

Plant-Derived Exosome-Like Nanovesicles in Chronic Wound Healing

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Abstract: The incidence of chronic wounds is steadily increasing each year, yet conventional treatments for chronic wounds yield unsatisfactory results. The delayed healing of chronic wounds significantly affects patient quality of life, placing a heavy burden on patients, their families, and the healthcare system. Therefore, there is an urgent need to find new treatment methods for chronic wounds. Plant-derived exosome-like nanovesicles (PELNs) may be able to accelerate chronic wound healing. PELNs possess advantages such as good accessibility (due in part to high isolation yields), low immunogenicity, and good stability. Currently, there are limited reports regarding the role of PELNs in chronic wound healing and their associated mechanisms, highlighting their novelty and the necessity for further research. This review aims to provide an overview of PELNs, discussing isolation methods, composition, and their mechanisms of action in chronic wound healing. Finally, we summarize future opportunities and challenges related to the use of PELNs for the treatment of chronic wounds, and offer some new insights and solutions.

Keywords: exosomes, exosome-like nanovesicles, plants, chronic wound healing

Introduction

Chronic wounds are wounds that persist with incomplete restoration of skin anatomy and function and are typically difficult to heal.^{1,2} With population ageing and lifestyle changes, the incidence of chronic wounds, such as diabetic ulcers,³ venous ulcers⁴ and pressure ulcers,⁵ is increasing annually.⁶ The clinical treatment efficacy for chronic wounds is still unsatisfactory, and the delayed healing of chronic wounds significantly affects patient quality of life,⁷ imposing substantial burdens on patients, their families, and the healthcare system.^{8,9} Therefore, there is an urgent need to find new, more effective treatment methods for chronic wounds. Conventional treatments for chronic wounds include physical therapy, surgical intervention, and pharmacological therapy. Physical therapy methods include heat therapy, cold therapy, and ultrasound therapy.^{10,11} Surgical treatments include wound debridement, tissue repair, and skin grafting.^{12,13} Pharmacological therapy entails the use of medication to promote healing, control infections, or alleviate symptoms.¹⁴ However, these conventional treatments still have drawbacks, such as unsatisfactory therapeutic effects and high costs. Research indicates that certain plant-derived compounds are effective for treating chronic wounds,^{15–17} but the complexity of their composition presents a challenge.

In 1987, researchers studying erythrocyte maturation discovered nanoscale vesicles and proposed the concept of exosomes.¹⁸ Exosomes are small vesicles that are formed within cells and then secreted into the extracellular space and are capable of transferring information between cells and regulating various biological processes.^{19,20} Exosomes exist not only in animal cells but also in plant cells.^{21,22} Animal-derived exosomes play important roles in intercellular communication, immune regulation, and disease development. However, animal-derived exosomes are inherently immunogenic, difficult to obtain, and prone to degradation.^{23–25} Therefore, plant-derived exosome-like nanovesicles (PELNs) isolated

from natural plants have become a hot research topic. PELNs may play a certain role in chronic wound healing. Compared with animal-derived exosomes, PELNs have the advantages of being readily obtainable, having low immunogenicity, and having good stability.^{26,27} Compared to animal-derived exosomes, there are limited reports regarding the role of PELNs in chronic wound healing and their associated mechanisms, highlighting their novelty and the necessity for further research. In this review, methods for the isolation of PELNs and their components are summarized, and the mechanisms of their effects on chronic wound healing are analysed through recent studies. Finally, we summarize the future opportunities and challenges of PELNs in the treatment of chronic wounds and present several new insights and solutions.

Overview of PELNs

PELNs were first isolated by researchers in 2009.²⁸ PELNs are exosome-like nanosized vesicles, encapsulated by a lipid bilayer membrane, that are released by plant cells. Their diameter typically ranges from 30 to 150 nanometres, although larger PELNs more similar in size to animal exosomes have also been observed.²⁹ PELNs have been isolated from various plant parts, including roots,^{30–32} leaves,^{33,34} fruits^{35–38} and seeds.³⁹ PELNs contain bioactive molecules that participate in biological processes and regulate various cell functions, such as those of stem cells,⁴⁰ tumour cells,^{41,42} fibroblasts⁴³ and osteoblasts.^{44,45} Research indicates that PELN activities can modulate various diseases, such as cancers,^{41,42} autoimmune diseases,⁴⁶ liver diseases,⁴⁷ and inflammatory diseases.^{48,49} PELNs are also considered to have significant potential applications in the field of chronic wound healing.⁵⁰

Isolation of PELNs

In 2023, the International Society for Extracellular Vesicles (ISEV) provided some guidance on methods for the isolation of extracellular vesicles based on yield and specificity.⁵¹ Currently, there is no single method for PELNs isolation that simultaneously achieves the highest yield and specificity. Therefore, the isolation method should be chosen based on the specific requirements of the intended research or application. The commonly used methods for isolating PELNs, as reported in the literature, are summarized in Table 1.

Table 1 The Isolation Methods for PELNs

Isolation Method	Plant Sources	Advantages	Disadvantages	References
dUC	<i>Pueraria lobata</i> Apple Yam <i>Phellinus linteus</i>	Simple operation; High isolation efficiency	Need special centrifugal equipment; Easy to break the vesicles	[52] [53] [44] [54]
Density gradient centrifugation	<i>Momordica charantia</i> <i>Portulaca oleracea</i> L Ginseng <i>Artemisia annua</i>	More pure; Preservation integrity	Complex operation; Large loss	[55] [56] [31] [57]
PEG precipitation	Blueberry <i>Physalis peruviana</i>	Simple operation; Lower costs	PEG residue	[58] [59]
Ultrafiltration	<i>Pueraria lobata</i>	Simple operation; Without complex equipment; Not introduce foreign substances	Some limitations for large scale application and high purity	[60]
SEC	Cabbage	High purity; Mild conditions	Complex operation; High equipment requirements; Sample loss; Low recovery rate	[61]
C-CP	Fruit and vegetable	High throughput; More efficient; miniaturization	Lack of standardization; Low recovery rate	[62]

Abbreviations: PELNs, plant-derived exosome-like nanovesicles; dUC, differential ultracentrifugation; PEG, polyethylene glycol; SEC, size exclusion chromatography; C-CP, capillary channel polymer.

Differential Ultracentrifugation

Differential ultracentrifugation (dUC) is a technique that is commonly employed for PELNs isolation. This method utilizes the centrifugal force generated by a centrifuge to separate and purify PELNs based on their density and size. Differential ultracentrifugation effectively removes large particles, dead cells, sticky proteins, fibers, and cell debris by sequentially applying low-speed, medium-speed, and high-speed centrifugation, followed by ultracentrifugation to obtain PELNs. PELNs can be obtained from *Pueraria lobata*,⁵² apples,⁵³ yams,⁴⁴ and *Phellinus linteus*⁵⁴ by dUC. In general, differential ultracentrifugation enables the effective separation of PELNs based on their density and size, offering advantages such as simplicity, ease of operation, and high separation efficiency. However, this approach also requires specialized equipment and meticulous handling to prevent sample degradation.

Density Gradient Centrifugation

Density gradient centrifugation is another method commonly used for separating PELNs. This method utilizes centrifugation in density gradient media, such as sucrose or iodixanol, to separate plant samples. PELNs precipitate in layers of different densities, thereby achieving separation. Density gradient centrifugation effectively removes large particles, dead cells, sticky proteins, fibers, and cell debris by sequentially applying low-speed, medium-speed, and high-speed centrifugation, subsequent sucrose gradient ultracentrifugation to collect vesicles between the sucrose layers to obtain PELNs. PELNs can be obtained from *Momordica charantia*,⁵⁵ Edible,⁵⁶ Ginseng,³¹ and *Artemisia annua*⁵⁷ by density gradient centrifugation. Overall, density gradient centrifugation enables effective separation of PELNs based on differences in density, yielding relatively pure and intact nanovesicles. This method is highly valuable for studying the composition and functions of PELNs. However, this process is complex and involves considerable loss of nanovesicles during multiple washing and transfer steps.

Polyethylene Glycol (PEG) Precipitation

Polyethylene glycol (PEG) precipitation is a commonly used method for isolating PELNs from plants. This method exploits the precipitating properties of PEG to concentrate PELNs in solution. For example, blueberry juice was subjected to differential centrifugation, and the supernatant was incubated with PEG overnight and then centrifuged at $10,000 \times g$ for 30 minutes to obtain blueberry-derived exosome-like nanovesicles.⁵⁸ Differential centrifugation of lantern fruit juice followed by overnight PEG incubation of the supernatant and subsequent low-speed centrifugation allowed the isolation of plant-derived exosome-like nanoparticles from *Physalis peruviana* fruit.⁵⁹ This method is relatively simple and cost-effective; hence, it is widely utilized in laboratory settings. However, importantly, this method may leave behind residual PEG, which could impact certain downstream experiments or therapeutic applications and must be carefully considered.

Ultrafiltration

Ultrafiltration is another commonly used technique for isolating PELNs from plants. This method utilizes membrane filters (with pore sizes typically ranging from 100 to 300 nanometres) to perform molecular sieving, removing cellular debris and larger particulate matter, thereby yielding relatively pure extracellular vesicles. Research in *Pueraria lobata* has shown that after the supernatant is filtered through a $0.22 \mu\text{m}$ membrane to remove large debris, followed by centrifugation, edible exosome-like nanovesicles can be obtained.⁶⁰ The ultrafiltration method is relatively simple to perform, requires no complex instrumentation, and does not introduce exogenous substances, as is the case for PEG precipitation. However, this method has certain limitations in terms of large-scale application and achieving high purity.

Size Exclusion Chromatography

Size exclusion chromatography (SEC) is a commonly used chromatographic technique that can also be employed to separate PELNs. Size exclusion chromatography separates molecules in a sample the basis of their size as they pass through a matrix, typically a porous gel or gel beads. Larger molecules are eluted more rapidly from the matrix, while smaller molecules experience greater hindrance and elute more slowly, establishing the basis of the molecular separation. Studies have shown that size exclusion chromatography can be used to isolate exosome-like nanovesicles from

cabbage.⁶¹ Size exclusion chromatography offers a method for the separation of high-purity PELNs under mild conditions. However, it has drawbacks such as operational complexity, the need for specialized equipment, and sample loss/low recovery rates.

Capillary Channel Polymer (C-CP) Separation

Capillary channel polymer (C-CP) technology is an emerging method used for the separation and capture of PELNs. This approach utilizes microfluidics technology and polymer materials to create microscale capillary channels, enabling the efficient separation of PELNs through these channels. The C-CP method has been used to isolate exosome-like nanovesicles from common *fruits* and *vegetables*.⁶² C-CP technology features high throughput, high efficiency, and miniaturization, allowing the separation and capture of PELNs at microscale. This method has potential applications in PELNs research, aiding researchers in gaining a better understanding of the composition and functions of exosomes; however, it exhibits low recovery rates. Importantly, as C-CP technology is still in the developmental stage, it lacks standardization, and further experimental validation and optimization are required to ensure its stability and reliability in the isolation of PELNs.

Each of these methods has its own advantages and disadvantages, and the choice of method depends on the nature of the PELNs of interest, the research objectives, and the laboratory's equipment and technical capabilities. When studying PELNs, it is usually necessary to consider each of these methods and select the most suitable separation method based on specific circumstances.

PELNs Composition

PELNs may contain a variety of biomolecules, and their composition varies depending on the plant species, tissue type, growth conditions, and physiological status. However, these components typically include the following constituents (Figure 1): i. Proteins. PELNs contain a variety of proteins, including structural proteins, signalling proteins, and regulatory proteins. These proteins play crucial roles in intercellular communication and signal transduction.^{63,64} ii. Nucleic acids. PELNs may contain nucleic acid molecules such as DNA, mRNA, and miRNA. These nucleic acids might play a role in regulating gene expression, thereby influencing cellular functions and biological processes.^{49,65,66} iii. Lipids. The lipid components of PELNs include membrane lipids and free phospholipids, which may play crucial roles in the organization and regulation of cell membrane structure and function.^{33,63,67} iv. Other small molecules. PELNs may also contain other small molecules, such as vitamin C, ions, polysaccharides, oligosaccharides, and metabolites.^{40,53,63,67,68}

The components of PELNs, such as proteins and lipids, exhibit a degree of similarity to those found in mammalian cells, which reduces the likelihood of their being recognized as foreign substances by the host immune system.⁶³ The bilayer lipid membrane of PELNs offers excellent physical stability, maintaining the integrity of their contents and allowing them to effectively withstand environmental changes.²⁷

The specific composition of PELNs varies among different types of plant cells and under different environmental conditions and greatly influences their biological functions and effects. Therefore, research on the composition of PELNs is of great importance.

Role of PELNs in Chronic Wound Healing

The current most common types of chronic wounds are as follows: i. Diabetic foot ulcers. These ulcers, which occur on the feet of diabetic patients, can easily develop into chronic wounds due to neuropathy, poor blood circulation, and infection associated with diabetes.^{69,70} ii. Venous leg ulcers. Poor venous circulation in the lower extremities causes tissue ischaemia and hypoxia, leading to ulcers that are difficult to heal.⁷¹ iii. Arterial leg ulcers. Inadequate blood supply to the lower limbs results in tissue ischaemia, necrosis, and the formation of ulcers that are difficult to heal.⁷² iv. Radiation ulcers. Skin damage and chronic wounds caused by radiation therapy are common in cancer patients who have undergone radiation treatment.⁷³ v. Pressure ulcers. Prolonged pressure on the skin and tissues in patients who are bedridden or confined to a wheelchair causes localized damage that does not heal, resulting in chronic wounds.⁷⁴ vi. Allergic eczema ulcers. Skin damage and ulceration caused by chronic skin inflammation or allergic reactions can have difficulty in healing.⁷⁵

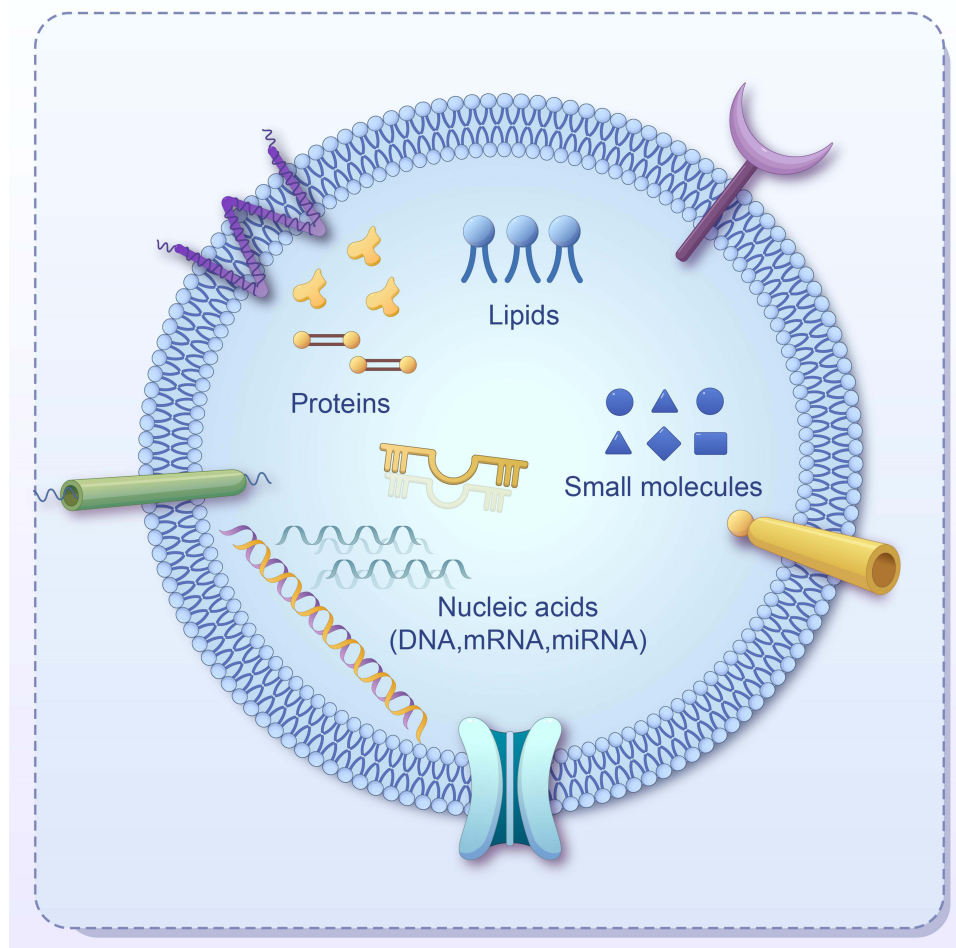


Figure 1 The composition of PELNs.

Abbreviation: PELNs, plant-derived exosome-like nanovesicles.

Chronic wound healing is a relatively complex biological process that requires a closely coordinated cascade of responses to restore and repair the damaged site. These processes include cell proliferation and migration, inflammatory response, angiogenesis, granulation tissue formation, extracellular matrix deposition, and remodelling, ultimately completing the healing process.^{76–78} The mechanisms of action of PELNs in chronic wound healing are still under investigation, but some studies have suggested that they may involve the following mechanisms (Figure 2 and Table 2).

Promotion of Healing

PELNs components such as proteins, lipids, and nucleic acids can promote cell proliferation, migration, and differentiation, aiding in the formation and repair of new tissue and thus accelerating the chronic wound healing process. One study showed that plant-derived exosome-like nanovesicles from *Physalis peruviana* fruit promote the proliferation and migration of human skin fibroblasts, upregulate the expression of type I collagen, and have the potential to promote the healing of chronic wounds.⁵⁹ Furthermore, exosome-like nanovesicles from aloe vera peels enhanced the proliferation and migration of human keratinocytes and human fibroblasts and reduced the levels of reactive oxygen species (ROS) in human keratinocytes, indicating antioxidant effects and the ability to promote the healing of chronic wounds.⁷⁹ *Solanum lycopersicum* (tomato)-derived exosome-like nanovesicles induced the migration of human keratinocytes and mouse fibroblasts, promoting wound healing, suggesting their potential therapeutic effects in chronic wound healing.⁸⁰ Finally, *Grapefruit*-derived exosome-like nanovesicles have been shown to increase cell viability and migration in human epidermal keratinocytes, indicating their potential to promote chronic wound healing.⁸¹

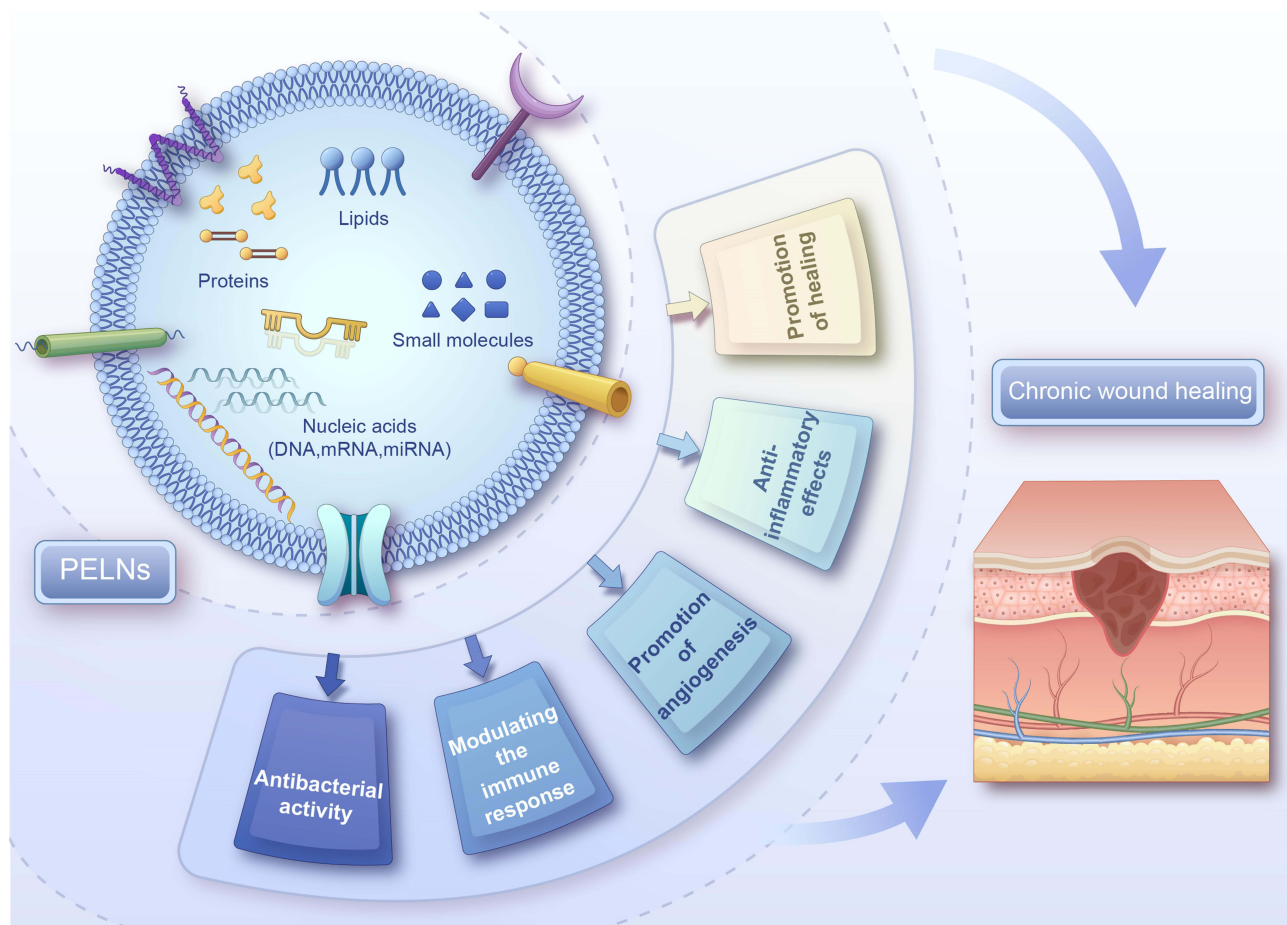


Figure 2 The role of PELNs in chronic wound healing.

Abbreviation: PELNs, plant-derived exosome-like nanovesicles.

Anti-Inflammatory Effects

Certain components of PELNs may possess anti-inflammatory properties, helping to reduce the inflammatory response in wounds and create a more favourable microenvironment for chronic wound healing. Currently, researches have shown that exosome-like nanovesicles derived from *Lemon*,⁸⁹ *Cabbage*,⁶¹ and *Turmeric*,⁹⁰ have anti-inflammatory effects. In addition, *Aloe vera peel*-derived exosome-like nanovesicles reduced the expression of the proinflammatory cytokines TNF α , IL-1 β , and IL-6, demonstrating anti-inflammatory properties that are beneficial for chronic wound healing,⁴³ and pomegranate-derived exosome-like nanovesicles have exhibited anti-inflammatory effects that are beneficial for the healing of chronic wounds.⁸² Further, *Dendrobium*-derived exosome-like nanovesicles can inhibit IL-1 β expression in mouse wounds, exhibiting anti-inflammatory effects and promoting wound healing.⁸³

Promotion of Angiogenesis

Certain components in PELNs may promote angiogenesis, which helps improve the blood supply to the wound and facilitates healing. Researches have shown that PELNs from *Grapefruit*,⁸¹ *Aloe saponaria*,⁵⁰ *Wheat*,⁸⁴ and *Ginseng*⁸⁵ enhance the tube formation capability of human umbilical vein endothelial cells (HUVECs), promote angiogenesis and exhibit the potential to facilitate the healing of chronic wounds.

Modulating the Immune Response

Some PELNs components may have immunomodulatory effects, helping to balance immune responses, reduce inflammatory reactions, and lower the risk of complications during the wound healing process. Research has shown that PELNs

Table 2 The Role of PELNs in Chronic Wound Healing

Mechanisms of role	Plant Sources	In Vitro and (or) Vivo	Effects	References
Promotion of healing	<i>Physalis peruviana</i>	HDF	Elevated HDF proliferation and migration; Upregulated collagen I	[59]
	<i>Aloe</i>	HDF; HaCaT	Reduced ROS levels in HaCaT; Enhanced migration ability of HaCaT and HDF	[79]
	<i>Tomato</i>	HaCaT; mouse fibroblasts (NIH-3T3)	Increased cell migration of HaCaT and NIH-3T3	[80]
	<i>Grapefruit</i>	HaCaT	Increased cell migration of HaCaT	[81]
Anti-inflammatory effects	<i>Aloe</i>	RAW264.7 macrophages; HaCaT	Anti-inflammatory potential in macrophages and keratinocytes; Decreased the secretion of pro-inflammatory cytokines TNF α , IL-1 β , and IL-6.	[43]
	<i>Pomegranate</i>	Monocytic cell (THP-1); Intestinal cell (Caco-2)	Anti-inflammatory effects in vitro cultures of THP-1 and Caco-2 cell lines	[82]
	<i>Dendrobium</i>	C57BL/6j mice	Suppressing IL-1 β expression	[83]
Promotion of angiogenesis	<i>Grapefruit</i>	HUVECs	Increased the tube formation capabilities of HUVECs	[81]
	<i>Aloe</i>	HUVECs	Enhanced tube formation in HUVECs	[50]
	<i>Wheat</i>	HUVECs	Increased tube-like structure formation of the HUVECs	[84]
	<i>Ginseng</i>	HUVECs; ICR mice	Enhanced the migration and angiogenesis in HUVECs; Facilitated skin wound healing in mouse	[85]
Modulating the immune response	<i>Catharanthus roseus</i>	RAW264.7 macrophages; primary spleen lymphocytes; BALB/c mice	Promoted the polarization of macrophages and lymphocyte proliferation; Alleviated white blood cell reduction and bone marrow cell cycle arrest in immunosuppressive mice	[86]
	<i>Pueraria lobata</i>	Peritoneal macrophages	Promote M2 macrophage polarization	[60]
	<i>Turmeric</i>	RAW 264.7 macrophages; C57BL/6j mice	Regulate macrophage polarization and advance the healing process	[87]
Antibacterial activity	<i>Dandelion</i>	Staphylococcus aureus; mouse RBCs; ICR mice	Binding to Staphylococcus aureus exotoxins; Showing detoxification effect in vivo	[88]

Abbreviations: PELNs, plant-derived exosome-like nanovesicles; HDF, human dermal fibroblast; HaCaT, Human keratinocytes; HUVECs, Human umbilical vein endothelial cells; RBCs, red blood cells; ROS, reactive oxygen species; TNF α , tumor necrosis factor α ; IL-1 β , interleukin-1 β ; IL-6, interleukin-6.

from *Catharanthus roseus* can promote macrophage polarization and lymphocyte proliferation, alleviate leukopenia and bone marrow cell cycle arrest in immunosuppressed mice, and have an immunomodulatory effect.⁸⁶ Other studies showed that PELNs from edible *Pueraria lobata*⁶⁰ and *Turmeric*⁸⁷ can promote M2 polarization of macrophages, exerting immunomodulatory and anti-inflammatory effects, and subsequently facilitate wound healing in diabetes.

Antibacterial Activity

Certain PELNs components may possess antibacterial activity, helping to reduce bacterial infections in chronic wounds and thereby improve the success rate of wound healing. Research has shown that dandelion-derived exosome-like nanovesicles possess antibacterial activity and can neutralize staphylococcal exotoxins, reduce bacterial infections, and expedite the healing of chronic wounds.⁸⁸

These mechanisms of action may interact through multiple pathways, contributing to the improvement of chronic wound healing, accelerating the healing process, reducing healing time, and minimizing the occurrence of complications. Notably, there are still unknowns regarding the possible role of PELNs in chronic wound healing. Further research is also needed to elucidate the underlying molecular mechanisms involved.

Conclusions and Future Perspective

PELNs can promote the healing of chronic wounds through mechanisms such as enhancing cell proliferation and migration, exerting anti-inflammatory effects, promoting angiogenesis, modulating immune responses, and providing antibacterial activity. Additionally, factors such as the isolation and purification methods used and the variations in PELNs components can influence their ability to promote chronic wound healing. PELNs hold great promise for research and applications in chronic wound healing; however, there remain challenges such as standardization, purity, yield of isolation and storage, as well as a lack of clarity regarding their functional mechanisms and biosafety.^{86,91,92} Collaborating with the International Organization for Standardization to promote the standardization of PELNs, thereby establishing globally recognized standards, is considered a viable approach for the standardization of PELNs.⁹³ Optimizing extraction techniques, improving separation methods, and automating production for scale-up are strategies to enhance the purity and yield of PELNs.⁹⁴ PELNs can be refrigerated at 4°C, which is suitable for temporary storage.^{51,95} PELNs are stored in a -80°C to maintain their activity and stability.^{51,96} Lyophilization of PELNs is considered beneficial for long-term preservation and reducing the risk of degradation during transport and storage.^{51,97} Clarifying the functional mechanisms and biosafety of PELNs requires long-term scientific research and assessment.⁹⁸ In the future, with continuous research efforts and technological advancements, we anticipate that these challenges will be gradually overcome, harnessing the immense potential of PELNs in the field of chronic wound healing and enabling their widespread application.

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Disclosure

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