Future applications of exosomes delivering resolvins and cytokines in facilitating diabetic foot ulcer healing

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Abstract

Type 2 diabetes mellitus (T2DM) increases the risk of many lethal and debilitating conditions. Among them, foot ulceration due to neuropathy, vascular disease, or trauma affects the quality of life of millions in the United States and around the world. Physiological wound healing is stalled in the inflammatory phase by the chronicity of inflammation without proceeding to the resolution phase. Despite advanced treatment, diabetic foot ulcers (DFUs) are associated with a risk of amputation. Thus, there is a need for novel therapies to address chronic inflammation, decreased angiogenesis, and impaired granulation tissue formation contributing to the non-healing of DFUs. Studies have shown promising results with resolvins (Rv) and anti-inflammatory therapies that resolve inflammation and enhance tissue healing. But many of these studies have encountered difficulty in the delivery of Rv in terms of efficiency, tissue targetability, and immunogenicity. This review summarized the perspective of optimizing the therapeutic application of Rv and cytokines by pairing them with exosomes as a novel strategy for targeted tissue delivery to treat non-healing chronic DFUs. The articles discussing the T2DM disease state, current research on Rv for treating inflammation, the role of Rv in enhancing wound healing, and exosomes as a delivery vehicle were critically reviewed to find support for the proposition of using Rv and exosomes in combination for DFUs therapy. The literature reviewed suggests the beneficial role of Rv and exosomes and exosomes loaded with anti-inflammatory agents as promising therapeutic agents in ulcer healing.

Key Words: Diabetic foot ulcer; Chronic inflammation; Amputation; Exosomes; Cytokines; Resolvins

Core Tip: Nonhealing diabetic foot ulcers (DFUs) are a debilitating condition with the risk of amputation despite the advanced treatment strategies. Chronic inflammation, decreased granulation tissue formation, and decreased angiogenesis underlies the pathogenesis of nonhealing. Targeted delivery of therapeutics targeting immune cell infiltration and chronic inflammation with loaded exosomes may increase the efficacy of treatment. We herein discuss the potential of exosomes loaded with resolvins and drugs targeting inflammatory cytokines to promote DFUs healing.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is an acquired metabolic disease characterized by insufficient insulin release from pancreatic β-cells and the reduced inability of tissues to respond to insulin stemming from increased insulin resistance. Blood glucose homeostasis is mediated by insulin release from pancreatic beta islet cells which facilitate the storage of glucose in muscles and adipose tissue. However, impaired insulin secretion and insulin sensitivity and increased insulin resistance cause increased blood glucose persistently leading to diabetes mellitus. Type I diabetes is due to the destruction of insulin-secreting β-cells resulting in insulin deficiency while T2DM is due to decreased insulin sensitivity, insulin resistance, or decreased insulin receptors[1,2]. Insulin resistance is defined as the inability of a known quantity of insulin to increase glucose uptake in diabetics compared to control subjects[3]. The metabolic dysfunction in diabetes leads to a plethora of complications, including retinopathy, neuropathy, heart disease, peripheral vasculopathy, tissue inflammation, and ulceration[4]. Diabetes causes severe disruption to the patient's quality of life and burdens the healthcare system. According to the 2022 National Diabetes Statistic Report from the Centers for Disease Control, more than 130 million adults in the United States live with diabetes or prediabetes[5]. There is an ever-present need to treat and manage diabetes and its related complications.

Diabetic foot ulceration is a debilitating complication that stems from chronic inflammation interfering with the process of tissue healing. Diabetic foot ulcers (DFUs) increase morbidity and mortality in diabetic patients. The lifetime incidence of ulceration for diabetic patients is estimated to be between 15%-25% and has a 30%-40% reoccurrence rate in prior patients. With ulceration, severe infections may develop which eventually lead to amputation[6,7]. Amputations in diabetic patients are preceded by DFUs in approximately 85% of cases[8]. In a study of diabetic foot infections and amputations, major amputations drastically reduced the 5-year survival rate to 8.3%[9]. Despite the advancement in DFU care with wound debridement, off-loading, applications of medication, and bandaging to prevent infection, there is a risk of lower limb amputation with a worldwide prevalence of 8.8%, with over half of the major leg amputations performed every year in the United States attributable to diabetes mellitus and peripheral artery disease[10-12]. Thus, an utmost need to develop better treatment strategies for nonhealing DFUs. Along with the local treatment, attenuating systemic inflammation or administering a therapeutic agent systemically to target a specific protein of interest should be considered while treating complicated non-healing DFUs. Exosomes, administered systemically, have been examined in numerous immunomodulatory studies and have been proposed as a treatment for the inflammatory dysregulation and delayed wound healing witnessed in T2DM[13,14]. Additionally, due to exosomes' immune privilege, specific tissue binding, and targetability, they have been widely studied as carriers for therapeutic compounds to enhance drug applications[15]. A potential candidate for exosomal delivery may be resolvins (Rv) and mediators targeting inflammation. Studies have shown promising results with Rv and anti-inflammatory cytokines that resolve inflammation and enhance tissue healing. Many of these studies, however, have encountered difficulty in the delivery of Rv in terms of efficiency, tissue targetability, and immunogenicity[16]. This article seeks to critically review the perspective of optimizing the therapeutic application of Rv and anti-inflammatory mediators by pairing them with exosomes as a method for targeted tissue delivery to treat non-healing chronic DFUs.

DIABETES AND INFLAMMATION

Chronic inflammation is a related complication in T2DM. Obesity is a risk factor for T2DM and obesity along with diabetes makes this worse in inducing chronic inflammation[17,18]. Adipose tissue hypertrophy and hyperplasia result in increased secretion of leptins and inflammatory cytokines

including interleukin (IL)-6, IL-1, and tumor necrosis factor (TNF)-α from adipocytes with a concurrent decrease in adiponectin. The secreted cytokines induce the recruitment of inflammatory immune cells which secrete chemokines and further enhance the recruitment of immune cells[19-21]. These changes induce inflammation in diabetes (Figure 1). However, inflammation is not an exclusively detrimental process and in normal physiologic functions, it plays an important role in wound healing. With the initial injury and damage to tissues, healing is conducted in a series of distinct phases. Acute damage is addressed in an inflammatory phase, followed by a resolution phase, and lastly with a phase of tissue remodeling and regeneration and then the return to homeostasis. Healing from acute tissue damage begins with activation of inflammatory factors leading to inflammation, hemostatic accumulation of lymphatic fluid, immune cells like macrophages and neutrophils infiltration into tissues, accumulation of blood and platelets at the site of damage, and constriction of arteries, and thrombosis at the damage site. In the inflammatory phase, the infiltration of neutrophils, monocytes, and macrophages activates targeted apoptosis of damaged cells and the clearance of cellular debris. A resolution phase follows with the release of growth factors, anti-inflammatory cytokines, and a return to homeostasis. The resolution of the inflammatory phase is an active process that involves the clearance of pro-inflammatory signals and a return to homeostasis[16,22,23] (Figure 1). But in diabetes, hyperglycemia, persistent recruitment of inflammatory immune cells, and increased expression of inflammatory cytokines like IL-6, IL-1, IL-8, and TNF-α produce a chronic inflammatory environment and prevent the progression of the inflammatory phase to the resolution phase[16,24]. Persistent inflammation not only holds the ulcer in the inflammatory phase but also negatively affects angiogenesis and granulation tissue formation, the requisite for proper healing[25,26]. Diabetic microangiopathy and abnormal response to hypoxia in diabetes cause hypoxia-related cell death and increased secretion of monocyte chemoattractant protein-1, chemokines from keratinocytes, and IL-6, IL-1, and TNF-α from infiltrating immune cells. This ultimately leads to increased secretion of inflammatory cytokines mediating chronic inflammation, decreased angiogenesis, and decreased extracellular matrix remodeling ultimately mediating impaired healing. Activated fibroblasts acquiring myofibroblasts phenotype actively contribute to granulation tissue formation and angiogenesis, however, persistent inflammation and altered fibroblast function impairs wound healing through attenuated angiogenesis and granulation tissue formation[27,28].

Hyperglycemia and inflammation have a multifactorial etiology which may lead to non-healing DFUs in an estimated 25% of diabetic patients with an increased risk for lower limb amputation. There is a disruption to cytoskeletal keratin proteins (K2, K6, and K10) which hinders keratinocyte development and negatively impacts re-epithelialization[29]. Reduction of laminin-5 α3 chain precursor protein hampers the binding of epithelial cells to basement membranes. Reduced activity of antioxidant enzymes, glutathione peroxidase, and super-oxidase dismutase, leads to free radical-associated damage. Further, diabetes-related atherosclerosis and reduced angiogenesis and revascularization result in decreased nutrients and oxygen reaching the wound site[29]. Epidemiological studies have demonstrated a correlation between T2DM-associated chronic inflammation and the presence of numerous inflammatory biomarkers. Inflammation is a likely contributor to the insulin resistance witnessed in T2DM and may be intensified by the hyperglycemia that it contributes to. With prolonged hyperglycemia, adipose tissue will increase insulin resistance through inflammatory mechanisms such as releasing free fatty acids and adipokine deregulation[1,30]. Inflammation can be recognized by an increase in circulating pro-inflammatory cytokines such as IL-6, IL-8, TNF-α, and leptin, which modulate the insulin signaling pathway and alters immune response[1,30]. These molecules activate different intracellular Ser/Thr kinases which catalyze serine phosphorylation of insulin receptor substrate 1, inhibiting its ability to recruit phosphatidylinositol-3-kinase and Akt, interfering with the metabolic pathway of insulin[31].

The presence of pro-inflammatory molecules can also cause the degradation of the insulin-producing β-cells of the pancreas[4]. In test settings, the induction of hypothalamic inflammation is sufficient to trigger diabetes-like features, demonstrating that inflammation is a key contributing factor to the pathophysiology seen in T2DM[31]. Increased leptin production is associated with immune system dysfunction in T2DM. It modulates both innate and adaptive immune responses, including promoting T-cell responses, activation of monocytes and neutrophils, and increasing the induction of pro-inflammatory mediators[30]. Evidence suggests that heightened immune cell infiltration in intermyocellular and perimuscular adipose tissue contributes to myocyte inflammation and increased insulin resistance[1].

The chronicity of inflammation contributes to the non-healing pattern of DFUs (Figure 1) and targeting this positive feedback of chronic inflammation may be a viable strategy for treating DFU[1,30]. Resolving chronic inflammation offers a therapeutic avenue to treat diabetes-associated ulceration [16,24] to improve the quality of life and diminish the economic burden on patients. Therapeutics that attenuate chronic inflammation may force the progression of inflammatory phase towards resolution and tissue remodeling and regeneration phase and E and D series Rv including RvE1, RvE2, RvE3, RvD1, and RvD2 have been proven beneficial[16]. This makes Rv attractive therapeutics in DFUs.
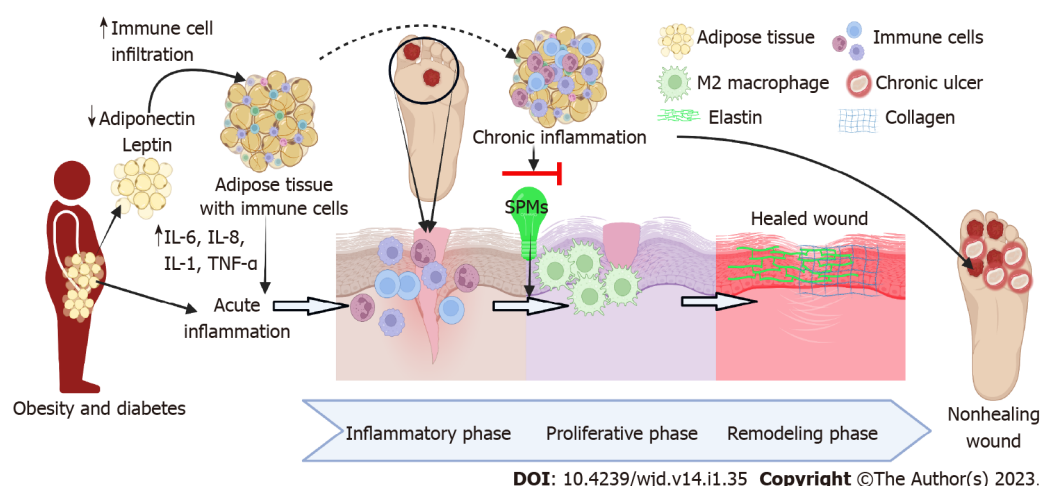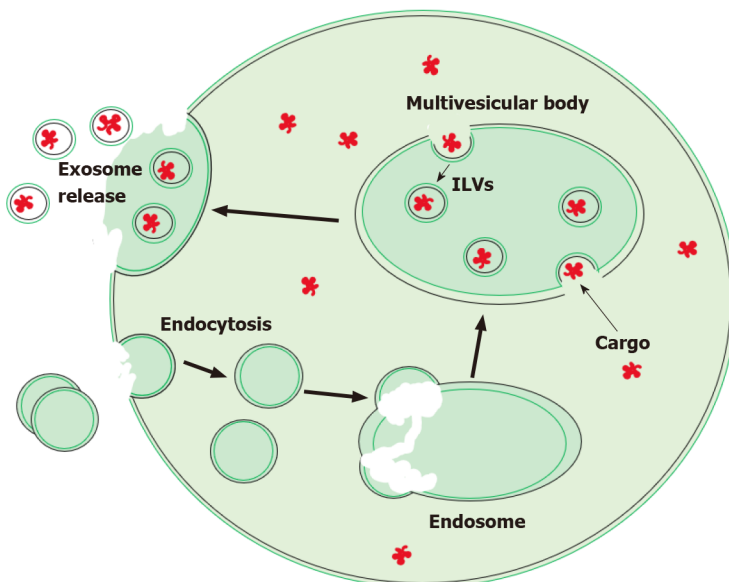
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Figure 1 Inflammation-mediated pathogenesis of diabetic foot ulcer, the role of resolvins, and phases of wound healing. Resolvins [specialized pro-resolving mediators (SPMs)] facilitate the resolution of inflammation and progression of the wound to the resolution phase followed by remodeling and healing (SPMs shown in green). However, persistent infiltration of immune cells and increased secretion of cytokines mediate chronic inflammation and hold the wound in the inflammation phase without progressing to resolution or proliferative phase (red arrow). This leads to the chronicity of inflammation and nonhealing of diabetic foot ulcers. ILV: Intraluminal vesicle.

INFLAMMATION-RESOLVING MEDIATORS

Rv, along with protectins, maresins, and lipoxins, are a class of specialized pro-inflammation-resolving mediators (SPMs). Rv are a class of small lipid molecules that are endogenously derived from Ω-3 essential fatty acids: Eicosapentaenoic acid and docosahexaenoic acid[16]. The role of Rv in the treatment of inflammation in T2DM and DFU *via* inhibition of neutrophil and macrophage infiltration, decreased secretion of inflammatory cytokines IL-6, IL-1, IL-8, and TNF-α, promoting apoptosis of activated immune cells and clearance of cellular debris, and downregulating platelet activation[16,22, 24]. The application of Rv and other SPMs has become a recognized therapeutic avenue to suppress pro-inflammatory disease states and offers promise in treating inflammation associated with T2DM and other diseases. The beneficial effects of Rv in attenuating inflammation in various chronic inflammatory conditions are suggestive of their potential in enhancing wound healing in DFUs[32-34], a chronic inflammatory condition, by suppressing inflammation and mediating progression to resolution phase of healing[16]. SPMs address several factors involved with the inflammation of T2DM. It inhibits polynuclear neutrophil infiltration of tissues, inhibits the production of pro-inflammatory mediators like chemokines and cytokines, promotes apoptosis in activated immune cells, promotes the uptake of cellular debris and chemokines by macrophages, downregulates platelet activation, suppresses nuclear factor-κβ activation, and improves insulin sensitivity[16,22].

In diabetes, the healing process is chronically arrested at the inflammation phase, delayed, and associated with decreased tissue regeneration. Applications of Rv offer attractive means of resolving the inflammatory phase and initiation of the resolution and regenerative phase for promoting tissue healing (Figure 1). Currently, animal models have supported the benefits of SPMs in the management of numerous chronic inflammatory conditions including arthritis, periodontitis, colitis, allergy airway infections, skin infections, neurodegenerative vascular disease, cardiovascular disease, T2DM, and more [16,22,35]. Numerous laboratory studies have demonstrated the benefit of the exogenous application of Rv, including inhibiting neutrophil migration, the clearance of apoptotic cells and wound debris, accelerating wound healing in cutaneous and intestinal tissue, and healing diabetes-associated wounds [22]. Animal and tissue models have supported the application of SPMs or Rv in the treatment of diabetic ulcers. Unfortunately, Rv are oxidatively unstable due to their 1,4-diene (skipped diene) structures and so clinical utility would require more stable Rv to be produced[36]. Other means of improving resolvin stability may be in better transport systems. Exosomes or microvesicles have been proposed as transporting systems for drug delivery in the treatment of DFUs[37]. However, below the adequate levels of circulating Rv, bioavailability, and stability are common limitations and issues while using Rv and biopolymers[35,38]. Thus, there is a need to improve the delivery techniques to enhance the bioavailability and stability of Rv as well as their therapeutic efficacy. In the next section, we will discuss exosomes and their role in enhancing the efficacy of Rv.

Figure 2 Exosome formation through the endosomal pathway. Endocytosis produces endocytic vesicles which will fuse to form early endosomes. Endosomes mature into multivesicular bodies (MVBs) and parts of their membranes endocytose to form intraluminal vesicles (ILVs) within themselves. With 2 stages of endocytosis, the orientation of the bilaminar membrane of the ILVs will possess the same orientation as the cell's membrane. The MVBs fuse to the cellular membrane to release the ILVs now referred to as exosomes. IL: Interleukin; TNF: Tumor necrosis factor.

EXOSOMES

Exosomes are a type of extracellular vesicle (EV) that carries many functions as carriers of many biological pathway factors. They are 40-150 nm-sized membrane-derived microvesicles that are produced by most of the body's cell types and have been detected in all fluids produced by the human body. The formation of exosomes differs from other EVs in that they are not direct buds of the parent cells' plasma membranes. Exosomes are formed through the endosomal pathway[39,40] (Figure 2).

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Exosome formation begins as endocytic vesicles which are invaginations of the cellular membrane. These endocytic vesicles fuse to form early endosomes. As the endosomes mature and grow, their membranes can invaginate further, forming ILVs within the maturing endosome, now referred to as a MVB[39]. This additional invagination within the cytoplasm of the cell allows the MVB to intersect with other EVs and organelles, adding to the diversity of its constituents. The MVB may fuse with a lysosome to degrade its contents or fuse with the cell's plasma membrane to release the ILVs, now called exosomes. Through this 2-step invagination process, the membrane of the exosome more closely resembles the orientation of the parent cell, whereas direct buds of the plasma membrane would be oriented inside-out in comparison. The cargo that exosomes carry includes transcription factors, cytosolic and nuclear proteins, RNA, microRNA (miRNA), mRNA, metabolites, and cytosolic and plasma membrane surface proteins[40]. The physiological role of exosomes is an ongoing topic of research. Among its proposed roles, it is speculated that exosomes assist in maintaining homeostasis by removing unnecessary cellular constituents, regulating intercellular communication modulating the immune response, altering disease progression, and more. Clinically, exosomes have recognized utility as diagnostic carriers of biomarkers and are being explored as therapeutics and vehicles for therapeutics [39,40].

Therapeutic uses of exosomes

Exosomes may have beneficial effects in disease states and have therapeutic implications in various diseases including the role in DFU wound healing[37,41-43]. Because of the ability of exosomes to modulate complex intercellular pathways, exosomes are thought to have therapeutic utility in treating many diseases. For instance, mesenchymal stem cell (MSC) derived exosomes have been demonstrated to be agents for the immunomodulation of inflammation[44] (Table 1).

The effect of exosomes depends on the cargo that it carries and the systems that the cargo modulates. For example, exosomes containing high concentrations of the circular RNA mmu-circ-0000250 have been demonstrated to enhance wound repair in diabetic ulcers while those transporting the miRNA

Table 1 Exosomes have therapeutic potential in inflammatory diseases and enhance wound healing		
Pathology	Source of exosome	Outcome
Inflammatory diseases[44]	Adipose-derived mesenchymal stem cells	Exosomes displayed an inhibitory effect in the activation, differentiation, and proliferation of T-cells and inhibit IFN-γ release
Impaired wound healing in diabetes[45]	Whole blood serum	Serum-derived exosomes promoted angiogenesis and extracellular matrix formation
Diabetic wound healing[46]	Bone marrow and adipose tissue	In mice models, adipose tissue-derived EVs promoted wound healing while those that were bone-derived did not
Diabetic wound healing[47]	Macrophages	Macrophage-derived exosomes inhibited the secretion of pro-inflammatory enzymes and cytokines in a rat model
Diabetic wounds[48]	Human umbilical cord mesenchymal stem cells	Exosomes accelerated cutaneous wound healing and reduced the effects of oxidative stress and promoted angiogenesis
Diabetic wounds[49]	Human amniotic epithelial cells	Exosomes promoted angiogenesis and fibroblast function *via* activation of the PI3K-Akt-mTOR pathway

IFN: Interferon; EVs: Extracellular vesicles; PI3K: Phosphatidylinositol-3-kinase; mTOR: Mechanistic target of rapamycin.

miR-20b-5p slow wound healing and angiogenesis[13,50]. Adipocyte stem cell-derived exosomes have been shown to promote angiogenesis and proliferation of cells in hyperglycemic environments[15]. Studies have shown a myriad of physiological benefits that exosomes provide, including improving immune responses in both infectious diseases and anti-tumor responses[40]. Exosomes from cardio-sphere-derived cells possess anti-inflammatory, anti-oxidative, anti-apoptotic, anti-fibrotic, and cardiomyogenic effects[51,52]. MSC-derived exosomes provide neuroprotective effects in stroke and exosomes from endothelial cells have been suggested as a method to treat atherosclerosis[40]. Exosomes may also play a role in disease pathogenesis. In studies of atherosclerosis, exosomes of patients contributed to endothelial cell dysfunction and vascular calcification while exosomes of healthy patients conferred atheroprotective effects. Thus, clinical use of exosomes will benefit from a greater understanding of how the exosomal cargo contributes to their effects and how modifications to that cargo can augment their therapeutic utility[53,54].

However, the perspective is that the diseases may also influence the cargo carried by exosomes compared to non-afflicted populations. This suggests that deficiencies in endogenous production and transportation of anti-inflammatory factors can be addressed by supplementing such factors through the application of anti-inflammatory-loaded exosomes (Table 2). The tissue and cell sources of the exosomes appear to play a role in their morphology, stability, and immunomodulatory ability[37] and thus, these aspects should be considered while modifying or pretreating exosomes to enhance their efficacy, stability, and bioavailability.

Exosomes in drug delivery and loaded exosomes
Exosomes loaded with therapeutic drugs, or loaded exosomes, have been shown to have improved performance over free drugs. Studies have examined the effect and potential benefit of modifying exosomal cargo to treat diseases and the results suggest the beneficial effects of loading exosomes with therapeutic agents. The contents of exosomes, either modified or natively expressed, have a great influence on disease progression[59-65]. Additionally, that pre-treatment or lading exosomes with the desired drug enhance efficacy of exosomes[53,54] and this strategy may be of significance in enhancing wound healing in chronic nonhealing DFUs by loading exosomes with anti-inflammatory agents of small molecules or drugs targeting a specific factor. The literature on using loaded exosomes for DFU healing is scarce and thus, need more focused research.

There are endogenous and exogenous methods that have been proposed and used to load therapeutics into exosomes to enhance efficacy and precision. Exogenous routes manipulate exosomes after collection and endogenous routes affect the parent cell's content and thus the content of the exosome during its biogenesis. An exogenous strategy is to incubate naïve exosomes with lipophilic small molecules. One limitation is that incubation is likely only a viable method for small hydrophobic molecules, which can spontaneously diffuse across the exosome membrane[14]. Another strategy is to manipulate the parent cells which will then produce exosomes containing the therapeutics. The drug can be directly loaded with the therapeutic agent, or the parent cell may be transfected with DNA encoding for the therapeutic and subsequent exosomes will then be carriers[14]. Commercially available transfecting kits using transfection agents such as lipofectamine have been shown to effectively incorporate small interfering RNAs (siRNAs) into exosomes. Electroporation or electro-permeabilization is another method to load siRNA, miRNA, DNA, drugs, *etc.* into exosomes. Electroporation is the process of applying an external electric field which increases cell membrane permeability. Electro-poration would be an advantageous method for loading hydrophilic agents which would otherwise not

Table 2 Strategies to enhance stability and bioavailability of exosomes

Pathology	Exosomes modification	Source of exosomes	Strategy and outcomes
Impaired diabetic wound healing[13]	MiR-20b-5p-upregulated exosomes	Isolated from diabetic and non-diabetic patient blood	Exosomes derived from diabetics delayed wound healing and angiogenesis compared to exosomes sourced from non-diabetic patients in mice wounds
Diabetic foot ulcer [15]	Nrf2-rich exosomes	ADSCs (human and rat)	Increased granulation tissue formation, angiogenesis, and growth factor levels and reduced levels of inflammation and oxidative stress with exosomes in a rat model
Diabetic wound[55]	Pioglitazone pre-treated exosomes	MSCs	PGZ-treated exosomes promoted angiogenesis and enhanced wound healing in a rat model
Diabetic foot ulcers [56]	LncRNA H19-overex-pressed exosomes	MSCs	LncRNA h19-rich exosomes prevented apoptosis and inflammation of fibroblasts and stimulated wound healing in the mice model
Diabetic wounds[57]	Deferoxamine precondi-tioned exosomes	Human bone marrow	The preconditioned exosomes promoted angiogenesis and wound healing in diabetic rats
Diabetic wounds[58]	Exosomes with a bioactive nano-dressing	Adipose stromal cells	The nanodressing-conjugated exosomes significantly enhanced tissue remodeling and re-epithelialization

miRNA: MicroRNA; Nrf2: Nuclear factor E2-related factor 2; lncRNA: Long non-coding RNA; ADSC: Adipose-derived stem cell; MSC: Mesenchymal stem cell.

readily cross the exosomal membranes. Tests with exosomes loaded with siRNAs are highly efficient and specific carriers for delivering to neuronal cell lines, microglia, and oligodendrocytes. In similar tests with other, non-exosomal, EVs, there was limited effective loading potential with nucleic acid cargos larger than miRNA or siRNA[66-68]. Understanding the influence of binding proteins in the physiological loading of EVs may provide a method for improved loading methods. Sonication, the application of low-frequency ultrasound to produce pores, has been used in loading siRNA into EVs and so may be a viable method for exosome loading[69]. However, sonication, along with extrusion and freeze-thawing has been shown to cause aggregation with EVs and their cargo, limiting their success [70].

Advantages and limitations of exosomes

Compared to exosomes, other types of EVs, and nanoparticles possess limitations in clinical use. For instance, synthetic nanoparticles have been developed as a means of drug delivery but administering synthetic drug-loaded nanoparticles into the bloodstream has unearthed two vexing issues: Toxicity and rapid phagocytic clearance. Compared to drug delivery with PEGylated nanoparticles, allogenic exosomes collected from patients' tissues and blood seem to have immune privilege, decreasing the chances of toxicity and immune reactions while decreasing the rate at which exosomes and their cargo are cleared by the mononuclear phagocyte system[14]. Liposomes are another EV that have been used for drug delivery. They are derived from cholesterol and formed from hydrated phospholipids and hold drawbacks that exosomes do not. Liposomes have limited clinical capacity due to their higher risk of toxicity, low target specificity, short half-life, low solubility, and risk of aggregation during storage[71-73]. Exosomes hold many advantages over other kinds of nanoparticles and even their parent cells in terms of immunoreactivity and targetability. Based on their tissue origin, exosomes possess surface adhesion proteins, vector ligands, and specific cell tropisms that can be utilized for highly specific targeting of the tissues of interest. Exosomes address the issues faced by other nanoparticle alternatives as they are immunologically inert and can pass through the blood-brain barrier and mucosal barriers and decrease the incidence of drug resistance development[14,54,59]. Additionally, exosomes are highly stable due to their rigid lipid membrane that is resistant to bursting in freeze-thawing cycles in the hypotonic environment[74].

While the advantages of using exosomes for drug delivery are many, some limitations need to be considered. The loading capacity of exosomes has been presented as a potential issue with exosome drug delivery. As exosomes naturally possess proteins and nucleic acids, they may hold lower capacity relative to other nanoparticle drug delivery methods. Studies have observed a low range of roughly 3% to highs of 26%, involving factors such as the drug in question, the methods of loading drugs into the exosomes, and the types of tissues that the exosomes were sourced from[14,71]. EVs have been shown to have a limited ability to carry nucleic acid cargo larger than siRNAs or miRNAs[66]. This limitation may potentially apply to exosomes. The potential for exosomes as a drug delivery method is promising. However, the information on loaded exosomes to treat nonhealing DFUs is limited and insight from other fields to explore the best effective strategy for effective delivery of a therapeutic molecules will be helpful.

Site-specific targetability of exosomes

The therapeutic efficacy and efficiency of an agent are decreased by lower bioavailability and off-target effects and have been a major hurdle while developing novel therapies and site-specific delivery of a therapeutic agent may be an answer[75]. Site-specific or organ-specific delivery of a therapeutic agent can be used by using a linker enhancing attachment of the agent with its ligand as in the case of protease-targeted chimeras (PROTACs) but the large size of PROTACs is a concern[76]. While exosomes due to their small size have an advantage for site- or organ-specific delivery because of their low molecular weight[75]. Low molecular weight, small size, and ease of manufacturability bolster the use of exosomes as a suitable agent to enhance drug efficacy by site-specific delivery. The cellular origin and membrane composition of the exosomes and the pathological condition of the host determine the biodistribution of exosomes. Along with this, the rapid clearance of exosomes from circulation and internalization of exosomes in a high cell type-specific manner are issues while administering exosomes systemically. Thus, site-specific delivery of exosomes will enhance therapeutic efficacy. The modifications to increase site-specificity of exosomes may be passive using natural tropism of exosomes or active *via* surface engineering of exosome membrane. Biological or chemical modification of the exosome's membrane proteins composition, covalent and non-covalent modification of the surface of exosomes, presenting the targeting peptides conjugated with exosome membrane-associated domains such as lysosome-associated membrane glycoprotein 2b, C1C2 domain of lactadherin, CD63, and CD47 improves site-specific targeting. Exosome-liposome hybridization, genetically modifying the exosome-producing cells, loading exosomes miRNA, short-interfering RNA, and therapeutic agent targeting desired proteins, PEGylation, mixing exosomes with micelles, and use of click chemistry are other strategies to enhance exosome targetability[77-79]. Therapeutic specificity and sensitivity of exosomes can also be enhanced by fine-tuning the isolation methods as discussed for NanoPoms[80]. Increasing the site-specificity of exosomes may have implications to target a specific protein of interest to promote wound healing of non-healing DFU. Recent reports suggesting increased expression of C-X-C motif chemokine 8, hypoxia-inducible factor 1 alpha, Chitinase-3 like-protein-1, TNF stimulated gene-6, matrix metallopeptidase (MMP)1, MMP3, and MMP11 in association with nonhealing diabetic ulcers[81, 82] suggest that these proteins might be novel therapeutic targets to promote wound healing in chronic DFUs using armed exosomes for targeted delivery to regulate their expression[83].

Exosomes and pro-Rv for diabetic ulcers

Exosomes have been demonstrated to be effective carriers of a myriad of bioactive factors including growth factors, nucleic acids, proteins, and antibiotics. Loading exosomes with therapeutic agents have been shown to benefit healing in diabetic skin diseases. One such study demonstrated that exosomes pre-treated with a statin, atorvastatin, or all-terrain vehicle (ATV), accelerated wound healing and angiogenesis in diabetic rats *via* the upregulation of the AKT/eNOS signaling pathway[84]. The wound-healing ability of these ATV-treated exosomes was compared to exosomes derived from bone marrow MSCs. In terms of cell proliferation and vascularization, the pretreated exosomes resulted in significantly accelerated healing compared to their non-treated counterparts. The usage of exosomes offers the possibility of enhancing the effect of a multitude of therapeutic agents, with many studies demonstrating that this method outperforms the benefits of non-loaded exosomes and exosome-free drug applications. Rvs have likewise gained attention in treating a myriad of inflammatory conditions and diseases. Though there has been some recognition that Rvs are unstable due to their vulnerability to oxidation[85]. Exosomes have been shown to be stable and efficient in targeting specific tissues while protecting their cargo from degradation[14,59]. So, exosomes may have great potential in transporting Rv and other SPMs and addressing the weaknesses in the clinical utility of SPMs on their own. Loaded exosomes may have great potential in enhancing healing in chronic nonhealing DFUs but there are only a few published studies (Table 3) that translate the potential of loaded exosomes warranting further research.

FUTURE PERSPECTIVES

Prolonged immune cell infiltration is an established factor in the disease process of nonhealing diabetic wounds. Pro-inflammatory cytokines including IL-6, IL-8, IL-1, and TNF-α promote the infiltration of CD8 T cells, neutrophils, and macrophages to the ulcer. Increased secretion of IL-8 in DFUs is further facilitated by an increased expression of toll-like receptor 9. A persistently increased infiltration of immune cells and secretion of pro-inflammatory cytokines characterizing nonhealing DFUs indicate that targeting the inflammatory pathway may offer a promising avenue to enhance healing in DFUs[24]. The beneficial results with the use of loading exosomes with anti-inflammatory miRNAs and cytokines, and exosomes-mediated increased stability to the anti-inflammatory mediator vulnerable to endogenous degradation[54,86] support the notion of using exosomes to enhance loaded exosomes in promoting healing in DFUs. Of note, the association of increased expression of inflammatory cytokine IL-8 in nonhealing DFU suggests that loading exosomes targeting IL-8 and its downstream signaling will have therapeutic significance[24,81,82]. Targeting inflammatory cytokines and the downstream signaling in

Table 3 Loaded exosomes in the treatment of diabetic wounds

Pathology	Source of exosome	Modification	Outcome
Diabetic ulcerative wounds [15]	Adipose-derived stem cells	Nrf2	Treatment of animal models with exosomes high in Nrf2 expression significantly reduced ulceration area and promoted angiogenesis
Diabetes-associated impaired wound healing[50]	Adipose-derived mesenchymal stem cells	mmu_circ_0000250	Exosomes modified to contain more mmu_circ_0000250 had a greater effect than unmodified exosomes in endothelial repair in diabetic rats
Diabetes-associated impaired wound healing[84]	Mesenchymal stem cells	ATV	ATV-loaded exosomes enhanced angiogenesis and tissue repair in animal models compared to unmodified exosomes
Diabetic wounds[86]	Mesenchymal stem cells	MiR-155 inhibitor	Loaded exosomes promoted anti-inflammatory action and enhanced re-epithelialization
Diabetic wounds[87]	Adipose stem cells	MiR-21-5P	Loaded exosomes promoted re-epithelialization and angiogenesis. MiR-21-5P was protected from degradation

Studies have shown that exosomes may carry a myriad of therapeutic cargo to end chronic inflammation, enhance wound healing, and promote angiogenesis and re-epithelialization. miRNA: MicroRNA; Nrf2: Nuclear factor E2-related factor 2; ATV: Atorvastatin.

DFUs is supported by the role of immunomodulation, antimicrobials, modulation of cytokine production, and targeting inflammation using loaded exosomes in chronic inflammatory diseases[88-92].

CONCLUSION

Various studies have discussed that attenuating chronic inflammation can be an effective strategy to enhance healing in DFUs. Despite the current treatment in practice, the risk of amputation persists. Exosomes have been demonstrated to be a potential therapeutic agent for a wide variety of inflammatory conditions and diseases. As a therapeutic vehicle, exosomes enhance the therapeutic efficacy of a host of drugs and anti-inflammatory factors. To this end, using exosomes for the delivery of inflammation resolving mediators including Rv and pro-inflammatory cytokine targeted therapy offers promise in ending chronic inflammation and enhancing wound healing with the benefits of target-specificity, non-immunogenicity, and easier handling compared to similar delivery methods.

FOOTNOTES

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