Application Progress of Plant-derived Exosome-like Nanoparticles in Diabetic Ulcers

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Abstract: Diabetes foot ulcers (DFU) is a serious complication of diabetes with high morbidity and mortality. The existing treatment methods have bottlenecks such as large trauma, high cost, and long recovery period. Plant derived extracellular vesicle like nanoparticles (PENPs) have become a novel candidate strategy for DFU treatment due to their natural low immunogenicity, high content of active ingredients such as polyphenols and miRNA, easy large-scale extraction, and good biocompatibility. This article systematically reviews the core mechanisms by which PENPs regulate DFU healing, including exerting anti-inflammatory and antioxidant effects by activating the Nrf2/HO-1 pathway, regulating the gut microbiota immune axis to improve systemic metabolism, promoting angiogenesis through glycolysis reprogramming and PI3K/Akt pathways, regulating insulin signaling to improve glucose metabolism, and directly promoting accelerated proliferation, migration, and re epithelialization of keratinocytes and fibroblasts; Simultaneously reviewing the research progress of 9 types of PENPs, including red onions, lemons, ginseng, and wheat, in cell or animal models, confirming their multi-target healing promoting activity. This article provides theoretical and experimental basis for expanding the field of extracellular vesicle therapy and developing safe and effective natural biological treatment strategies for DFU.
***Keywords*:** diabetes foot ulcer, plant exocrine, extracellular vesicle like nanoparticles

1. Introduction

DFU is one of the common and serious complications of diabetes, with a high incidence rate and disability rate, which seriously affects the quality of life and prognosis of patients, and is closely related to the significantly increased risk of death[1,2]. At present, the treatment of diabetes foot ulcer mainly includes debridement, decompression, infection control, blood circulation reconstruction, and the use of various dressings and growth factors. It is traumatic, costly, and has a long recovery period after surgery, which seriously affects the quality of life of patients [2].Therefore, it is urgent to develop more efficient, safe, and controllable new treatment strategies to break through existing treatment bottlenecks.

At present, research on extracellular vesicles mainly focuses on animal derived, especially stem cell-derived extracellular vesicles. In contrast, PENPs have gradually attracted attention due to their natural origin, low immunogenicity, abundant active ingredients (such as polyphenols, flavonoids, miRNAs, etc.), and good biocompatibility [3].These plant nano vesicles are similar to mammalian exosomes in structure and function, with good stability and targeting, and can promote the healing of diabetes ulcers by regulating oxidative stress, inflammatory reaction, cell proliferation, angiogenesis and other mechanisms. In addition, nano vesicles derived from plants are easy to extract on a large scale, and vesicles from some plants such as grapes, ginger, citrus, etc. have shown good tissue repair ability in animal models [3,4].Therefore, a systematic summary of the research progress of plant derived nano vesicles in the healing of diabetes ulcers will not only help expand the research field of exocrine therapy, but also provide theoretical basis and experimental basis for the development of new, safe and effective natural biological treatment strategies.

2. Mechanism of PENPs affecting ulcer healing in diabetes

PENPs regulate the healing microenvironment of diabetes ulcer through multiple targets and pathways, and its mechanisms include anti-inflammatory and antioxidant, regulating intestinal flora, promoting angiogenesis, improving metabolic disorder and enhancing cell repair ability, as follows:

2.1 Anti inflammatory and antioxidant: reshaping the local microenvironment of ulcers

Diabetes ulcer is in a chronic inflammatory state for a long time, and excessive reactive oxygen species and proinflammatory factors accumulate to hinder the healing process. PENPs break this pathological cycle through multiple pathways. On the one hand, PENPs can activate antioxidant signaling pathways. For example, PENPs derived from mung bean sprouts can activate the Nrf2/heme oxygenase-1 (HO-1) pathway, increase glutathione peroxidase (GSH Px) and

superoxide dismutase (SOD) activity, reduce liver malondialdehyde (MDA) levels, alleviate lipid peroxidation damage, protect liver cell function, and indirectly improve the adverse effects of systemic metabolic disorders on ulcer healing [5]. Composite System of PENPs and Selenium Nanoparticles Derived from Rumex Acetylosa (TB-ELNs@SeNPs) Similarly, through Nrf2 mediated signal pathway, it can significantly increase the level of SOD and GSH in diabetes mice, and inhibit oxidative stress [6]. On the other hand, PENPs can directly regulate the release of inflammatory factors. Ginger derived PENPs can downregulate pro-inflammatory factors such as tumor necrosis factor - α (TNF - α) and interleukin-6 (IL-6), upregulate anti-inflammatory factor IL-10, and their engineered platform (HMS/A @ GE) can reduce ROS accumulation in liver and pancreatic tissues, restore GSH content, and alleviate oxidative stress damage [7,8]. After embedding lemon derived PENPs into biological functional hydrogel, they can induce M2 macrophages to polarize, down regulate the expression of inducible nitric oxide synthase (eNOS) and TNF - α, up regulate arginase 1 (Arg-1) and IL-10 levels, inhibit nuclear factor - κ B (NF - κ B) signaling pathway, and reshape the local inflammatory microenvironment of ulcers [9]. Garlic derived PENPs can also interact with the outer membrane vesicles released by Akkermansia muciniphila, enhance intestinal tight junction function, reduce circulating pro-inflammatory cytokine levels, and indirectly alleviate local inflammation in ulcers [10].

2.2 Regulating gut microbiota immune axis: improving systemic metabolism and immune balance

The imbalance of intestinal flora is an important cause of diabetes and its complications. PENPs can indirectly promote the healing of diabetes ulcers by regulating the composition of intestinal flora and metabolites. TB-ELNs@SeNPs It can significantly enrich short chain fatty acid (SCFA) producing bacteria (such as Dubosiella and Lachnospiraceae), inhibit pathogenic bacteria (such as Helicobacter), increase SCFA concentration in feces, and improve metabolic indicators [11]. Ginger derived HMS/A @ GE platform can promote the proliferation of Lactobacillus bacteria, regulate intestinal tryptophan metabolism, increase the levels of aryl hydrocarbon receptor (AhR) ligands (such as indole and indole-3-acetic acid), activate the AhR-IL-22 axis, enhance intestinal tight junction protein expression, improve intestinal barrier integrity, inhibit local inflammation, and regulate glucose metabolism [8]. PENPs derived from orange peel can regulate bile acid metabolism, promote SCFA production, reshape gut microbiota, reduce systemic inflammation, and improve insulin sensitivity and lipid metabolism in db/db mice [12]. In addition, mdo-miR7267-3p from ginger derived PENPs can target the ycnE gene in Lactobacillus, promote indole-3-aldehyde production, stimulate host epithelial cell expression of anti-inflammatory cytokine IL-22, alleviate intestinal inflammation, and indirectly create a favorable systemic environment for ulcer healing; The ath-miR167a it contains can also inhibit bacterial spaC genes, reduce microbial adhesion, and further regulate gut microbiota balance [13].

2.3 Promoting angiogenesis: repairing ischemic tissue through glycolysis reprogramming and activation of signaling pathways

Diabetes ulcer is often accompanied by local ischemia. PENPs can promote angiogenesis and improve local blood supply through metabolic reprogramming and signal pathway regulation. Ginseng derived PENPs are rich in specific miRNAs and ginsenosides (Rg1, Re, Rb1). In high glucose stimulated human umbilical vein endothelial cells (HUVECs), they can significantly upregulate the expression of glycolytic enzymes (such as phosphofructokinase muscle type PFKM, phosphoglycerate kinase 1 PGK1, enolase 1 ENO1), enhance anaerobic glycolysis, increase intracellular ATP levels, inhibit mitochondrial oxidative phosphorylation, and improve endothelial cell energy metabolism and vascular function. In the wound model of db/db diabetes mice, GExos can significantly increase the expression of vascular endothelial growth factor (VEGF) and CD31 at the wound site, and increase the microvessel density by 2.72 times compared with the control group [14]. The lemon derived PENPs functional hydrogel can promote the expression of endothelial type (eNOS) and the production of nitric oxide (NO) in HUVECs, improve the function of endothelial cells, and synergistically promote angiogenesis [9]. In addition, PENPs can also exert their effects through classical angiogenesis signaling pathways, such as grapefruit derived PENPs, which can upregulate the expression of proliferation and migration related genes in HUVECs and enhance their ability to form tubular structures [15]; Wheat derived PENPs promote endothelial cell angiogenesis by activating the ERK and Akt/mTOR pathways [16]; Ginseng derived PENPs can also enhance endothelial cell migration and tubular structure formation through the PI3K/Akt signaling pathway, further accelerating angiogenesis in ulcer sites [17].

2.4 Regulating glucose metabolism and insulin signaling: improving local and systemic metabolic disorders

PENPs can improve insulin resistance and blood glucose level in diabetes patients by regulating insulin signaling pathway and glucose transport, and provide a basis for ulcer healing. In high-fat diet and streptozotocin (STZ) induced diabetes mice, mung bean sprout derived PENPs can promote glucose uptake and glycogen synthesis by regulating PI3K/

Akt/GLUT4/GSK-3 β pathway, reduce fasting blood glucose, total cholesterol and triglyceride levels, improve oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) performance, reduce liver lipid deposition, and protect the morphology and function of pancreatic beta cells [5]. TB-ELNs@SeNPs It can activate the PI3K/Akt and AMPK pathways, downregulate gluconeogenic enzymes (PEPCK, G6Pase) and lipid regulatory factors (SREBP-1c, FAS), inhibit liver glucose production, and restore metabolic homeostasis [11]. Ginger derived PENPs upregulate the Foxa2 signaling axis, enhance intestinal glucose transporter 2 (GLUT2) expression, promote glucose transport, and alleviate HFD induced insulin resistance [7]; When the HMS/A @ GE platform co delivers PENPs with molecular hydrogen (H_2), it can further enhance PI3K/Akt signaling activity, increase glucose uptake in liver and muscle tissues, and reduce the insulin resistance index (HOMA-IR) [8]. Orange peel derived PENPs can restore the expression of peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1 alpha), insulin receptor substrate 1 (IRS-1), and GLUT4 in db/db mice, reduce liver steatosis and inflammation factor production, and improve insulin signaling [12]. Garlic derived PENPs interact with Akkermansia muciniphila to stimulate intestinal secretion of glucagon like peptide-1 (GLP-1), activate the GLP-1 receptor pathway, upregulate IRS1/2 and Akt expression, and repair HFD induced insulin signaling damage [10].

2.5 Enhancing cell proliferation and migration: accelerating ulcer re epithelialization and tissue repair

PENPs can directly act on local cells in ulcers (such as keratinocytes and fibroblasts), promoting their proliferation and migration, accelerating re epithelialization and tissue regeneration. Aloe derived PENPs enhance the migration ability of human keratinocytes (HaCaT) and human dermal fibroblasts (HDF) by scavenging free radicals and ROS, while promoting the formation of tubular structures in HUVECs and increasing local blood supply [18,19,20]. Wheat derived PENPs can promote the proliferation and migration of HDFs, HUVECs and HaCaT cells in a dose-dependent manner, up regulate the transcription level of type I collagen, promote the angiogenesis of endothelial cells through ERK and Akt/mTOR pathways, and accelerate the repair of full-thickness diabetes skin ulcers [16, 21, 22]. Ginger derived PENPs can promote HaCaT cell proliferation and migration, upregulate gene expression related to skin cell proliferation, and accelerate damaged skin repair [17]. Pomelo derived PENPs can enhance the vitality and migration ability of human epidermal keratinocytes (HaCaT), providing impetus for ulcer re epithelialization [15]. In addition, PENPs can also protect cell function by regulating apoptosis related pathways. For example, PENPs derived from bitter gourd can inhibit matrix metalloproteinase-9 (MMP-9), activate the AKT/GSK3 β signaling pathway, alleviate high glucose induced cell apoptosis, protect neuronal and endothelial cell functions, and indirectly promote ulcer healing [23].

3. Research progress of PENPs in ulcer models (classified by plant source)

3.1 Red Onion

As a traditional medicinal plant, the extract of red onion has been proven to have antioxidant, anti-inflammatory, and wound healing effects due to its high content of flavonoids such as quercetin and kaempferol. In recent years, PENPs separated from red onion juice by differential centrifugation have become potential candidates for the treatment of diabetes ulcers due to their advantages of enriching plant derived bioactive ingredients, low immunogenicity, easy large-scale preparation, etc. At present, the research on RDNVs in the diabetes ulcer model has not directly constructed the diabetes animal model, but through the mechanism analysis of the normal mouse full-thickness skin wound model (such as anti-inflammatory, antioxidant, regulating macrophage polarization, etc.), it has provided key evidence for its application in diabetes ulcers (the nuclear and cardiac pathological characteristics are chronic inflammation, oxidative stress, and abnormal repair cell function) [24].

3.2 Lithospermum

PENPs, derived from the callus tissue of the medicinal plant Lithospermum, were isolated and purified through methods such as grinding, filtration, differential centrifugation, and tangential flow-through filtration. These PENPs are an emerging plant derived nanovesicle with good biocompatibility and anti-inflammatory and reparative abilities. It significantly promotes healing in inflammatory wound models, suggesting that it has potential application prospects in the treatment of chronic wounds related to diabetes. Future research can further verify its efficacy in diabetes models, and explore its active ingredients and mechanism of action [25].

3.3 Citrus limon

Citrus limon derived PENPs are isolated from lemon juice by ultracentrifugation. As a natural vesicle derived from plants, they have multiple functions such as antioxidant, anti-inflammatory, promoting angiogenesis, and tissue repair. After

loaded with hydrogel, it showed significant healing promoting effect in the chronic wound model of diabetes, especially in regulating the immune microenvironment and promoting tissue regeneration. This strategy provides a new idea and experimental basis for the clinical transformation of plant exosomes in the treatment of diabetes wounds [9].

3.4 Triticum aestivum

PENPs derived from Triticum aestivum juice combined with Exo spin through differential centrifugation ™ The extracellular vesicle like nanocapsules isolated from the extraction kit are an emerging plant derived repair factor with good biocompatibility and multi-target healing activity. Although the current research is still limited to the in vitro model, its role in promoting proliferation, migration, angiogenesis and collagen synthesis at the cellular level has laid a solid foundation for its further application in the treatment of diabetes wounds. In the future, it is necessary to carry out the verification of diabetes animal models and in-depth research on the mechanism to promote its clinical transformation [16].

3.5 Ginseng

Ginseng source PENPs are extracted from the root of Changbai Mountain ginseng by differential centrifugation and ultracentrifugation. As a plant exocrine derived from traditional Chinese medicine, they have the advantages of natural, safe, degradable, easy to ingest, etc., and show significant ability to promote angiogenesis and tissue repair in the treatment of diabetes wounds. It effectively reverses endothelial dysfunction induced by high glucose through glycolysis reprogramming mechanism, provides a new plant-based nanotherapy strategy for chronic wounds of diabetes, and has good clinical transformation prospects [14].

3.6 Solanum lycopersicum

Tomato Solanum lycopersicum derived PENPs can significantly promote the migration of keratinocytes and fibroblasts, enhancing the process of wound re epithelialization. Daniello et al. (2023) found that tomato PELNs accelerate in vitro wound closure and promote collagen deposition by activating cell migration related signaling pathways such as MAPK/PI3K. Although its efficacy has not yet been verified in diabetes models, its significant role in cell migration and tissue regeneration suggests that it can be used as a potential adjuvant treatment for diabetes wound repair [26].

3.7 Grapefruit

Grapefruit derived PENPs have significant pro angiogenic, antioxidant, and cell migration promoting effects. Savci et al. (2021) found that grapefruit PELNs can enhance the angiogenic ability of human umbilical vein endothelial cells (HUVECs), promote neovascularization, and increase the migration activity of keratinocytes. These functions have important intervention value for ischemia, hypoxia and angiogenesis disorders common in diabetes wounds [15].

3.8 Dandelion

Dandelion derived PENPs exhibit excellent antibacterial and detoxifying effects. Tan et al. (2024) found that dandelion derived nanocapsules can neutralize Staphylococcus aureus exotoxins, reduce the risk of bacterial infection, and accelerate healing in a mouse infectious wound model. Considering that diabetes wounds are prone to secondary infection, the antibacterial properties of dandelion PELNs provide a theoretical basis for their application in diabetes wound management [27].

3.9 Curcuma longa

PELNs derived from turmeric exhibit significant anti-inflammatory and immune regulatory functions due to their high content of curcumin compounds. Wu et al. (2024) constructed a turmeric PELNs functional hydrogel, and found that it can promote the polarization of macrophages to M2 type, inhibit the expression of inflammatory factors, and accelerate the healing of full-thickness skin wounds in mice. Although it has not been verified in the diabetes model, its ability to regulate the immune microenvironment has important intervention significance for the common chronic inflammatory state in diabetes wounds [28].

4. Conclusion and prospect

PENPs demonstrate unique value in DFU treatment due to their natural safety, multi-component synergistic functionality, and ease of industrial preparation advantages. Existing research has confirmed that it can break through the pathological cycle of "chronic inflammation oxidative stress blood supply deficiency" in DFU, and PENPs such as red onions, lemons, and ginseng have been validated for their healing promoting effects in the model, laying the foundation for the transformation of basic research into practical applications. However, the macroscopic efficacy, targeting of active ingredients (such as specific miRNAs) to specific targets, and cross organ pathways (gut skin axis) have not been elucidated;

Moreover, existing research mainly focuses on animal experiments, lacking multi center large sample studies on DFU patients, and the pharmacokinetics and long-term safety are unclear; Moreover, the extraction processes of different PENPs (differential centrifugation, tangential flow filtration) vary greatly, and the consistency between particle size and active ingredients is poor, with no unified standard. In the future, it is necessary to strengthen mechanism analysis, promote clinical research, optimize preparation and delivery, and 4 Explore combination therapy. Further develop safe and effective natural biological treatment strategies for DFU.

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