

Extracellular vesicles in burn injury: roles, mechanisms, and applications

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Abstract

The morbidity and mortality rate from burn injuries is substantial, and patients might suffer irreparable harm from the several pathophysiological processes that severe burns cause, including metabolic alterations, distributive shock, and inflammatory responses. Prevention of infections, reduction of inflammation, alleviation of pain, and regulation of growth factors are all essential components of burn wound care, yet there are still major challenges. Multiple biological activities rely on extracellular vesicles (EVs), which are lipid bilayer vesicles released by a wide range of cell types (including prokaryotes, plant cells, and mammals). Among these processes are immunomodulation, neovascularization, and cell survival. Due to their rich composition, EVs may carry a variety of active substances, including nucleic acids and proteins, and hence play a pivotal role in many subcellular levels during wound healing. In addition, EVs may be found in a variety of burn damage patients' biofluids, making them potential new biomarkers for tracking the efficacy of treatments and making prognoses assumptions. An overview of the biological functions of EVs and the ways in which they alleviate burn injuries is provided in this paper. Additionally, we cover the likelihood of EVs being used in burn wound treatment in the future. This study aims to inspire and direct future comprehensive research on the use of EVs in burn wound healing, introduce a novel treatment for burn wounds, and serve as a resource for anybody interested in using EVs for clinical wound healing.

Keywords: Extracellular vesicles; Different sources; Biomarker; Burn injury; Wound healing

Highlights

- This article reviews the characteristics and clinical treatment of burn wounds and expounds on extracellular vesicles' advantages and biological functions.
- This study systematically summarizes the research achievements and mechanisms of extracellular vesicles in burns.
- EVs provide new insights into the treatment of burn injury and the current challenges, as well as pending questions in EVs application are also involved.

Background

It is common practice to describe skin injury as a burn when the victim comes into direct touch with an object that is extremely hot, contains certain chemicals, or is indirectly exposed to extremely high radiation. As a result of their complexity and severity, burns have surpassed all other traumas to become the fourth most frequent traumatic illness in the world [1]. The World Health Organization reports that 180000 people die each year as a direct result of burns, with a global burn-related death rate reaching 18.27% [2]; This occurrence is most prevalent in residential and commercial settings. Despite the fact that burn-related deaths and injuries are on the decline globally [3], due to the ongoing nature of military warfare, burns among soldiers and other special experts continue to be serious. A significant decrease in the survival rate of burnt troops might occur if wounded soldiers who participated in the combat were not treated promptly and adequately. The survival rates of burn patients have improved over the last

several decades because to continuous medical development, but there are still numerous obstacles to face. Debridement and skin grafts are the mainstays of conventional burn wound treatment [4], and pharmacologic therapy [5]. We need to find better therapies because these ones have their limits in some cases (like when there's a chance of infection or not enough donors).

An innovative tissue engineering treatment strategy, EVs produced from stem cells have recently garnered a lot of interest [6, 7]. Various physiologically active substances, including proteins and nucleic acids, are included in EVs, which are small vesicles released by cells. These vesicles have the ability to influence cell signaling, promote healing, and reduce inflammation [8, 9]. Dressings, gadgets, drugs, surgical procedures, and biological methods are all sufficient ways to heal wounds; nevertheless, they can be costly and lead to adverse effects such infection, allergies, and rejection [10]. The three primary and intricate steps in the healing of a burn wound

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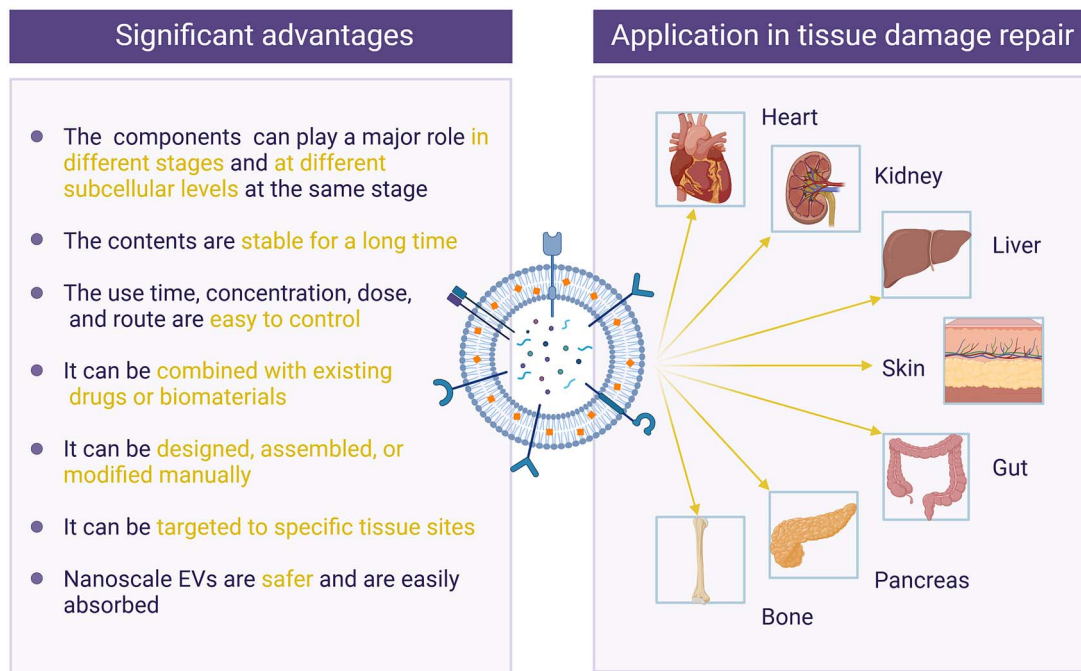


Figure 1. The advantages of EVs and their application in tissue damage repair (created with BioRender.com)

are inflammation, proliferation, and remodeling [11]. One major drawback of these treatments is that they can only start the healing process; they can't stimulate the reconstruction phase that follows. Electric vehicles provide several benefits: 1) According to Professor Sluijter, a leading authority on exosomes and tissue regeneration, the protein components of exosomes can exert a substantial impact at various subcellular levels throughout the repair of damaged tissue, and these components frequently collaborate via multiple pathways [12], which have various repair effects, such as repairing skin scars, stretching marks, and pockmarks. 2) Protected by lipid bilayer membranes, the contents are stable for a long time. 3) The use time, concentration, dose, and route are easy to control. 4) It can be combined with existing drugs or biomaterials. 5) It can be designed, assembled, or modified manually to improve the curative effect. 6) By making certain technical adjustments, it may be directed to particular areas of tissue. 7) Nanoscale electric vehicles could be less harmful and easier for the skin to absorb than nanomaterials. As a result of their distinct benefits, EVs have recently attracted interest as potential new therapeutic instruments for burn therapy.

The ability of EVs, especially EVs produced from stem cells, to greatly aid in tissue regeneration and repair has been demonstrated in a number of studies. Research on stem cell-derived EVs for scar-free wound healing has recently exploded in the skin damage repair field. Here we take a look at how EVs made from stem cells can help skin wounds (including diabetic wounds) and chronic wounds (such as cuts and scrapes) recover [13–22]. On the other hand, there hasn't been a comprehensive analysis of the mechanism and possible uses of EVs in burn wounds that focuses on the intricacy of burn wounds. Because burn wounds may be so complicated and dangerous, this article provides a concise overview of clinical treatment options. Burn wounds treated with EVs show promise. This study focused on the mechanism and application of EVs from different sources in burn wounds

(Figure 1). This article also discusses the therapeutic strategies of engineered EVs for burn wounds and the challenges associated with their practical clinical application.

Review

Burn injury

Burns, while being one of the most prevalent traumas, have received surprisingly little research attention. Burns cause a cascade of inflammatory responses that injure the skin and make the wound more vulnerable to harmful bacterial infections. Serious burns not only harm the skin but also trigger immune system dysregulation and systemic inflammation, which in turn induce infections like bacteremia and sepsis. These consequences can result in organ failure or even death. Bacterial infections can cause disease, such as those from *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus miris*, are common and are the main causes of death resulting from prolonged burn wounds [23].

According to the severity of the burn, that is, the depth of the wound, burns can be qualitatively divided into degrees I ~ III, and second-degree burns can be divided into shallow degree II and profound degree II burns [24]. The outer dermal layer is the most common site of first-degree burns. Most blisters may heal on their own since the germinal layer is living and doesn't create them. Blisters, which often do not leave scars after healing, can be caused by superficial second-degree burns, injuries to the dermal papilla and germinal layers of the epidermis, and other similar injuries. The dermis under the papillary layer is affected by deep second-degree burns; the pain is delayed, and scar hyperplasia is a common aftereffect. Even while the localized discomfort at the burn site may go away, third-degree burns affect the entire skin, muscles, and even bones beneath the surface (Figure 2) [25].

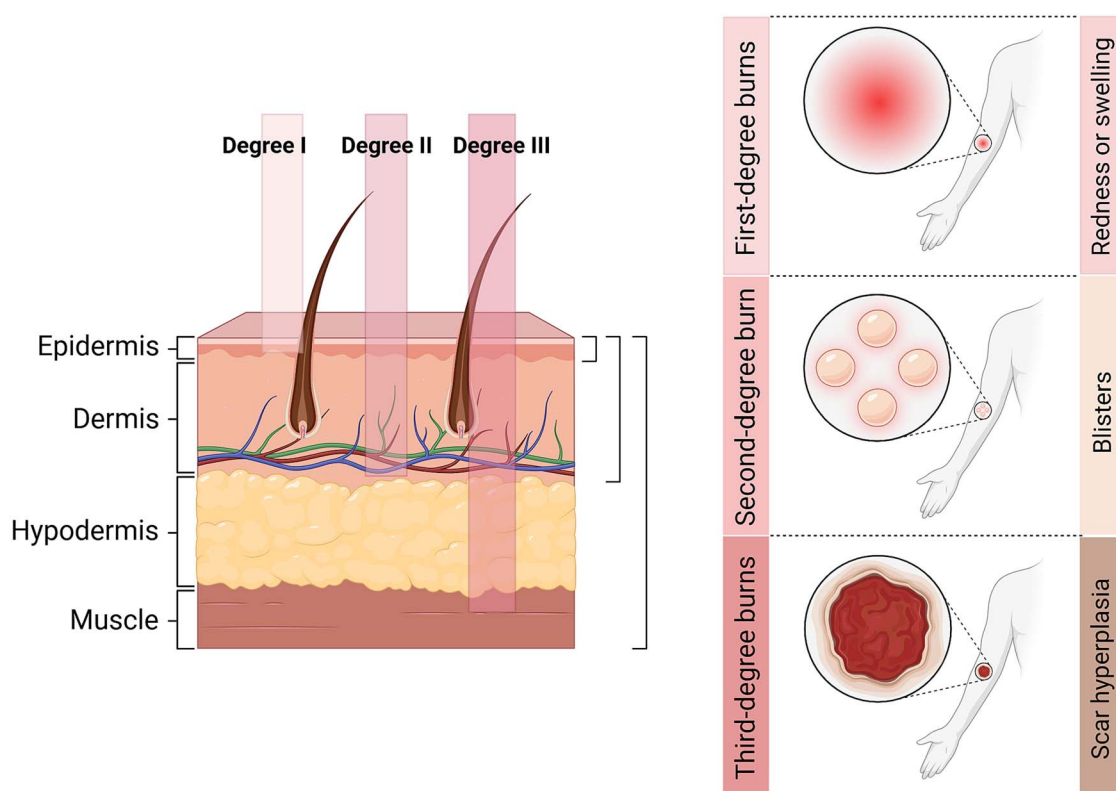


Figure 2. Burn grading diagram (created with BioRender.com)

The healing process of burn wounds

It takes a lot of time for burn wounds to heal. Healing a wound typically entails four steps: coagulation, inflammation, proliferation, and remodeling [26]. This is an overlapping and ever-changing process. Wound bleeding and coagulation are common symptoms of the damage-limiting period, which occurs immediately after a burn injury. This does double duty: it temporarily covers the tissue gap, closes the wound, and stops more blood loss. It also brings the initial wave of reactive cells to the wound site, which helps the healing process progress to the next inflammatory stage [27]. Phase two involves a rise in blood flow to the injured area, which triggers an immune response by monocytes and neutrophils via vasodilation and fluid extravasation at the injury site. Macrophages then bring in chemokines to keep the process going, which involves the removal of damaged and irreparable necrotic tissue, the protection of tissues with specific biological activity, and the prevention of pathogenic microorganism colonization of the wound. The third and final step, proliferation, begins once inflammatory cells have entered the wound environment to a sufficient degree. This phase is concomitant with the inflammatory phase. Fibroblasts and keratinocytes are activated by growth factors and cytokines at this stage [28]. Keratinocytes reorganize the blood vessel network and finish the wound's closure as they migrate into the wound. This stage is crucial for the wound-healing process. An important phase in this process is controlled by the communication network that exists between immune cells, endothelial cells, and stromal cells. Even while it may seem that the healing process is coming to a close after a wound is closed, the remodeling phase, which lasts the longest, really begins at this point [29]. At this point,

scar tissue forms when fibroblasts alter the fibrous tissue that previously filled the incision (Figure 3).

Treatments for burn wounds

Nonsurgical treatment. If the burn is superficial, nonsurgical therapy may be an option. Burns can be treated with medications, and wounds can be covered with artificial skin, allogeneic skin, or any number of other materials. The amount of local burn damage is determined by the growth direction of the stagnation zone tissue, according to relevant research [30, 31]. When the stagnation zone gets worse and spreads to the edges, it causes full-thickness skin necrosis, which makes wound healing even more challenging. Clinical treatments for swimming bands exist now, including local applications of thrombolytic agents, epidermal growth factor, and nerve growth factor (prevalent medications before the 21st century included sulfamethoxine silver cream and moisturizers). Wounds can heal more quickly with the help of these medications.

Surgical treatment. Surgical treatment is an important method for treating deep burn wounds and is widely used in clinical practice. Specifically, these methods include mixed skin transplantation, microparticle skin transplantation, large autologous skin transplantation with preserved degenerated dermis, and skin flap transplantation [32].

Mixed skin grafting

The mixed skin grafting method, which appeared in the 1960s, is a surgical treatment used mainly to repair large-scale deep burn wounds. It involves transplanting a mixture

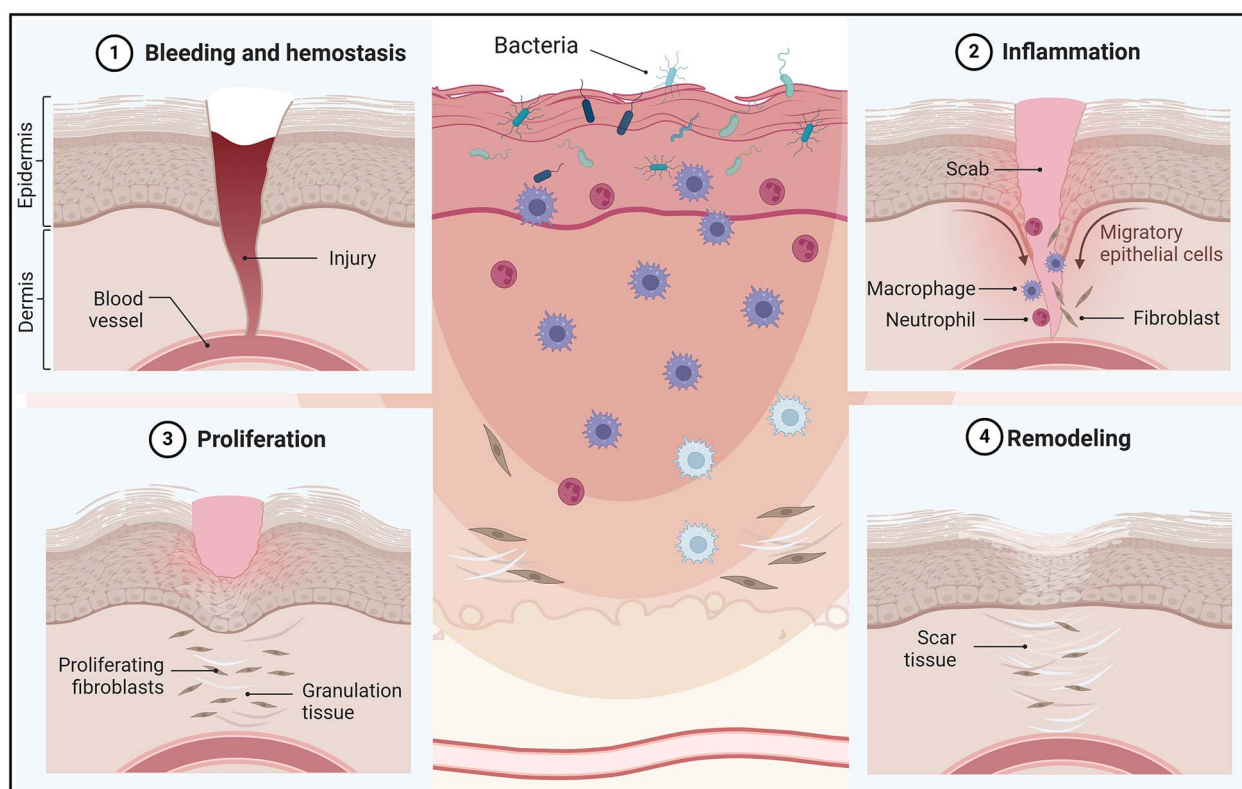


Figure 3. The healing process of burn wounds. Four stages are commonly associated with wound healing: Hemostasis, inflammation, proliferation, and remodeling (created with [BioRender.com](https://www.biorender.com))

of autologous and allogeneic skin to the burn wound [33]. Although this repair method solves the problem of insufficient autologous skin sources, it is ineffective for extensive and deep burn wounds.

Microskin transplantation

Microskin transplantation refers to a surgical treatment method involving a mixture of autologous microskin and allogeneic skin for transplantation into burn wounds. It is mainly used to solve the problem of early coverage of extensive large and deep burn wounds. This technology has been widely used in China and has achieved good repair and treatment effects [34, 35]. However, relevant studies have shown that although this surgical treatment method can repair large-area burn wounds, the long-term effect on functional areas needs further research, and further clinical exploration is needed to improve this technology.

Large autologous skin transplantation

Repairing burn wounds in functional regions like the face, neck, and hands might be a challenge, but there is a solution: large autologous skin transplantation with maintained deteriorated dermis. Since 1999, it has been utilized in China. Improving the functionalities of a deteriorated dermis is possible despite the fact that it has diminished cell functions and disturbed tissue cell metabolism [36]. The dermis in this area is not necrotic. It is possible to restore its function and look by preserving it and covering it with autologous skin. According to research, the deteriorated dermis can be seen for up to seven days following surgery. After 2 months, the biomechanics were nearly identical to normal skin, and the

remaining deteriorated dermis's form and thickness went back to normal after 21 days. While the results from this technique have been excellent, more research is needed to clarify the mechanisms of scar-free development and the repair of cell molecules in the deteriorated dermis.

Skin flap transplantation

In the 1980s, skin flap transplantation was a common procedure. Repairing burns that have caused profound tissue damage is the primary purpose of this therapy. Avoiding amputation and restoring function to a burnt region are both made possible by skin grafting. Although it is ideal to undertake skin flap transplantation as soon as possible, the urgency of the procedure depends on the patient's burn status. Surgery must be performed without delay in the event that the blood vessel is injured [37]. It is important to take into account the degree of damage to the deep tissue from an infection and its function following a burn injury while dealing with injured tissue. Excision of infected or severely damaged tissue, including necrotic skin, muscles, and interstitial muscles, may be necessary. The circumstances at both the donor and recipient locations must be carefully evaluated before skin flap transplantation may be carried out [38]. For safe and effective surgery, it is important to adhere to basic criteria ranging from the most basic to the most complicated when choosing skin flaps; the best skin flaps come from the area immediately surrounding the burn.

Biomedical engineering technology. This innovation is a relatively new method of skin tissue manufacturing that has been gaining a lot of traction recently. Its introduction and use alleviate the need on autologous skin for repair treatments and

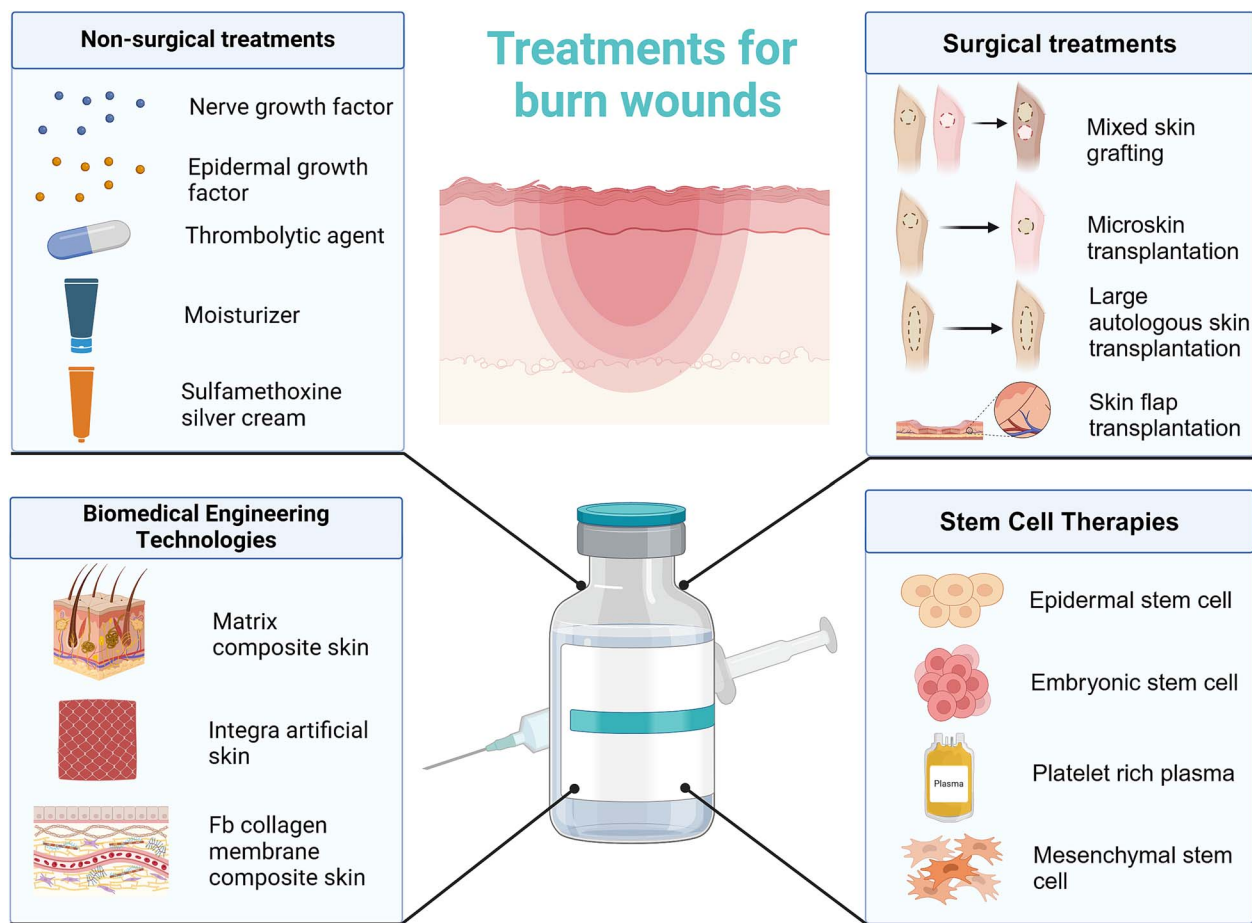


Figure 4. Treatments for burn wounds (created with BioRender.com)

streamline burn therapy [39]. The most critical thing is that severely injured skin may really have its structure and function restored. Many different kinds of tissue-engineered skin, such as matrix composite skin, integra artificial skin, Fb collagen membrane composite skin, etc., have recently entered clinical practice with the use of biomedical engineering technologies. The manufactured skin of these tissues has shown to be an effective treatment for burns. We anticipate that this technology will continue to advance, and that its skin restoration materials will eventually resemble natural skin even more closely, bringing the promise of restorative healing one step closer to fruition.

Stem cell therapy. Stem cell research idea has evolved over the past several years, and medicines based on this theory have been used more and more frequently. At this time, epidermal stem cell transplantation is the mainstay of stem cell treatment procedures utilized in clinical settings. Improved skin microenvironment and hair regeneration can be achieved by cell transplantation, which includes procedures such as embryonic stem cell transplantation, platelet-rich plasma transplantation, and epidermal stem cell transplantation. On the other hand, transplanting mesenchymal stem cells from bone marrow can speed up the healing process and lessen the likelihood of scarring [40, 41]. When implanted into a human body, embryonic stem cells have the capacity to proliferate indefinitely, which means they can aid in the survival and regeneration of injured nerves [42]. The field of stem cell treatment is continually evolving, and with it, come debates

around the use of these cells. Nevertheless, when targeted differentiation technology develops further, its dependability and safety will be greatly enhanced (Figure 4).

Extracellular vesicles

Natural membrane vesicles with a size of nanophotomicro-meters, or EVs, serve an essential role in cellular communication. Encased in a phospholipid bilayer, they can be actively secreted into the extracellular space by live cells or shed into the extracellular space by dying cells [43]. Various cell-released membrane structure subtypes are grouped together under the umbrella term "EVs." These subtypes include apoptotic bodies, microvesicles, exosomes, ectosomes, big dense core vesicles, membrane blebs, microparticles, oncosomes, prosta-somes, and many more [44]. Bring a wealth of bioactive substances. There are a number of mechanisms that control the complicated biological processes of EV production and secretion, which differ according on the cellular state and the surroundings. There is a wide range of size, composition, and function among secreted EVs [45]. Their importance in intercellular communication and information transfer cannot be overstated. In recent years, EVs have attracted extensive attention and research in biology, medicine, and drug delivery, highlighting the significance of your research in this field.

Origin and composition

EVs are secreted and formed by many types of cells, such as tumor, neural, and stem cells, and are widely found in various body fluids, such as plasma, saliva, breast milk, urine,

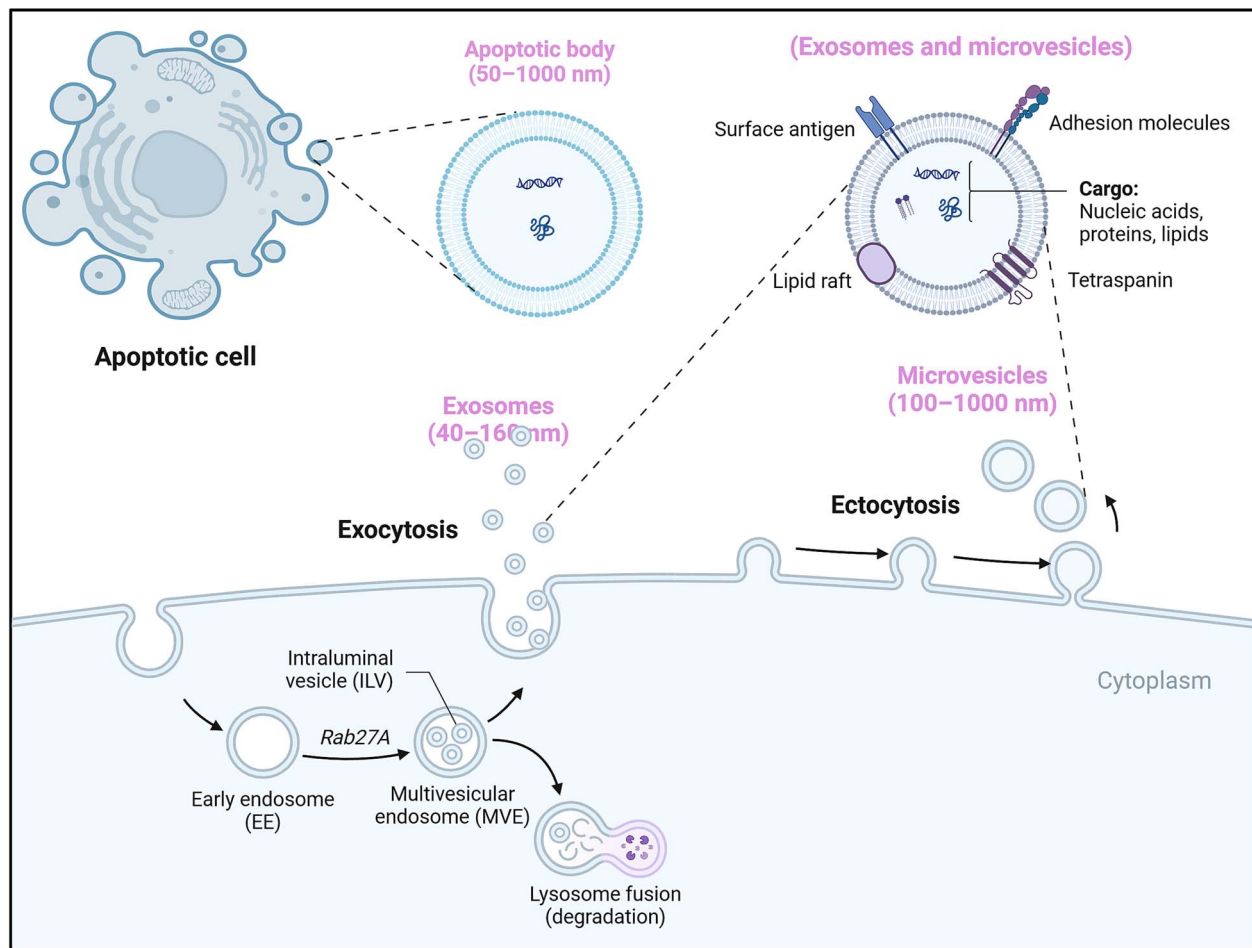


Figure 5. Schematic diagram of the biogenesis and composition of EVs (created with BioRender.com)

amniotic fluid, and ascites [46, 47]. Based on differences in the biological origin, size, density, and composition of EVs, they can be classified into three main categories: apoptotic vesicles, microvesicles (MVs), and exosomes. The International Society for Extracellular Vesicles (ISEV) has published a new document stating that small extracellular vesicles (sEVs) are a class of EVs 30–200 nm in diameter and that exosomes are the most abundant subtype of vesicles in sEVs [44]. Exosomes are generally 40–160 nm in diameter and are derived mainly from the endoplasmic reticulum and Golgi apparatus. These organelles undergo invagination to form intraluminal vesicles (ILVs), which form and gradually fuse into multivesicular bodies (MVBs) [48]. Finally, MVBs fuse with the cell membrane under the regulation of RABase, a family of GTPases, to form exosomes [49]. The membrane surface of exosomes contains specialized proteins released outside the cell by fusion with the cell membrane through the vesicle membrane. Exosomes are composed mainly of membrane proteins, lipid bilayers, and bioactive molecules encapsulated within the membrane. The lipid bilayer of exosomes contains a variety of components, such as cholesterol, diglycerides, sphingomyelin, ceramides, and phosphatidylcholine (PC) [50]. Microvesicles have a diameter of ~100–1000 nm and are formed when cells are stimulated by activation, stress, inflammation, or apoptosis, and the cell membrane swells to form protrusions that are eventually shed [51, 52].

The composition of microvesicles, including membrane proteins, lipid bilayers, and some intracellular components, is

similar to that of exosomes. However, the membrane composition of microvesicles may be enriched with more components, such as PC. Apoptotic vesicles are ~50 nm to 2000 nm in diameter and are small vesicles formed during apoptosis. Apoptosis is a controlled cell death process in which cells are actively involved and closely linked to physiological and developmental processes. In the later stages of the apoptotic process, fragments of the nucleus and cytoplasm are encapsulated in membranes to form apoptotic vesicles. Apoptotic vesicles contain fragments of damaged apoptotic cells, such as organelles, cell membrane fragments, and remnants of the cytoplasm and nucleus [53]. Like vesicles, apoptotic vesicles are usually encapsulated by membranes, and their outer membranes often contain phospholipids and membrane proteins. Exosomes are the most abundant and contain a variety of biologically active components, such as nucleic acids (gDNA, mtDNA, mRNA, miRNA, circRNA, etc.), proteins, lipids, and metabolites [54] (Figure 5).

Biological functions

Various bioactive molecules, such as mRNAs, microRNAs (miRNAs), cytokines, chemokines, and immunomodulatory factors, are enriched in EVs [55–57]. The active substances contained in EVs from different sources are different, and EVs generally have cell species characteristics compatible with the cells from which they are derived. EVs from regular cell sources can remove metabolic waste and mediate intercellular information transduction. EVs secreted by specialized cells

can perform specific biological functions; for example, EVs of antigen-presenting cell origin have more histocompatibility complexes. EVs secreted by tumor cells have immunological marker functions [58]. Moreover, including EVs can also reflect physiological and pathological changes in the cells of origin [59]. EVs can exert their biological effects by mediating intercellular communication. EVs mainly communicate with cells in three ways: directly recognizing target cells, fusing with the target cell membrane, and releasing inclusions to recognize the target cells [13]. Recently, EVs have been shown to play important roles in various physiopathological processes, such as intercellular information transduction, immune regulation, inflammation suppression, and maintenance of tissue homeostasis [60, 61]. EVs mediate local and intersystemic communication and signaling through the proteins, RNAs, and DNAs they carry, which deliver information to target cells [62, 63]. Studies have shown that EVs carry specific cellular components that make their functions complex and diverse and have a certain degree of targeting ability. Specific protein molecules on the surface of EVs can directly activate receptors on the surface of target cells, generate signaling complexes, and activate intracellular signaling pathways, including membrane transporter proteins and membrane fusion proteins (RAB, flotillin), heat shock proteins (HSP60, HSP70, and HSP90), tetrameric cross-linking proteins (HSP60, HSP70, and HSP90), and members of the four-molecule crosslinker family (CD9, CD63, and CD81) [64].

EVs in burn injury

Burns are among the most common causes of traumatic tissue injury and can disrupt the skin barrier, resulting in symptoms such as redness, blistering, pain, and, in severe cases, the involvement of subcutaneous tissue, muscle, and bone tissue [23]. The treatment of burn wounds is the key to improving burn injuries. Increasing evidence has revealed that EVs are promising therapeutics and drug delivery platforms [65]. Circulating EVs in serum or plasma serve as biomarkers and mediators of the burn healing process or postburn response. Many studies have shown that EVs from different sources have promising therapeutic effects on burn wound repair, with sEVs derived from mesenchymal stem cells (MSC-sEVs) being the most studied. MSC-EVs are promising candidates for facilitating and accelerating burn wound healing. How do EVs from different sources participate in burn injury repair? What is the mechanism by which EVs function? To explore the role of EVs in burn wounds and to provide a clinical basis and ideas for diagnosing and treating burn wounds (Table 1).

EVs as biomarkers for burn injury

Research into and reports of EVs as diagnostic indicators for a range of disorders are extensive, due to the fact that they carry a number of physiologically active nucleic acids or proteins. Once released into circulation, EVs translocate cargo contents and carry out pleiotropic tasks, reflecting the physiological state of the parent cells in their generation and molecular composition. Therefore, circulating EVs serve as both indicators of illness and mediators of damage responses. As an example, sEVs may be a novel tool for the therapeutic treatment of cancer patients and noninvasive melanoma indicators due to their plasma expression of CD63 and caveolin-1 [66]. Amniotic fluid sEVs with messenger RNA (mRNA) expressing the zinc finger protein ZFY can also be utilized for fetal sex determination [67]. Endometrial

cancer (EC) is associated with increased levels of plasma-derived EV miR-15a-5p, miR-106b-5p, and miR-107 [68]. In order to create a model for metastatic risk scores, Sun et al. [69] extracted miR-21, miR-451, and miR-636 from prostate cancer patients' urine EVs. Compared to the preoperative PSA level and clinical Gleason score, the model's ability to forecast metastasis was much higher. Early gastric cancer diagnosis and disease progression monitoring using circulating EV lncRNA-GC1 has been demonstrated. With a specificity of 84.97% and a sensitivity of 84.77%, lncRNA-GC1 outperforms CEA, CA72-4, and CA19-9 [70]. Gastric cancer (GC) patients are likely to have circSHKBP1 and circATP8A1 as diagnostic and prognostic biomarkers in their blood-derived EVs. Elevated levels of these proteins are linked to advanced TNM stage and poor prognosis [71, 72].

EVs can also reflect the degree and severity of burn progression. Studies have shown that exosomes stimulate dermal fibroblast proliferation and apoptosis in the serum of both burn patients and healthy individuals, suggesting that exosome profiles may be important signals affecting burn wound healing. However, further studies are needed to understand how exosomes regulate fibroblast wound healing [73]. Burn injury significantly increases the number of circulating EVs. It promotes the production of EVs containing unique cargo proteins in a time-dependent manner, a response that correlates with injury severity and worsening clinical outcomes. Burn-derived EVs function act as potent mediators that reduce endothelial barrier resistance and compromise junctional integrity, a pathophysiological process that underlies burn-related tissue dysfunction. Therefore, further in-depth characterization of circulating EVs will contribute to developing new prognostic tools or therapeutic targets for advanced burn care [74]. Circulating burn-induced EVs may also modulate inflammation, and studies have demonstrated that plasma EVs released after severe burn injury modulate the macrophage phenotype and function [75]. S100A9-containing serum exosomes obtained from burn injury patients promote myocardial cell pyroptosis through NLRP3 [76] and promote the permeability of pulmonary microvascular endothelial cells through the zonula occludens (ZO-1) and occludin proteins and the p38 signaling pathway [77]. In addition, platelet-activating factors and microvesicle particles are potential mediators of the toxicity associated with intoxicated thermal burn injury [78].

Changes in the contents of EVs may explain the use of EVs as biomarkers for burn injury. On the one hand, burn injury causes changes in miRNAs in EVs. Next-generation sequencing of plasma exosome-derived miRNAs and target gene analysis of a microarray database of thermally injured skin samples revealed key miRNA-mRNA axes involved in potential blood-tissue interactions at the early burn stage, suggesting that plasma exosome-derived miRNAs may impact the alteration patterns of gene expression in burn wounds [79]. EVs were extracted from the serum of rats with severe burns (30% of the total body surface area, III°, the miRNA profiles of EVs in severe burns during the shock stage). There are significant differences between the downregulated and upregulated genes [80].

On the other hand, burn injury caused changes in specific proteins in EVs, and gene ontology analysis of serum exosomes from burn patients revealed that the ITGA2B and ITGB3 proteins were significantly upregulated in the burn group and were involved mainly in the PI3K/AKT signaling

Table 1. Roles of extracellular vesicles from different sources in burn injury

EV sources	Model diseases	Cargoes	Target genes pathways	Effects	Ref.
Serum exosomes	Burn injury	–	–	Stimulated dermal fibroblast proliferation and apoptosis	[66]
Plasma EVs	–	ZO-1	–	Reduced endothelial barrier resistance and impaired junction integrity	[67]
Plasma EVs	Severe burn injury	HMGB1, IL-1 β	–	Modulated macrophage phenotype and function	[68]
S100A9-containing serum exosomes	–	–	NLRP3	Promoted myocardial cell pyroptosis through NLRP3	[69]
S100A9-containing serum exosomes	–	S100A9	ZO-1 and occludin, p38 signaling	Promoted permeability of pulmonary microvascular endothelial cells	[70]
MVP	Thermal burn injury	PAF	–	Induced immunosuppression	[71]
Plasma exosomes	–	miRNA	–	Key miRNA–mRNA axes in potential blood-to-tissue interactions at early burn stage	[72]
Serum EVs	–	miRNA	–	The miRNA profiles of EVs in severe burns during the shock stage	[73]
Serum exosomes	–	ITGA2B and ITGB3	PI3K/AKT signaling pathway	Participated in injury repair	[74]
Plasma MVs	–	HMGB1/IL-1 β	–	Modulated immune responses and MVs are identified as a novel reservoir	[75]
Early mice	–	CRP and SAA1	–	Promoted proinflammatory responses in cultured splenic macrophages	[76]
BMSC-Exos	Corneal wound	–	p44/42 MAPK pathway	Promoted the proliferation and migration of HCEs and inhibited alkali burn-induced inflammation, fibrosis, and vascularization	[77]
ADSC-Exos	Cornea alkali burns	miRNA 24-3p	–	Accelerated corneal epithelial defect healing, and reduced corneal stromal fibrosis and macrophage activation	[78]
ADSCs-Exos	Burns	Circ_0075932	–	Exhibited burn-associated infection in obese rats by suppressing the expression of circ_0075932	[79]
BMSC-EVs	Burn-induced corneal damage	–	–	Modulate cell death, inflammation, and angiogenic programs	[80]
HucMSC-Ex	Deep second-degree burn injury	Angiopoietin-2 (Ang-2)	–	Accelerated skin wound healing and angiogenesis	[81]
HucMSC-Ex	Deep second-degree burn rats	14–3–3 ζ	Wnt/ β -catenin signaling	Promoted skin injury repair by Wnt/ β -catenin signaling, and modulated YAP to orchestrate controlled cutaneous regeneration	[82]
HucMSC-Ex	Burn-induced excessive inflammation	miR-181c	TLR4 signaling pathway	Alleviated inflammation factors and inflammatory reaction in burned rats	[83]
hMenSC-sEVs	Third-degree burn	–	–	Enhanced wound closure and increasing neoangiogenesis	[84]
iPSCs-MVs	Deep second-degree burn	miR-16-5p	p38/MARK pathway	Promoted migration of keratinocytes	[85]
MSC-MVs and PRP	Murine burn wound	miRNA203	–	Improved burn wound healing by regulating scar formation and antioxidant mechanism	[86]
HucMSC-Ex	Burn-induced ALI	miR-451	TLR4/NF- κ B pathway	Decreased inflammation in rats after burn	[87]
HucMSC-Ex	Burn-induced ALI	miR-451	PI3K/AKT signaling pathway	Alleviated ALI by modulating macrophage M2 polarization by regulating the MIF-PI3K-AKT signaling pathway	[88]
iPSCs-KCs-Exos	Deep second-degree burn	miR-762	ITGB1	Mediated migration of keratinocytes and endothelial cells	[90]
HTSF-Ex	–	–	–	Altered the molecular pattern of NHKs, and EMT markers differently	[91]
hAEC-EVs	Corneal alkali burns	–	Focal adhesion signaling pathway	Promoted ECM reorganization and cell adhesion by activating the focal adhesion signaling pathway	[92]
LF-FVs	A rat burn model	–	–	Improved the quality of wound healing, and decreased scar formation	[93]
AVpNVs	Burn wounds	–	–	Diminished myofibroblast differentiation and contraction	[100]
S. eongatus	Burn wounds	–	IL-6	Promoted angiogenesis and wound healing by regulating IL-6 expression	[105]

Table 2. Similarities and differences between EVs of different origins

Sources	Similarities	Differences	Effects on wound healing	Limitations
Stem cells	Size distribution Surface charge Surface morphology Content composition	High safety Low immunogenicity Low tumorigenicity Easy storage and transportation	Improved granulation tissue [89, 90] Promoted the formation of new blood vessels [89] Promoted re-epithelialization [91] Reduced scar formation [90]	Quality standards and control Translation of clinical research Supervision of EVs products
Skin cells		Real participants More likely to express intercellular communication receptors	Promoted keratinocyte and endothelial cell migration [99] Promoted wound healing	
hAECs		Widely used to treat corneal alkali burn and other ocular surface diseases [106]	Antibacterial Anti-inflammation Promoted wound healing and re-epithelialization Reduced scar formation [103–105]	
Plants		From edible plants, the source is abundant [118] The membrane bilayer is composed of PA, PC, DGDG, and MGDG Easier to produce on a large scale High stability low immunogenicity [119]	Promoted transdermal absorption Sustained drug release system Antioxidation Inhibited oxidative stress Anti-aging Anti-inflammation Inhibited scar formation [111–115]	
<i>Synechococcus elongatus</i> PCC 7942		Prokaryotic origin Easy to obtain Scalable at large scale [120]	Promoted angiogenesis of the wound	

pathway [81]. One study revealed that HMGB1 was enriched in plasma microvesicles after considerable burn injury (LBI), modulating immune responses and that microvesicles are a novel reservoir for these immune mediators [82]. In a sample of 50 patients with significant burn injuries, EV SAA1 and CRP were correlated with TBSA injury in both sexes. They were correlated with the length of hospital stay in women, suggesting that EVs are drivers of immune responses after burn injury and that their content may predict the hospital course [83]. These findings suggest that EVs possess diagnostic and prognostic potential.

EVs derived from mammalian cells in burn injury

Burns, especially severe burns, in which skin function is not fully restored by the normal wound healing process, are often accompanied by immune and inflammatory responses, which can lead to potentially serious complications such as infections, cardiac arrhythmias, electrolyte disturbances, and shock [23]. Therefore, completing wound healing with minimal delay after injury is crucial. Stem cells, especially mesenchymal stem cells, have broad clinical applications in skin tissue regeneration because of their wide range of sources, easy *in vitro* expansion, and low rejection after *in vivo* transplantation (Figure 6).

EVs derived from stem cells in burn injury

Stem cell therapy can promote burn wound healing and effectively inhibit the inflammatory response through cytokines secreted by stem cells, promote the recovery of skin function, and create a suitable repair microenvironment. Several studies have shown that stem cell-derived EVs can promote burn wound healing. For example, exosomes from bone marrow-derived mesenchymal stem cells (BMSCs) facilitate corneal wound healing by regulating the p44/42 MAPK pathway [84] (Figure 7a). miRNA 24-3p-rich adipose-derived mesenchymal stem cell-derived exosome-functionalized DEGMA-modified hyaluronic acid hydrogels for cornea alkali burn wound

healing [85] (Figure 7b). In addition, oxaliplatin-derived stem cell (ADSC)-exo-lenti_circrna_0075932 shRNA exhibited a therapeutic effect on burn-associated infection in obese rats by suppressing the expression of circ_0075932 [86]. Bone marrow-derived MSC-derived EVs (BMSC-EVs) modulate cell death, inflammation, and angiogenesis in injured tissue, thus leading to faster recovery in a murine model of alkali burn-induced corneal damage [87] (Figure 7c). Human umbilical cord MSCs (HucMSCs) and their HucMSC-derived exosomes (HucMSC-Ex) accelerated deep second-degree burn wound healing and angiogenesis in a rat model, resulting in a greater wound closure rate and increased expression of CD31 [88] (Figure 7d). Components are abundant components within EVs, and protein components in EVs can play significant roles at different stages of tissue damage repair, at different subcellular levels during the same stage, and often work together through multiple components [12]. Studies have shown that hucMSC-Exs function not only as an "accelerator" of Wnt/ β -catenin signaling to repair damaged skin tissue but also as "brakes" of signaling by modulating YAP to orchestrate controlled cutaneous regeneration in a deep second-degree burn rat model [89] (Figure 7e). In addition, hucMSC-Exs play a pivotal role in regulating inflammation and can mediate the miR-181c-mediated attenuation of burn-induced excessive inflammation [90] (Figure 7f). Human menstrual blood-derived mesenchymal stem cell (MenSC) sEVs can enhance wound closure and increase neoangiogenesis in a mouse model of third-degree burn [91] (Figure 7g). In addition, induced pluripotent stem cell-derived microvesicles accelerate deep second-degree burn wound healing in mice through the miR-16-5p-mediated promotion of keratinocyte migration [92] (Figure 7h). MVs from MSCs with platelet-rich plasma (PRP) significantly downregulated miRNA203 and alpha-smooth muscle actin gene expression and collagen types I and III. They upregulated the antioxidant Nrf2 and other mechanisms regulating scar formation and antioxidant mechanisms to promote burn wound healing

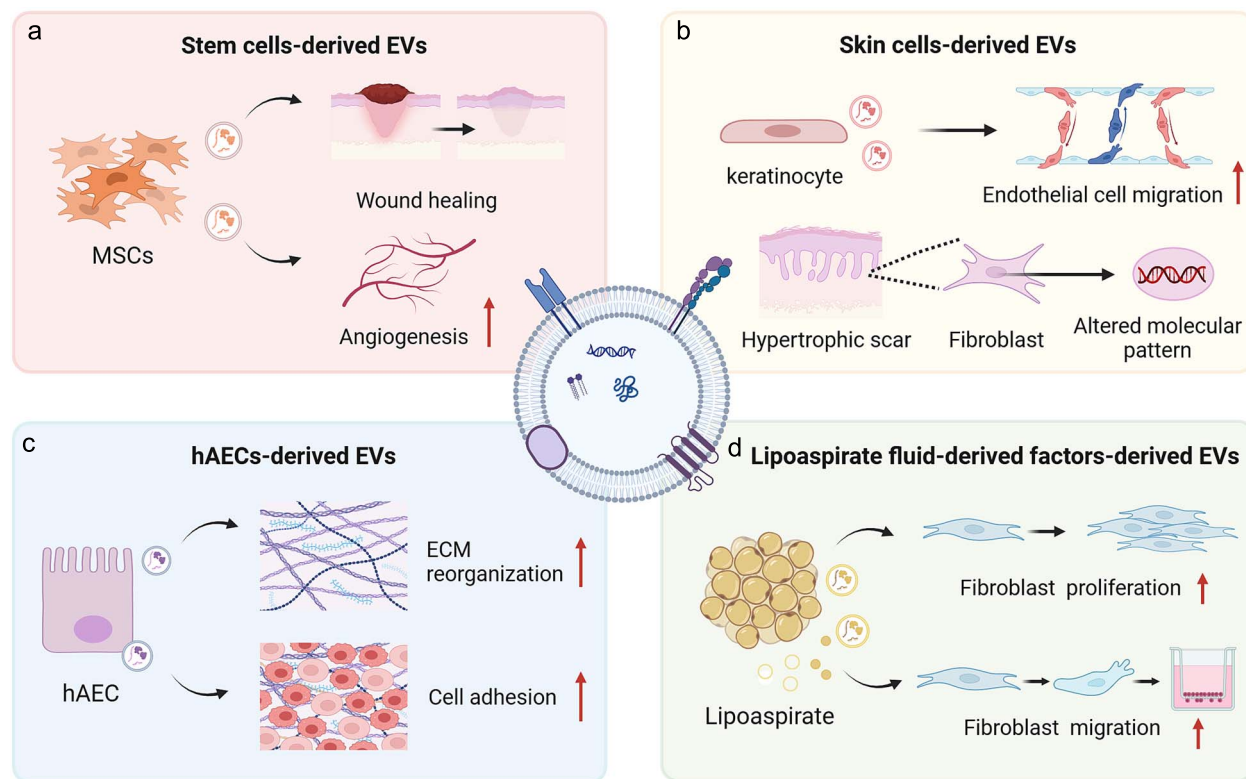


Figure 6. EVs derived from mammalian cells in burn injury(created with BioRender.com). (a) Stem cell derived EVs facilitate wound healing and angiogenesis. (b) Skin cell-derived EVs promote keratinocyte and endothelial cell migration and modulate the molecular patterns of fibroblasts. (c) hAEC-derived EVs promote ECM reorganization and cell adhesion. (d) Lipoaspirate fluid-derived factors and EVs augment the proliferation and migration of fibroblasts

[93]. Studies have demonstrated that MSC-EVs can also treat burn-induced acute lung injury (ALI), and exosomal miR-451 from hucMSCs attenuates ALI via the TLR4/NF- κ B pathway [94]. HucMSCs-Ex alleviated ALI by modulating macrophage M2 polarization by regulating the MIF-PI3K-AKT signaling pathway [95]. These findings suggest that EVs from MSCs play an essential role in burn wound healing.

MSC-based therapy is safe [96, 97] and can improve granulation [96, 97], promote neovascularization and re-epithelialization [96, 98], reduce scar formation [97], and reduce side effects (pain, infection, and blisters). MSC-derived EVs can secrete various bioactive molecules, including cytokines, chemokines, growth factors, and lipid mediators [99], and can regulate the response of surrounding cells [100]. As a new type of cell-free therapy, MSC-EVs are highly safe [56, 101]. In addition, MSC-EVs can prevent tumorigenicity, immune compatibility, infection transmission, complex material storage, and other problems caused by cell transplantation [102]. Therefore, it has broad application prospects in the treatment of burn wounds.

EVs derived from skin cells in burn injury

In the repair process, different cell populations cooperate to promote the coordination of individual events and temporary and spatial control [103]. Monocytes, platelets, inflammatory, epidermal, and dermal cells interact to promote wound healing. These cells are interconnected with the production of various cytokines and regulatory growth factors [104], fight infection at the injury site, form new tissues, and ultimately lead to wound closure [105]. Exosomes from human-induced

pluripotent stem cell-derived keratinocytes accelerate burn wound healing through miR-762-mediated promotion of keratinocyte and endothelial cell migration [106] (Figure 8a). Exosomes derived from hypertrophic scar fibroblasts (HTSFs) differentially altered proliferation, activation, differentiation, apoptosis, and proliferation/differentiation regulators of NHKs and EMT markers [107] (Figure 8b). EVs from skin cells or tissues play unique roles in burn wound repair. These findings better reflect the cross-talk between cells in the wound-healing environment. Further research will aid in understanding their contribution to wound healing in more detail and may explain the different clinical outcomes.

Compared with MSCs, MSCs can act as "external assistance," and these cells are "real participants". In the complex microenvironment of wound healing, skin tissue EVs are more likely to express receptors for intercellular communication. Due to their different sources, EVs may have different characteristic molecular expression profiles and can act selectively on different target cells. For example, exosomes expressing the transmembrane surface protein network tspan8-alpha4 are more likely to enter endothelial and pancreatic cells [108]. Exosomes expressing CD47 are not quickly taken up by monocytes [109]. Skin cell-derived EVs and MSC-derived EVs may have different molecular expression profiles. It is speculated that EVs derived from specific skin cells may selectively act on target cells.

EVs derived from other burn injury sources

EVs have diverse sources; in addition to MSCs, specific skin cells and other cellular or humoral sources of EVs also have

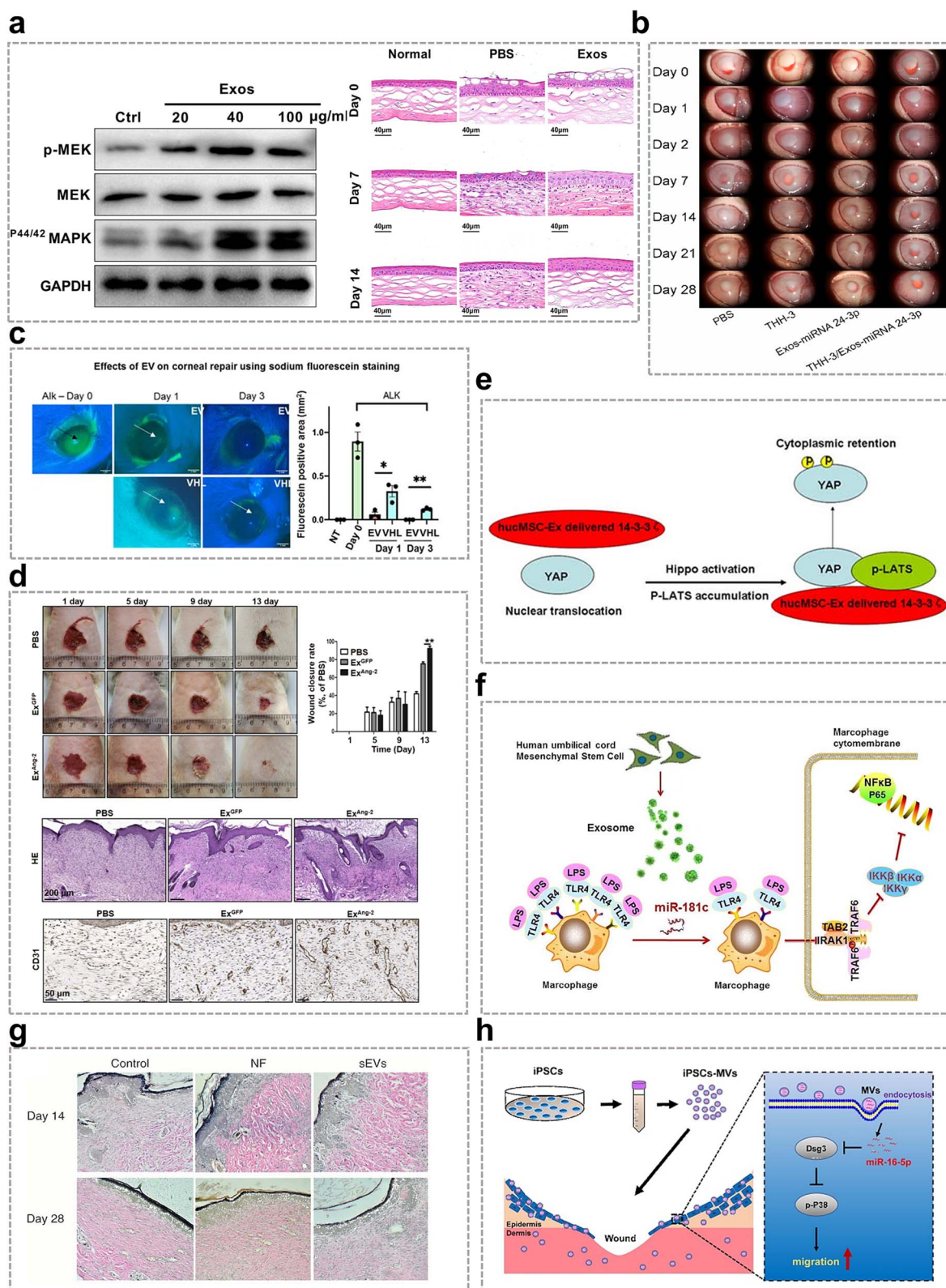


Figure 7. EVs derived from stem cells in burn injury. **(a)** EVs derived from BMSCs. Reproduced with permission from ref. [84]. Copyright 2022, springer. **(b)** EVs are derived from adipose-derived mesenchymal stem cells. Reproduced with permission from ref. [85]. Copyright 2022, Elsevier. **(c)** EVs derived from BMSCs. Reproduced with permission from ref. [87]. Copyright 2022, MDPI. **(d)** EVs derived from HucMSCs. Reproduced with permission from ref. [88]. Copyright 2020, springer. **(e)** EVs derived from HucMSCs. Reproduced with permission from ref. [89]. Copyright 2016, Oxford university press. **(f)** EVs derived from HucMSCs. Reproduced with permission from ref. [90]. Copyright 2016, Elsevier. **(g)** EVs are derived from adipose tissue versus stem cells. Reproduced with permission from ref. [91]. Copyright 2021, Taylor & Francis. **(h)** EVs derived from induced pluripotent stem cells. Reproduced with permission from ref. [92]. Copyright 2020, Ivyspring international Publisher

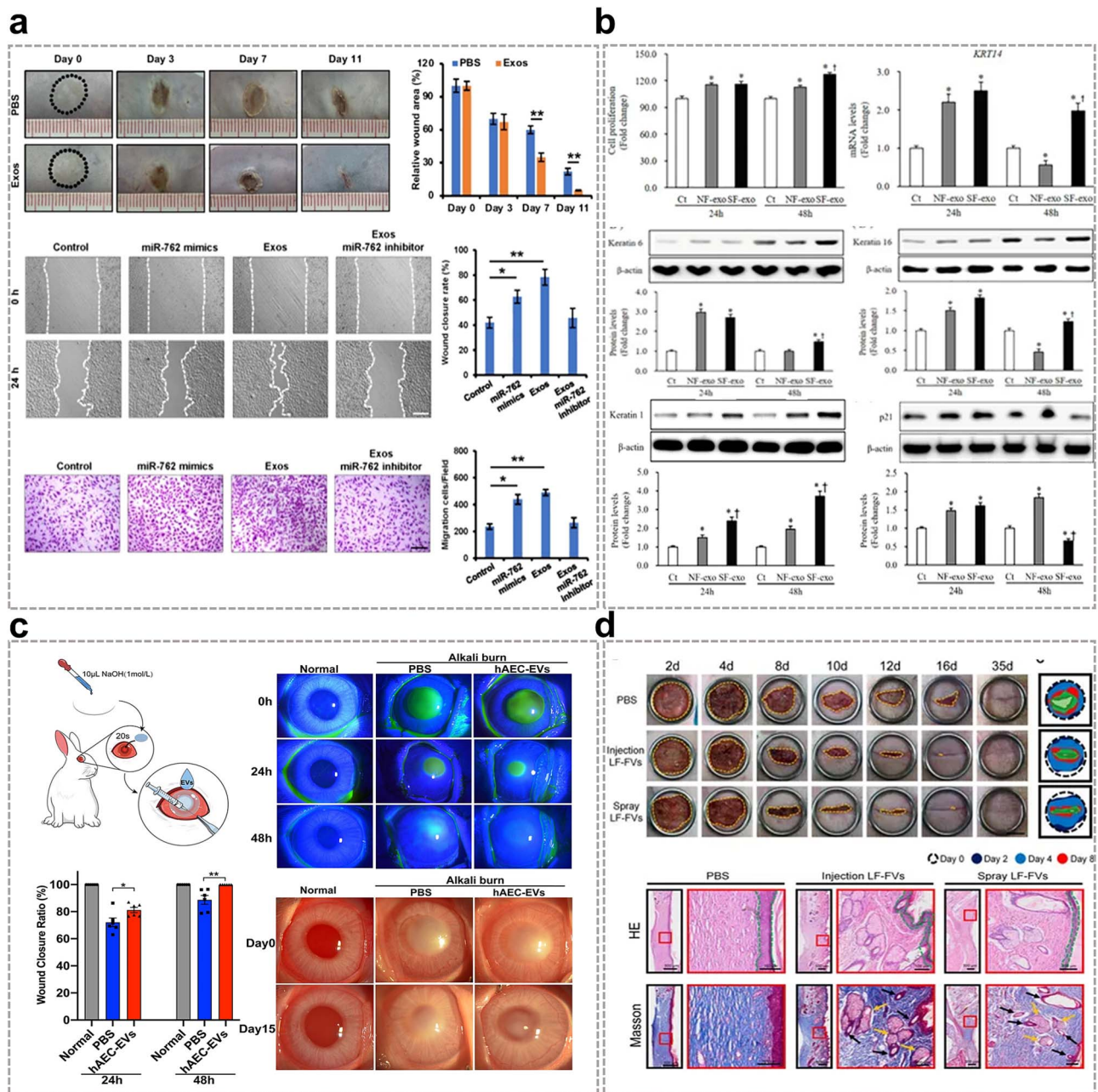


Figure 8. EVs are derived from skin cells and other burn injury sources. **(a)** EVs are derived from human induced pluripotent stem cells. Reproduced with permission from ref. [106]. Copyright 2022, springer nature. **(b)** EVs are derived from adipose-derived mesenchymal stem cells. Reproduced with permission from ref. [107]. Copyright 2023, MDPI. **(c)** EVs are derived from hypertrophic scar fibroblasts. Reproduced with permission from ref. [114]. Copyright 2023, Frontiers media. **(d)** EVs derived from lipoaspirate fluid. Reproduced with permission from ref. [115]. Copyright 2023, sage

reparative effects on burn wounds. Many studies have shown that the human amniotic membrane secretes a series of growth factors and immunosuppressive factors. The human amniotic membrane also has antibacterial and anti-inflammatory properties [110, 111], promotes wound healing and re-epithelialization [112], and reduces scar formation. Amniotic membrane transplantation is widely used to treat ocular surface diseases, including corneal alkali burns, and amniotic membrane patch transplantation is one of the most effective methods for treating corneal trauma [113]. Human amniotic epithelial cells (hAECs) have the characteristics of embryonic stem cells, such as proliferation and differentiation, immune regulatory functions, and easy access. They have shown

sufficient effectiveness in treating lung injury, liver injury, and ocular surface diseases in animals and have no hemolytic reactions, toxicity, or allergic reactions, with systemic safety. In skin injury, hAEC-EVs can increase the migration and proliferation of fibroblasts, inhibit the excessive deposition of collagen, induce the formation of orderly collagen fibers, and reduce scar formation, thus promoting skin wound healing. A previous study indicated that human amniotic epithelial cell-derived extracellular vesicles (hAEC-EVs) promoted ECM reorganization and cell adhesion of target tissues or cells via orderly activation of the focal adhesion signaling pathway [114] (Figure 8c). Lipoaspirate fluid-derived factors and EVs augment the proliferation and

migration of fibroblasts and accelerate wound healing in a rat burn model [115] (Figure 8d). However, more EVs from different sources have yet to be discovered and studied in burn wounds.

EVs derived from plants with burn injury

EVs can be derived not only from animal cells but also from plant cells. Plant cells secrete membrane-enclosed micrometer- and nanometer-sized vesicles in response to various biotic and abiotic environmental stresses (e.g. pathogen infection and attack). Like EVs released by mammals or bacteria, plant-derived vesicles are rich in composition, carry complex proteins, nucleic acids, lipids, and other macromolecules, and are an important storage form for a wide range of active substances (e.g. vitamins, minerals, plant polysaccharides, and secondary metabolites) [116]. Plant-derived vesicles play significant regulatory roles in the field of regenerative medicine. Studies have shown that wheat-derived exosomes can promote wound repair [117, 118] (Figure 9a). Ginseng-derived exosomes can ameliorate the senescence phenotype of human skin fibroblasts by downregulating senescence-associated molecules and melanogenesis-associated proteins as well as pigmentation phenotypes associated with the senescence of human melanocytes exposed to ultraviolet radiation [119] (Figure 9b). Exosomes derived from *aloe vera* can upregulate the mRNA expression of Nrf2, HO-1, CAT, and SOD, indicating potential antioxidant properties [120] (Figure 9c). Strawberry juice is rich in anthocyanins, folic acid, vitamin C, and other components, and exosomes derived from strawberries can inhibit cellular oxidative stress [121] (Figure 9d). *Aloe vera* peel-derived nanovesicles (AVpNVs) act as inflammatory modulators while inhibiting myofibroblast differentiation and contraction, and new strategies for burn wound treatment seek to reduce scarring at the cellular and molecular levels during the early stages of wound healing, making AVpNVs promising candidates for future plant vesicle-based therapies [122] (Figure 9e).

Like animal-derived EVs, plant-derived EVs have a similar size distribution, surface charge, surface morphology, content composition, etc. Vesicles contain RNA, proteins, lipids, and other molecules that regulate physiological processes. They can serve as a transport carrier and have biological functions [123, 124]. However, they are also different. One difference is that plant EVs can be extracted from many edible plants, and the source is sufficient.

In contrast, the extraction of animal-derived EVs is time-consuming and laborious. The second difference lies in the different compositions of the lipid bilayer. The bilayer of animal-derived EVs is composed of cholesterol, glycolipids, ceramide, and phosphatidylserine, whereas that of plant-derived EVs is composed of phosphatidic acid (PA), PC, di-galactosyl diacylglycerol (DGDG), and mono-galactose diacylglycerol (MGDG). Phosphatidic acid can target and stimulate mTOR, thereby regulating autophagy in animal cells. In addition, plant-derived EVs are more straightforward to produce on a large scale, with high stability and low immunogenicity [125, 126] (Table 2).

EVs derived from *Synechococcus elongatus* in burn injury

S. elongatus PCC 7942 is a naturally occurring photoautotrophic cyanobacterium that is easy to obtain and amplify on a large scale. *Synechococcus elongatus* PCC 7942 is the

simplest prokaryotic clock model organism, and studying its clonal gene expression pattern is of great scientific importance [127]. Studies have shown that this cyanobacterium has a better therapeutic effect on acutely burned mice, and the secretion of EVs was a key mechanism in the cyanobacterium-induced regulation of wound healing. *Synechococcus elongatus* PCC 7942 significantly promoted angiogenesis and burn wound repair in mice under light and dark conditions, mainly through delivering functional EVs but not through their photosynthetic activity. Promoting IL-6 expression may be one of the mechanisms underlying the proangiogenic and wound-healing effects induced by cyanobacteria and their EVs [128] (Figure 9f). These findings suggest that *Synechococcus elongatus* PCC 7942 is a potentially promising strategy for therapeutic angiogenesis and wound healing (Figure 10).

Open questions and future directions

This review discusses the effects of EVs from diverse sources, such as plasma, serum, MSCs, skin cells, plants, and *Synechococcus elongatus*, on burn wound repair. Circulating EVs in plasma or serum are biomarkers for burn injuries in the specific context of burn injuries and are closely related to burn condition and healing. EVs from different sources effectively repair burn wounds and provide new ideas and methods for treating burn wounds. EVs also have therapeutic effects on postburn complications, such as ALI. In addition, tailoring EVs and apoptotic extracellular vesicles (apoEVs) may be new therapeutic strategies for burn injury. However, many challenges and opportunities exist from bench to bedside (Figure 11).

Tailoring EVs as new therapeutics for burn injury

Enhancing the wound-healing effect of EVs is essential for expanding their clinical application, improving their cost-effectiveness, and promoting individualized drug use. The modification of EVs in skin wound treatment is considered mainly for bioengineering modifications and the combination of EVs with biomaterials [129–131]. According to the different stages of bioengineering, EVs can be divided into pre-engineered EVs obtained via parental cell treatment and post-engineered EVs obtained via self-modification. In addition, the combination of EVs and biomaterials provides a platform for optimizing the interaction between EVs and the surrounding microenvironment [132].

Pre-engineered EVs. The pre-engineering strategy was the earliest engineering strategy. Its main goal is to hijack the biosynthetic process of cells through gene manipulation technology to produce functional EVs. Plasmids and viral vectors are standard molecular biology tools used to express genes of interest in target cells. This method promotes the overexpression of proteins, nucleic acids, and other molecules in donor cells' interior or membrane surface. These molecules can subsequently be encapsulated inside or outside EVs via a molecular synthesis mechanism [133, 134]. For example, EVs secreted by bone marrow MSCs overexpressing TSG-6 significantly enhanced anti-inflammatory capacity and attenuated the formation of pathological scar injuries [135]. ADSC-EVs significantly promoted granulation tissue formation and angiogenesis and markedly facilitated wound healing [136]. The lncRNA Hox transcription antisense RNA (HOTAIR) can promote angiogenesis in various

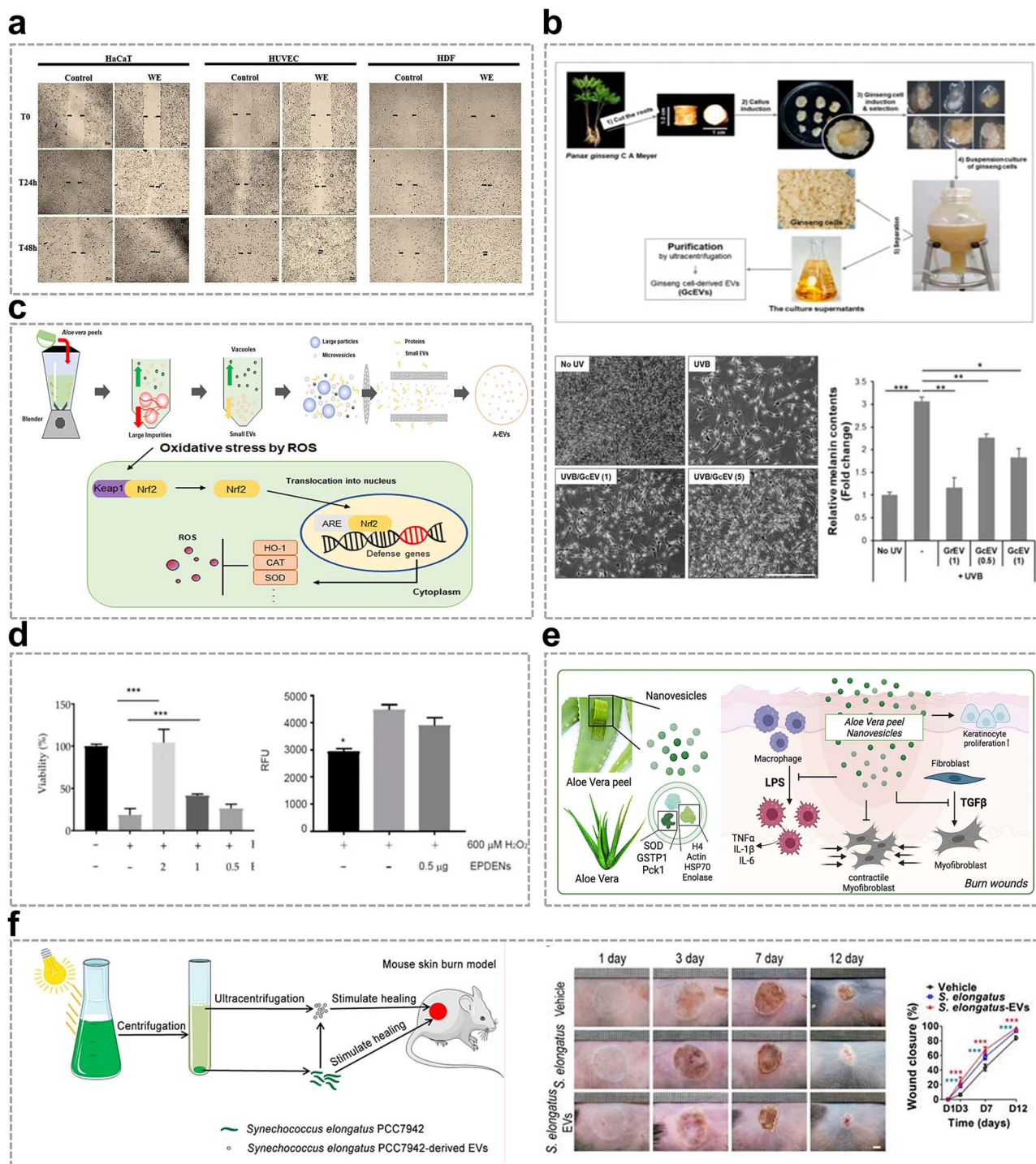


Figure 9. EVs are derived from skin cells and other burn injury sources. (a) EVs are derived from wheat. Reproduced with permission from ref. [117]. Copyright 2018, springer. (b) EVs are derived from *Panax ginseng*. Reproduced with permission from ref. [119]. Copyright 2021, MDPI. (c) EVs are derived from *aloe vera* peels. Reproduced with permission from ref. [120]. Copyright 2021, springer. (d) EVs are derived from strawberries. Reproduced with permission from ref. [121]. Copyright 2021, MDPI. (e) EVs are derived from *aloe vera* peels. Reproduced with permission from ref. [122]. Copyright 2024, Elsevier. (f) EVs derived from *Synechococcus elongatus* PCC7942. Reproduced with permission from ref. [128]. Copyright 2019, Ilyspring international Publisher

environments. The content of HOTAIR in EVs derived from MSCs transfected with PCMV increased, effectively promoting promoted angiogenesis and improved wound healing in diabetic (db/db) mice [137]. Although genetically engineered cells are a feasible method to improve the loading of specific molecules into EVs, they carry the risk of changing

genetic information, which may lead to unpredictable changes in the cargo composition of EVs. Therefore, altered EVs may lead to poor or potentially harmful molecule delivery to target cells, leading to tumors or other adverse outcomes. Therefore, safety issues, especially the clinical application of EVs, represent a significant concern in genetic engineering. Future

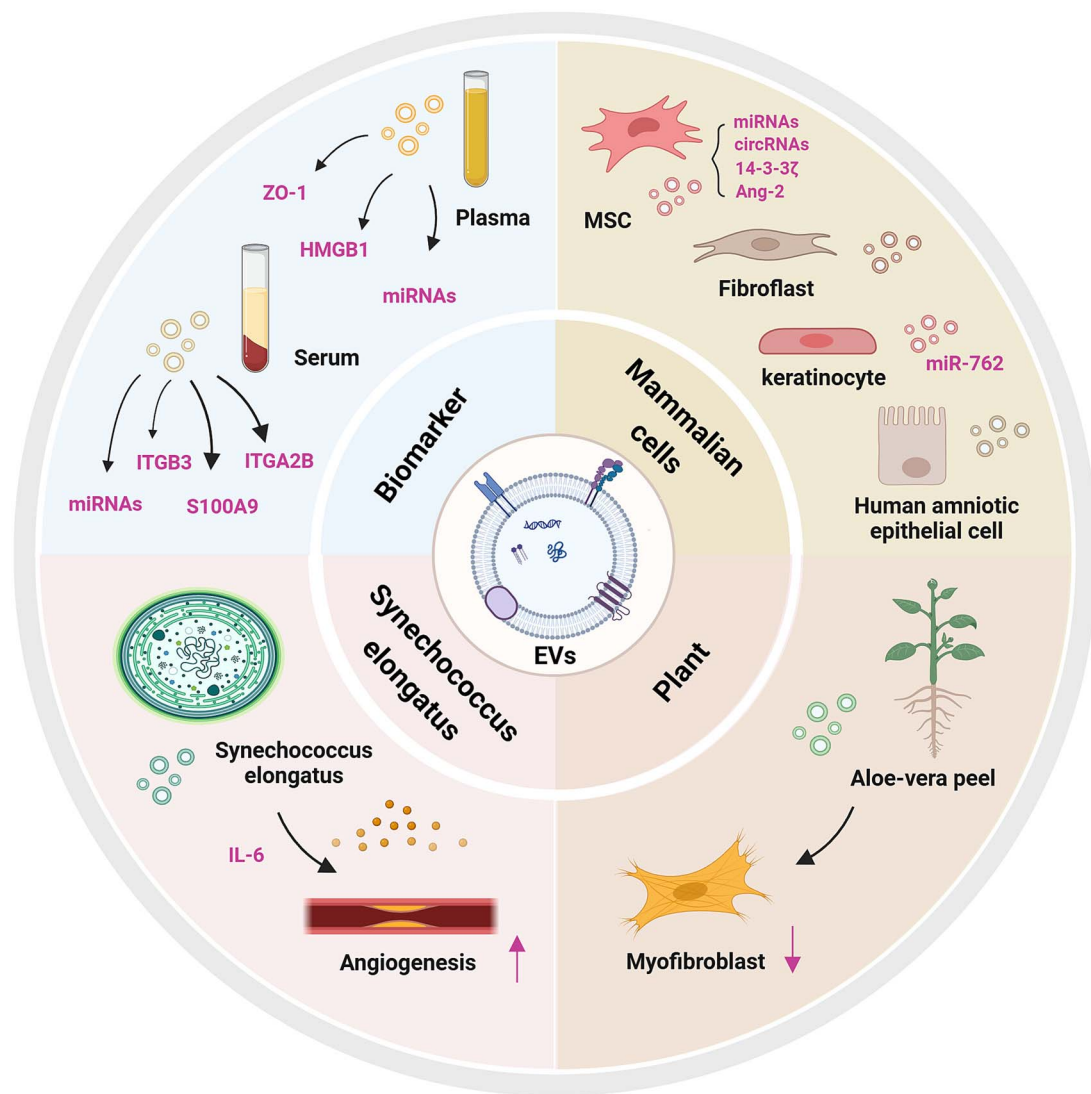


Figure 10. Diagrammatic illustration of EVs from different sources, including plasma, serum, mammalian cells, plants, and *Synechococcus elongatus*. They are related to burn wound healing (created with BioRender.com)

gene editing technologies, such as CRISPR-Cas9, are expected to provide more precise and controllable methods for modifying EVs.

Post-engineered EVs. The post-engineering strategy mainly includes passive loading and active loading. Passive loading refers to the direct incubation of EVs with exogenous substances or EVs collected after incubation with donor cells. Active loading refers to the use of membrane permeation strategies, such as electroporation, ultrasonication, extrusion, surfactant permeation, freeze/thaw cycles, hypotonic dialysis or chemical methods, such as commercial transfection kits, liposome-mediated membrane fusion, metabolic labeling, click chemistry and the absorption of exogenous substances by cells. The choice of loading method depends on the specific characteristics of the cargo, the expected release mechanism, and the expected effect.

Piyush Gongliya and colleagues cocultured MSC-EVs with a miR-155 inhibitor. They reported that MSC-EVs loaded with a miR-155 inhibitor synergistically promoted keratinocyte migration, potentially enhancing the management of chronic nonhealing diabetic wounds [138]. EVs or donor cells

are co-incubated with drugs. This method is relatively simple, does not require adding active agents to the culture medium, and can passively load exogenous cargo into EVs without damaging their structure. However, the loading efficiency of this method is relatively low, and it is not easy to control the amount of cargo loaded by EVs. The loading efficiency of drugs in EVs depends on the hydrophobicity of the drugs. Hydrophobic drugs can interact with the lipid membrane of EVs, making them easier to enter.

Compared with passive loading, active loading is more efficient. Electroporation, which can directly cross the exogenous therapeutic entity through the EVs lipid membrane, is currently the most widely used membrane penetration strategy. The miRNAs and siRNAs were transfected into parental cells and then encapsulated into the exosome via electroporation or liposomal transfection. For example, miR-21-5p mimics were transferred into EVs derived from ADSCs by electroporation, and engineered EVs showed superior therapeutic efficacy in promoting re-epithelialization, collagen remodeling, and angiogenesis in diabetic wounds [139]. Zhou et al. [140] loaded miR-146a-5p into MSC-EVs via electroporation and reported accelerated re-epithelialization and

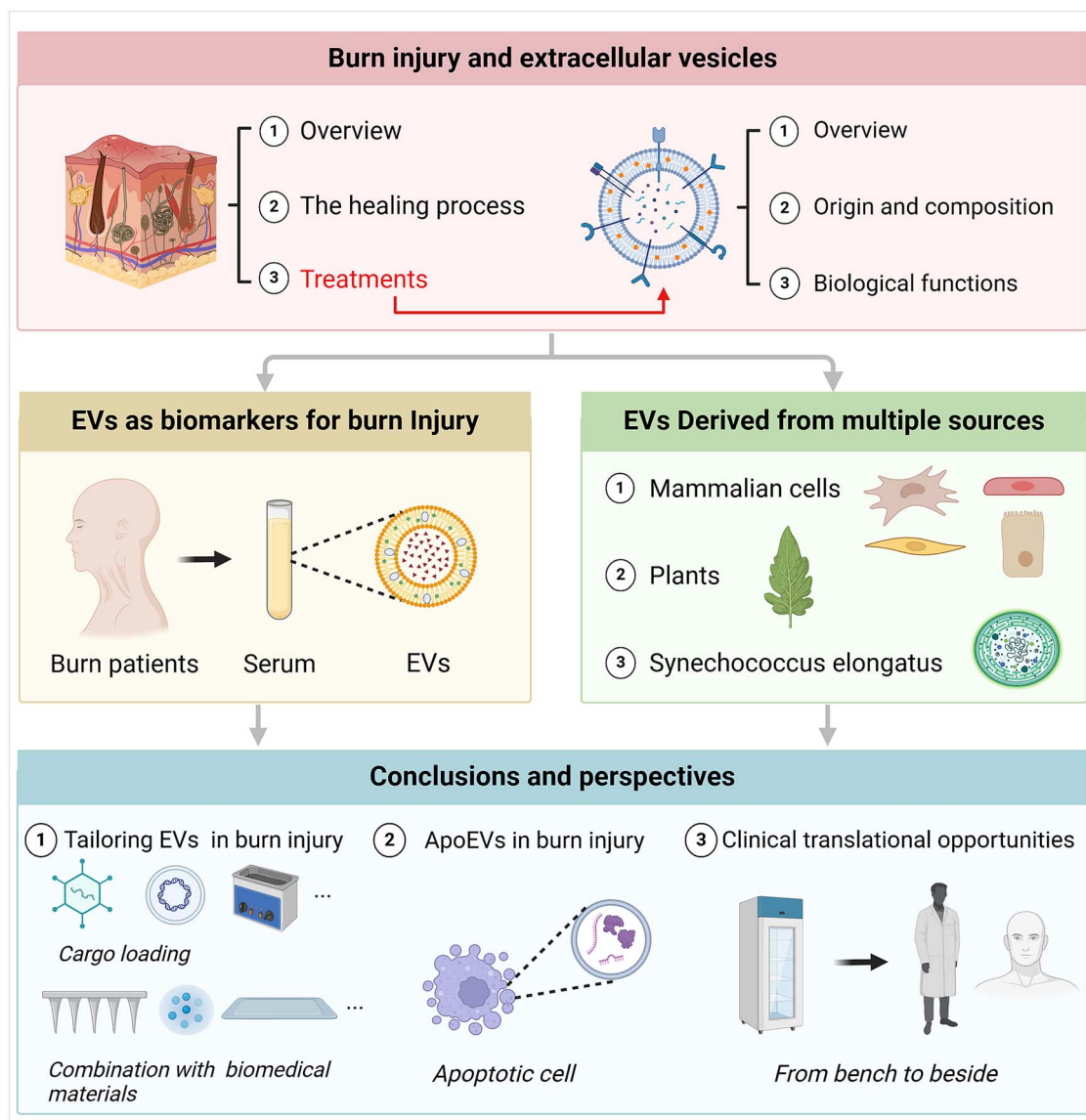
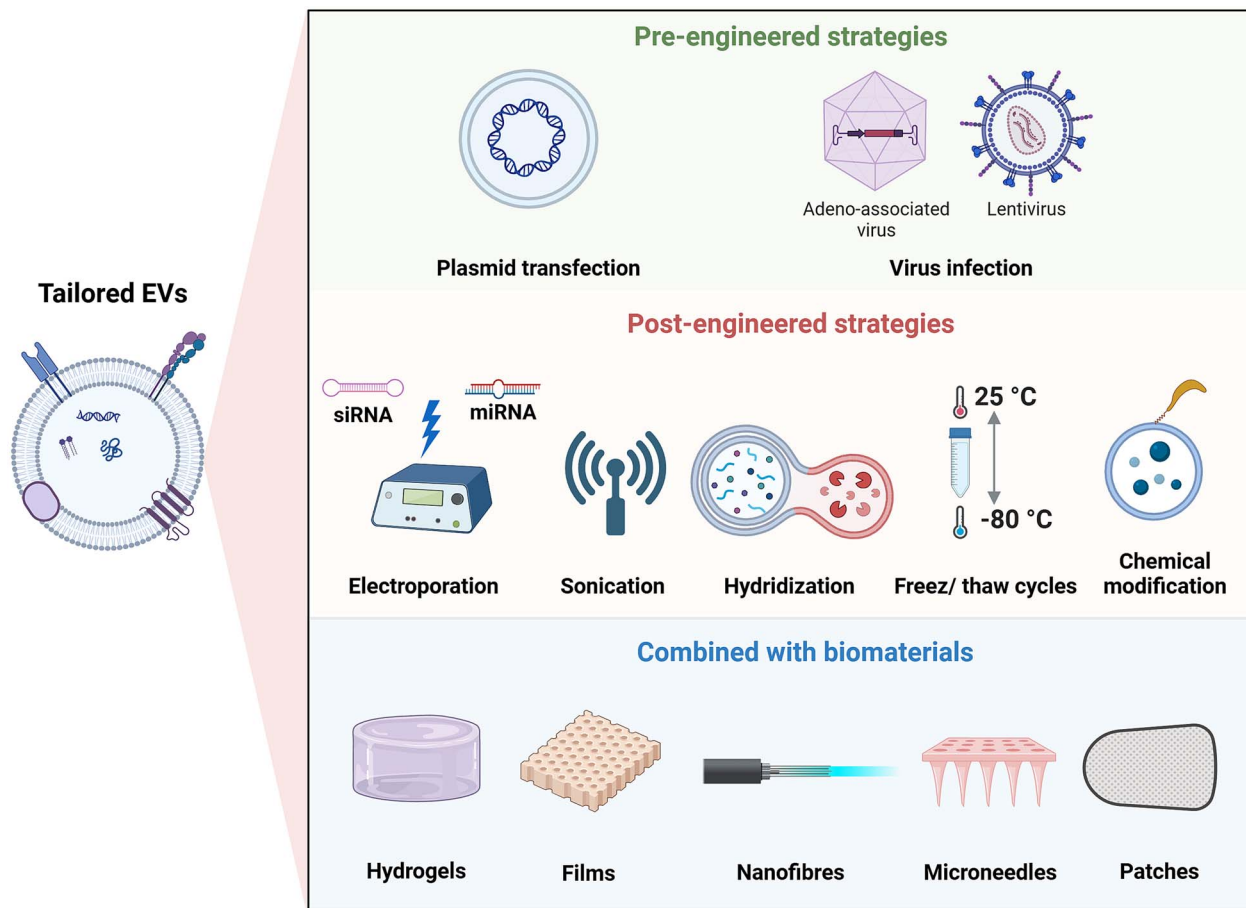


Figure 11. Conclusion of the review (created with BioRender.com)

wound healing in chronic wounds. In addition, developing new transformation methods according to the characteristics of the goods and the intended loading purpose is feasible. Electroporation technology may affect the EVs' zeta potential and colloidal stability and deform organic molecules. Nevertheless, electroporation is still the most promising EV loading strategy. Ultrasound is the second most common membrane permeation strategy. Although ultrasound often produces effective loads, it has several limitations. During ultrasonication, some drugs may adhere to the outer layer of the membrane, thereby affecting its release. In addition, the mechanical shear force generated by ultrasound damages the integrity of the EV membrane, resulting in a significant decrease in its microviscosity. Chemical, such as liposomes, are among the most commonly used transfection reagents to deliver nucleotides to EVs, but their cytotoxicity is a matter of concern. Transfection reagents may reconstitute EV membranes, disrupt delivery, or cause immunogenicity [141]. Mechanical extrusion can repeatedly pass a mixture of cargo molecules and EVs through an Avanti microextruder equipped with a heating module and polycarbonate

films with different nanodiameters for encapsulating synthetic nanoparticles or chemotherapy drugs in EVs. EVs modified by this method have significantly enhanced therapeutic effects, but continuous mechanical extrusion may change EVs' membrane protein structure and membrane potential, resulting in adverse effects [142]. Surface permeation is another membrane permeation strategy for EV cargo loading, but its cytotoxic activity limits its application to a certain extent. The freezing/thawing method can mediate the membrane fusion of liposomes with EVs and promote the loading of macromolecules into EVs [143]. Freeze-thaw technology avoids membrane contamination by harmful chemicals produced by calcium- or polyethylene glycol-mediated chemical fusion methods. Ultrasound and extrusion have higher loading efficiencies than incubation, whereas surface penetration and freeze-thaw cycle efficiency are generally achieved. One limiting factor affecting the drug loading efficiency of different methods is the hydrophilicity and hydrophobicity of the drug itself, which an electroporation strategy can greatly alleviate. Freezing/thawing cycles, extrusion, and sonication significantly increased the size of EVs, but incubation at room



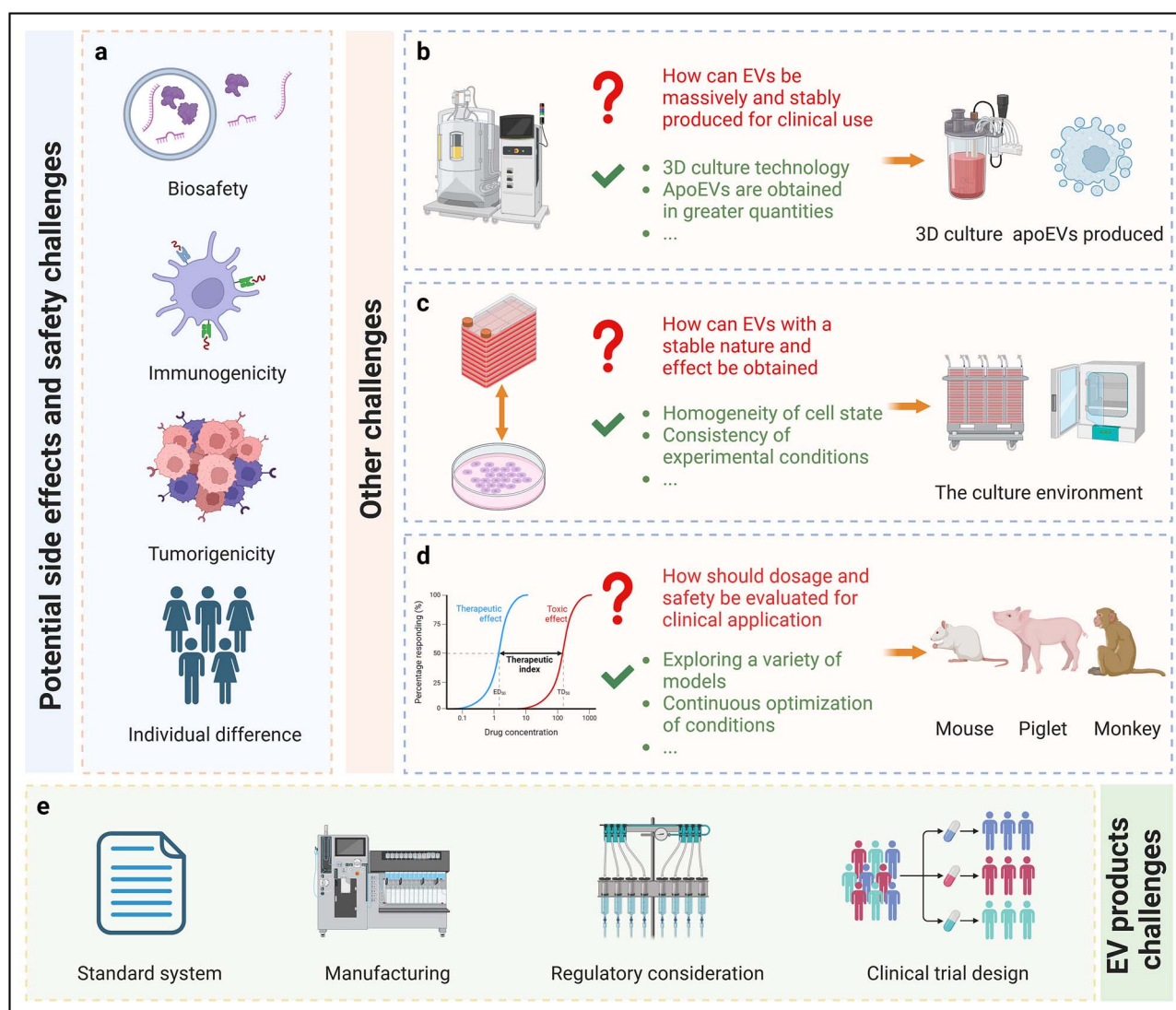


Figure 13. Clinical translational challenges of EVs (created with BioRender.com)

to investigate the safety and efficacy of bone marrow EVs in patients with deep second-degree skin burns ([ClinicalTrials.gov](https://clinicaltrials.gov/Identifier/NCT05078385) Identifier NCT05078385). The potential side effects and safety issues related to EV treatment cannot be ignored. On the one hand, the signaling molecules carried by EVs, such as proteins, nucleic acids, lipids, and metabolites, may cause potential biosafety problems in some cases. To address this challenge, researchers are conducting studies to remove some contents from EVs by hypotonic lysis selectively. This may be a promising strategy to improve the biosafety of EVs.

On the other hand, EVs of allogeneic or xenogeneic origin may be at risk of immunogenicity and tumorigenicity. Masawa, Maimonah Eissa, et al. [158] reported that 57 studies (83.8%) administered human sEVs to immunized normal animals, 11 studies (16.2%) used allogeneic sEVs, and only one study used autologous sEVs (from the same subject). One study compared the efficacy of xenogeneic and isogenic-derived sEVs [159], and another compared autologous sEVs with isogenic-derived sEVs [160]. Lu et al. reported a mild immune response to allogeneic iPSCs but did not observe an

immune response to their sEVs. However, in treated tissues, the viability of allogeneic sEVs was lower than that of their autologous counterparts [160]. These findings are generally consistent with previous studies supporting the safety of sEVs in treating various diseases [161]. The side effects of EVs may vary according to individual differences. The genetic background, disease status, immune system function, and other factors of different patients affect the effects of EVs. Although hundreds of animal studies and clinical trials have demonstrated the biosafety of EVs, safety assessment is an integral part of developing novel therapeutic products. In the absence of relevant risk assessment guidelines, verifying each product's safety is necessary. This requires an assessment of the potential risks at each stage, from source selection to providing treatment to patients [162].

There are several other challenges. (i) How can EVs be massively and stably produced for clinical use? 3D culture technology has been developed in which three-dimensional (3D) culture can more realistically mimic the *in vivo* environment, which not only expands the cell population in large quantities but also improves the yield and activity of EVs and

alters the content of EVs to improve their therapeutic effects [163, 164]. In addition, compared with normal-extracted EVs, apoEVs are obtained in more significant quantities [165], and an in-depth investigation of the role of apoEVs has broad application prospects. (ii) How can EVs with a stable nature and effect be obtained? The therapeutic effect of EVs extracted in experiments, such as MSC-derived EVs and EVs, is often affected by the status of the MSCs. (iii) How should dosage and safety be evaluated for clinical application? Systemic application of EVs can induce apoptosis in some cells, including immunomodulation [166]. Generally, wounds are applied topically, but EVs are readily and rapidly cleared systemically and locally [167]. EVs have shown excellent therapeutic efficacy in regenerative medicine; however, most applications are still experimental. A more straightforward, efficient, safe, and high-yield production method for EVs must be developed to provide better EVs for future clinical applications. In summary, further research on EVs and their use in progressive clinical trials for burn wounds is promising (Figure 13).

Conclusions

This review discusses the effects of EVs derived from different sources, such as mammalian cells and plants, on burn injury. Many open questions remain regarding tailored EVs and their clinical applications. Mechanistically, it would be interesting to elucidate further how EVs from different sources function. Therefore, further research is needed to clarify the regulation of EVs during the burn wound healing process and to translate these results to clinical settings.

Abbreviations

EV: Extracellular vesicle; MV: Microvesicle; ISEV: International Society for Extracellular Vesicles; sEVs: Small extracellular vesicles; ILVs: Intraluminal vesicles; MVBs: Multivesicular bodies; miRNAs: MicroRNAs; MSC-sEVs: sEVs derived from mesenchymal stem cells; ZO-1: Zonula occludens; LBI: Large burn injuries; ADSCs: Adipose-derived stem cells; HucMSCs: Human umbilical cord MSCs; HucMSC-Exs: HucMSC-derived exosomes; hMenSCs: Human menstrual blood-derived mesenchymal stem cells; PRP: Platelet-rich plasma; HTS: Postburn hypertrophic scar; HTSF: Hypertrophic scar fibroblast; iPSCs-KCs-Exos: Induced pluripotent stem cells; iPSCs-KCs-Exos: Exosomes produced by iPSC-derived keratinocytes; LBI: Large burn injuries; PAF: Platelet-activating factor; MVP: Microvesicle particles; ALI: Acute lung injury; hMenSC: Human menstrual blood-derived mesenchymal stem cells; sEVs: Small extracellular vesicles; ITGB1: Integrin beta1; HTSF-Ex: Hypertrophic scar fibroblast-derived exosomes; NHKs: Normal human keratinocytes; EMT: Epithelial-mesenchymal transition.

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Author contributions

Min Wang, Xinyu Zhao, and Yuyu Cui (Writing—original draft), Hengshuo Gui (Investigation), Shuai Wang (Methodology), Zhuang Liu (Supervision), Xianwen Wang (Conceptualization).

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agree to publish this review.

Conflicts of interest

All authors declare no conflicts of interest.

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Data availability

Not applicable.

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