑ERK signaling pathway, ↓β-catenin signaling pathway	Inhibit melanin production, downregulate melanogenesis-related gene expression and melanosome transport-related protein expression	[46]
Macrophages	Wnt3a and Wnt7b	Hair-depilated mice	↑Wnt/β-catenin signaling pathway, ↑Axin2, ↑Lef1	Increase the number of hair follicles and the thickness of the dermis, elongate hair shaft	[47]
EDs	Undefined	Skin wound mice	↓YAP phosphorylation, ↑PI3K/Akt/mTOR pathway	Accelerate skin wound healing, increase microvascular density, increase collagen deposition	[48]
Apple	Undefined	HDFs	↓NF-κB signaling pathway	Increase collagen synthesis, downregulate metalloproteinase production	[49]
Aloe saponaria	Undefined	RAW264.7 macrophages, HDFs, and HUVECs	Undefined	Facilitate angiogenesis	[50]

(Continued)

Table 1. Continued

Origins	Cargos in EVs	Models	Mechanisms	Functions	Ref.
<i>Aloe vera</i>	Undefined	HaCaT cells and HDFs	Undefined	Increase antioxidant gene expression	[51]
Grapefruit	Undefined	HaCaT cells and HUVECs	Undefined	Reduce intracellular ROS production, facilitate angiogenesis	[52]
<i>Dendropanax morbifer</i>	Undefined	A reconstituted human epidermis model	↓ MITF, ↓ TYR, TRP-1, and TRP-2	Inhibit the production of melanin	[53]
<i>Sargassum fusiforme</i>	Undefined	A three-dimensional model of human epidermis	Undefined	Reduce the production of melanin	[54]
<i>L. plantarum</i>	LTA	Human monocyte THP1 cells	TLRs	Induce the transition of monocytes to macrophages and polarization of macrophages towards M2b state	[55]
<i>L. plantarum</i>	Undefined	Subjects (women in their 50s)	Undefined	Inhibit the formation of wrinkles and pigmentation	[56]
<i>L. druckerii</i>	Undefined	Scleroderma mice and excisional wound healing mice	↑ MAPK signaling pathway	Inhibit the formation of hypertrophic scars, promote wound healing	[57]
<i>S. elongatus</i>	Undefined	Burn wound mice	↑ IL-6	Promote angiogenesis and wound healing	[58]
<i>S. aureus</i>	α-hemolysin	Hairless mice	↑ Necrotic cell death	Destroy skin barrier, promote the development of AD-like skin inflammation	[59]
Milk	miR-2478	Melanoma cells and melanocytes	↓ Rap1a, ↑ Akt-GSK3β signal pathway	Inhibit melanogenesis and melanogenesis-related gene expression	[66]
Milk	Undefined	Female volunteers	Undefined	Preserve moisture and reduce wrinkles	[67]
Colostrum	Undefined	UVC-irradiated cells	Undefined	Improve skin aging and damage	[69]
Colostrum	Undefined	Hair-depilated mice	↑ Wnt/β-catenin signaling pathway	Promote hair regeneration	[70]
Colostrum	miR-21	Excisional wound mice	TGF-β/Smad signaling pathway, Smad7-Smad2/3-elastin signaling pathway	Promote re-epithelialization, activate angiogenesis, enhance extracellular matrix maturation	[71]

ADSCs: adipose-derived mesenchymal stem cells; miRNAs: microRNAs; UVB: ultraviolet B; HDFs: human dermal fibroblasts; TIMP-1: tissue inhibitor of metalloproteinase-1; TGF-β1: transforming growth factor-β1; AD: atopic dermatitis; JAK/STAT: Janus kinase/signal transducers and activators of transcription; AGA: androgenetic alopecia; DHF: dihydrotestosterone; TNF-α: tumor necrosis factor-α; PDGF: platelet-derived growth factor; VEGF: vascular endothelial growth factor; NF-κB1: nuclear factor kappa-light-chain-enhancer of activated B cells 1; ROS: reactive oxygen species; IL-17RA: interleukin 17 receptor A; hucMSCs: human umbilical cord mesenchymal stem cells; GPX-1: glutathione peroxidase 1; FGF-7: fibroblast growth factor 7; BMP-4: bone morphogenetic protein 4; AA: alopecia areata; BMSCs: bone marrow-derived mesenchymal stem cells; Akt/ERK: protein kinase B/extracellular signal-regulated kinase; IGF-1: insulin-like growth factor 1; P-MSCs: placental mesenchymal stem cells; CDKN1A: cyclin-dependent kinase inhibitor 1A; hASCs: human amniotic stem cells; MITF: microphthalmia-associated transcription factor; DSCCs: dermal sheath cup cells; DPCs: dermal papilla cells; BMP2: bone morphogenetic protein 2; ORSCs: outer root sheath cells; ERK: extracellular signal-regulated kinase; EDs: endothelial cells; YAP: Yes-associated protein; PI3K/Akt/mTOR: phosphoinositide 3-kinase/protein kinase B/mechanistic target of rapamycin; HUVECs: human umbilical vein endothelial cells; TYR: tyrosinase; TRP: tyrosinase-related protein; *L. plantarum*: lactobacillus plantarum; LTA: lipoteichoic acid; TLRs: toll-like receptors; *L. druckerii*: *Lactobacillus druckerii*; MAPK: mitogen-activated protein kinase; *S. elongatus*: synechococcus elongatus; IL-6: interleukin-6; *S. aureus*: staphylococcus aureus; Akt/GSK3β: protein kinase B/glycogen synthase kinase-3β; UVC: ultraviolet C; Smad: small mother against decapentaplegic; ↑: increase; ↓: decrease

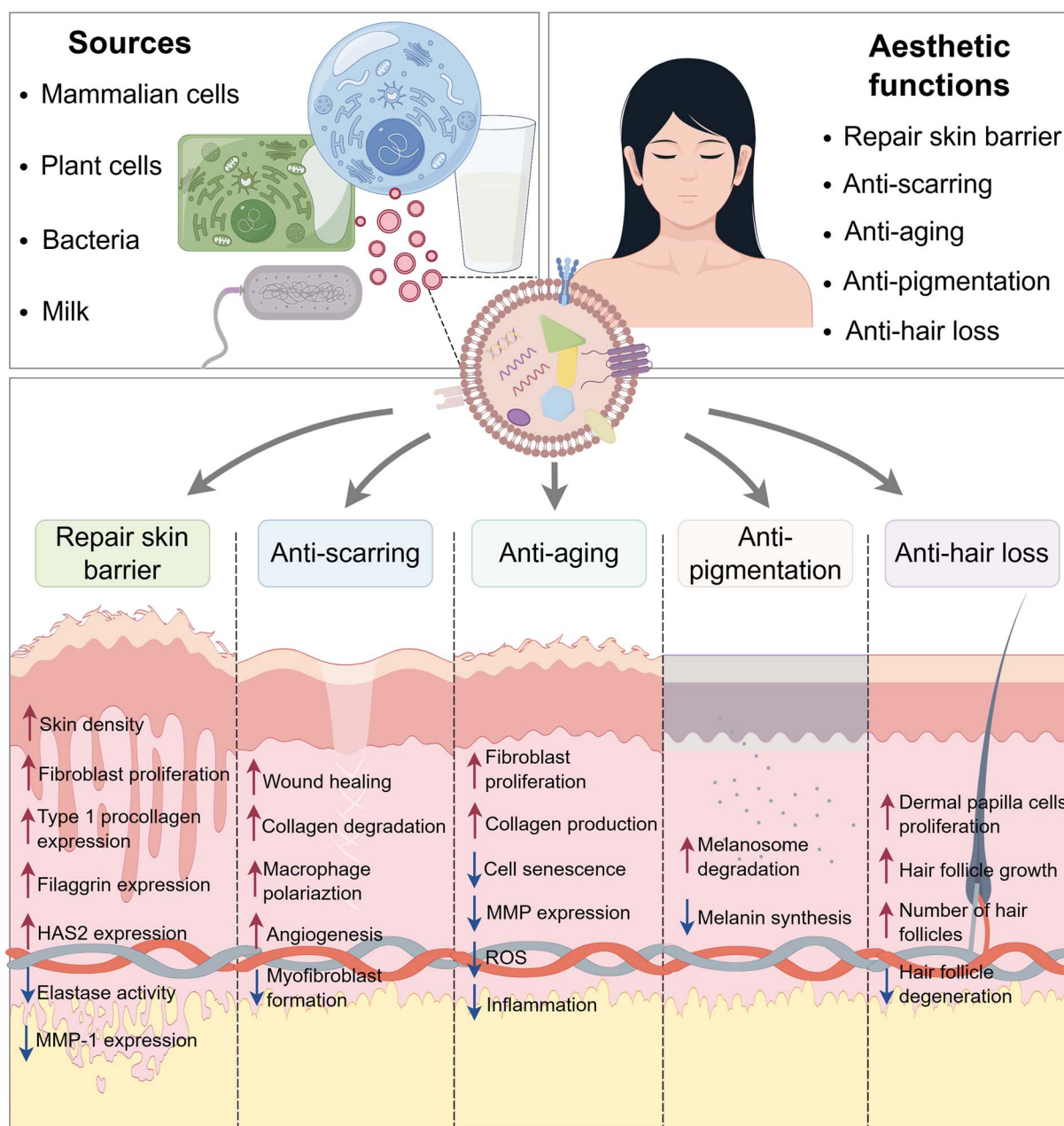


Figure 2. The functions of EVs from different sources in medical aesthetics (by Figdraw). EVs from different sources have similar effects in medical aesthetics, including repairing skin barrier, anti-scarring, anti-aging, anti-pigmentation, and anti-hair loss. HAS2: hyaluronan synthase 2; MMP-1: matrix metalloproteinases 1; ROS: reactive oxygen species; ↑: increase; ↓: decrease

Wnt/ β -catenin signaling pathway to accelerate hair regrowth [45]. In pigmented mice, fibroblast-derived EVs inhibited melanin production through various mechanisms, including the downregulation of melanin generation-related gene expression and melanosome transport-related protein expression to affect melanin transport [46]. Although fibroblast-derived EVs have advantages in medical aesthetics, numerous critical concerns warrant careful consideration, especially the similarity and uniqueness of fibroblasts from different organs (such as the skin, lungs, heart, and skeletal muscle).

EVs derived from other mature somatic cells, such as macrophages and endothelial cells, have also been used as therapeutic strategies for hair growth and wound healing. For example, Wnt proteins in EVs from macrophages activated the Wnt/ β -catenin signaling pathway, which induced

dermal papilla cell activation *in vitro* and increased the number of hair follicles and dermis thickness *in vivo* [47]. In addition, plasma endothelial cell-derived EVs (ED-EVs) have demonstrated the ability to impede the premature aging of skin fibroblasts and promote wound healing in diabetic mice by activating the PI3K/Akt/mTOR pathway and YAP nuclear translocation, making ED-EVs a new therapeutic tool for wound healing in diabetes [48] (Table 1).

Plant cell-derived EVs

Although EVs have been obtained from carrot cells as early as 1967, plant-derived EVs have not been as well studied as mammalian cell-derived EVs. Plant-derived EVs exhibit numerous similarities to mammalian cell-derived EVs and present significant prospects in the cosmeceutical domain.

For example, apple-derived EVs could play an anti-aging role by increasing collagen synthesis and downregulating metalloproteinase production via inhibiting the NF- κ B pathway [49]. *Aloe* has anti-inflammatory, anti-aging, and antibacterial properties and is one of the most widely used sources of antioxidants in the cosmetic and medical fields. Recent research has indicated that *Aloe*-derived EVs decrease proinflammatory gene expression, increase antioxidant gene expression, and promote cell proliferation and migration, revealing that they can promote skin rejuvenation and regeneration by activating antioxidant defense mechanisms and promoting wound healing [50, 51]. Similarly, Savci *et al.* [52] found that grapefruit-derived EVs reduced intracellular ROS production and facilitated angiogenesis. Plant-derived EVs can also modulate melanogenesis. EVs extracted from *Dendropanax morbifera* reduce melanin content and can be used as anti-melanin components in cosmeceutical preparations [53]. EVs from *Sargassum fusiforme* have been shown to possess anti-melanogenic properties by suppressing melanogenesis in the basal layer of the epidermis [54]. These results indicated that plant-derived EVs possess cytocompatibility, anti-aging, whitening, and pro-healing properties that are suitable for cosmeceutical applications. Nonetheless, there are geographical and seasonal differences among plants, and the stability and consistency of plant-derived EVs obtained from different regions and seasons must be systematically evaluated. Furthermore, many plant-derived EVs are currently obtained from fresh juice, and the preservation of the extracted plant-derived EVs is a limiting factor in determining their suitability for large-scale processing (Table 1).

Bacteria-derived EVs

The rapid reproduction of bacteria facilitates the acquisition and cultivation of substantial quantities of supernatants for the large-scale production of EVs, garnering the interest of researchers. The advantages of probiotic-derived EVs in cutaneous medical aesthetics have been reported several times. *Lactobacillus plantarum*-derived EVs induce monocyte-to-macrophage transition and macrophage polarization to the M2b state, thus improving hyperinflammatory skin conditions [55]. *L. plantarum*-derived EVs obtained from the skin of young women can improve skin aging [56]. In addition, *Lactobacillus delbrueckii*-derived EVs have been reported to promote wound healing and inhibit hypertrophic scar fibrosis via multiple pathways [57]. Based on the many health-promoting effects of probiotics, EVs carrying bacterial bioactive components are expected to be a new and safe therapeutic strategy for clinical applications. In addition to probiotics, Yin *et al.* [58] found that a particular bacterium, *Synechococcus elongatus* PCC7942, stimulates neovascularization, promotes burn healing, and reduces scar formation in mice, primarily by delivering functional EVs that promote IL-6 expression, rather than through its photosynthetic activity. Bacteria can also use EVs as a biological shuttle system to deliver virulence factors to host cells, thereby increasing bacterial pathogenicity. For example, α -hemolysin secreted by *Staphylococcus aureus* could be efficiently delivered by EVs to initiate the necrotic pathway to induce skin barrier impairment and atopic dermatitis (AD)-like skin inflammation [59]. In addition, skin fungus *Malassezia sympodialis*-derived EVs have also been reported to spread allergens, leading to skin barrier degradation or immune regulation, and play an important role

in the pathogenesis of AD [60, 61]. Because of their unique properties, EVs not only play a role in accelerating disease progression but, more importantly, also have the characteristic of inducing protection against both bacterial and fungal infections [62] and have significant potential in clinical diagnosis, treatment, and prevention. Although bacteria-derived EVs have not been extensively studied, these early studies have provided a valuable foundation for the development of safe and effective drugs and therapies (Table 1). Currently, research on EVs of fungal origin is still in its infancy, particularly in medical aesthetics. In addition to the relationship between EVs derived from *Malassezia* and skin diseases such as AD, researchers have recently discovered that EVs from a typical medicinal fungus, *Phellinus linteus*, can inhibit UV-induced skin photoaging by suppressing the expression of Mical2 in the skin through cross-species regulation, which confirms the research value of EVs of fungal origin in medical aesthetics [63].

Milk-derived EVs

Compared with EVs from cell lines or other biological fluids, milk-derived EVs have the advantages of superior safety and high yield, suggesting their potential utility as drug delivery vehicles and their development as a novel class of prebiotics [64, 65]. Bae *et al.* [66] found that miR-2478 derived from milk-derived EVs inhibits melanogenesis and melanogenesis-related gene expression by directly targeting Rap1a. In addition, a human clinical trial demonstrated the safety of milk-derived EVs for dermal applications and their role in retaining moisture and reducing wrinkles in humans [67]. Colostrum contains higher concentrations of immunological and bioactive components than mature milk, and its therapeutic efficacy has been substantiated in multiple clinical trials [68]. Therefore, some researchers have focused on the use of colostrum EVs in the skin, discovering their roles in repairing UV-exposed skin aging and damage [69], promoting hair regrowth [70], and facilitating wound healing [71]. More importantly, colostrum EVs showed significant resistance to freeze-drying, preserving their inherent characteristics and effectiveness in wound repair after freeze-drying [71]. The results regarding the exceptional stability and superior activity of milk-derived EVs suggest their significant potential for development and clinical translation (Table 1).

Preconditioning and engineering strategies for improving the efficacy of EVs in medical aesthetics

EVs' biological content and therapeutic effectiveness are strongly correlated with their extracellular environment and cellular state. Three-dimensional culture, cytokine preconditioning, and several other cell preconditioning methods improve the biological activity of EVs while preserving or increasing their yields. In addition, engineering strategies such as the encapsulation of bioactive molecules and membrane modification, which may modify or improve the biological function and targeting of EVs, have also received extensive attention from researchers. In the following section, we focus on the advantages and limitations of utilizing these two strategies (preconditioning and engineering) to enhance the clinical application of EVs in medical aesthetics (Figure 3).

Extracellular environment preconditioning

3D culture environment. Traditionally, cells are produced using static adherent culture (2D culture) in the presence

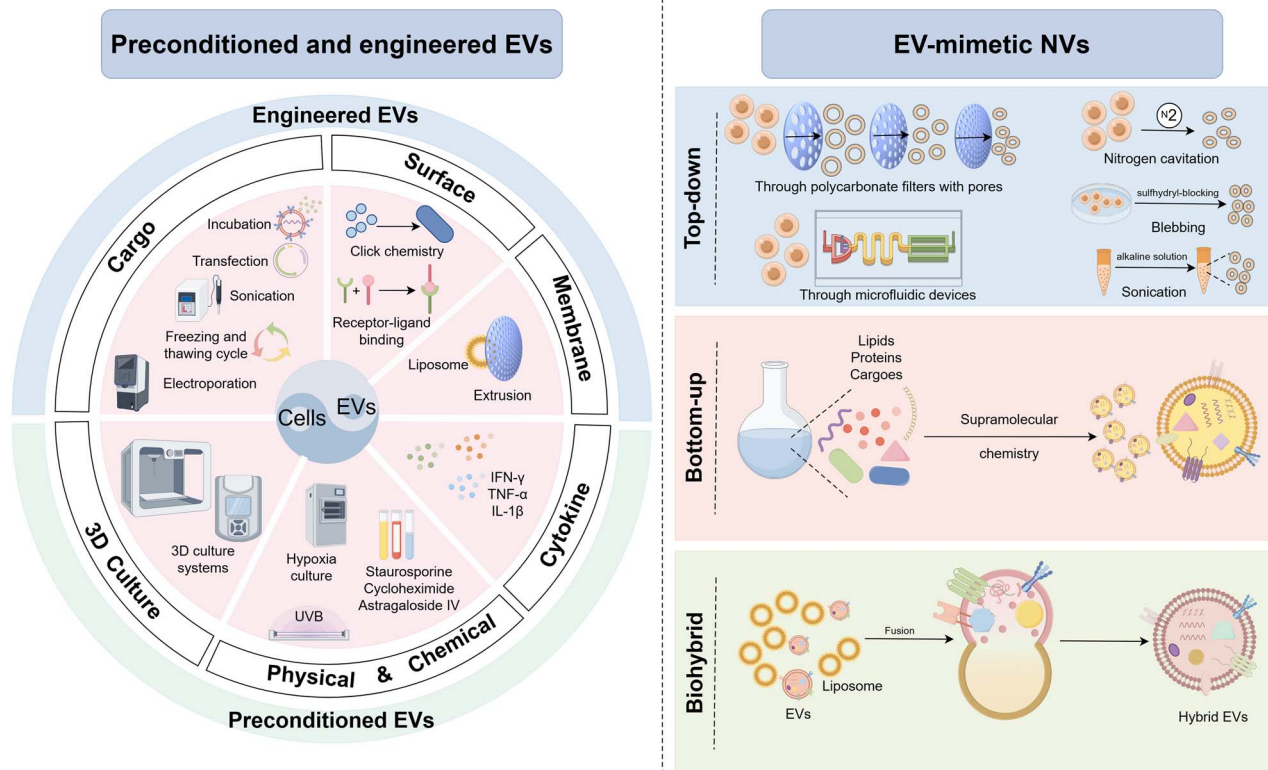


Figure 3. Schematic diagram of the main strategies for obtaining preconditioned and engineered EVs and EV mimetics (by Figdraw). The preconditioning strategies for EVs mainly include 3D culture, physical and chemical treatment, and cytokine treatment. The engineering strategies for EVs mainly include modifications to the membranes, surfaces, and cargoes. The methods for obtaining EV mimetics mainly include top-down, bottom-up, and biohybrid methods. EVs: extracellular vesicles; 3D: three-dimensional; UVB: ultraviolet B; IFN- γ : interferon- γ ; TNF- α : tumor necrosis factor- α ; IL-1 β : interleukin-1 β

of fetal bovine serum (FBS). However, cells cultured in 2D media may gradually lose their original characteristics, morphology, structure, and function, thereby affecting the yield and therapeutic properties of EVs and failing to meet the expected demands of future cell-free therapies. Compared with traditional 2D culture, 3D culture more realistically reproduces the interactions between cells and between cells and the extracellular matrix (ECM), more accurately simulates the actual microenvironment of cells in tissues, and the characteristics of cellular behaviors are closer to the state of existence in organisms [72–74]. Currently, 3D cell culture technologies fall into two main types: scaffold-free and scaffold-based. Scaffold-free 3D cell culture (mainly forced floating, hanging drop, magnetic levitation, and stirring spinner flask culture) refers to the self-assembly of cells to form nonadherent cell aggregates called spheroids [75]. Spheroids mimic solid tissues by secreting their own ECM and displaying different nutrient availabilities. Scaffold-based 3D cell culture (including natural, synthetic, and hybrid scaffolds) refers to the use of scaffolds to mimic ECM components, in which cells are grown in the presence of a support [76]. 3D culture enables large-scale production of stem cells and enhances their ability to secrete EVs [77–79]. For example, Yan *et al.* [80] found that a hollow fiber bioreactor-based 3D culture system resulted in a ~7.5-fold increase in the total yield of MSC-EVs compared with 2D culture. 3D culture also enhances the biological activity of EVs, and EVs from 3D culture sources demonstrate superior effects in delivering siRNA, promoting wound healing, and anti-inflammation, than EVs from 2D culture sources [77, 79,

81]. In addition, 3D cultures can alter the content of EVs and have demonstrated efficacy in enriching certain proteins and miRNAs in the secretome [82]. For example, 3D-cultured dermal papilla cells (DPCs)-derived EVs enriched in miR-218-5-p could promote hair follicle development by modulating the β -catenin signaling pathway [83]. However, the hanging-drop method and low-adhesion technology, which are widely used in 3D cell culture, continue to exhibit difficulties in clinical applications [84, 85]. Therefore, Wang *et al.* [86] developed novel 3D-DPC spheroids (tdDPCs) as a simple and innovative optimization strategy and demonstrated that tdDPC-EVs significantly promote angiogenesis and accelerate the speed and efficiency of wound healing by upregulating KLF4/VEGFA. Interestingly, in another study, the authors combined physical preconditioning with 3D culture and found that miR-22-5p was markedly upregulated and targeted the inhibition of GDF11 expression in EVs from UVB-irradiated HDFs. Targeted suppression of miR-22-5p in 3D-cultured HDF-EVs efficiently modulated HDFs to promote skin repair and improve skin photoaging [87]. Although 3D cell culture technology has demonstrated excellent performance in medical aesthetics research, its clinical translational application remains distant, given its high requirements, slow growth rate, high resource and maintenance costs, comparative difficulties in standardization, and lack of applicable detection technologies (Table 2).

Physical and chemical preconditioning. Cells are sensitive to the external environment, and physical signals, such as light, temperature, and oxygen partial pressure, as well as various

Table 2. The effects of preconditioned and engineered EVs in medical aesthetics

Strategies	Methods	Origins	Models	Functions	Ref.
3D culture environment	Three-dimensional culture	DPCs	Hair-depilated mice	Promote hair regeneration	[83]
3D culture environment	Three-dimensional culture	DPCs	Full-thickness skin wound models	Enhance wound-healing efficiency and speed	[86]
3D culture environment	Three-dimensional culture	HDFs	UVB-induced skin photoaging mice	Reduce inflammatory response and ECM degradation, promote collagen synthesis	[87]
Physical preconditioning	Hypoxia	ADSCs	UVB-induced skin damage mice	Reduce ROS, inflammatory cytokine expression, and excessive collagen degradation	[94]
Chemical preconditioning	Staurosporine	HUVECs	Full-thickness skin wound models	Promote wound healing	[95]
Chemical preconditioning	Cycloheximide	DFs	DNCB stimulated HaCaT cells	Restore skin barrier function	[96]
Cytokine preconditioning	IFN- γ	iMSCs	DNCB-induced AD mice	Suppress inflammation, facilitate skin barrier restoration	[104]
Endogenous cargo loading	Plasmids	Keratinocytes	Melanocytes	Inhibit melanin production	[108]
Endogenous cargo loading	Viral vectors	HucMSCs	Full-thickness dorsal skin wound models	Promote wound healing of diabetes	[109]
Endogenous cargo loading	Viral vectors	ADSCs	UVB-induced skin photoaging mice	Inhibit photoaging	[110]
Endogenous cargo loading	Plasmids	BMSCs	Excisional wound models	Promote angiogenesis and wound healing	[111]
Endogenous cargo loading	Viral vectors	hADSCs	UVA-irradiated HDFs	Ameliorate skin photoaging	[112]
Endogenous cargo loading	Viral vectors	ADSCs	Subcutaneous wounds of diabetic nude mice	Promote fibroblast proliferation and migration, increase the production of ECM components and various growth factors	[113]
Endogenous cargo loading	Plasmids	HDFs	Excisional wound mice	Induce faster wound closure	[114]
Endogenous cargo loading	Co-incubation	ADSCs	Full-thickness excisional skin wound models	Promote wound healing	[116]
Exogenous cargo loading	Co-incubation	ESCs	Full-thickness skin wound models	Promote angiogenesis and accelerate wound healing	[118]
Exogenous cargo loading	Co-extrusion	Milk	Full-thickness skin wound models	Promote angiogenesis and inhibit inflammation	[119]

3D: three-dimensional; DPCs: dermal papilla cells; HDFs: human dermal fibroblasts; UVB: ultraviolet B; ECM: extracellular matrix; ADSCs: adipose-derived mesenchymal stem cells; ROS: reactive oxygen species; HUVECs: human umbilical vein endothelial cells; DFs: dermal fibroblasts; DNCB: 1-chloro-2,4-dinitrobenzene; IFN- γ : interferon- γ ; iMSCs: induced pluripotent stem cell-derived mesenchymal stem cells; AD: atopic dermatitis; hucMSCs: human umbilical cord mesenchymal stem cells; BMSCs: bone marrow-derived mesenchymal stem cells; hADSCs: human adipose-derived stem cells; UVA: ultraviolet A; ESCs: epidermal stem cells

chemical signal stimuli, may significantly alter their phenotype, thus affecting the amount and content of secreted EVs, which have wide applications in tissue regeneration and immune modulation [88]. Moreover, these methods are inexpensive and do not require special instrumentation, making them suitable for clinical translation. Consistent with previous findings, UVB preconditioning affected the miRNA content of EVs and significantly regulated melanocyte pigmentation [89, 90]. Another study found that blue light irradiation increased the pro-angiogenic capacity of MSC-EVs *in vitro* and *in vivo*, effectively increasing the therapeutic potential of EVs [91]. Hypoxic preconditioning refers to the brief, non-lethal, hypoxic acclimatization of cells that makes them tolerant to the subsequent hypoxic environment [92]. Hypoxic preconditioning enhances the proliferative capacity and paracrine effects of MSCs, which might contribute to enhancing the therapeutic potential of MSC-EVs [93]. For example, EVs from hypoxic-pretreated ADSCs can alleviate UV-induced skin damage by delivering GLRX5 and inhibiting ferroptosis [94]. Pretreatment with other chemicals may significantly enhance the biological functions of EVs. Apoptotic EVs (ApoEVs), isolated after inducing apoptosis in human umbilical vein endothelial cells (HUVECs) using staurosporine (STS), promote wound healing by enhancing cell

proliferation, migration, and angiogenesis [95]. Similarly, EVs harvested from the conditioned medium of cycloheximide-treated fibroblasts showed increased levels of skin epidermal barrier proteins, indicating their potential to restore skin barrier function [96]. These results indicate that diverse physical and chemical stimuli may affect the quantity and content of EV secretion, enhancing their therapeutic potential. However, the optimal dosage and duration of pretreatment require further investigation because the specific mechanism has not been fully elucidated and there is heterogeneity of cells from different sources (Table 2).

Cytokine preconditioning. In addition to 3D culture, physical and chemical stimuli, cytokines including TNF- α [97, 98], IL-1 β [99, 100], and IFN- γ [101] are frequently used in preconditioning strategies. Cytokine stimulation has been shown to modulate the production of different potential therapeutic factors in MSC and improve their paracrine efficiency [102]. EVs from proinflammatory cytokine-preconditioned MSCs induced an anti-inflammatory phenotype in macrophages; however, MSC-EVs under standard culture conditions had no positive effects, confirming that cytokine pretreatment enhanced the biological function of MSCs, thereby increasing their therapeutic applicability [103]. Similarly, Kim *et al.*

[104] stimulated iPSC-derived MSC (iMSC) by IFN- γ and found that iMSC-EVs were enriched with a protein set associated with the regulation of interferon response and inflammatory pathways, effectively suppressed inflammation, and promoted skin barrier repair, thereby contributing to the amelioration of AD. In addition, cytokine stimulation could upregulate the expression of miRNAs associated with the inhibition of inflammation in EVs, thereby enhancing the anti-inflammatory effects of EVs [98, 105]. The biological functions and properties of preconditioned EVs have been studied and validated in medical aesthetics with favorable results. Further investigation of the unique processes and functional conditions of pretreatment is required to formulate an adaptive strategy that enhances the yield and biological functions of EVs (Table 2).

Engineering EVs for improving therapeutic potential

Various engineering strategies for EVs, including cargo loading and membrane modification, have been explored to compensate for their inherent limitations of natural EVs and enhance their therapeutic potential [106]. In medical aesthetics, we will focus on and present strategies for cargo loading. Cargo-loading strategies are widely used to load endogenous or exogenous molecules into the lumen of EVs and enhance their therapeutic capabilities. There are two types of cargo-loading strategies for EVs. Endogenous cargo loading is achieved using biological tools, such as plasmids and viral vectors, to genetically manipulate cells or by directly co-incubating cargo with donor cells to promote the expression of endogenous molecules, thereby encapsulating these endogenous molecules in EVs using the cell's biological molecular synthesis mechanism [107]. Gene transfection is a common strategy used for endogenous cargo loading. Specific cargo-loaded EVs can be obtained from the transfected cells via rapid isolation and purification. Increasing evidence has indicated that EVs mediate the therapeutic effects of parental cells in various disease models via miRNA delivery. Liu *et al.* [108] transfected miR-330-5p mimetics into keratinocytes using plasmids and successfully isolated EVs overexpressing miR-330-5p, which inhibited TYR expression in melanocytes and suppressed pigmentation. Furthermore, Wei *et al.* [109] obtained EVs overexpressing miR-17-5p by lentiviral transfection and demonstrated that they could restore the cellular functions of diabetic wound skin repair cells by inhibiting senescence and promoting angiogenesis. Similarly, Gao *et al.* [110] found that EVs overexpressing miR-1246 could effectively reverse ROS production, collagen degradation, wrinkle formation, and epidermal thickening in mice by regulating the MAPK/AP-1, NF- κ B, and TGF- β /Smad pathways. In addition to miRNAs, the loading of long-stranded non-coding RNAs [111], circRNAs [112], and endogenous proteins [113, 114] has increased the prospects of EVs in medical aesthetics. Although gene transfection is simple and feasible and does not compromise the membrane integrity of EVs, it has limitations such as low loading efficiency, possible induction of apoptosis in donor cells, and impairment of biological responses [115]. Further studies are required to determine the ideal donor cells and improve loading efficiency. Co-incubation is another common strategy for endogenous cargo loading [116] and is applicable to the loading of compounds, especially small-molecule drugs, which can be introduced into EVs by co-incubation with donor cells under different conditions. Currently, most relevant studies have been conducted to load

anticancer drugs, such as adriamycin, into EVs. Similarly, although this method is simple and does not significantly affect the structure and content of EVs, it has poor specificity, may be cytotoxic, and its loading efficiency is influenced by details such as drug properties and incubation protocols [117]. Although endogenous cargo loading strategies ensure the integrity of EVs, their low load efficiency cannot be ignored. In comparison, exogenous cargo-loading strategies are a more direct approach for certain nucleotides (miRNA and siRNA). Exogenous cargo loading is the direct loading of cargo into EVs via co-incubation, electroporation, sonication, extrusion, freezing/thawing cycles, and low-permeability dialysis. Wang *et al.* [118] co-incubated EVs with VH298 to successfully introduce exogenous VH298 into EVs (VH-EVs). VH-EVs could promote wound healing of diabetes through HIF-1 α mediated enhanced angiogenesis. Similarly, Fan *et al.* [119] prepared milk-derived EVs containing polydopamine by co-extrusion, which showed remarkable biocompatibility and pro-angiogenic and antioxidant capacities. Co-incubation is the simplest and most cost-effective method; however, hydrophobic EV membranes make co-incubation more commonly used for hydrophobic drugs, and the loading efficiency depends on the hydrophobicity of the drug and the chemical lipid composition of the EVs [120]. Electroporation is stable and efficient; however, high-voltage pulses result in large amounts of EV cleavage, only partial membrane repair is possible, and recovery is inefficient. Therefore, excessive amounts of EVs need to be invested. Ultrasonication and extrusion have higher loading efficiencies than co-incubation and electroporation but result in the aggregation of EVs, compromised membrane integrity, and changes in size [121, 122]. Therefore, different loading methods may be used for different molecules or the same type of molecule, and the most suitable loading method must be selected based on the requirements. The appropriate modification of EV membranes enhances targeted delivery. Chemical modification, genetic engineering, and membrane fusion are the three most widely used methods of EV membrane modification. Chemical modifications display a variety of natural and synthetic ligands or receptors on the membrane surface through covalent or non-covalent modifications but may alter the activity of membrane proteins [123]. Genetic engineering fuses the gene sequence of a guide protein or peptide with the gene sequence of a selected EV membrane protein, so that it can be efficiently displayed on the EV surface [106]. Membrane fusion involves extrusion to fuse EVs with other membrane structures [124, 125]. Its production process is controlled but has low homogeneity. Although current engineering strategies still have their own shortcomings and need to be improved further, the extensive exploration of engineered EVs with unique advantages will make them a potential next-generation nanomedicine therapeutic platform (Table 2).

Application of EV mimetics in medical aesthetics

Although engineering modifications of natural EVs can enhance their targeted delivery ability and therapeutic potential, the lack of standardized separation and purification protocols is insufficient to ensure adequate production. EV mimics are potential alternatives to EVs because of their high yield and similarity to EVs in terms of physical features, protein and lipid composition, and *in vitro* and *in vivo* behaviors [126]. Synthesis methods for EV mimics can be divided into three categories (Figure 3). (i) The top-down

Table 3. The effects of EV mimetics in medical aesthetics

Strategies	Methods	Origins	Comparison to natural EVs	Functions	Ref.
Top-down strategies	Filtration-based method	hucMSCs	Similar to EVs in terms of physical characteristics, protein and lipid composition	Promote cell proliferation, migration, and angiogenesis	[128]
Top-down strategies	Extrusion-based approaches	ADSCs	Favorable biomimetic properties similar to EVs	Promote hair regeneration	[130]
Top-down strategies	Extrusion-based approaches	Fibroblasts	Similar morphology, size distribution and protein markers	Induce hair growth	[131]
Top-down strategies	Extrusion-based approaches	Neural progenitor cells	Similar morphology and size distribution	Enhance hair follicle cells proliferation and promote telogen-to-anagen transition of hair follicles	[132]
Top-down strategies	Extrusion-based approaches	HaCaT cells	Improve productivity	Inhibit inflammation	[133]
Top-down strategies	Extrusion-based approaches	HEK293 cells	Easy to separate and high yield	Suppress local inflammation, mast cell infiltration, and fibrosis	[134]
Bottom-up strategies	NPs embedded with specific protein	Liposomes coated chitosan	Similar size and specific protein marker	Drug delivery	[136]

hucMSCs: human umbilical cord mesenchymal stem cells; EVs: extracellular vesicles; ADSCs: adipose-derived mesenchymal stem cells; NPs: nanoparticles

strategy generates EV mimics by inducing cell disruption using methods such as extrusion [127], filtration [128], and microfluidic devices [129] to enable cells to act as precursors for plasma membrane fragments. Among them, the extrusion method has been used for hair regrowth [130–132] and skin barrier improvement [133, 134]. For example, Park *et al.* [133] successfully prepared EV mimics (AEV_{P-MEL}) by continuously extruding PEGylated mannosylerythritol lipids (P-MEL) and keratinocytes. They found that AEV_{P-MEL} treatment significantly restored immune homeostasis in skin affected by PM-induced inflammation and enhanced intrinsic skin barrier function. (ii) The bottom-up strategy involves using individual molecules (lipids, proteins, and cargo) as building blocks and progressively assembling them into EV mimics with pure and well-defined compositions [135, 136]. (iii) The biohybrid strategy is another technique for developing EV mimics by merging synthetic NPs and natural vesicles. The preparation methods include extrusion [137], freeze–thaw [138], and warm incubation [139]. For example, Lin *et al.* [139] briefly incubated EVs with liposomes expressing CRISPR/Cas9 for 12 hours at 37°C to create EV and liposome hybrid NPs containing expression vectors encapsulating the CRISPR/Cas9 system. EV mimics developed using nanobiotechnology hold great promise for advanced drug delivery, because they combine the advantages of natural and synthetic NPs (Table 3).

Advanced delivery strategy for EVs

The modes of EV administration include smear, oral, subcutaneous, intraperitoneal, and tail vein injections. Different modes of administration not only influence the distribution of EVs in the body but also directly correlate with their therapeutic efficacy and safety. Because EVs injected intraperitoneally are mainly enriched in the gastrointestinal tract and lymph nodes [140], smear, oral, subcutaneous, and intravenous injections are relatively more common modes of administration in medical aesthetics. Smear administration is simple, convenient, and suitable for various applications in medical aesthetics. However, research indicates that, although

topically administered EVs can be absorbed by the skin, the vast majority of EVs are confined to the stratum corneum only, exhibiting no detectable migration in the granular layer over 24 h [141, 142]. Although oral administration is the preferred route of administration, oral EVs may be ineffective for skin improvement owing to the strongly acidic conditions in the stomach and degradation conditions in the gut. Subcutaneous injections can penetrate the epidermis to deliver EVs and are indicated for skin rejuvenation and scar-free wound healing. However, the uneven delivery of EVs can lead to their accumulation in specific areas of the dermis and subcutaneous tissues [143]. In addition, the local injection of EVs around the wound can disrupt the wound and inevitably disrupt the wound healing process. More importantly, the rapid clearance of EVs induced by local injections requires repeated administration, which increases patient suffering. Conversely, it has been shown the intravenous administration of EVs has a relatively long half-life and results in faster wound closure than subcutaneous injections [144]. However, the higher dose and lower targeting efficacy of the intravenous injection of EVs may lead to unnecessary waste and potential organ toxicity. Therefore, exploring emerging dermal delivery strategies such as microneedles (MNs), hydrogels, and scaffolds is essential for reducing patient pain and maximizing the efficacy of locally applied EVs. Currently, MNs are primarily used for skin rejuvenation and hair regrowth, whereas hydrogels are primarily used to promote scar-free wound healing. Below, we highlight the applications of these advanced delivery strategies in medical aesthetics (Figure 4).

Physical penetration

MNs, an innovative drug delivery system comprising a series of microscale needles, have been extensively utilized to breach the stratum corneum barrier of the skin such that drugs can reach the dermis directly because of their safety, painlessness, localized controlled drug delivery, and diverse cargo-loading capabilities. In addition, although MNs were originally designed for transdermal drug administration, other novel applications have recently been developed, such as

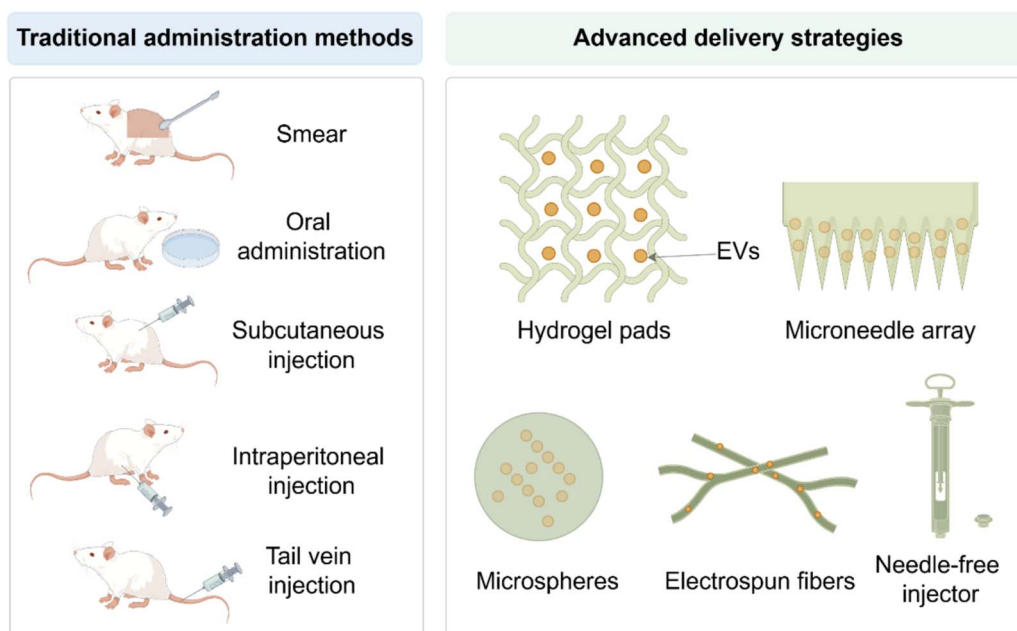


Figure 4. The administration strategies of EVs (by Figdraw). The traditional administration methods of EVs include smear, oral, subcutaneous, intraperitoneal, and tail vein injection. At present, the advanced delivery strategies of EVs have been explored, including microneedles, hydrogels, microspheres, etc. EVs: extracellular vesicles

promoting hair regrowth [145, 146], wound healing [147], and anti-skin aging [15, 143]. For example, Shi *et al.* [145] prepared a detachable MN patch composed of chitosan lactate (CL) and EVs, which not only activates the Wnt signaling pathway for hair regrowth by sustained release of EVs but also avoids potential infections due to the inherent antimicrobial properties of CL. Similarly, You *et al.* [143] delivered EVs encapsulating collagen mRNA via hyaluronic acid MN patches so that EVs could be more efficiently distributed in the dermis, resulting in long-lasting collagen implantation and improved treatment of wrinkles in photoaging skin. In another study, the authors prepared a novel MN, marine sponge *Haliclona* sp. spicules (SHSs), which significantly increased the absorption of EVs into the skin, reduced micro-wrinkles, and increased collagen gene expression [148]. Intriguingly, Hu *et al.* [82] used a needleless jet injector that utilizes a high-pressure jet of fluid medication to move EVs through the epidermis and deeper into the dermis, which not only reduces pain and provides greater penetration and absorption but also reduces wrinkles and increases collagen synthesis in EV-treated skin. These results suggest that EVs can be useful for medical aesthetics via various delivery modes. However, in terms of medical aesthetics, we suggest transdermal administration. In contrast to direct application, transdermal delivery enables EVs to penetrate the epidermis and reach the dermis, thereby augmenting their therapeutic efficacy. Compared with injected EVs, MN, SHSs, and needleless jet injectors are more convenient, have fewer side effects, are safer, and have significant promise for future use in medical aesthetics (Table 4).

Hydrogel/biomaterials-based dressings

In addition to penetration efficiency, sustained delivery of EVs is essential for optimizing their therapeutic efficacy in the skin. Hydrogels, which are formed when hydrophilic polymer chains undergo cross-linking in water, are highly porous, biocompatible, and biodegradable, making them suitable for

drug delivery. Hydrogels, including chitosan (CS) [149], fibrin, gelatin [150], and alginate (Alg) [151], have been explored to facilitate the sustained release of EVs. Zhao *et al.* [152] found that a CS hydrogel not only prolonged EV release and significantly increased body retention but also showed rejuvenation of aged skin tissue after treatment with subcutaneous injections. Fibrin gel (FG) is also an ideal carrier for EVs. A previous study showed that FG enhances the retention of EVs at the wound site and might facilitate scarless wound healing by accelerating the rate of wound closure and promoting collagen formation and maturation [44]. Similarly, gelatin methacryloyl (GelMA) hydrogels are widely used for wound protection because of their structural similarity to the ECM and customizable physical properties [153]. Alongside skin aging and scar-free wound healing, hair regeneration has been a topic that has garnered the interest of scholars. Researchers integrated EVs with a partially oxidized sodium alginate (OSA) hydrogel to create an injectable microgel. OSA hydrogel enhanced the retention and stability of the EVs, greatly promoting the proliferation of hair matrix cells [154]. Furthermore, HydroMatrix, a peptide nanofiber 3D scaffold that self-assembles into 3D hydrogels in response to variations in temperature and ionic strength, has been used as a vehicle for preserving EVs at their designated locations [155]. Duan *et al.* [156] found that HydroMatrix loaded with epidermal stem cell-derived EVs (EPSC-EVs) accelerated wound healing and reduced scar formation. The combination of EVs with hydrogels or biomaterial-based scaffolds promotes scar-free wound healing, anti-aging effects, and hair regeneration, demonstrating their great potential in medical aesthetics. However, single-layer hydrogel pads cannot achieve the multistep release of EVs for scar-free wound healing. Therefore, Shen *et al.* [17] developed a hydrogel pad with a heterogeneous structure in which the upper and lower layers were loaded with two types of EVs, which contributed to the sequential release of EVs, promoted wound healing,

Table 4. Advanced delivery strategy for EVs

Strategies	Materials	Origins	Administration	Functions	Ref.
MN patch	Chitosan lactate, hyaluronic acid, and polyvinyl alcohol	ADSCs	Topical application	Prevent infection and promote hair regeneration	[145]
MN patch	Keratin, UK5099-loaded PLGA nanoparticles, and HA	BMSCs	Topical application	Promote pigmentation and hair regrowth	[146]
MN patch	HA	HDFs	Topical application	Reduce skin wrinkles caused by photoaging	[143]
MN roller	Undefined	ADSCs	Topical application	Promote collagen expression and reduce wrinkle formation	[15]
SHSs	Explants of marine sponge <i>Haliclona</i> sp.	HucMSCs	Topical application	Promote skin absorption of EVs and reduce micro wrinkles and epidermal thickness	[148]
Needle-free injector	Undefined	HDFs	Needle-free injection	Promote the expression of collagen protein	[82]
CS-glycerol based hydrogel	CS, acetic acid, and glycerol	hEnSCs	Topical application	Promote wound healing	[149]
CS hydrogel	CS, acetic acid, and β -GP	hP-MSCs	Subcutaneous injection	Skin rejuvenation	[152]
FG	Undefined	Fibroblasts	Topical application	Promote scarless wound healing	[44]
GelMA hydrogel	Gelatin and methacrylic anhydride	HUVECs	Topical application	Wound repair and regeneration	[150]
GelMA hydrogel	Porcine skin-derived gelatin and MA	UCMSCs	Topical application	Promote wound healing of diabetes	[153]
Alg hydrogel	Sodium-Alg and CaCl_2	ADSCs	Topical application	Promote wound healing	[151]
OSA hydrogel	Sodium alginate and sodium periodate	DPCs	Subcutaneous injection	Promote hair regeneration	[154]
HydroMatrix	Undefined	PVCs	Local injection	Promote wound healing	[155]
HydroMatrix	Undefined	EPSCs	Local injection	Promote scarless wound healing	[156]

MN: microneedle; ADSCs: adipose-derived mesenchymal stem cells; HA: hyaluronic acid; BMSCs: bone marrow-derived mesenchymal stem cells; HDFs: human dermal fibroblasts; SHSs: marine sponge *Haliclona* sp. Spicules; hucMSCs: human umbilical cord mesenchymal stem cells; EVs: extracellular vesicles; CS: chitosan; hEnSCs: human endometrial stem cells; β -GP: β -glycerophosphate; hP-MSCs: human placental mesenchymal stem cells; FG: fibrin gel; GelMA: gelatin methacryloyl; HUVECs: human umbilical vein endothelial cells; MA: methacrylic anhydride; UCMSCs: umbilical cord mesenchymal stem cells; Alg: alginate; OSA: oxidized sodium alginate; DPCs: dermal papilla cells; PVCs: perivascular cells; EPSCs: epidermal stem cells

and inhibited scar formation. More clinical studies are needed to demonstrate the safety and efficacy of this combination in dermatological aesthetic applications to achieve perfect skin repair (Table 4).

Current therapeutic and commercial landscape of EVs

Despite the growing quantity of registered clinical trials evaluating the role of EVs in tissue repair and regeneration. However, to date, there are only a limited number of studies based on the registration of clinical trials related to EVs in skin improvement ([ClinicalTrials.gov](https://clinicaltrials.gov): NCT05969717, NCT02565264, NCT05078385, NCT04134676, NCT05243368, NCT05475418, NCT05813379, NCT06221787, NCT06677931, NCT06539273, and NCT06482541) (Table 5). An ongoing clinical trial is evaluating the safety, tolerability, and preliminary efficacy of iPSC-derived EVs in the treatment of AD by applying varying doses of iPSC-derived EVs to a 2–4 cm² area of the affected skin in patients with AD twice a day for 14 consecutive days and using saline as a control. Other clinical trials have included the application of EVs or a broader range of stem cell-conditioned media for the treatment of ulcerated wounds, burn wounds, skin rejuvenation, melasma, and hair loss. The recruitment of NCT02565264 for these trials remains unknown. This trial applied autologous plasma-derived EVs to subjects' ulcers for 28 days and assessed the effect of autologous plasma-derived EVs on wound healing in patients with recalcitrant skin ulcers based on wound length, width, and depth. The

disadvantage is that the separation of EVs by filters and the lack of a purification step for the EVs result in EVs that may be highly heterogeneous, rendering any positive results observed in this experiment inconclusively claimed to be EV-mediated. In addition, in a clinical registration trial related to skin aging, which started in February 2022 and was expected to be completed in August 2023, MSC-EVs were injected at each standardized injection point in a superficial manner. The injection points were along the inferior border of the cheek, mid-cheek, and temple, followed by 10–15 minutes of icing. However, the trial is still recruiting and may be related to its failure to provide information about the dose administered or the purity of the EV formulation, as well as the current skepticism about the safety of EVs. Of these clinical registry trials, only three evaluated the safety of EVs, including safety in the treatment of AD, burn wounds, and melasma. Therefore, there has been insufficient validation of the safety and potential side effects of EVs in clinical applications. Owing to the highly heterogeneous nature of EVs, parental cell source and status, different isolation techniques, and even different parameters can lead to huge differences in the characteristics and contents of EVs, complicating reproducibility and comparative studies between different batches of EVs and causing great difficulties in validating their safety. Tumor cell-derived EVs can promote tumor development, invasion, and metastasis by loading tumor-associated signaling molecules. Therefore, there is a need to ensure the safety of EV sources in clinical applications. Second, although previous studies have shown that some EV sources (e.g. MSCs) are hypoinmunogenic and, therefore,

EVs derived from them are not expected to be immunogenic, recent studies have shown that EVs secreted by virally infected cells or immune cells can deliver cell-specific molecules over long distances to trigger an immune response [157]. In particular, EVs of xenogeneic or allogeneic may pose a risk of immune rejection. It is important to consider the dose-related toxicity of EVs. There is no standardized dosage for the administration of EVs, and the dosage of EVs is not always better at higher concentrations. When saturation is reached, increasing the concentration will not lead to further positive effects and may even cause toxic side effects. Some clinical registration trials do not provide information on the dosage and purity of the administered EV preparations, which is undesirable. In addition, nonspecific uptake of EVs by off-target cells may have uncertain and potentially negative consequences, even though EVs cannot differentiate or form tumors per se. Although MSC-EVs are currently the most widely used type of EVs, their procoagulant activity and risk of thrombosis, as well as the lack of assessment of the safety of long-term multiple uses in humans, may limit their clinical safety. The potential side effects of EVs include aberrant activation of the immune system, off-target effects in targeted delivery, and risk of thrombosis, making a multidimensional risk assessment framework necessary for the clinical translation of EVs. The safety, efficacy, and dosage of EVs must be determined in sufficiently robust human clinical trials.

The results of *in vitro* and preclinical investigations have highlighted the huge commercial potential of EVs as therapeutics. Currently, more than 30 international companies have EV products in the research and development phases. These EV products belong to three main categories: cell-derived EVs, engineered EVs (in which the therapeutic molecule of interest is loaded into the parental cell using an endogenous loading strategy), and EV mimics (natural EVs combined with synthetic lipid NPs) [158]. For example, ExoCoBio (Korea) produces lyophilized human ADSC-EVs under serum-free conditions and claims that they can positively affect the epidermal barrier by reducing local inflammatory cytokine levels and inducing the *de novo* production of ceramides following injection in an AD model [159]. However, the effects of lyophilization on the long-term integrity and efficacy of EV formulations require further investigation. Another example is ExoWound (Exogenous Therapeutics), which accelerates wound closure in diabetic mice by combining hydrogels with EV from human umbilical cord blood monocytes [160, 161]. In addition, Infusio (Germany) developed anti-aging therapies based on the discovery in mouse models that the infusion of EVs from the plasma of young mice into older mice extends their lifespan [162]. However, no injectable EV therapies have been approved by the Food and Drug Administration (FDA) at this time. The use of EVs in dermatology is limited to topical administration (sometimes with microneedling to increase penetration), following the current FDA regulations. Therefore, the safety of EV therapies needs to be further evaluated, and caution should be exercised when using EVs as an adjunctive therapy. Moisturizing creams and serums-containing stem cell-derived EVs are a growing trend in beauty products. However, these products often lack the isolation and characterization of the EVs used. EVs have great potential in medical aesthetics, but their extensive and homogenous use in clinical practice requires that their limitations be considered and addressed.

The medical aesthetics field is experiencing a transition from traditional fine chemicals and plant extracts to the development of biotechnology and regenerative medicine, especially with emerging ingredients such as EVs, which bring huge market potential to the medical aesthetics field while simultaneously posing a challenge to the regulation of the global cosmetics industry because of the different regulatory frameworks in different national markets. The regulatory pathways for different medicines (or therapies) usually depend on their regulatory classification; however, the wide range of sources and types of EVs makes their regulatory classification in different countries ambiguous and difficult to categorize and study uniformly. The diversity of production technologies and the difficulty in ensuring the purity and consistency of EVs make their standardization challenging, leading to a fragmented regulatory landscape. Different countries have different standards for evaluating the safety and validating the efficacy of EVs, adding to the complexity of regulations. In addition, different countries have different ethical reviews and legal frameworks for EVs, which affect their market access and commercialization. The regulatory standards for EVs vary from region to region. In the United States and Europe, it is based on whether the EV content can affect physiological functions. Japan, South Korea, and Taiwan differ in how EVs are obtained [163]. The United States encourages scientific and technological development and attaches importance to the cosmetics market. According to the FDA regulations, the management of cosmetics in the United States differs from that of drugs and medical devices. Cosmetics do not require FDA pre-approval, and manufacturers are responsible for ensuring the safety of their products and the authenticity of labeling information. Novel cosmetic ingredients such as EVs of various origins must be filed with the International Cosmetic Ingredient Dictionary (INCI) manual of the Personal Care Products Council. In INCI's official database, the newly registered EV sources were mainly plants and MSCs. The United States is currently the country with the largest number of EV cosmetic products on the market, with a variety of product types, including frozen liquids, freeze-dried powders, and essence, and there are already stem cell EV companies in the market. The European Union has strict ethical regulations on cosmetic ingredients, with an emphasis on risk assessment. The European Union regulations explicitly prohibit the use of human tissue- and animal-derived ingredients in cosmetics, prohibit animal testing of cosmetics, and require all cosmetics to undergo a safety assessment prior to marketing, as well as ingredient information must be made public. In Japan, cosmetics do not have drug-like therapeutic or preventive effects; however, Japanese regulations recognize cosmeceutical products. Cell derivatives can be used in cosmetics, but the use of such ingredients is subject to strict safety, labeling regulations, and ethical standards, and may be subject to additional scrutiny similar to that of pharmaceuticals. Korea encourages the development of the cosmetic and medical aesthetic industries, and human cells and tissue cultures that meet safety standards within the regulations can be used as cosmetic ingredients. According to Taiwan's latest guidelines, EVs from human cells, tissues, or products of human origin can be used in cosmetics in compliance with regulations and under compliant routes. China encourages and supports innovative research on new cosmetic ingredients but attaches importance to the quality and safety of cosmetics. Considering that the safety of EVs has not been sufficiently verified, they

Table 5. Registration of clinical trials related to skin improvement based on EVs

Identifier	Condition	Participants	Inclusion criteria	Intervention/treatment	Status	Location
NCT05969717	Atopic dermatitis	20	18–70 years, mixed sex, BMI between 18 and 35 kg/m ² , atopic dermatitis, IGA score of 2 to 3, a history of subacute or chronic atopic dermatitis symptoms for at least 6 months, BSA of atopic dermatitis involvement of ≤5% at screening and baseline	iPSC-derived EVs	Early phase 1	China
NCT02565264	Intractable cutaneous wound ulcer	5	No age criteria, mixed sex, intractable cutaneous ulcers	Autologous plasma-derived EVs	Early phase 1	Japan
NCT05078385	Second degree burn wounds	10	>18 years, mixed sex, deep second degree thermal burn wounds, index burn injury within the prior 72 hours, maximum index wound size limited to single or multiple wounds cumulatively ≤600 cm ² , minimum wound size: 30 cm ²	BMSC-derived EVs	Phase 1	United States
NCT04134676	Chronic ulcer wounds	38	18–80 years, mixed sex, chronic skin ulcers (<10 × 15 cm) unresponsive to therapy after 1 month	Stem cell-conditioned medium	Phase 1	Indonesia
NCT05243368	Cutaneous ulcers in diabetics	30	50–80 years, mixed sex, diagnosis of diabetes mellitus for more than 1 year, HbA1c < 9%, category 5 in the Rutherford-Becker classification	MSC-derived EVs	Not applicable	Spain
NCT05475418	Wounds and injuries	5	18–60 years, mixed sex, the time between operation and amputation must be more than 30 days, the wound size after debridement was ≥1 and ≤20 cm ² , ankle-brachial index ≥0.7, subjects completed 2 weeks of initiation and wound reduction was 30%	Adipose tissue derived EVs	Not applicable	China
NCT05813379	Anti-aging	20	35–65 years, females, patients of all undergoing facial rejuvenation	MSC-derived EVs	Phase 1 Phase 2	Iran, Islamic Republic of
NCT06221787	Melasma	80	Adult, mixed sex, patients diagnosed with chloasma who meet the clinical diagnostic standards and efficacy standards (revised version) of chloasma and have skin lesions on the face	HucMSC-derived EVs	Not applicable	China
NCT06677931	Melasma	60	18–60 years, mixed sex, diagnosed with melasma according to clinical diagnostic criteria and efficacy standards (revised edition), with facial skin lesions	UCMSC- derived EVs	Not applicable	China
NCT06539273	AGA	30	22–65 years, males, with hair type III-VI according to the Norwood-Hamilton scale	MSC-derived EVs	Phase 3	Turkey
NCT06482541	AGA	100	Adult, mixed sex, participant is experiencing hair loss and is diagnosed with AGA	MSC-derived EVs	Phase 1	United States

BMI: body mass index; IGA: investigator overall assessment; BSA: body surface area; iPSC: induced pluripotent stem cell; EVs: extracellular vesicles; BMSC: bone marrow-derived mesenchymal stem cell; MSC: mesenchymal stem cell; hucMSC: human umbilical cord mesenchymal stem cell; UCMSCs: umbilical cord mesenchymal stem cells; AGA: androgenetic alopecia

cannot be added to cosmetics as ingredients. When using EVs as new ingredients in cosmetics, it is important to ensure that informed consent is obtained from the donor, that his or her privacy is protected, and that the donor's health is adequately exposed. Because obtaining ingredients from the human body can raise fairness and traceability issues, traceability information systems are required. Products containing human-derived components have stringent regulatory requirements. The novelty and wide range of uses of EVs mean that clear regulatory categorization and jurisdictional boundaries are necessary. There is an urgent need to strengthen international cooperation to promote the development of harmonized regulatory standards for the industry.

Perspectives and challenges

In recent years, numerous preclinical studies have applied EVs for skin barrier improvement, wound healing, scar removal, anti-aging, anti-pigmentation, and anti-hair loss, with promising therapeutic results, and clinical trials related to EVs for skin improvement are increasing. However, EVs still face significant challenges for large-scale applications. First, the yield of EVs is very low. Conventional 2D culture is inefficient, and cultured cells secrete very low amounts of EVs. Therefore, researchers are exploring the use of techniques such as 3D culture, physical and chemical stimulation, and genetic manipulation (overexpression of core proteins that regulate EV biogenesis) [164] to scale up the production of EVs. In addition, EV mimics have emerged as promising alternatives to EVs, owing to their high yield, low production time, low cost, and similar biological properties to EVs.

Second, different methods of separation, purification, and preservation have significant effects on the yield, purity, and active ingredients of EVs. Some existing methods for separating EVs, including ultracentrifugation, ultrafiltration, and precipitation, may lead to the co-separation of lipoproteins or other contaminants with physicochemical properties similar to those of EVs. In addition, these methods may damage the structural and biological integrity of EVs due to external forces. To improve separation efficiency and purity, new EV enrichment techniques such as microfluidics, non-contact sorting, and immunoaffinity enrichment have emerged. In the future, it may be better to select an appropriate separation method or apply a combination of different separation methods according to the requirements. Moreover, the storage of EVs limits their large-scale application in clinical settings. EVs were more stable at -80°C . However, this increases the storage and transport risks. Lyophilization enhances the stability of EVs for storage at room temperature or 4°C , but its effect on EV membranes and efficacy may require further confirmation.

Third, EVs are highly heterogeneous and standardized procedures for their utilization are lacking. The function of EVs is greatly affected by variations in parental cell sources and culture conditions. Therefore, it is imperative to establish quality control standards to identify the parental cell status of EVs. In addition, there is a need to investigate the pharmacokinetics, pharmacodynamics, and biodistribution of various sources of EVs *in vivo*, as well as to determine the appropriate dosage and administration method for each disease and target tissue. Different adaptive concentrations of EVs exist at the cellular, organ, and *in vivo* levels. The dosage of EVs is not always better at higher concentrations, which may be related to the fact that there are only a limited

number of receptors on effector cells and that once saturation occurs, increasing the concentration yields no additional benefits. As previously summarized, EVs are available in a variety of delivery modes; however, in medical aesthetics, we recommend transdermal delivery. MNs enhance the efficiency of transdermal drug delivery. However, microneedling is a minimally invasive procedure that cannot be used frequently. Therefore, further development of transdermal drug delivery methods with minimal skin damage (e.g. needle-free injectors) is required. A comprehensive understanding of EV differences and the establishment of standardized procedures would facilitate the selection of the most appropriate type of EVs, dose, and mode of administration in different clinical situations.

Finally, there is insufficient validation and regulation of safety in the application of EVs. Although studies have shown that EVs can be safely applied topically and do not cause inflammation or skin sensitization, there is a lack of monitoring of the adverse effects of long-term application of EVs. In addition, contamination and residues from FBS in mammalian cell-derived EVs are unavoidable, and the development of alternative serum-free media is necessary. As previously summarized, EVs have potential side effects, including aberrant activation of the immune system, off-target effects in targeted delivery, and the risk of thrombosis, which may also be the main reason for the low acceptance of EVs by patients, making it difficult to recruit subjects for various clinical trials. Therefore, a thorough study of the bioactive components of EVs is required to rule out potential safety risks prior to their clinical application. In addition, it is important to emphasize that although countries differ in their regulations of EVs, they are all conservative regarding EVs of human origin. Plant-derived EVs have several distinct advantages owing to their natural origin and longstanding dietary habits. First, EVs from most edible plants have been shown to have no detectable toxicity or immunogenicity and are more readily accepted by patients. Second, plants are free of human disease-causing pathogens and may be safer. Finally, plant-derived EVs are less expensive and do not have ethical concerns associated with human-derived components. Plant-derived EVs may have greater commercial prospects in medical aesthetics.

Conclusions

EVs can be used to improve skin barrier function, promote scar-free wound healing, anti-aging, anti-hyperpigmentation, and anti-hair loss. However, current research focuses on small EVs released from stem cells. Therefore, further exploration of other sources (plants and microorganisms), as well as large EVs (MVs and apoptotic bodies) may be one of the future directions. In addition, the development of various emerging isolation and purification techniques, characterization techniques, and engineering modification strategies to improve the yield, purity, and efficacy of EVs is an important research topic. Meanwhile, the combination of large-scale preparation technology and long-term effective preservation technology is also a worthy research direction. Many studies have highlighted the effectiveness of adjunctive procedures (MNs, needle-free injectors, and hydrogels) to enhance EV penetration or modulate their release kinetics. However, studies on the *in vivo* effects of topical and injected EV have been limited to mouse models. The human skin is relatively thick, which may negatively affect EV penetration and therapeutic efficacy in clinical trials, further increasing the need

to combine topical EV delivery with adjuvant treatments that enhance skin absorption. In summary, EVs are an emerging powerhouse in dermatological aesthetics with great prospects for development. In the future, if the shortcomings of high heterogeneity, low yield, and biosafety of EVs can be overcome, they can be converted into cosmeceuticals to promote their use in the facial aesthetic industry.

Acknowledgements

All the figures in the manuscript were drawn in Figdraw (<https://www.figdraw.com/>).

Authors' contributions

Zixuan Sun (Conceptualization [equal], Funding acquisition [equal], Project administration [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal]), Jiali Li (Conceptualization [equal], Funding acquisition [equal], Writing—original draft [lead], Writing—review & editing [lead]), Zhijing Wu (Writing—review & editing [equal]), Jiaxin Zhang (Writing—review & editing [equal]), Tangrong Wang (Writing—review & editing [equal]), Yuzhou Zheng (Writing—review & editing [equal]), Xuezhong Xu (Writing—review & editing [equal]), Wei Ding (Writing—review & editing [equal]), Hui Qian (Funding acquisition [equal], Project administration [equal], Supervision [equal]), Yulin Tan (Funding acquisition [equal], Project administration [equal], Supervision [equal]).

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors reviewed and approved the final manuscript.

Conflict of interest

The authors confirm that there are no conflicts of interest regarding the publication of this article.

Funding

This work was supported by the National Natural Science Foundation of China [82003379], the Postgraduate Research & Practice Innovation Program of Jiangsu Province [KYCX23_3767], Zhenjiang Key Laboratory of High Technology Research on Exosomes Foundation and Transformation Application [SS2018003], The Priority Academic Program Development of Jiangsu Higher Education Institutions [Phase IV, Clinical Medicine], Open Project of Jiangsu Provincial Key Laboratory of Laboratory Medicine [JSKLM-Z-2024-002], and Changzhou High-Level Medical Talents Training Project [2022CZBJ105].

Data availability

Not applicable.

References

- Martínez-González MC, Martínez-González RA, Guerra-Tapia A. Key communication skills in cosmetic dermatology: a 3-pillar model. *Actas Dermosifiliogr (Engl Ed)* 2019;110:794–9. <https://doi.org/10.1016/j.ad.2019.01.010>.
- Xiong M, Zhang Q, Hu W, Zhao C, Lv W, Yi Y. *et al.* The novel mechanisms and applications of exosomes in dermatology and cutaneous medical aesthetics. *Pharmacol Res* 2021;166:105490. <https://doi.org/10.1016/j.phrs.2021.105490>.
- Odrobińska J, Mielańczyk Ł, Neugebauer D. 4-n-Butylresorcinol-based linear and graft Polymethacrylates for Arbutin and vitamins delivery by micellar systems. *Polymers (Basel)* 2020;12:330. <https://doi.org/10.3390/polym12020330>.
- Gazitaeva ZI, Drobintseva AO, Prokopov AY, Sidorina AN, Leonteva DO, Kvetnoy IM. Signaling molecules of human skin cells as the targets for injection cosmetology. *Clin Cosmet Investig Dermatol* 2021;14:1473–80. <https://doi.org/10.2147/ccid.S321104>.
- Shekhter AB, Fayzullin AL, Vukolova MN, Rudenko TG, Osipchewa VD, Litvitsky PF. Medical applications of collagen and collagen-based materials. *Curr Med Chem* 2019;26:506–16. <https://doi.org/10.2174/0929867325666171205170339>.
- Naik PP. Utilities of Botulinum Toxins in dermatology and cosmetology. *Clin Cosmet Investig Dermatol* 2021;14:1319–30. <https://doi.org/10.2147/ccid.S332247>.
- Yan D, Li SH, Zhang AL, Xiao Y, Huang ZC. A clinical study of platelet-rich fibrin combined with autologous high-density fat transplantation in augmentation Rhinoplasty. *Ear Nose Throat J* 2023;102:598–604. <https://doi.org/10.1177/01455613211016902>.
- Aziz ZAA, Mohd-Nasir H, Ahmad A, Mohd Setapar SH, Peng WL, Chuo SC. *et al.* Role of nanotechnology for design and development of cosmeceutical: application in makeup and skin care. *Front Chem* 2019;7:739. <https://doi.org/10.3389/fchem.2019.00739>.
- Li J, Wang T, Hou X, Li Y, Zhang J, Bai W. *et al.* Extracellular vesicles: opening up a new perspective for the diagnosis and treatment of mitochondrial dysfunction. *J Nanobiotechnology*. 2024;22:487. <https://doi.org/10.1186/s12951-024-02750-8>.
- Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B. *et al.* Minimal information for studies of extracellular vesicles (MISEV2023): from basic to advanced approaches. *J Extracell Vesicles*. 2024;13:e12404. <https://doi.org/10.1002/jev2.12404>.
- Zhu J, Huang J, Sun Y, Xu W, Qian H. Emerging role of extracellular vesicles in diabetic retinopathy. *Theranostics*. 2024;14:1631–46. <https://doi.org/10.7150/thno.92463>.
- Jacquin-Porretaz C, Cordonnier M, Nardin C, Boullerot L, Chanteloup G, Vautrot V. *et al.* Increased levels of interleukin-17A exosomes in psoriasis. *Acta Derm Venereol* 2019;99:1143–7. <https://doi.org/10.2340/00015555-3300>.
- Wang ZY, Yan BX, Zhou Y, Chen XY, Zhang J, Cai SQ. *et al.* miRNA profiling of extracellular vesicles reveals biomarkers for psoriasis. *J Invest Dermatol* 2021;141:185–189.e4. <https://doi.org/10.1016/j.jid.2020.04.021>.
- Belhadj Z, He B, Deng H, Song S, Zhang H, Wang X. *et al.* A combined “eat me/don't eat me” strategy based on extracellular vesicles for anticancer nanomedicine. *J Extracell Vesicles*. 2020;9:1806444. <https://doi.org/10.1080/20013078.2020.1806444>.
- Cao Z, Jin S, Wang P, He Q, Yang Y, Gao Z. *et al.* Microneedle based adipose derived stem cells-derived extracellular vesicles therapy ameliorates UV-induced photoaging in SKH-1 mice. *J Biomed Mater Res A* 2021;109:1849–57. <https://doi.org/10.1002/jbm.a.37177>.
- Naruskaitė D, Vydmantaitė G, Rusteikaitė J, Sampath R, Rudaitytė A, Stašytė G. *et al.* Extracellular vesicles in skin wound healing. *Pharmaceuticals (Basel)* 2021;14:811. <https://doi.org/10.3390/ph14080811>.
- Shen Y, Xu G, Huang H, Wang K, Wang H, Lang M. *et al.* Sequential release of small extracellular vesicles from Bilayered Thiolated alginate/polyethylene glycol Diacrylate hydrogels for Scarless wound healing. *ACS Nano* 2021;15:6352–68. <https://doi.org/10.1021/acsnano.0c07714>.

18. Xu P, Xin Y, Zhang Z, Zou X, Xue K, Zhang H. *et al.* Extracellular vesicles from adipose-derived stem cells ameliorate ultraviolet B-induced skin photoaging by attenuating reactive oxygen species production and inflammation. *Stem Cell Res Ther* 2020;11:264. <https://doi.org/10.1186/s13287-020-01777-6>.
19. Zhang H, Xiao X, Wang L, Shi X, Fu N, Wang S. *et al.* Human adipose and umbilical cord mesenchymal stem cell-derived extracellular vesicles mitigate photoaging via TIMP1/Notch1. *Signal Transduct Target Ther* 2024;9:294. <https://doi.org/10.1038/s41392-024-01993-z>.
20. Cho BS, Lee J, Won Y, Duncan DI, Jin RC, Lee J. *et al.* Skin brightening efficacy of exosomes derived from human adipose tissue-derived stem/stromal cells: a prospective, split-face, randomized placebo-controlled study. *Cosmetics*. 2020;7:90. <https://doi.org/10.3390/cosmetics7040090>.
21. Kwon HH, Yang SH, Lee J, Park BC, Park KY, Jung JY. *et al.* Combination treatment with human adipose tissue stem cell-derived exosomes and fractional CO2 laser for acne scars: a 12-week prospective, double-blind, randomized, split-face study. *Acta Derm Venereol* 2020;100:adv00310. <https://doi.org/10.2340/00015555-3666>.
22. Dong L, Wang Y, Zheng T, Pu Y, Ma Y, Qi X. *et al.* Hypoxic hUCMSC-derived extracellular vesicles attenuate allergic airway inflammation and airway remodeling in chronic asthma mice. *Stem Cell Res Ther* 2021;12:4. <https://doi.org/10.1186/s13287-020-02072-0>.
23. Di Trapani M, Bassi G, Midolo M, Gatti A, Kamga PT, Casaro A. *et al.* Differential and transferable modulatory effects of mesenchymal stromal cell-derived extracellular vesicles on T, B and NK cell functions. *Sci Rep* 2016;6:24120. <https://doi.org/10.1038/srep24120>.
24. Lee H, Cha H, Park JH. Derivation of cell-engineered Nanovesicles from human induced pluripotent stem cells and their protective effect on the senescence of dermal fibroblasts. *Int J Mol Sci* 2020;21:343. <https://doi.org/10.3390/ijms21010343>.
25. Zhu YZ, Hu X, Zhang J, Wang ZH, Wu S, Yi YY. Extracellular vesicles derived from human adipose-derived stem cell prevent the formation of hypertrophic scar in a rabbit model. *Ann Plast Surg* 2020;84:602–7. <https://doi.org/10.1097/SAP.0000000000002357>.
26. Choi JS, Cho WL, Choi YJ, Kim JD, Park HA, Kim SY. *et al.* Functional recovery in photo-damaged human dermal fibroblasts by human adipose-derived stem cell extracellular vesicles. *J Extracell Vesicles*. 2019;8:1565885. <https://doi.org/10.1080/20013078.2019.1565885>.
27. Deng M, Yu TZ, Li D, Wang X, Zhou G, Liu W. *et al.* Human umbilical cord mesenchymal stem cell-derived and dermal fibroblast-derived extracellular vesicles protect dermal fibroblasts from ultraviolet radiation-induced photoaging in vitro. *Photochem Photobiol Sci* 2020;19:406–14. <https://doi.org/10.1039/c9pp00421a>.
28. Rajendran RL, Gangadaran P, Bak SS, Oh JM, Kalimuthu S, Lee HW. *et al.* Extracellular vesicles derived from MSCs activates dermal papilla cell in vitro and promotes hair follicle conversion from telogen to anagen in mice. *Sci Rep* 2017;7:15560. <https://doi.org/10.1038/s41598-017-15505-3>.
29. Su J, Wei Q, Ma K, Wang Y, Hu W, Meng H. *et al.* P-MSC-derived extracellular vesicles facilitate diabetic wound healing via miR-145-5p/CDKN1A-mediated functional improvements of high glucose-induced senescent fibroblasts. *Burns Trauma* 2023;11:tkad010. <https://doi.org/10.1093/burnst/tkad010>.
30. Liu F, Liu S, Luo X, Fan Z, Huang S, Deng F. *et al.* Combatting ageing in dermal papilla cells and promoting hair follicle regeneration using exosomes from human hair follicle dermal sheath cup cells. *Exp Dermatol* 2024;33:e14948. <https://doi.org/10.1111/exd.14948>.
31. Chen Y, Huang J, Liu Z, Chen R, Fu D, Yang L. *et al.* miR-140-5p in small extracellular vesicles from human papilla cells stimulates hair growth by promoting proliferation of outer root sheath and hair matrix cells. *Front Cell Dev Biol* 2020;8:593638. <https://doi.org/10.3389/fcell.2020.593638>.
32. Cho B, Kim J, Ha D, Yi Y. Exosomes derived from mesenchymal stem cells alleviate atopic dermatitis by suppressing inflammation and improving skin barrier function. *Cytotherapy*. 2019;21:E4–4. <https://doi.org/10.1016/j.jcyt.2019.04.014>.
33. Kim SY, Yoon TH, Na J, Yi SJ, Jin Y, Kim M. *et al.* Mesenchymal stem cells and extracellular vesicles derived from canine adipose tissue ameliorates inflammation, skin barrier function and pruritus by reducing JAK/STAT Signaling in atopic dermatitis. *Int J Mol Sci* 2022;23:4868. <https://doi.org/10.3390/ijms23094868>.
34. Ye CX, Zhang YQ, Su Z, Wu SX, Li YX, Yi JL. *et al.* hMSC exosomes as a novel treatment for female sensitive skin: an in vivo study. *Front Bioeng Biotechnol* 2022;10:1053679. <https://doi.org/10.3389/fbioe.2022.1053679>.
35. Wang XY, Guan XH, Yu ZP, Wu J, Huang QM, Deng KY. *et al.* Human amniotic stem cells-derived exosomal miR-181a-5p and miR-199a inhibit melanogenesis and promote melanosome degradation in skin hyperpigmentation, respectively. *Stem Cell Res Ther* 2021;12:501. <https://doi.org/10.1186/s13287-021-02570-9>.
36. Tang X, Cao C, Liang Y, Han L, Tu B, Yu M. *et al.* Adipose-derived stem cell exosomes antagonize the inhibitory effect of Dihydrotestosterone on hair follicle growth by activating Wnt/beta-catenin pathway. *Stem Cells Int* 2023;2023:5548112. <https://doi.org/10.1155/2023/5548112>.
37. Li Y, Wang G, Wang Q, Zhang Y, Cui L, Huang X. Exosomes secreted from adipose-derived stem cells are a potential treatment agent for immune-mediated alopecia. *J Immunol Res* 2022;2022:7471246. <https://doi.org/10.1155/2022/7471246>.
38. Wu J, Yang Q, Wu S, Yuan R, Zhao X, Li Y. *et al.* Adipose-derived stem cell exosomes promoted hair regeneration. *Tissue Eng Regen Med* 2021;18:685–91. <https://doi.org/10.1007/s13770-021-00347-y>.
39. Li Y, Zhang J, Shi J, Liu K, Wang X, Jia Y. *et al.* Exosomes derived from human adipose mesenchymal stem cells attenuate hypertrophic scar fibrosis by miR-192-5p/IL-17RA/Smad axis. *Stem Cell Res Ther* 2021;12:221. <https://doi.org/10.1186/s13287-021-02290-0>.
40. Wang L, Hu L, Zhou X, Xiong Z, Zhang C, Shehada HMA. *et al.* Exosomes secreted by human adipose mesenchymal stem cells promote scarless cutaneous repair by regulating extracellular matrix remodelling. *Sci Rep* 2017;7:13321. <https://doi.org/10.1038/s41598-017-12919-x>.
41. Zhang W, Bai X, Zhao B, Li Y, Zhang Y, Li Z. *et al.* Cell-free therapy based on adipose tissue stem cell-derived exosomes promotes wound healing via the PI3K/Akt signaling pathway. *Exp Cell Res* 2018;370:333–42. <https://doi.org/10.1016/j.yexcr.2018.06.035>.
42. Wang M, Wang C, Chen M, Xi Y, Cheng W, Mao C. *et al.* Efficient angiogenesis-based diabetic wound healing/skin reconstruction through bioactive antibacterial adhesive ultraviolet shielding Nanodressing with exosome release. *ACS Nano* 2019;13:10279–93. <https://doi.org/10.1021/acsnano.9b03656>.
43. Hu S, Zhang J, Ji Q, Xie S, Jiang J, Ni H. *et al.* Exosomes derived from uMSCs promote hair regrowth in alopecia areata through accelerating human hair follicular keratinocyte proliferation and migration. *Cell Biol Int* 2024;48:154–61. <https://doi.org/10.1002/cbin.12099>.
44. Oh EJ, Gangadaran P, Rajendran RL, Kim HM, Oh JM, Choi KY. *et al.* Extracellular vesicles derived from fibroblasts promote wound healing by optimizing fibroblast and endothelial cellular functions. *Stem Cells* 2021;39:266–79. <https://doi.org/10.1002/stem.3310>.
45. Rajendran RL, Gangadaran P, Kwack MH, Oh JM, Hong CM, Sung YK. *et al.* Human fibroblast-derived extracellular vesicles promote hair growth in cultured human hair follicles. *FEBS Lett* 2021;595:942–53. <https://doi.org/10.1002/1873-3468.14050>.
46. Lee JM, Lee JO, Kim Y, Jang YN, Yeon Park A, Kim SY. *et al.* Anti-melanogenic effect of exosomes derived from human dermal

- fibroblasts (BJ-5ta-ex) in C57BL/6 mice and B16F10 melanoma cells. *Pigment Cell Melanoma Res* 2024;37:232–46. <https://doi.org/10.1111/pcmr.13135>.
47. Rajendran RL, Gangadaran P, Seo CH, Kwack MH, Oh JM, Lee HW. *et al.* Macrophage-derived extracellular vesicle promotes hair growth. *Cells*. 2020;9:856. <https://doi.org/10.3390/cells9040856>.
 48. Wei F, Wang A, Wang Q, Han W, Rong R, Wang L. *et al.* Plasma endothelial cells-derived extracellular vesicles promote wound healing in diabetes through YAP and the PI3K/Akt/mTOR pathway. *Aging (Albany NY)* 2020;12:12002–18. <https://doi.org/10.18632/aging.103366>.
 49. Trentini M, Zanolla I, Zanotti F, Tiengo E, Licastro D, Dal Monego S. *et al.* Apple derived exosomes improve collagen type I production and decrease MMPs during aging of the skin through downregulation of the NF-kappaB pathway as mode of action. *Cells*. 2022;11:3950. <https://doi.org/10.3390/cells11243950>.
 50. Kim M, Park JH. Isolation of Aloe saponaria-derived extracellular vesicles and investigation of their potential for chronic wound healing. *Pharmaceutics*. 2022;14:1905. <https://doi.org/10.3390/pharmaceutics14091905>.
 51. Kim MK, Choi YC, Cho SH, Choi JS, Cho YW. The antioxidant effect of small extracellular vesicles derived from Aloe vera peels for wound healing. *Tissue Eng Regen Med* 2021;18:561–71. <https://doi.org/10.1007/s13770-021-00367-8>.
 52. Savci Y, Kirbas OK, Bozkurt BT, Abdik EA, Tasli PN, Sahin F. *et al.* Grapefruit-derived extracellular vesicles as a promising cell-free therapeutic tool for wound healing. *Food Funct* 2021;12:5144–56. <https://doi.org/10.1039/d0fo02953j>.
 53. Lee R, Ko HJ, Kim K, Sohn Y, Min SY, Kim JA. *et al.* Anti-melanogenic effects of extracellular vesicles derived from plant leaves and stems in mouse melanoma cells and human healthy skin. *J Extracell Vesicles* 2020;9:1703480. <https://doi.org/10.1080/20013078.2019.1703480>.
 54. Jang B, Chung H, Jung H, Song HK, Park E, Choi HS. *et al.* Extracellular vesicles from Korean Codium fragile and Sargassum fusiforme negatively regulate melanin synthesis. *Mol Cells* 2021;44:736–45. <https://doi.org/10.14348/molcells.2021.2167>.
 55. Kim W, Lee EJ, Bae IH, Myoung K, Kim ST, Park PJ. *et al.* Lactobacillus plantarum-derived extracellular vesicles induce anti-inflammatory M2 macrophage polarization in vitro. *J Extracell Vesicles* 2020;9:1793514. <https://doi.org/10.1080/20013078.2020.1793514>.
 56. Jo CS, Myung CH, Yoon YC, Ahn BH, Min JW, Seo WS. *et al.* The effect of lactobacillus plantarum extracellular vesicles from Korean women in their 20s on skin aging. *Curr Issues Mol Biol* 2022;44:526–40. <https://doi.org/10.3390/cimb44020036>.
 57. Han F, Wang K, Shen K, Wang J, Han S, Hu D. *et al.* Extracellular vesicles from lactobacillus druckerii inhibit hypertrophic scar fibrosis. *J Nanobiotechnology* 2023;21:113. <https://doi.org/10.1186/s12951-023-01861-y>.
 58. Yin H, Chen CY, Liu YW, Tan YJ, Deng ZL, Yang F. *et al.* Synechococcus elongatus PCC7942 secretes extracellular vesicles to accelerate cutaneous wound healing by promoting angiogenesis. *Theranostics*. 2019;9:2678–93. <https://doi.org/10.7150/thno.31884>.
 59. Hong SW, Choi EB, Min TK, Kim JH, Kim MH, Jeon SG. *et al.* An important role of alpha-hemolysin in extracellular vesicles on the development of atopic dermatitis induced by Staphylococcus aureus. *PLoS One* 2014;9:e100499. <https://doi.org/10.1371/journal.pone.0100499>.
 60. Gehrmann U, Qazi KR, Johansson C, Hultenby K, Karlsson M, Lundeberg L. *et al.* Nanovesicles from Malassezia sympodialis and host exosomes induce cytokine responses—novel mechanisms for host-microbe interactions in atopic eczema. *PLoS One* 2011;6:e21480. <https://doi.org/10.1371/journal.pone.0021480>.
 61. Johansson HJ, Vallhov H, Holm T, Gehrmann U, Andersson A, Johansson C. *et al.* Author correction: extracellular nanovesicles released from the commensal yeast Malassezia sympodialis are enriched in allergens and interact with cells in human skin. *Sci Rep* 2019;9:15014. <https://doi.org/10.1038/s41598-019-43724-3>.
 62. Vallhov H, Johansson C, Veerman RE, Scheynius A. Extracellular vesicles released from the skin commensal yeast Malassezia sympodialis activate human primary keratinocytes. *Front Cell Infect Microbiol* 2020;10:6. <https://doi.org/10.3389/fcimb.2020.00006>.
 63. Han J, Wu T, Jin J, Li Z, Cheng W, Dai X. *et al.* Exosome-like nanovesicles derived from Phellinus linteus inhibit Mical2 expression through cross-kingdom regulation and inhibit ultraviolet-induced skin aging. *J Nanobiotechnology*. 2022;20:455. <https://doi.org/10.1186/s12951-022-01657-6>.
 64. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Lett* 2016;371:48–61. <https://doi.org/10.1016/j.canlet.2015.10.020>.
 65. Zemleni J, Sukreet S, Zhou F, Wu D, Mutai E. Milk-derived exosomes and metabolic regulation. *Annu Rev Anim Biosci* 2019;7:245–62. <https://doi.org/10.1146/annurev-anima-1-020518-115300>.
 66. Bae IS, Kim SH. Milk exosome-derived MicroRNA-2478 suppresses Melanogenesis through the Akt-GSK3beta pathway. *Cells*. 2021;10:2848. <https://doi.org/10.3390/cells10112848>.
 67. Lu L, Bai W, Wang M, Han C, Du H, Wang N. *et al.* Novel roles of bovine milk-derived exosomes in skin antiaging. *J Cosmet Dermatol* 2024;23:1374–85. <https://doi.org/10.1111/jocd.16112>.
 68. Struff WG, Sprotte G. Bovine colostrum as a biologic in clinical medicine: a review—Part II: clinical studies. *Int J Clin Pharmacol Ther* 2008;46:211–25. <https://doi.org/10.5414/cpp46211>.
 69. Han G, Kim H, Kim DE, Ahn Y, Kim J, Jang YJ. *et al.* The potential of bovine colostrum-derived exosomes to repair aged and damaged skin cells. *Pharmaceutics*. 2022;14:307. <https://doi.org/10.3390/pharmaceutics14020307>.
 70. Kim H, Jang Y, Kim EH, Jang H, Cho H, Han G. *et al.* Potential of colostrum-derived exosomes for promoting hair regeneration through the transition from Telogen to Anagen phase. *Front Cell Dev Biol* 2022;10:815205. <https://doi.org/10.3389/fcell.2022.815205>.
 71. Kim H, Kim DE, Han G, Lim NR, Kim EH, Jang Y. *et al.* Harnessing the natural healing power of colostrum: bovine milk-derived extracellular vesicles from colostrum facilitating the transition from inflammation to tissue regeneration for accelerating cutaneous wound healing. *Adv Healthc Mater* 2022;11:e2102027. <https://doi.org/10.1002/adhm.202102027>.
 72. Ravi M, Paramesh V, Kaviya SR, Anuradha E, Solomon FD. 3D cell culture systems: advantages and applications. *J Cell Physiol* 2015;230:16–26. <https://doi.org/10.1002/jcp.24683>.
 73. Fontoura JC, Viezzer C, Dos Santos FG, Ligabue RA, Weinlich R, Puga RD. *et al.* Comparison of 2D and 3D cell culture models for cell growth, gene expression and drug resistance. *Mater Sci Eng C Mater Biol Appl* 2020;107:110264. <https://doi.org/10.1016/j.msec.2019.110264>.
 74. Pampaloni F, Reynaud EG, Stelzer EH. The third dimension bridges the gap between cell culture and live tissue. *Nat Rev Mol Cell Biol* 2007;8:839–45. <https://doi.org/10.1038/nrm2236>.
 75. Mohandas S, Gayatri V, Kumaran K, Gopinath V, Paulmurugan R, Ramkumar KM. New Frontiers in three-dimensional culture platforms to improve diabetes research. *Pharmaceutics*. 2023;15:725. <https://doi.org/10.3390/pharmaceutics15030725>.
 76. Marchini A, Gelain F. Synthetic scaffolds for 3D cell cultures and organoids: applications in regenerative medicine. *Crit Rev Biotechnol* 2022;42:468–86. <https://doi.org/10.1080/07388551.2021.1932716>.
 77. Haraszi RA, Miller R, Stoppato M, Sere YY, Coles A, Didiot MC. *et al.* Exosomes produced from 3D cultures of MSCs by tangential flow filtration show higher yield and improved activity. *Mol Ther* 2018;26:2838–47. <https://doi.org/10.1016/j.ymthe.2018.09.015>.
 78. Jeske R, Liu C, Duke L, Canonico Castro ML, Muok L, Arthur P. *et al.* Upscaling human mesenchymal stromal cell production in a novel vertical-wheel bioreactor enhances extracellular vesicle

- cle secretion and cargo profile. *Bioact Mater* 2023;25:732–47. <https://doi.org/10.1016/j.bioactmat.2022.07.004>.
79. Kim JY, Rhim WK, Cha SG, Woo J, Lee JY, Park CG. *et al.* Bolstering the secretion and bioactivities of umbilical cord MSC-derived extracellular vesicles with 3D culture and priming in chemically defined media. *Nano Conver* 2022;9:57. <https://doi.org/10.1186/s40580-022-00349-z>.
 80. Yan L, Wu X. Exosomes produced from 3D cultures of umbilical cord mesenchymal stem cells in a hollow-fiber bioreactor show improved osteochondral regeneration activity. *Cell Biol Toxicol* 2020;36:165–78. <https://doi.org/10.1007/s10565-019-09504-5>.
 81. Gobin J, Muradia G, Mehic J, Westwood C, Couvrette L, Stalker A. *et al.* Hollow-fiber bioreactor production of extracellular vesicles from human bone marrow mesenchymal stromal cells yields nanovesicles that mirrors the immuno-modulatory antigenic signature of the producer cell. *Stem Cell Res Ther* 2021;12:127. <https://doi.org/10.1186/s13287-021-02190-3>.
 82. Hu S, Li Z, Cores J, Huang K, Su T, Dinh PU. *et al.* Needle-free injection of exosomes derived from human dermal fibroblast spheroids ameliorates skin Photoaging. *ACS Nano* 2019;13:11273–82. <https://doi.org/10.1021/acsnano.9b04384>.
 83. Hu S, Li Z, Lutz H, Huang K, Su T, Cores J. *et al.* Dermal exosomes containing miR-218-5p promote hair regeneration by regulating beta-catenin signaling. *Sci Adv* 2020;6:eaba1685. <https://doi.org/10.1126/sciadv.aba1685>.
 84. Fitzgerald SJ, Cobb JS, Janorkar AV. Comparison of the formation, adipogenic maturation, and retention of human adipose-derived stem cell spheroids in scaffold-free culture techniques. *J Biomed Mater Res B Appl Biomater* 2020;108:3022–32. <https://doi.org/10.1002/jbm.b.34631>.
 85. Ryu NE, Lee SH, Park H. Spheroid culture system methods and applications for mesenchymal stem cells. *Cells*. 2019;8:8. <https://doi.org/10.3390/cells8121620>.
 86. Wang Y, Shen K, Sun Y, Cao P, Zhang J, Zhang W. *et al.* Extracellular vesicles from 3D cultured dermal papilla cells improve wound healing via Kruppel-like factor 4/vascular endothelial growth factor a -driven angiogenesis. *Burns Trauma* 2023;11:tkad034. <https://doi.org/10.1093/burnst/tkad034>.
 87. Wu H, Wang J, Zhao Y, Qin YY, Chen X, Zhou YT. *et al.* Extracellular vesicles derived from human dermal fibroblast effectively ameliorate skin photoaging via miRNA-22-5p-GDF11 axis. *Chem Eng J* 2023;452:139553. <https://doi.org/10.1016/j.cej.2022.139553>.
 88. Mirabel C, Puente-Massaguer E, Del Mazo-Barbara A, Reyes B, Morton P, Godia F. *et al.* Stability enhancement of clinical grade multipotent mesenchymal stromal cell-based products. *J Transl Med* 2018;16:291. <https://doi.org/10.1186/s12967-018-1659-4>.
 89. Lo Cicero A, Delevoye C, Gilles-Marsens F, Loew D, Dingli F, Guere C. *et al.* Exosomes released by keratinocytes modulate melanocyte pigmentation. *Nat Commun* 2015;6:7506. <https://doi.org/10.1038/ncomms8506>.
 90. Yoon JH, Jo CS, Hwang JS. Comprehensive analysis of Exosomal MicroRNAs derived from UVB-irradiated keratinocytes as potential Melanogenesis regulators. *Int J Mol Sci* 2024;25:3095. <https://doi.org/10.3390/ijms25063095>.
 91. Yang K, Li D, Wang M, Xu Z, Chen X, Liu Q. *et al.* Exposure to blue light stimulates the proangiogenic capability of exosomes derived from human umbilical cord mesenchymal stem cells. *Stem Cell Res Ther* 2019;10:358. <https://doi.org/10.1186/s13287-019-1472-x>.
 92. Ma T, Grayson WL, Frohlich M, Vunjak-Novakovic G. Hypoxia and stem cell-based engineering of mesenchymal tissues. *Biotechnol Prog* 2009;25:32–42. <https://doi.org/10.1002/btpr.128>.
 93. Pulido-Escribano V, Torrecillas-Baena B, Camacho-Cardenosa M, Dorado G, Galvez-Moreno MA, Casado-Diaz A. Role of hypoxia preconditioning in therapeutic potential of mesenchymal stem-cell-derived extracellular vesicles. *World J Stem Cells* 2022;14:453–72. <https://doi.org/10.4252/wjsc.v14.i7.453>.
 94. Liu Y, Wang Y, Yang M, Luo J, Zha J, Geng S. *et al.* Exosomes from hypoxic pretreated ADSCs attenuate ultraviolet light-induced skin injury via GLRX5 delivery and ferroptosis inhibition. *Photochem Photobiol Sci* 2024;23:55–63. <https://doi.org/10.1007/s43630-023-00498-y>.
 95. Liu J, Dong J, Pei X. Apoptotic extracellular vesicles derived from human umbilical vein endothelial cells promote skin repair by enhancing angiogenesis: from death to regeneration. *Int J Nanomedicine* 2024;19:415–28. <https://doi.org/10.2147/IJN.S441453>.
 96. Yoo K, Thapa N, Lee J, Jang Y, Lee JO, Kim J. Dermal fibroblast cell-derived exosomes for atopic dermatitis: In-vitro test. *Skin Res Technol* 2023;29:e13382. <https://doi.org/10.1111/srt.13382>.
 97. Nakao Y, Fukuda T, Zhang Q, Sanui T, Shinjo T, Kou X. *et al.* Exosomes from TNF-alpha-treated human gingiva-derived MSCs enhance M2 macrophage polarization and inhibit periodontal bone loss. *Acta Biomater* 2021;122:306–24. <https://doi.org/10.1016/j.actbio.2020.12.046>.
 98. Zhang S, Jiang L, Hu H, Wang H, Wang X, Jiang J. *et al.* Pretreatment of exosomes derived from hUCMSCs with TNF-alpha ameliorates acute liver failure by inhibiting the activation of NLRP3 in macrophage. *Life Sci* 2020;246:117401. <https://doi.org/10.1016/j.lfs.2020.117401>.
 99. Liu K, Cai GL, Zhuang Z, Pei SY, Xu SN, Wang YN. *et al.* Interleukin-1beta-treated mesenchymal stem cells inhibit inflammation in hippocampal astrocytes through exosome-activated Nrf-2 Signaling. *Int J Nanomedicine* 2021;16:1423–34. <https://doi.org/10.2147/IJN.S289914>.
 100. Song Y, Dou H, Li X, Zhao X, Li Y, Liu D. *et al.* Exosomal miR-146a contributes to the enhanced therapeutic efficacy of interleukin-1beta-primed mesenchymal stem cells against sepsis. *Stem Cells* 2017;35:1208–21. <https://doi.org/10.1002/stem.2564>.
 101. Yang R, Huang H, Cui S, Zhou Y, Zhang T, Zhou Y. IFN-gamma promoted exosomes from mesenchymal stem cells to attenuate colitis via miR-125a and miR-125b. *Cell Death Dis* 2020;11:603. <https://doi.org/10.1038/s41419-020-02788-0>.
 102. Gorgun C, Ceresa D, Lesage R, Villa F, Reverberi D, Balbi C. *et al.* Dissecting the effects of preconditioning with inflammatory cytokines and hypoxia on the angiogenic potential of mesenchymal stromal cell (MSC)-derived soluble proteins and extracellular vesicles (EVs). *Biomaterials*. 2021;269:120633. <https://doi.org/10.1016/j.biomaterials.2020.120633>.
 103. Tolomeo AM, Castagliuolo I, Piccoli M, Grassi M, Magarotto F, De Lazzari G. *et al.* Extracellular vesicles secreted by mesenchymal stromal cells exert opposite effects to their cells of origin in murine sodium dextran Sulfate-induced colitis. *Front Immunol* 2021;12:627605. <https://doi.org/10.3389/fimmu.2021.627605>.
 104. Kim J, Lee SK, Jung M, Jeong SY, You H, Won JY. *et al.* Extracellular vesicles from IFN-gamma-primed mesenchymal stem cells repress atopic dermatitis in mice. *J Nanobiotechnology* 2022;20:526. <https://doi.org/10.1186/s12951-022-01728-8>.
 105. Liang YC, Wu YP, Li XD, Chen SH, Ye XJ, Xue XY. *et al.* TNF-alpha-induced exosomal miR-146a mediates mesenchymal stem cell-dependent suppression of urethral stricture. *J Cell Physiol* 2019;234:23243–55. <https://doi.org/10.1002/jcp.28891>.
 106. Wu P, Zhang B, Ocansey DKW, Xu W, Qian H. Extracellular vesicles: a bright star of nanomedicine. *Biomaterials*. 2021;269:120467. <https://doi.org/10.1016/j.biomaterials.2020.120467>.
 107. Ding JY, Chen MJ, Wu LF, Shu GF, Fang SJ, Li ZY. *et al.* Mesenchymal stem cell-derived extracellular vesicles in skin wound healing: roles, opportunities and challenges. *Mil Med Res* 2023;10:36. <https://doi.org/10.1186/s40779-023-00472-w>.
 108. Liu Y, Xue L, Gao H, Chang L, Yu X, Zhu Z. *et al.* Exosomal miRNA derived from keratinocytes regulates pigmentation in melanocytes. *J Dermatol Sci* 2019;93:159–67. <https://doi.org/10.1016/j.jdermsci.2019.02.001>.
 109. Wei Q, Su J, Meng S, Wang Y, Ma K, Li B. *et al.* MiR-17-5p-engineered sEVs encapsulated in GelMA hydrogel facilitated

- diabetic wound healing by targeting PTEN and p21. *Adv Sci (Weinh)* 2024;11:e2307761. <https://doi.org/10.1002/adv.202307761>.
110. Gao W, Yuan LM, Zhang Y, Huang FZ, Gao F, Li J. *et al.* miR-1246-overexpressing exosomes suppress UVB-induced photoaging via regulation of TGF-beta/Smad and attenuation of MAPK/AP-1 pathway. *Photochem Photobiol Sci*. 2023;22:135–46. <https://doi.org/10.1007/s43630-022-00304-1>.
 111. Born LJ, Chang KH, Shoureshi P, Lay F, Bengali S, Hsu ATW. *et al.* HOTAIR-loaded mesenchymal stem/stromal cell extracellular vesicles enhance angiogenesis and wound healing. *Adv Healthc Mater*. 2022;11:e2002070. <https://doi.org/10.1002/adhm.202002070>.
 112. Zhang Y, Zhang M, Yao A, Xie Y, Lin J, Sharifullah F. *et al.* Circ_0011129 encapsulated by the small extracellular vesicles derived from human stem cells ameliorate skin Photoaging. *Int J Mol Sci* 2022;23:15390. <https://doi.org/10.3390/ijms232315390>.
 113. Wang J, Wu H, Zhao Y, Qin Y, Zhang Y, Pang H. *et al.* Extracellular vesicles from HIF-1alpha-overexpressing adipose-derived stem cells restore diabetic wounds through accelerated fibroblast proliferation and migration. *Int J Nanomedicine* 2021;16:7943–57. <https://doi.org/10.2147/IJN.S335438>.
 114. Rincon-Benavides MA, Mendonca NC, Cuellar-Gaviria TZ, Salazar-Puerta AI, Ortega-Pineda L, Blackstone BN. *et al.* Engineered Vascuogenic extracellular vesicles drive nonviral direct conversions of human dermal fibroblasts into induced endothelial cells and improve wound closure. *Adv Ther (Weinh)* 2023;6:2200197. <https://doi.org/10.1002/adtp.202200197>.
 115. Ridder K, Sevko A, Heide J, Dams M, Rupp AK, Macas J. *et al.* Extracellular vesicle-mediated transfer of functional RNA in the tumor microenvironment. *Oncoimmunology*. 2015;4:e1008371. <https://doi.org/10.1080/2162402X.2015.1008371>.
 116. Gao L, Feng Q, Cui B, Mao Y, Zhao Z, Liu Z. *et al.* Loading Nanoceria improves extracellular vesicle membrane integrity and therapy to wounds in aged mice. *ACS Biomater Sci Eng* 2023;9:732–42. <https://doi.org/10.1021/acsbomaterials.2c01104>.
 117. Smyth T, Kullberg M, Malik N, Smith-Jones P, Graner MW, Anchordoquy TJ. Biodistribution and delivery efficiency of unmodified tumor-derived exosomes. *J Control Release* 2015;199:145–55. <https://doi.org/10.1016/j.jconrel.2014.12.013>.
 118. Wang Y, Cao Z, Wei Q, Ma K, Hu W, Huang Q. *et al.* VH298-loaded extracellular vesicles released from gelatin methacryloyl hydrogel facilitate diabetic wound healing by HIF-1alpha-mediated enhancement of angiogenesis. *Acta Biomater* 2022;147:342–55. <https://doi.org/10.1016/j.actbio.2022.05.018>.
 119. Fan L, Ma X, Liu B, Yang Y, Yang Y, Ren T. *et al.* Antioxidant-engineered milk-derived extracellular vesicles for accelerating wound healing via regulation of the PI3K-AKT Signaling pathway. *Adv Healthc Mater*. 2023;12:e2301865. <https://doi.org/10.1002/adhm.202301865>.
 120. Fuhrmann G, Serio A, Mazo M, Nair R, Stevens MM. Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. *J Control Release* 2015;205:35–44. <https://doi.org/10.1016/j.jconrel.2014.11.029>.
 121. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z. *et al.* Exosomes as drug delivery vehicles for Parkinson's disease therapy. *J Control Release* 2015;207:18–30. <https://doi.org/10.1016/j.jconrel.2015.03.033>.
 122. Kim MS, Haney MJ, Zhao Y, Mahajan V, Deygen I, Klyachko NL. *et al.* Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. *Nanomedicine*. 2016;12:655–64. <https://doi.org/10.1016/j.nano.2015.10.012>.
 123. Salunkhe S, Dheeraj BM, Chitkara D, Mittal A. Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: strategies and significance. *J Control Release* 2020;326:599–614. <https://doi.org/10.1016/j.jconrel.2020.07.042>.
 124. Liang Y, Duan L, Lu J, Xia J. Engineering exosomes for targeted drug delivery. *Theranostics*. 2021;11:3183–95. <https://doi.org/10.7150/thno.52570>.
 125. Lee JR, Kyung JW, Kumar H, Kwon SP, Song SY, Han IB. *et al.* Targeted delivery of mesenchymal stem cell-derived Nanovesicles for spinal cord injury treatment. *Int J Mol Sci* 2020;21:4185. <https://doi.org/10.3390/ijms21114185>.
 126. Goh WJ, Zou S, Ong WY, Torta F, Alexandra AF, Schifferles RM. *et al.* Bioinspired cell-derived Nanovesicles versus exosomes as drug delivery systems: a cost-effective alternative. *Sci Rep* 2017;7:14322. <https://doi.org/10.1038/s41598-017-14725-x>.
 127. Park KS, Svennerholm K, Shelke GV, Bandeira E, Lasser C, Jang SC. *et al.* Mesenchymal stromal cell-derived nanovesicles ameliorate bacterial outer membrane vesicle-induced sepsis via IL-10. *Stem Cell Res Ther* 2019;10:231. <https://doi.org/10.1186/s13287-019-1352-4>.
 128. Neupane YR, Handral HK, Alkaff SA, Chng WH, Venkatesan G, Huang C. *et al.* Cell-derived nanovesicles from mesenchymal stem cells as extracellular vesicle-mimetics in wound healing. *Acta Pharm Sin B* 2023;13:1887–902. <https://doi.org/10.1016/j.apsb.2022.10.022>.
 129. Yoon J, Jo W, Jeong D, Kim J, Jeong H, Park J. Generation of nanovesicles with sliced cellular membrane fragments for exogenous material delivery. *Biomaterials*. 2015;59:12–20. <https://doi.org/10.1016/j.biomaterials.2015.04.028>.
 130. Xiong J, Liu Z, Jia L, Sun Y, Guo R, Xi T. *et al.* Bioinspired engineering ADSC nanovesicles thermosensitive hydrogel enhance autophagy of dermal papilla cells for androgenetic alopecia treatment. *Bioact Mater* 2024;36:112–25. <https://doi.org/10.1016/j.bioactmat.2024.02.023>.
 131. Rajendran RL, Gangadharan P, Kwack MH, Oh JM, Hong CM, Jeyaraman M. *et al.* Engineered Nanovesicles from fibroblasts modulate dermal papillae cells in vitro and promote human hair follicle growth ex vivo. *Cells*. 2022;11:11. <https://doi.org/10.3390/cells11244066>.
 132. Cao L, Tian T, Huang Y, Tao S, Zhu X, Yang M. *et al.* Neural progenitor cell-derived nanovesicles promote hair follicle growth via miR-100. *J Nanobiotechnology*. 2021;19:20. <https://doi.org/10.1186/s12951-020-00757-5>.
 133. Park S, Lim J, Kim S, Jeon M, Baek H, Park W. *et al.* Anti-inflammatory artificial extracellular vesicles with notable inhibition of particulate matter-induced skin inflammation and barrier function impairment. *ACS Appl Mater Interfaces* 2023;15:59199–208. <https://doi.org/10.1021/acsami.3c14377>.
 134. Kim YS, Go G, Yun CW, Yea JH, Yoon S, Han SY. *et al.* Topical Administration of Melatonin-Loaded Extracellular Vesicle-Mimetic Nanovesicles Improves 2,4-dinitrofluorobenzene-induced atopic dermatitis. *Biomolecules*. 2021;11:1450. <https://doi.org/10.3390/biom11101450>.
 135. Garcia-Manrique P, Matos M, Gutierrez G, Pazos C, Blanco-Lopez MC. Therapeutic biomaterials based on extracellular vesicles: classification of bio-engineering and mimetic preparation routes. *J Extracell Vesicles*. 2018;7:1422676. <https://doi.org/10.1080/20013078.2017.1422676>.
 136. Lu M, Zhao X, Xing H, Liu H, Lang L, Yang T. *et al.* Cell-free synthesis of connexin 43-integrated exosome-mimetic nanoparticles for siRNA delivery. *Acta Biomater* 2019;96:517–36. <https://doi.org/10.1016/j.actbio.2019.07.006>.
 137. Sun L, Fan M, Huang D, Li B, Xu R, Gao F. *et al.* Clodronate-loaded liposomal and fibroblast-derived exosomal hybrid system for enhanced drug delivery to pulmonary fibrosis. *Biomaterials*. 2021;271:120761. <https://doi.org/10.1016/j.biomaterials.2021.120761>.
 138. Cheng L, Zhang X, Tang J, Lv Q, Liu J. Gene-engineered exosomes-thermosensitive liposomes hybrid nanovesicles by the blockade of CD47 signal for combined photothermal therapy and cancer immunotherapy. *Biomaterials*. 2021;275:120964. <https://doi.org/10.1016/j.biomaterials.2021.120964>.

139. Lin Y, Wu J, Gu W, Huang Y, Tong Z, Huang L. *et al.* Exosome-liposome hybrid nanoparticles deliver CRISPR/Cas9 system in MSCs. *Adv Sci (Weinh)* 2018;5:1700611. <https://doi.org/10.1002/adv.201700611>.
140. Patel S, Schmidt KF, Farhoud M, Zi T, Jang SC, Dooley K. *et al.* In vivo tracking of [(89)Zr]Zr-labeled engineered extracellular vesicles by PET reveals organ-specific biodistribution based upon the route of administration. *Nucl Med Biol* 2022;112-113:20-30. <https://doi.org/10.1016/j.nucmedbio.2022.06.004>.
141. Zhang B, Lai RC, Sim WK, Choo ABH, Lane EB, Lim SK. Topical application of mesenchymal stem cell exosomes alleviates the Imiquimod induced psoriasis-like inflammation. *Int J Mol Sci* 2021;22:720. <https://doi.org/10.3390/ijms22020720>.
142. Kim YJ, Yoo SM, Park HH, Lim HJ, Kim YL, Lee S. *et al.* Exosomes derived from human umbilical cord blood mesenchymal stem cells stimulates rejuvenation of human skin. *Biochem Biophys Res Commun* 2017;493:1102-8. <https://doi.org/10.1016/j.bbrc.2017.09.056>.
143. You Y, Tian Y, Yang Z, Shi J, Kwak KJ, Tong Y. *et al.* Intradermally delivered mRNA-encapsulating extracellular vesicles for collagen-replacement therapy. *Nat Biomed Eng* 2023;7:887-900. <https://doi.org/10.1038/s41551-022-00989-w>.
144. Hu L, Wang J, Zhou X, Xiong Z, Zhao J, Yu R. *et al.* Exosomes derived from human adipose mesenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci Rep* 2016;6:32993. <https://doi.org/10.1038/srep32993>.
145. Shi Y, Zhao J, Li H, Yu M, Zhang W, Qin D. *et al.* A drug-free, hair follicle cycling Regulatable, separable, antibacterial microneedle patch for hair regeneration therapy. *Adv Healthc Mater* 2022;11:e2200908. <https://doi.org/10.1002/adhm.202200908>.
146. Yang G, Chen Q, Wen D, Chen Z, Wang J, Chen G. *et al.* A therapeutic microneedle patch made from hair-derived keratin for promoting hair regrowth. *ACS Nano* 2019;13:4354-60. <https://doi.org/10.1021/acsnano.8b09573>.
147. Barnum L, Samandari M, Schmidt TA, Tamayol A. Microneedle arrays for the treatment of chronic wounds. *Expert Opin Drug Deliv* 2020;17:1767-80. <https://doi.org/10.1080/17425247.2020.1819787>.
148. Zhang K, Yu L, Li FR, Li X, Wang Z, Zou X. *et al.* Topical application of exosomes derived from human umbilical cord mesenchymal stem cells in combination with sponge spicules for treatment of Photoaging. *Int J Nanomedicine* 2020;15:2859-72. <https://doi.org/10.2147/IJN.S249751>.
149. Nooshabadi VT, Khanmohammadi M, Valipour E, Mahdipour S, Salati A, Malekshahi ZV. *et al.* Impact of exosome-loaded chitosan hydrogel in wound repair and layered dermal reconstitution in mice animal model. *J Biomed Mater Res A* 2020;108:2138-49. <https://doi.org/10.1002/jbm.a.36959>.
150. Zhao D, Yu Z, Li Y, Wang Y, Li Q, Han D. GelMA combined with sustained release of HUVECs derived exosomes for promoting cutaneous wound healing and facilitating skin regeneration. *J Mol Histol* 2020;51:251-63. <https://doi.org/10.1007/s10735-020-09877-6>.
151. Shafei S, Khanmohammadi M, Heidari R, Ghanbari H, Taghdiri Nooshabadi V, Farzamfar S. *et al.* Exosome loaded alginate hydrogel promotes tissue regeneration in full-thickness skin wounds: an in vivo study. *J Biomed Mater Res A* 2020;108:545-56. <https://doi.org/10.1002/jbm.a.36835>.
152. Zhao X, Liu Y, Jia P, Cheng H, Wang C, Chen S. *et al.* Chitosan hydrogel-loaded MSC-derived extracellular vesicles promote skin rejuvenation by ameliorating the senescence of dermal fibroblasts. *Stem Cell Res Ther* 2021;12:196. <https://doi.org/10.1186/s13287-021-02262-4>.
153. Tang L, Zhao C, Liu Y, Zhou J, Dong Y, Huang J. *et al.* GelMA hydrogel loaded with extracellular vesicles derived from umbilical cord mesenchymal stem cells for promoting cutaneous diabetic wound healing. *ACS Omega* 2023;8:10030-9. <https://doi.org/10.1021/acsomega.2c07291>.
154. Chen Y, Huang J, Chen R, Yang L, Wang J, Liu B. *et al.* Sustained release of dermal papilla-derived extracellular vesicles from injectable microgel promotes hair growth. *Theranostics*. 2020;10:1454-78. <https://doi.org/10.7150/thno.39566>.
155. Kim MH, Chung C, Oh MH, Jun JH, Ko Y, Lee JH. Extracellular vesicles from a three-dimensional culture of perivascular cells accelerate skin wound healing in a rat. *Aesth Plast Surg* 2021;45:2437-46. <https://doi.org/10.1007/s00266-021-02254-y>.
156. Duan M, Zhang Y, Zhang H, Meng Y, Qian M, Zhang G. Epidermal stem cell-derived exosomes promote skin regeneration by downregulating transforming growth factor-beta1 in wound healing. *Stem Cell Res Ther* 2020;11:452. <https://doi.org/10.1186/s13287-020-01971-6>.
157. Hong S, Ruan S, Greenberg Z, He M, McGill JL. Development of surface engineered antigenic exosomes as vaccines for respiratory syncytial virus. *Sci Rep* 2021;11:21358. <https://doi.org/10.1038/s41598-021-00765-x>.
158. Davies OG, Williams S, Goldie K. The therapeutic and commercial landscape of stem cell vesicles in regenerative dermatology. *J Control Release* 2023;353:1096-106. <https://doi.org/10.1016/j.jconrel.2022.12.025>.
159. Shin KO, Ha DH, Kim JO, Crumrine DA, Meyer JM, Wakefield JS. *et al.* Exosomes from human adipose tissue-derived mesenchymal stem cells promote epidermal barrier repair by inducing de novo synthesis of ceramides in atopic dermatitis. *Cells*. 2020;9:680. <https://doi.org/10.3390/cells9030680>.
160. Cardoso RMS, Rodrigues SC, Gomes CF, Duarte FV, Romao M, Leal EC. *et al.* Development of an optimized and scalable method for isolation of umbilical cord blood-derived small extracellular vesicles for future clinical use. *Stem Cells Transl Med* 2021;10:910-21. <https://doi.org/10.1002/sctm.20-0376>.
161. Rodrigues SC, Cardoso RMS, Freire PC, Gomes CF, Duarte FV, Neves RPD. *et al.* Immunomodulatory properties of umbilical cord blood-derived small extracellular vesicles and their therapeutic potential for inflammatory skin disorders. *Int J Mol Sci* 2021;22:9797. <https://doi.org/10.3390/ijms22189797>.
162. Prattichizzo F, Giuliani A, Sabbatinelli J, Mensa E, De Nigris V, La Sala L. *et al.* Extracellular vesicles circulating in young organisms promote healthy longevity. *J Extracell Vesicles*. 2019;8:1656044. <https://doi.org/10.1080/20013078.2019.1656044>.
163. Wang CK, Tsai TH, Lee CH. Regulation of exosomes as biologic medicines: regulatory challenges faced in exosome development and manufacturing processes. *Clin Transl Sci* 2024;17:e13904. <https://doi.org/10.1111/cts.13904>.
164. Ortega FG, Roefs MT, de Miguel PD, Kooijmans SA, de Jong OG, Sluijter JP. *et al.* Interfering with endolysosomal trafficking enhances release of bioactive exosomes. *Nanomedicine*. 2019;20:102014. <https://doi.org/10.1016/j.nano.2019.102014>.