International Journal of Nanomedicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

REVIEW

Role of Exosomes in Pharyngucutaneous Fistula After Total Laryngectomy

Li Chen[1,*], Maohua Wang[2,*], Zhenhua Zhong[3,*], Baoxu Liu[1], Wentao Zhang[4], Bin Zhu[4], Cheng Jiao[4], Chenjie Yu[5], Bing Guan[4]

[1]Department of Otolaryngology, Head and Neck Surgery, Dalian Medical University, Dalian, 116000, People's Republic of China; [2]Department of Otolaryngology, Head and Neck Surgery, The First People's Hospital of Foshan, Foshan, 528000, People's Republic of China; [3]Department of Otolaryngology, Head and Neck Surgery, The Affiliated Hospital of Yangzhou University, Yangzhou University, Yangzhou, 225000, People's Republic of China; [4]Department of Otolaryngology, Head and Neck Surgery, Clinical Medical College, Yangzhou University, Yangzhou, 225001, People's Republic of China; [5]Department of Otolaryngology, Head and Neck Surgery, Affiliated Drum Tower Hospital of Nanjing University Medical School, Jiangsu Provincial Key Medical Discipline (Laboratory), Nanjing, 210008, People's Republic of China

*These authors contributed equally to this work

Correspondence: Chenjie Yu, Department of Otolaryngology, Head and Neck Surgery, Affiliated Drum Tower Hospital of Nanjing University Medical School, Jiangsu Provincial Key Medical Discipline (Laboratory), Nanjing, 210008, People's Republic of China, Tel/Fax +86-13705168089, Email entphd@163.com; Bing Guan, Department of Otolaryngology, Head and Neck Surgery, Clinical Medical College, Yangzhou University, Yangzhou, 225001, People's Republic of China, Tel/Fax +86-18051063666, Email aliceguan0685@sina.com

Abstract: Pharyngocutaneous fistula is the most common complication after total laryngectomy and is difficult to heal. Although conservative treatment and surgical repair are effective, they often take longer and additional trips to the operating room, which undoubtedly increases the financial burden on patients. Especially in combination with diseases such as diabetes and hypertension, which affect the efficacy of surgery. Adding growth factors into the repair material can promote fibroblast proliferation, angiogenesis, and accelerate wound healing. A substantial number of studies have shown that a type of nanoscale extracellular vesicle, called exosomes, facilitates organization repair by promoting blood vessel production, protein polysaccharides, and collagen deposition, thereby representing a new type of cellular therapy. At present, there is little research on the application of exosomes in pharyngocutaneous fistula regeneration after total laryngectomy. In this review, we summarize the biological characteristics of exosomes and their application in biomedical science, and highlight their application prospects in pharyngocutaneous fistula regeneration after total laryngectomy.

Keywords: exosomes, extracellular vesicle, head and neck squamous cell carcinoma, pharyngocutaneous fistula, tissue repair and regeneration, bioengineering

Introduction

Pharyngocutaneous fistula (PF) occurs when saliva stored under the skin or under the incision of tissue forms a pus cavity to break to the skin or incision edge, so that the pharynx, esophagus, and skin connect into a sinus tract. PF is a common complication of total laryngectomy, with an incidence rate of 3–65% because of marginal vascular damage according to recent reports (Figure 1).[1] Usually, wound healing occurs in four phases, including hemostasis, inflammation, repair, and shaping (Figure 2) and requires a series of complex molecules and cellular events, including cellularization, cell proliferation, angiogenesis, extracellular matrix deposition, and tissue remodeling.[2–4] Most scholars believe that infection is the root cause of PF. Elderly age, multiple underlying diseases, poor nutrition, and low body resistance are factors known to increase the incidence of PF. The destruction of the submucosal vascular bed by an electrosurgical knife and the damage of normal tissue by radiation therapy also affect PF healing. Clinically, the preferred treatment of PF is conservative, including intravenous antibiotics, local antibiotic irrigation, local injection of botulinum toxin A,[5] and nasogastric tube feeding until the closure of the PF. However, these strategies have limited efficacy, with slow, or even absent healing. Patients with poor conservative treatment may be treated with pedicled flap, surgical sealant,[6] fibrin

Received: 29 April 2022
Accepted: 1 September 2022
Published: 12 September 2022

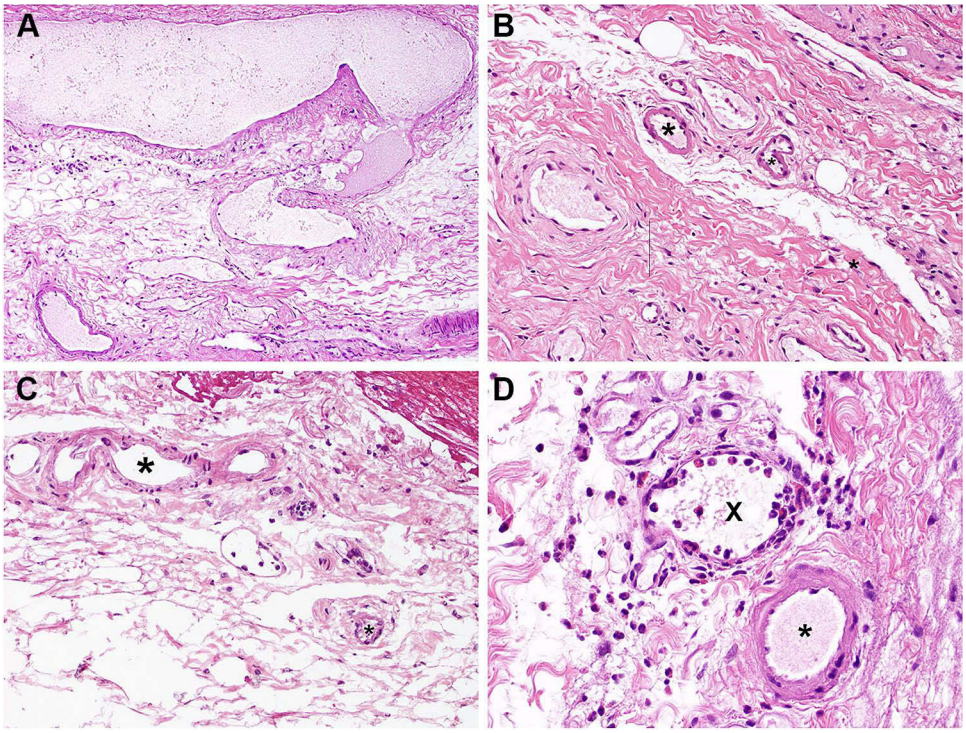

Figure 1 Histological features of marginal blood vessels after total laryngectomy. Used with permission of Spring Nature BV, from Abouyared, M., et al, Abnormal Microvasculature in Laryngectomy Mucosal Margins may be Associated with Increased Risk of Fistula. Head Neck Pathol, 2019. 13(3): p. 364-370, permission conveyed through Copyright Clearance Centre, Inc.[11] (**A**) Lymphatic vessels with marked dilation within the submucosal tissues. (**B**) Eosinophilic substances in the walls of blood vessels result in transparent and thickened arterioles. (**C**) Analysis of the frozen sections corresponding to B shows that transparent arterioles can be detected histologically. (**D**) Dilated capillaries (X); transparent arterioles (*). These histological features are more common in patients with postoperative fistula.

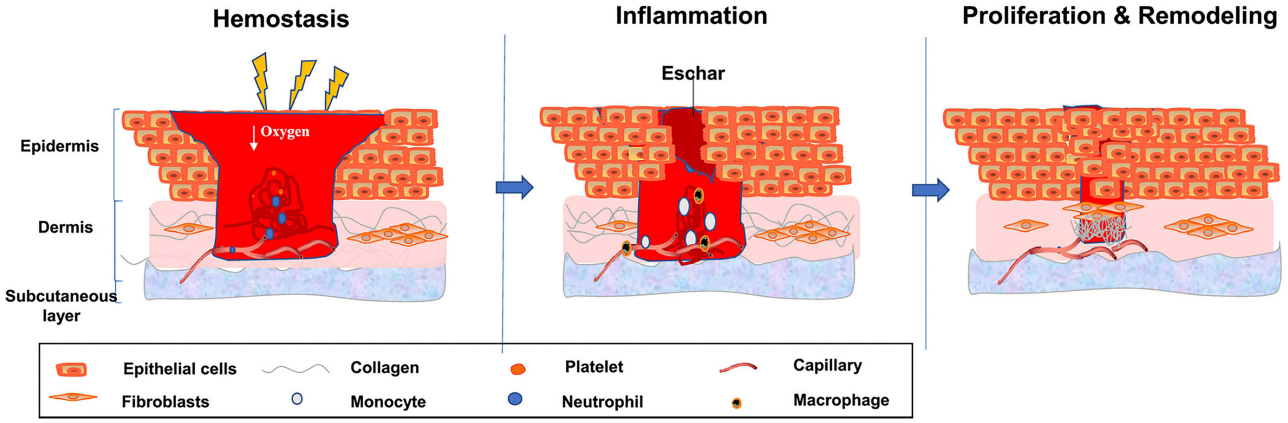

Figure 2 The four main stages of wound healing: hemostasis, inflammation, proliferation, and remodeling. Reproduced from Liu Y, Yang X, Liu Y, et al. NRF2 signalling pathway: new insights and progress in the field of wound healing. J Cell Mol Med. 2021;25(13):5857–5868. © 2021 The Authors. Journal of Cellular and Molecular Medicine published by Foundation for Cellular and Molecular Medicine and John Wiley & Sons Ltd. Creative Commons CC BY licens.[12] Inflammatory stages include the activation of inflammatory cells and the release of proinflammatory factors; proliferative stages include fibroblasts proliferation and angiogenesis; and remodeling includes myofibroblast shrinkage of the wound and barrier repair.

binder,[7] or autologous fat.[8] Recently, some scholars have proposed that nanoscale extracellular vesicles termed exosomes[9,10] can be directly delivered to the wound or defect to accelerate wound healing. The therapeutic purpose is achieved by direct injection, intraperitoneal injection, hydrogel complex, or drug carrier.

Most cells can release vesicles, including microbubbles, apoptosis, and exosomes,[13] to transmit information between cells. The term "exosome" was first proposed by Trams et al in 1981[14] to describe nanosized (30–150 nm) vesicles generated by endosomes to form a multivesicular body (MVB) (Figure 3). MVBs fuse with lysosomes to degrade and recycle their

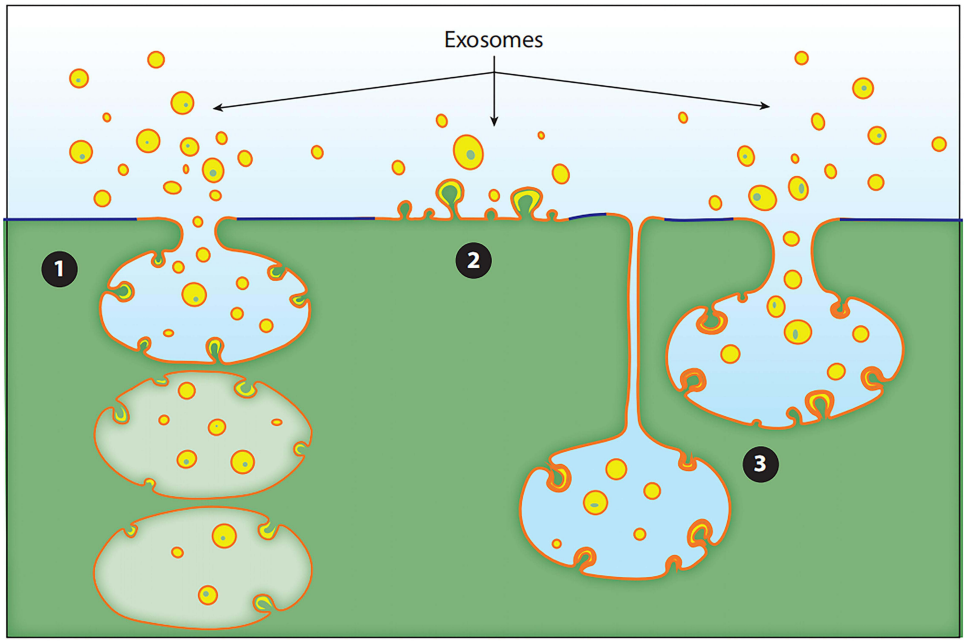

Figure 3 The three ways of exosome formation. Used with permission of Annual Reviews, Inc, from Pegtel DM, Gould SJ. Exosomes. Annu Rev Biochem. 2019; permission convyed through Copyright Clearance Center, Inc.[20] 1. Multivesicular bodies release exosomes upon plasma membrane fusion. 2. Exosomes released by budding from the plasma membrane. 3. Delayed release by budding at the intracellular plasma membrane-connected compartments.

contents by autophagy.[15] However, when the MVB fuses with the plasma membrane, proteins, lipids, and nucleic acids can be transferred to receptor cells by paracrine, autocrine, and endocrine mechanisms.[16] Exosomes protect the contents from degradation, which is conducive to intercellular communication. It has been reported that exosomes are secreted by lymphocytes[17] and dendritic cells (DCs),[18] with the function of immune regulation and molecular messengers, as achieved by exposing major histocompatibility complexes (MHC) and costimulatory molecules. Indeed, exosomes form mature DCs carrying B7-2 and ICAM-1 have been shown to directly interact with T cells to activate the immune system. Moreover, immature DC-derived exosomes can present antigens to receptor cells to indirectly induce T-cell activation.[19] Almost all cells secrete exosomes (see Table 1 for specific sources), including macrophages, T cells, B cells, mesenchymal stem cells, fat cells, and tumor cells. Exosomes are widely present in body fluids (blood, saliva, urine, cerebrospinal fluid, breast milk, amniotic fluid, ascites, semen), playing an important role in immunity, as messengers, and in disease diagnosis and treatment. Recently, the profitability of promoting wound healing has gained extensive attention. After tissue damage, abnormal repair mechanisms prevent wound healing or further develop to form a pharyngeal. During the inflammatory phase, the nuclear factor-E2-related factor 2 (Nrf2)-Ccl2-EGF signal axis is inhibited, affecting macrophage transport, re-epithelialization, and angiogenesis. Simultaneously, the release of inflammatory bodies, the production of apoptotic-associated spot-like protein (ASC), and the activation of pro-inflammatory caspases leads to cell pyroptosis and increased pathological autophagy. The release of inflammatory mediators promotes the downregulation of the Wnt/β-catenin signal pathway, blocking the phosphorylation of AKT and ERK1/2, affecting cell proliferation, and slowing wound healing. However, the high expression of transcription factor nuclear factor E2 related factor 2 in exosomes has been shown to accelerate diabetic foot ulcer healing. Various RNAs present in exosomes could regulate other physiological processes, such as transcription and translation, which are beneficial for angiogenesis, fibroblast migration, and tissue damage repair, representing a potential treatment strategy for difficult wounds. Therefore, in this review, we summarize the physiological characteristics, extraction methods, and clinical applications of exosomes, and highlight their application prospects in PF regeneration after total laryngectomy.

Table 1 Source and Function of Exosomes (for Reference)

Cells of Origin	Exosomal Cargo	Biofluids	Recipient Cells	Function	Reference
T cells	miR-198	Plasma	Tumor cells	Inhibit tumor occurrence	[21]
	CD-73	Plasma	T cells	Produce adenosine to suppress immunity	[22]
	Micr-155	Plasma	Th1 cells	Mediate immune suppression	[23]
Dendritic cells	miR-16	Plasma	Endotropical cells	Inhibit the inflammatory response	[24]
	miR-21	Plasma	Endotropical cells	Inhibit the inflammatory response	[24]
	Latent membrane protein-1	Plasma	EBV-infected cells	Suppress the immune response	[25]
Mesoplasmic stem cells	lncRNA H19	Plasma	Fibroblast	Stimulate the wound healing process in diabetic foot ulcer	[26]
	miR-let7, miR-21-5p	Plasma	M2 macrophage	Attenuate the progression of atherosclerotic plaques	[27]
	miR-130a		Endotropical cells	Promote angiogenesis	[28]
Tumor cells	miR-105	Plasma	Endotropical cells	Promote lung and brain metastasis of cancer cells	[29]
	miR-210	Plasma	Lung adenocarcinoma cells	Increase tumor occurrence	[30]
	miR-210	Plasma	JAK2/STAT3	Promote neoplastic angiogenesis	[31]
	ITGA3 & ITGB1	Serum	None	Suggest tumor metastasis	[32]
	lncRNA-MALAT-1	Serum	None	Prevent tumor cells apoptosis	[33]
Endotropical cells	miR-214	Plasma	Endotropical cells	Promote cells migration and angiogenesis	[34]
Macrophage	miR-21-3p, miR-146a, miR-146b	Serum	Inflammation cells	Inhibits overaction of the congenital immune response	[35]
	miR-155	Serum	Endotropical cells	Inhibits angiogenesis	[36]

Application of Exosomes in Biomedicine

Composition and Biological Characteristics of Exosomes

Exosomes are secreted by progenitor cells through the endosomal sorting complexes required for the transport (ESCRT) pathway, which contains most of the components of progenitor cells, which mainly include protein, DNA, mRNA, miRNA, micRNA, and lncRNA. The outer membrane is lipid biomolecules, mainly composed of ceramide, cholesterol, sphingomyelin, glycosphingolipid, and phosphatidylcholine. The components contained in different types of exosomes are distinct, but most of them are highly enriched in cholesterol, which is 2–3-fold more than that in parental cells. Interestingly, ether lipids account for a high proportion of membrane lipids, but there has been limited research into their roles. The research shows[37] that ether lipids include lecithin and phosphatidylethanolamine, mainly assuming functions of membrane transport, cell differentiation, and antioxidant activities to stabilize the cell structure and protect the internal components against degradation.[38–42] The proteins found in exosomes include tetrapeptide, transmembrane proteins, heat shock proteins (HSP60, HSP70, HSPA5, CCT2, and HSP90), lactadherin, and annexins[43] (I, II, IV, V, VI, VII and XI), all of which represent good biomarkers for separating and quantifying exosomes. Exosomal proteins also have differing roles. Tetrapeptide is an integrated outer membrane protein, and it has been demonstrated[20] that exosomes are highly

enriched in CD81, CD82, CD37, and CD63, which assist with the transportation and stability of other membrane proteins and may be used as specific biomarkers.[44] Heat shock proteins are highly conservative molecular partners, which promote the folding modification of other proteins, and antistress and adjustment of redox reactions. Annexins are mainly involved in membrane adhesion and fusion. Nucleic acids are other key substances, encompassing DNA, mi-RNA, mic-RNA, and IncRNA. The type and quantity of the nucleic acids contained in exosomes are achieved by an active sorting mechanism. Indeed, Guduric-Fuchs et al[45] found that miR-150, miR-142-3p, and miR-451 are given priority over the exosomes, mediating intercellular communication, immunity, antigen presence, and antigen transfer via transcription or translation. Non-encoded RNA mainly regulates gene expression, catalyzing specific RNA degradation, but whether DNA should be selectively classified to the exosomes is unclear. To summarize, exosomes are nanoscale vesicles derived by exocytosis, which contain proteins, lipids, and nucleic acids, all of which play a key role in cellular communication. Not only are exosomes involved in physiological processes, such as reproductive, immunization, transcription, translation, and organ development,[46–48] but they also mediate disease development, such as tumor invasion, inflammation, and cardiovascular disease.[49–51] Clinically, exosomes have been used in vaccine development, drug carriers, biocoupling, and wound-binders. The specific applications are shown in Table 2.

Separation and Extraction of Exosomes

Although the use of exosomes in treatment is highly regarded, it is difficult to use in clinical work due to difficult extraction technology, and high purity and storage requirements. Therefore, in future research, it will be important to simplify the extraction of exosomes and improve the yield. Commonly used exosome extraction methods include ultracentrifugation, ultrafiltration, precipitation, miniature exclusion chromatography, and affinity capture.

Table 2 Type and Clinical Application of Exosomes (for Reference)

Cargo Type	Exosomal Cargo	Target	Clinical Value	Reference
miR-RNA	miR-RNA126	Fibroblast	Promote epithelialization	[52]
	miR-100-5p	M-TOR autophagy pathway	Protect cartilage from damage	[53]
	miR-92a-3p	WNT5A mRNA	Enhance cartilage generation	[54]
	miR-16	Vascular endothelial growth factor	Prevent angiogenesis	[55]
	miR-92a	Leukemia cells	Mediate leukemia metastasis	[56]
	miR-21-3p	Cardiomyocytes	Induce cardiomyocyte hypertrophy	[57]
	miR-207	Astrocytes	Inhibits NF-κB and alleviates symptoms of depression	[58]
Inc-RNA	Inc-EGFR	Regulating T-cells	Promoting hepatocellular carcinoma immune evasion	[59]
	Inc-H19	miR-let-7	Promote tongue squamous cell carcinoma migration and invasion	[60]
Cir-RNA	Fil1 exonic	Small cell lung cancer cells	Promote tumor metastasis	[61]
Protein	Annexin 2 and L-plastin	Breast cancer cells	Prognosis for breast cancer	[62,63]
	PD-L1	Head and neck cancer cells	Indicate tumor progression	[64]
	Leucine rich alpha-2-glycoprotein 1	Cancer cells	A potential biomarker for diagnosing for NSCLC	[65]
	Latent membrane protein 1	T cells	Biomarker for diagnosing for nasopharyngeal carcinoma	[66]
	Tau proteins	Neurons and microglia	Promote progression of Parkinson's disease	[67]

Ultracentrifugation is the gold standard for the separation of exosomes, including differential ultracentrifugation and density gradient ultracentrifugation, among which, differential ultracentrifugation is the most commonly used. Under different centrifugal forces (up to 1,000,000×g), contaminants (300–400×g), cell debris (2000×g), and apoptotic bodies (10,000×g) are removed sequentially before exosomes are finally purified. The separation efficiency is related to the rotor acceleration, rotor characteristics (rotation radius, k-coefficient, sedimentation path length), and sample viscosity. Therefore, we should pay close attention to these aspects when adjusting the rotor parameters. Although differential ultracentrifugation is simple to operate and does not require specialized knowledge, prolonged operation (more than 4 h) can cause exosome damage and cannot completely separate exosomes from other components outside the cell. Ultrafiltration is faster than ultracentrifugation, but with lower purity. Currently, a new method of exosome extraction, microfluidics,[68] is being widely used in biomedical research, molecular biology, and analytical chemistry. In this technology, exosome membrane-binding proteins bind to protein antibodies on a microfluidic chip to achieve the effect of separation. ExoSearch microfluidic chips have been developed and applied clinically. The most recent research[69] found that two-phase separation was the most economical, fast, and promising exosome extraction method. In this method, polyethylene glycol and dextran are simultaneously dissolved in aqueous solution to form two independent phases; exosomes are preferentially deposited in the dextrose phase through chemical reaction, and other proteins will be deposited in the polyethylene glycol phase. Fresh polyethylene glycol solution is extracted repeatedly; the protein content continues to decrease, while the purity of the exosomes is elevated. Although the clinical application of exosomes in diagnosis, treatment, and prognosis has been established, their isolation and purification remain challenging. Therefore, further research is necessary to establish efficient, short-term, and convenient extraction methods to realize the advantages of exosome therapy.

Clinical Application of Exosomes

Based on their good physiological characteristics, exosomes have been used in the diagnosis of myocardial infarction,[70,71] psychiatric diseases,[72] cancer,[73–77] spinal cord injury, and wound healing in vivo.[78,79] In vitro, exosomes have been included in drug carriers,[80] where access to the lesion achieves the purpose of treatment through direct injection, intravenous delivery, intraperitoneal injection, and others. Hydrogels and drug carriers are the two most common modes of exosome transport.

Hydrogel

Hydrogels with three-dimensional crosslinking structures can support bioactive molecules, such as stem cells and antioxidants, with good biocompatibility, antibacterial, hemostasis, tissue adhesion, easy degradation, and injectable properties, and represent ideal wound dressings. Hydrogel has been used for diabetic chronic wounds,[81] bone regeneration,[82] spinal cord injury,[83] periodontitis,[84] and limb ischemia, and can improve the effectiveness of transdermal administration through hydrogel-formed microneedles.[85] As research has progressed, researchers have found that the hydrogel-exosome hybridization system was more conducive to wound healing than the use of hydrogel alone. Exosomes can be embedded in hydrogel or act as crosslinkers to construct a three-dimensional hydrogel network directly. In a mouse injury model, Nooshabadi et al covered the wound with a chitosan hydrogel containing stem cell-derived exosomes and demonstrated a wound closure capacity of nearly 83.6%, which was significantly higher than the control group (51.5%). These findings suggest that chitosan glycerol exosome hydrogel can be used for the repair of defective skin, and to promote wound healing and epithelialization.[86] Similarly, exosomes of the sodium alginate hydrogel are placed at the wound, which significantly improves wound closure, collagen synthesis and angiogenesis, and promotes the regeneration of the whole skin.[87] Besides, Wang et al[81] produced a new type of hydrogel consisting of Pluronic F127 (F127), oxidative hyaluronic acid (OHA), and EPL, known as FHE hydrogel. This hydrogel has advantages of being injectable, antibacterial, and self-healing, all of which can promote diabetic wound healing, angiogenesis, and skin regeneration via the joining of exosomes. Exosomes are loaded into hydrogels through electrostatic interaction with EPL and released in a weakly acidic environment. Recently, exosomal hydrogels derived from HucMSCs-exos have been used in bone transplantation with self-healing to extend the life and safety of the material.[88] Hydrogel is a good tissue repair material, and the addition of low immunogenic exosomes can greatly improve the repair efficiency in the context of

chronic erosion wounds, ulcers, and PF regeneration after total laryngectomy. Hydrogel-exosome hybridization system promotes tissue and mucosal regeneration, with the aim to replace the graft flap, which will represent a major step toward cell-free therapy.

Drug Carriers

In addition to their combination with hydrogels, exosomes can also act as drug carriers. In recent years, the use of nanoscale exosomes as tumor-targeted drug carriers has gained increasing attention. Compared to traditional drug carriers, such as ice flakes,[89] liposomes, and multimers, exosomes are favored for their stability and ability to penetrate the blood–brain barrier, protecting the payload from degradation. Sun et al first proposed the use of exosomes as drug delivery systems.[87] In terms of tumors, multiple studies have confirmed that exosome nanoparticles can be used for triple-negative breast cancer,[90–93] osteosarcoma,[87,92] glioblastoma. In terms of inflammation, exosome wrapping of the anti-inflammatory agent curcumin shows increased solubility and stability, as well as the ability to downregulate the $CD11b^{+}Gr\text{-}1^{+}$ cell population to control the inflammatory response.[94] The use of exosomes as drug delivery systems has also been reported in the application of rheumatoid arthritis,[95] lymphoma, septic shock, Parkinson's disease, and neuroinductive diseases. Exosomes can also be used as drug carriers in the context of wound healing, where they have advantages in their high biocompatibility and ability to prolong the drug action. Exosomes, which can be wrapped in centella asiatica, gallic acid, syringe, and orange bell to promote wound healing, represent a good material for PF regeneration after total laryngectomy, enhancing antioxidant properties and stimulating keratinocyte migration. Several alternative nanoparticles, such as organic nanoparticles, lipid nanoparticles, polymer nanoparticles, nano hydrogels, and nanofibers, have also been reported. The healing difficulties observed in PF are largely due to the lack of oxygen. Consequently, damaged blood vessels cannot provide sufficient oxygen to normal tissues, affecting fistula repair, and resulting in a vicious cycle of "hypoxia–necrosis–hypoxia." Oxygen-carrying nanodroplets are proposed as a promising tool for the treatment of chronic wounds, where they can serve to continuously release oxygen, improving wound hypoxia and promoting collagen deposition. Exosomes are better choices than chitosan as oxygen-carrying droplet carriers, and may be trialed for the repair of PF regeneration after total laryngectomy in the future, providing another option for the repair materials of PF.

Prospects for Exosomes in PF Regeneration

Mechanism of PF After Total Laryngeal Surgery

In summary, exosomes have high biocompatibility, low immunogenicity, non-toxicity, and low cost, thus wound dressing could be used to deliver bioactive exosomes for promoting wound healing, significantly increasing the wound healing rate. Wound healing is generally divided into four stages, including coagulation, inflammation, repair, and maturity, and obstruction at any stage may lead to wound non-healing. PF is particularly difficult to heal due to the low immunity and weak anti-infection ability of patients following total laryngectomy. The higher the tumor stage, the more nutrients the cancer cells plunder from the normal tissue, and the weaker the immune function of the normal tissue is. The normal immune function of the body is closely related to wound healing, mainly through the actions of neutrophils, monocytes, macrophages, and dendritic cells to trigger epithelial migration and proliferation. Previous studies[96] have shown that during the inflammatory phase, the Nrf2-Ccl2-EGF signal axis is inhibited, the secretion of nrf2 by epidermal keratinocytes is blocked, and the secretion of Ccl2-EGF is reduced, affecting macrophage transport, re-epithelialization, and angiogenesis (Figure 4). This process often occurs in chronic, unhealed wounds such as those observed in PF. Moreover, nrf2 can affect the transformation of M1 to M2 macrophages, hinder the production of anti-inflammatory factors, promote an inflammatory state, and slow wound repair; however, the specific effect mechanism has not yet been elucidated. New studies[12] suggest that chronic wounds lack nrf2, which stimulates the release of inflammasomes, the production of apoptosis-associated spot-like protein (ASC), and the activation of pro-inflammatory caspase, leading to cell pyroptosis (a new type of programmed apoptosis) and autophagy (Figure 5). Zeng et al[97] found that miR-106b-5p induces excessive autophagy of fibroblasts by inhibiting erk1/2 expression, reducing collagen production, and delaying wound healing. In addition, the increased release of matrix metalloproteinases, pro-

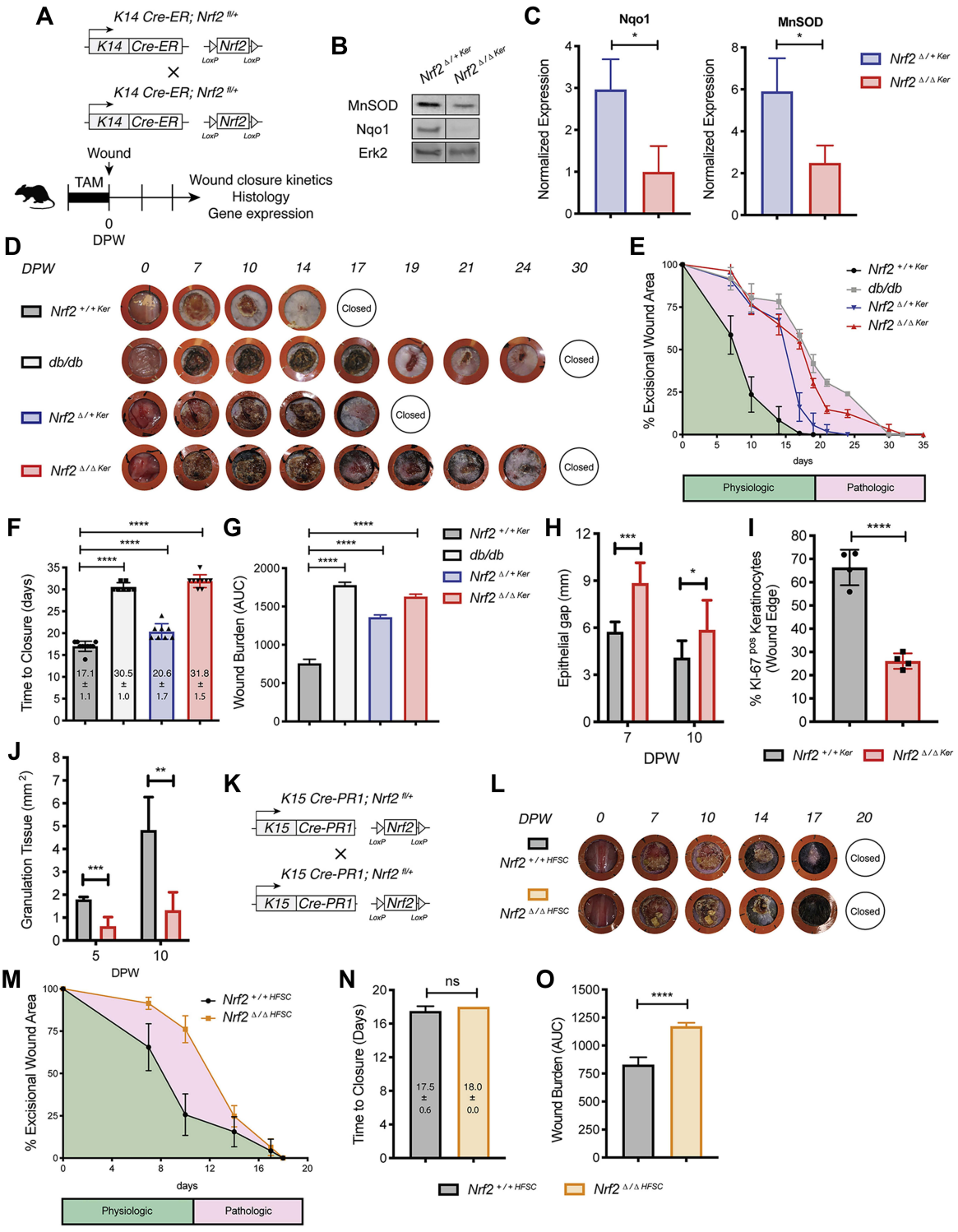

Figure 4 The lack of Nrf2 from epidermal keratinocytes impairs wound repair. Reproduced from Villarreal-Ponce, A., et al, Keratinocyte-Macrophage Crosstalk by the Nrf2/Ccl2/EGF Signaling Axis Orchestrates Tissue Repair. Cell Rep, 2020. 33(8): p. 108417. © 2020 The Authors. This article is available under the Creative Commons CC-BY-NC-ND license.[96] (**A**) Generation of $Nrf2^{\Delta/+Ker}$ and $Nrf2^{\Delta/\Delta ker}$ mice. (**B**) WB (Western-Blot) for MnSOD and Nqo1 on whole-wound lysates from $Nrf2^{\Delta/+Ker}$ and $Nrf2^{\Delta/\Delta ker}$ mice. (**C**) Quantification of (**B**). (**D**) Images of healing in $Nrf2^{+/+Ker}$, $Nrf2^{\Delta/+Ker}$, $Nrf2^{\Delta/\Delta ker}$. (**E**). Wound area over time in $Nrf2^{+/+Ker}$, $Nrf2^{\Delta/+Ker}$, $Nrf2^{\Delta/\Delta ker}$, db/db mice. (**F**). Wound closure in $Nrf2^{+/+Ker}$, $Nrf2^{\Delta/+Ker}$, $Nrf2^{\Delta/\Delta ker}$, db/db mice. (**G**) Wound burden analyses in $Nrf2^{+/+Ker}$, $Nrf2^{\Delta/+Ker}$, $Nrf2^{\Delta/\Delta ker}$, db/db mice. (**H**) Epithelial gap measurements in in $Nrf2^{+/+Ker}$, $Nrf2^{\Delta/\Delta ker}$. (**I**) Quantification of Ki-67+ keratinocytes at the epithelial wound edge in $Nrf2^{+/+Ker}$, $Nrf2^{\Delta/\Delta ker}$. (**J**) Granulation tissue measurements in $Nrf2^{+/+Ker}$, $Nrf2^{\Delta/\Delta ker}$. (**K**) Generation of $Nrf2^{\Delta/+HFSC}$ and $Nrf2^{\Delta/\Delta HFSC}$. (**L**) Images of $Nrf2^{+/+HFSC}$ and $Nrf2^{\Delta/\Delta HFSC}$. (**M**–**O**) Wound area over time (**M**), wound closure (**N**), and wound burden analyses (**O**) in $Nrf2^{+/+HFSC}$ and $Nrf2^{\Delta/\Delta HFSC}$ mice. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Abbreviation: ns, not significant.

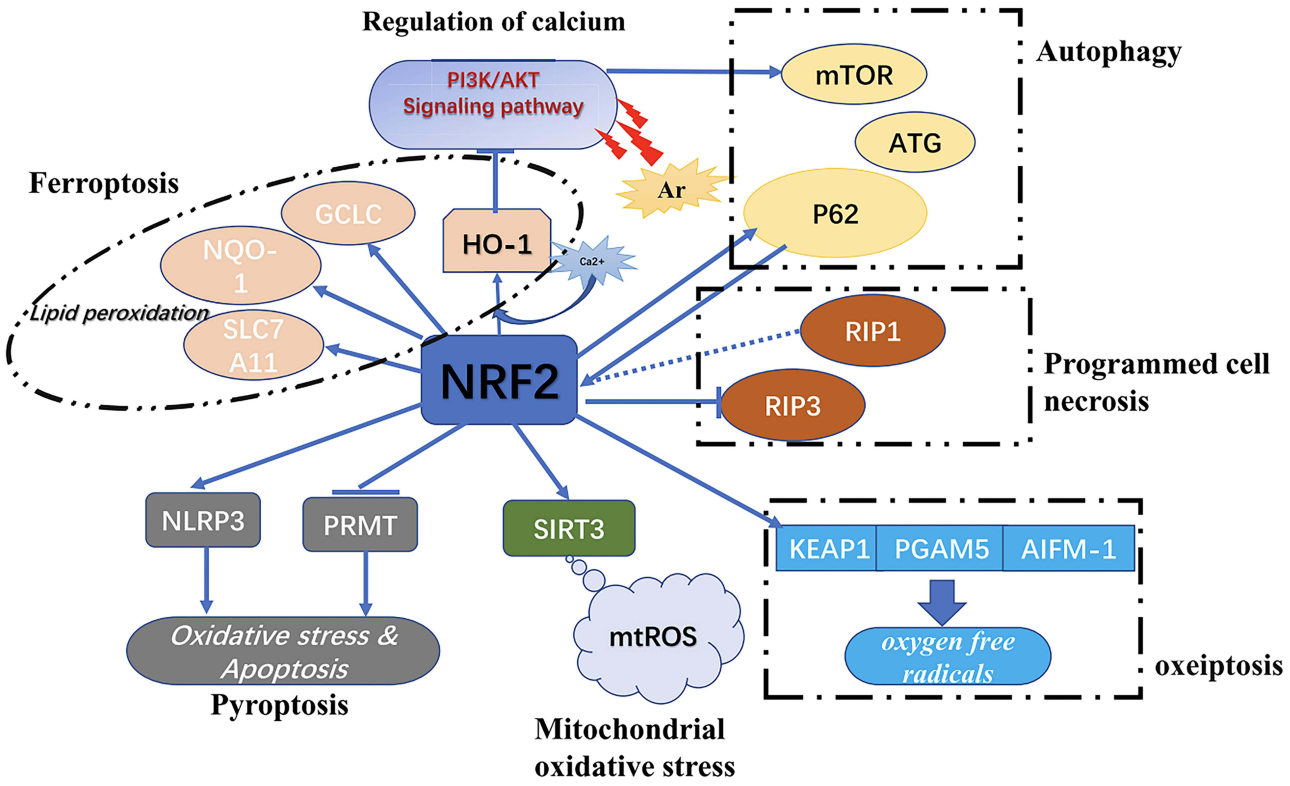

Figure 5 Several modes of regulation of the NRF2 signal pathway. Reproduced from Liu Y, Yang X, Liu Y, et al. NRF2 signalling pathway: new insights and progress in the field of wound healing. J Cell Mol Med. 2021;25(13):5857–5868. © 2021 The Authors. Journal of Cellular and Molecular Medicine published by Foundation for Cellular and Molecular Medicine and John Wiley & Sons Ltd. Creative Commons CC BY license.[12] Regulation of calcium ions (increased concentration of calcium ions can inhibit the transduction of the PI3K/AKT signal pathway due to the overexpression of HO-1), mitochondrial oxidative stress (SIRT3 expression is strengthen by the adjustment of NRF2, thereby optimizing the therapeutic effect of mesenchymal stem cells on skin wound healing), ferroptosis (lipid peroxidation is mainly achieved by the participation of NRF2 downstream target genes such as HO-1, GCLC, NQO-1, and SLC7A11), pyroptosis (activating the NRF2 pathway promotes apoptosis and inhibits the activation of NLRP3 - an inflammatory body), and autophagy (PI3K/AKT pathway activation influences the expression of NRF2 and mTOR, as well as P62 and ATG gene expression).

inflammatory factor TNF-α, serum procalcitonin, anti-angiogenic factor,[98] interleukins,[99] platelet-reactive proteins 1,[100] and reactive oxygen species inhibit cell migration and are not conducive to wound healing. Moreover, it has been reported[101] that the expression of IL-25 functional receptors (IL-17RB) is inhibited due to the lack of IL-25 in the wound. The low expression of β-catenin results in downregulation of the Wnt/β-catenin signal pathway and obstruction of AKT and ERK1/2 phosphorylation, decreasing cell proliferation, increasing apoptosis, and leading to delayed wound healing (Figure 6). Together, these mechanisms contribute to the poor healing observed in PF, although research is still relatively limited. Therefore, it is necessary to conduct more basic experiments to provide new treatment ideas for promoting wound healing.

Mechanism of Exosomes Repair Tissue Defect

Exosomes Activate Normal Immunity

As the name implies, PF is a tissue defect. Long-term mucosal necrosis and insufficient angiogenesis cause the tissue necrosis so that the wound does not heal. Typically, platelets are rapidly gathered in the damaged site after tissue damage, forming fibrino clots to promote hemostasis. Next, the release of inflammatory factors, such as pentin and histamine, results in higher capillary permeability, attracting inflammatory cells to the wound area, playing anti-inflammatory roles. Infiltrated monocytes differentiate into anti-inflammatory M2 macrophages, facilitating fibroblast proliferation, collagen deposition and tissue shaping. Previous studies[102–104] have shown that T cells secrete exosomes enriched in TCR-CD3 composites following stimulation with antigen or inflammatory factors, such as IL-12. Exosomes can directly activate CD8+ T cells to generate IFN-γ and granzyme, but also carry hazardous signals to activate dendritic cells, which starts the next cellular immunization. However, this has only been examined in vitro, and it remains unknown whether T cell–derived exosomes have the same influence in vivo or over long distances. In

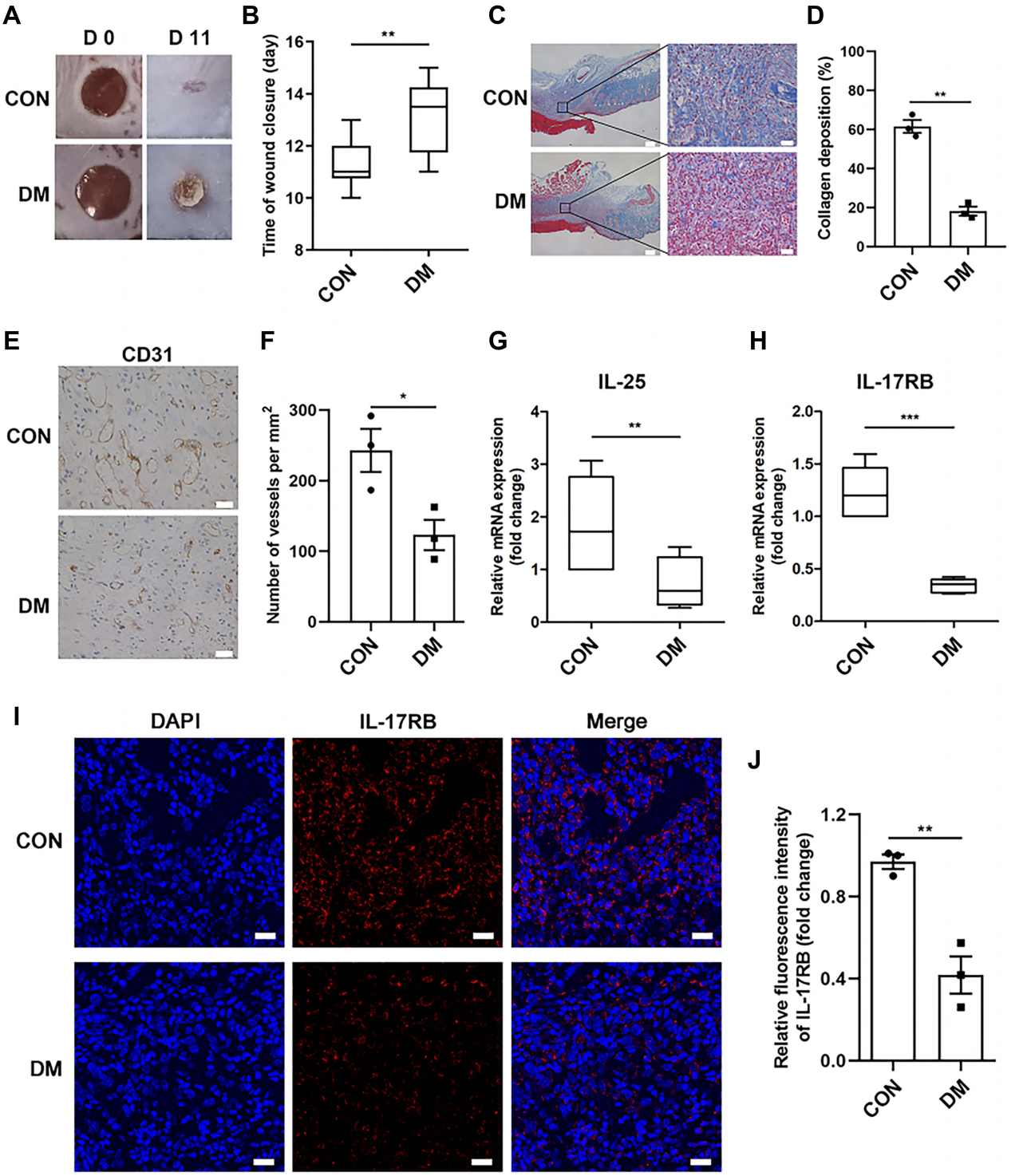

Figure 6 Downregulation of IL-17RB signaling pathway mediated by IL-25 delayed wound healing in diabetic mice. Reproduced from Zhang F, Liu Y, Wang S, et al. Interleukin-25-Mediated-IL-17RB upregulation promotes cutaneous wound healing in diabetic mice byimproving endothelial cell functions. Front Immunol. 2022;13:809755. Copyright © 2022 Zhang, Liu, Wang, Yan, Lin, Chen, Tan and Wu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY).[101] (**A**). Images of wounds on day 0 and day 11 after injury in the control (CON) group and diabetic mice (DM) group. (**B**) Quantitative analysis of wound closure time. (**C**) Masson trichrome staining of the wound skin sections from CON group and DM group. (**D**) Quantitative of collagen deposition in the CON group and DM group. (**E**). Angiogenesis analysis of CD31 in the skin sections from CON group and DM group through immunohistochemistry staining. (**F**) Quantitative analysis of angiogenesis. (**G-H**). Quantitative analysis of IL-25 and IL-17RB mRNA expression in the CON group and DM group. (**I**) Immunofluorescence staining of IL-17RB in the CON group and DM group. (**J**) Quantitative analysis of IL-17RB protein expression in the CON group and DM group. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bars, 200 μm (6C left), 25 μm (6C right), 25 μm (6E), 20 μm (6I).

addition, exosomes from dendritic cells can carry MHC-peptide complex to activate CD4+ and CD8+ T[105] cells directly, activating immune responses to resist virus or bacterial invasion. Nevertheless, exosomes derived from macrophages can affect the function of T cells and dendritic cells. For instance, macrophage-derived exosomes enriched in CD63+ and OX40L promote CD4+ Th2 cell proliferation and differentiation.[106] Exosomes derived from macrophage also upregulate the antigen-presenting ability of dendritic cells, leading to the initiation of an antigen-specific immune response.[107] Taken together, exosomes derived from immune cells have protective effects on the whole body, which can effectively activate the immune system, fight inflammation, and accelerate tissue regeneration. In patients with PF, the transformation from M1 to M2 macrophages is affected, and increasing inflammatory mediators suppress immunity and hinder fibroblast migration. Therefore, targeting exosomes secreted by immune cells may be a potential strategy for the treatment of PF, and, through artificial intervention, enhancing immunity will inevitably be conducive to subsequent damaged tissue repair.

Exosomes Regulate the Inflammatory Response

In addition to their participation in immune regulation, exosomes can regulate inflammatory response, as has been shown in skin wounds, diabetic foot ulcers, bone defects, scapula tendon injuries, and burns.[82,97,108–110] According to previous reports,[27,111–114] exosomes derived from mesenchymal stem cells promote the polarization of macrophages from M1 to M2, leading M2 to secret anti-inflammatory factors such as IL-10 and TGF-β, reducing inflammation and accelerating wound healing. Some exosomes are achieved by increasing arginase 1 (ARG1)(M2 macrophage marker) and decreasing inducible nitric oxide synthase (INOS) (M1 macrophage marker). Others downregulate toll-like receptor 4 (TLR4), nuclear factor κB (NF-κB), and phosphor (p)-p65 to induce M2 macrophages to secrete anti-inflammatory factors. A recent study[115] suggested that extracellular vesicle-loaded protein fragments could act as bait receptors, integrating with specific cytokines, such as TNF-α receptor 1 and IL-6 signal converters, to prevent them from exerting pro-inflammatory effects and promoting tissue damage repair. Schneide et al[116] confirmed through animal model experiments that in humans, anti-inflammatory effects are primarily achieved by activating CD8 T cells to secrete CD73-rich exosomes. There is growing evidence that exosomes can also contribute to the progression of inflammatory diseases, such as inflammatory bowel disease, arthritis, atherosclerosis, diabetes, and neurodegenerative diseases. The inflammatory response is controlled, which is conducive to the proliferation of fibroblasts, collagen deposition, and tissue shaping. The incidence of infection is reduced and the secretion of nrf2 is often hindered by epidermal keratinocytes in PF so that Ccl2-EGF is affected; this prevents the conversion from M1 to M2, hindering subsequent fibroblast proliferation, angiogenesis, and collagen deposition. Therefore, appropriate exosome supplementation is conducive to tissue damage repair. Exosome supplementation can be performed in various ways, including the following: implanting exosomes into PF repair materials combined with specific cytokines to reduce inflammation and play a role in promoting repair; or injecting specific exosomes at the wound edge to promote rapid tissue regeneration and wound healing. The delivery of exosomes through endogenous or exogenous pathway can provide a clinical basis for PF repair. Yet, there are insufficient studies on exosomes for PF repair, and a large number of basic studies are needed to further confirm.

Exosomes Promote the Proliferation and Invasion of Fibroblasts

After the inflammatory phase, fibroblast hyperplasia is the most important link in the repair phase. Fibroblasts secrete collagen, extracellular matrix, and collagenase, and participate in granulation tissue formation. Studies[117] have shown that exosomes derived from adipose stem cells can transport miRNA-125a and miRNA-31 to vascular endothelial cells, stimulating fibroblast proliferation, and regulating collagen remodeling; exosomes derived from human umbilical blood plasma are highly enriched with miR-21-3p, which promotes wound healing by inhibiting phosphatase and tension protein homologues, as well as bud-like homologies.[118] Furthermore, exosomes derived from mesenchymal stem cells deliver lncRNA H19 to fibroblasts, inhibiting miR-152-3p, promoting phosphatase synthesis gene expression, blocking the PI3K/AKT pathway to enhance the proliferation and migration of fibroblasts, inhibiting apoptosis, and accelerating DFU healing.[26] Exosomes can also inhibit the apoptosis of damaged cells and restore the vitality of senescent endothelial cells. The exosomes secreted by human embryonic stem cells are rich in miR-200a, which rejuvenates endothelial cells by downregulating the expression of Kelch-likeECH-associated protein 1 (Keap1) and activating nuclear factors (erythroid derivation 2).[119] Fibroblasts increase extracellular matrix and laminin production, reduce platelet production,

promote subsequent angiogenesis, and improve blood supply. Consequently, fibroblasts are lacking in PF; thus, targeted delivery of normal fibroblasts to the fistula may serve to compensate for the fibroblasts consumed by repeated infections and accelerate the healing rate. This process is expected to be achieved via exosomes. The above studies show that exosome-assisted wound therapy is a promising cell-free therapy, and a variety of cell-derived exosomes can stimulate the proliferation of fibroblasts to promote wound healing.

Exosomes Promote Angiogenesis

Wound healing requires both cell proliferation and adequate blood supply. Angiogenesis describes the new formation of blood vessels on the basis of the original capillaries or venules through the proliferation and migration of vascular endothelial cells. Under the regulation of pro-angiogenesis factors (alkaline fibroblast growth factor, vascular endothelial cell growth factor), a new basement membrane is formed, which subsequently forms a capillary network that can be remodeled. Previous studies[30,65] have reported that exosomes can promote angiogenesis by transmitting mi-RNA and protein signals. Exosomes derived from cardiomyocyte progenitor cells (CMPC) and mesenchymal stem cells highly express extracellular matrix metalloproteinase inducers, which mediate ERT/AKT pathway activation and promote angiogenesis. Knockout of the extracellular matrix metalloproteinase inducer in cardiomyocyte progenitor cells weakens its angiogenesis effect.[120] Studies have reported that patients with PF have elevated MMP-2, MMP-7, and MMP-9, which is not conducive to wound healing.[103] Similarly, Zhang et al[121] confirmed that exosomes derived from bone marrow mesenchymal stem cells activate the PI3K/AK pathway by transmitting miRNA-126, promoting angiogenesis, and accelerating wound healing. Secondly, exosomal miR-1260a could promote angiogenesis and osteogenesis by targeting HDAC7 and COL4A2, and the addition of low-dose nanomaterials Fe3O4 and SMF could enhance this promoting effect.[122] To summarize, most exosomes affect gene transcription by transmitting RNA signals, promoting protein factor synthesis, and mediating angiogenesis. Studies have shown that exosomes derived from human-induced mesenchymal stem cells can also promote angiogenesis and osteogenesis,[82,109,111] representing another option for treating bone defects. Exosomes secreted by adipose-derived stem cells (ADSCs) can facilitate the proliferation of endothelial progenitor cells and the production of vascular growth factor, reducing the expression of inflammation-related proteins and accelerating wound healing. Moreover, the high expression of the transcription factor Nrf2 can enhance these effects and can be used as a therapy for diabetic foot ulcers.[109] Given the ability of exosomes to promote angiogenesis and assist with wound healing in skin wounds, burns, and ulcers, the application of exosomes in PF repair is worth exploring.

Exosomes Promote the Deposition of Proteoglycan and Production of Collagen

The final step in wound healing is the formation of granulation tissue, ie, proteoglycan deposition and collagen production. Granulation tissue protects wounds and fills wounds and other tissue defects. However, patients are generally in poor condition after total laryngectomy, and when PF occurs, the peri-traumatic fibroblasts cannot migrate in an orderly manner, leading to insufficiency of new blood vessels. These blood vessels cannot provide adequate oxygen and nutrient supply, and cannot form hard granulation tissues, resulting in slow or poor-quality healing. Li Qian et al[123] determined the effect of exosomes derived from fat mesenchymal stem cells on the repair and healing of traumatic tissues by flow cytometry, reverse transcription quantitative polymerase chain reaction (RT-qPCR), and Western blotting. As a result, they found that lncRNA H19 (H19), microRNA 19b (miR-19b), and SRY-related high-mobility group protein cassette 9 (SOX9) played a major role. First, exosomes can inhibit SOX9 to activate the Wnt/β-catenin pathway and promote the proliferation, migration, and invasion of fibroblasts around the wound. Second, H19 in exosomes can be used as a signaling molecule to upregulate the expression of SOX9 by inhibiting miR-19b, promoting collagen synthesis and wound repair. Hence, exosomal H19 is a positive regulator of wound healing. Thus, targeted delivery of exosomal H19 may represent another option for PF repair. Moreover, exosomes derived from ADSCs can also directly stimulate the generation of collagen types I and III, increase the expression of N-cadherin and cyclin-1 genes, internalize fibroblast expression, promote their proliferation, migration, and hasten tissue healing.[124] The latest research[124] shows that miR-21-5p and miR-125b-5p carried by exosomes derived from cord blood mesenchymal stem cells inhibit the conversion of growth factor β receptor 2 and transforming growth factor β receptor 1, thereby inhibiting the TGF-β signal pathway to stimulate wound regeneration and healing, and reduce scarring. Likewise, transmitting Wnt4 to activate the Wnt/β-catenin and AKT pathways in skin cells is beneficial to wound healing by enhancing wound closure and inhibiting

apoptosis.[123,125,126] In patients with PF, the lack of β-catenin leads to the obstruction of AKT and ERK1/2 phosphorylation, affecting wound healing. These findings demonstrate that exosomes interfere with the normal metabolism of tissues by transmitting RNA or proteins, with the associated signaling pathways representing sites of action. Furthermore, exosomes derived from mesenchymal stem cells can replace stem cells for cartilage tissue regeneration and repair, mainly to reduce the production of inflammatory factors (eg TNF-α, IL-6 and IL-10), promoting the deposition of proteoglycans and type II collagen production.[54] In summary, a strong wall is needed to defend against external diseases, and strong granulation is needed to defend against inflammation. The difficulties with PF healing are mainly due to poor tissue regeneration and lack of blood supply, which together affect the formation of granulation tissue. While local antibiotic therapy is ineffective, and the survival rate of flap transplantation is low, the prognosis of patients remains poor. Combined with previous studies, exosome therapy may open new perspectives for PF repair, and targeted delivery, drug scaffolds, biological patches, and hydrogels are expected to be cell-free treatment options for PF repair.

Conclusion and Future Prospects

Wound healing has long caused issues following surgery, and poor healing tissue is not only vulnerable to infection but also reduces the effectiveness of surgery. Particularly, patients with cancer are prone to PF after total laryngectomy, which can persist for a long time. For patients, this not only increases financial burden, but also reduces the quality of life. Numerous studies[120,121,127] have shown that exosomes play an important role in promoting all phases of tissue repair, and can assist with help wound healing by enhancing anti-inflammatory factors, promoting fibroblast proliferation, and promoting angiogenesis. We have reason to believe that exosomes somehow influence the repair process of PF. The use of genetic engineering techniques for the repair of PF not only represents a future research direction, but also a major therapeutic strategy for PF in cell-free therapy. Nevertheless, optimizing the production and storage methods of exosomes remains an urgent problem to be solved.

Acknowledgments

We would like to thank Professors Bing Guan and Maohua Wang for reviewing and providing advice on the final search strategy. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Funding

This work was supported by grants from Jiangsu Province Natural Science Foundation (BK20201220), Doctoral Startup Foundation (2021BS-ZZH), Nanjing Medical Science and Technique Development Foundation (No.QRX17051) The Project of Invigorating Health Care through Science, Technology and Education (No.ZDXKB2016015).

Disclosure

The authors declare that they have no conflict of interest.

References

1. Wang M, Xun Y, Wang K, et al. Risk factors of pharyngocutaneous fistula after total laryngectomy: a systematic review and meta-analysis. *Eur Arch Otorhinolaryngol*. 2020;277(2):585–599. doi:10.1007/s00405-019-05718-9
2. Velnar T, Bailey T, Smrkolj V. The wound healing process: an overview of the cellular and molecular mechanisms. *J Int Med Res*. 2009;37(5):1528–1542.
3. Robson MC, Steed DL, Franz MG. Wound healing: biologic features and approaches to maximize healing trajectories. *Curr Probl Surg*. 2001;38(2):72–140. doi:10.1067/msg.2001.111167
4. Baum CL, Arpey CJ. Normal cutaneous wound healing: clinical correlation with cellular and molecular events. *Dermatol Surg*. 2005;31(6):674–86; discussion 686. doi:10.1097/00042728-200506000-00011
5. Guntinas-Lichius O, Eckel HE. Temporary reduction of salivation in laryngectomy patients with pharyngocutaneous fistulas by botulinum toxin A injection. *Laryngoscope*. 2002;112(1):187–189. doi:10.1097/00005537-200201000-00033
6. Saki N, Nikakhlagh S, Kazemi M. Pharyngocutaneous fistula after laryngectomy: incidence, predisposing factors, and outcome. *Arch Iran Med*. 2008;11(3):314–317. doi:10.08113/AIM.0013
7. Eryılmaz A, Demirci B, Gunel C, et al. Can tissue adhesives and platelet-rich plasma prevent pharyngocutaneous fistula formation? *Auris Nasus Larynx*. 2016;43(1):62–67. doi:10.1016/j.anl.2015.06.012
8. Sapundzhiev NR, Nikiforova LT, Spasova BH, et al. Endoscopic repair of pharyngocutaneous fistula following laryngectomy. *Cureus*. 2019;11(10):e5871. doi:10.7759/cureus.5871

9. Tao SC, Guo S-C, Li M, et al. Chitosan wound dressings incorporating exosomes derived from MicroRNA-126-overexpressing synovium mesenchymal stem cells provide sustained release of exosomes and heal full-thickness skin defects in a diabetic rat model. *Stem Cells Transl Med*. 2017;6(3):736–747. doi:10.5966/sctm.2016-0275
10. Li M, Ke Q-F, Tao S-C, et al. Fabrication of hydroxyapatite/chitosan composite hydrogels loaded with exosomes derived from miR-126-3p overexpressed synovial mesenchymal stem cells for diabetic chronic wound healing. *J Mater Chem B*. 2016;4(42):6830–6841. doi:10.1039/C6TB01560C
11. Abouyared M, Kerr DA, Burroway B, et al. Abnormal microvasculature in laryngectomy mucosal margins may be associated with increased risk of fistula. *Head Neck Pathol*. 2019;13(3):364–370. doi:10.1007/s12105-018-0974-7
12. Liu Y, Yang X, Liu Y, et al. NRF2 signalling pathway: new insights and progress in the field of wound healing. *J Cell Mol Med*. 2021;25(13):5857–5868. doi:10.1111/jcmm.16597
13. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. *Science*. 2020;367(6478). doi:10.1126/science.aau6977
14. Hariharan NC, Sugumar M, Muthukumar R. Pectoralis major Myocutaneous/Myofascial flap and split skin grafting for repair of post laryngectomy pharyngo cutaneous fistula: an undemanding and a reliable solution. *Indian J Otolaryngol Head Neck Surg*. 2019;71(Suppl 1):305–311. doi:10.1007/s12070-018-1288-5
15. Koritzinsky EH, Street JM, Star RA, et al. Quantification of exosomes. *J Cell Physiol*. 2017;232(7):1587–1590. doi:10.1002/jcp.25387
16. Yue B, Yang H, Wang J, et al. Exosome biogenesis, secretion and function of exosomal miRNAs in skeletal muscle myogenesis. *Cell Prolif*. 2020;53(7):e12857. doi:10.1111/cpr.12857
17. Raposo G, Nijman HW, Stoorvogel W, et al. B lymphocytes secrete antigen-presenting vesicles. *J Exp Med*. 1996;183(3):1161–1172. doi:10.1084/jem.183.3.1161
18. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells*. 2019;8(7):727. doi:10.3390/cells8070727
19. Tan L, Wu H, Liu Y, et al. Recent advances of exosomes in immune modulation and autoimmune diseases. *Autoimmunity*. 2016;49(6):357–365. doi:10.1080/08916934.2016.1191477
20. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem*. 2019;88:487–514. doi:10.1146/annurev-biochem-013118-111902
21. Villarroya-Beltri C, Gutiérrez-Vázquez C, Sánchez-Cabo F, et al. Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs. *Nat Commun*. 2013;4:2980. doi:10.1038/ncomms3980
22. Smyth LA, Ratnasothy K, Tsang JYS, et al. CD73 expression on extracellular vesicles derived from CD4 + CD25 + Foxp3 + T cells contributes to their regulatory function. *Eur J Immunol*. 2013;43(9):2430–2440. doi:10.1002/eji.201242909
23. Okoye IS, Coomes S, Pelly V, et al. MicroRNA-containing T-regulatory-cell-derived exosomes suppress pathogenic T helper 1 cells. *Immunity*. 2014;41(1):89–103. doi:10.1016/j.immuni.2014.05.019
24. Li X, Li X, Lin J, et al. Exosomes derived from low-intensity pulsed ultrasound-treated dendritic cells suppress tumor necrosis factor-induced endothelial inflammation. *J Ultrasound Med*. 2019;38(8):2081–2091. doi:10.1002/jum.14898
25. Uccini S, Al-Jadiry MF, Pepe G, et al. Follicular dendritic cells display microvesicle-associated LMP1 in reactive germinal centers of EBV+ classic Hodgkin lymphoma. *Virchows Arch*. 2019;475(2):175–180. doi:10.1007/s00428-019-02605-w
26. Li B, Luan S, Chen J, et al. The mesenchymal stem cell-derived exosomal long noncoding RNA H19 promotes wound healing in diabetic foot ulcers by up-regulating PTEN*via* MicroRNA-152-3p. *SSRN Electronic Journal*. 2019. doi:10.2139/ssrn.3408040
27. Li J, Xue H, Li T, et al. Exosomes derived from mesenchymal stem cells attenuate the progression of atherosclerosis in ApoE(-/-) mice via miR-let7 mediated infiltration and polarization of M2 macrophage. *Biochem Biophys Res Commun*. 2019;510(4):565–572.
28. Zhou B, Xu K, Zheng X, et al. Application of exosomes as liquid biopsy in clinical diagnosis. *Signal Transduct Target Ther*. 2020;5(1):144. doi:10.1038/s41392-020-00258-9
29. Zhang J, Li S, Li L, et al. Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinformatics*. 2015;13(1):17–24. doi:10.1016/j.gpb.2015.02.001
30. Cui H, Seubert B, Stahl E, et al. Tissue inhibitor of metalloproteinases-1 induces a pro-tumourigenic increase of miR-210 in lung adenocarcinoma cells and their exosomes. *Oncogene*. 2015;34(28):3640–3650. doi:10.1038/onc.2014.300
31. Fan J, Xu G, Chang Z, et al. miR-210 transferred by lung cancer cell-derived exosomes may act as proangiogenic factor in cancer-associated fibroblasts by modulating JAK2/STAT3 pathway. *Clin Sci (Lond)*. 2020;134(7):807–825. doi:10.1042/CS20200039
32. Bijnsdorp IV, Geldof AA, Lavaei M, et al. Exosomal ITGA3 interferes with non-cancerous prostate cell functions and is increased in urine exosomes of metastatic prostate cancer patients. *J Extracell Vesicles*. 2013;2:22097. doi:10.3402/jev.v2i0.22097
33. Zhang R, Xia Y, Wang Z, et al. Serum long non coding RNA MALAT-1 protected by exosomes is up-regulated and promotes cell proliferation and migration in non-small cell lung cancer. *Biochem Biophys Res Commun*. 2017;490(2):406–414. doi:10.1016/j.bbrc.2017.06.055
34. van Balkom BW, de Jong OG, Smits M, et al. Endothelial cells require miR-214 to secrete exosomes that suppress senescence and induce angiogenesis in human and mouse endothelial cells. *Blood*. 2013;121(19):3997–4006, s1-15. doi:10.1182/blood-2013-02-478925
35. McDonald MK, Tian Y, Qureshi RA, et al. Functional significance of macrophage-derived exosomes in inflammation and pain. *Pain*. 2014;155(8):1527–1539. doi:10.1016/j.pain.2014.04.029
36. Liu S, Chen J, Shi J, et al. M1-like macrophage-derived exosomes suppress angiogenesis and exacerbate cardiac dysfunction in a myocardial infarction microenvironment. *Basic Res Cardiol*. 2020;115(2):22. doi:10.1007/s00395-020-0781-7
37. Skotland T, Hessvik NP, Sandvig K, et al. Exosomal lipid composition and the role of ether lipids and phosphoinositides in exosome biology. *J Lipid Res*. 2019;60(1):9–18. doi:10.1194/jlr.R084343
38. Wallner S, Schmitz G. Plasmalogens the neglected regulatory and scavenging lipid species. *Chem Phys Lipids*. 2011;164(6):573–589. doi:10.1016/j.chemphyslip.2011.06.008
39. da Silva TF, Sousa VF, Malheiro AR, et al. The importance of ether-phospholipids: a view from the perspective of mouse models. *Biochim Biophys Acta*. 2012;1822(9):1501–1508. doi:10.1016/j.bbadis.2012.05.014
40. Dean JM, Lodhi IJ. Structural and functional roles of ether lipids. *Protein Cell*. 2018;9(2):196–206. doi:10.1007/s13238-017-0423-5
41. Honsho M, Fujiki Y. Plasmalogen homeostasis - regulation of plasmalogen biosynthesis and its physiological consequence in mammals. *FEBS Lett*. 2017;591(18):2720–2729. doi:10.1002/1873-3468.12743

42. Dorninger F, Forss-Petter S, Berger J. From peroxisomal disorders to common neurodegenerative diseases - The role of ether phospholipids in the nervous system. *FEBS Lett*. 2017;591(18):2761–2788. doi:10.1002/1873-3468.12788
43. Li P, Kaslan M, Lee SH, et al. Progress in exosome isolation techniques. *Theranostics*. 2017;7(3):789–804. doi:10.7150/thno.18133
44. Hemler ME. Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annu Rev Cell Dev Biol*. 2003;19:397–422. doi:10.1146/annurev.cellbio.19.111301.153609
45. Guduric-Fuchs J, O'Connor A, Camp B, et al. Selective extracellular vesicle-mediated export of an overlapping set of microRNAs from multiple cell types. *BMC Genom*. 2012;13(1):357. doi:10.1186/1471-2164-13-357
46. Korkut C, Ataman B, Ramachandran P, et al. Trans-synaptic transmission of vesicular Wnt signals through Evi/Wntless. *Cell*. 2009;139 (2):393–404. doi:10.1016/j.cell.2009.07.051
47. Robbins PD, Morelli AE. Regulation of immune responses by extracellular vesicles. *Nat Rev Immunol*. 2014;14(3):195–208. doi:10.1038/nri3622
48. Machtinger R, Laurent LC, Baccarelli AA. Extracellular vesicles: roles in gamete maturation, fertilization and embryo implantation. *Hum Reprod Update*. 2016;22(2):182–193. doi:10.1093/humupd/dmv055
49. Skog J, Würdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008;10(12):1470–1476. doi:10.1038/ncb1800
50. Jia G, Sowers JR. Targeting endothelial exosomes for the prevention of cardiovascular disease. *Biochim Biophys Acta Mol Basis Dis*. 2020;1866 (8):165833. doi:10.1016/j.bbadis.2020.165833
51. Sanwlani R, Gangoda L. Role of extracellular vesicles in cell death and inflammation. *Cells*. 2021;10(10):2663. doi:10.3390/cells10102663
52. Wang M, Wang C, Chen M, et al. Efficient angiogenesis-based diabetic wound healing/skin reconstruction through bioactive antibacterial adhesive ultraviolet shielding nanodressing with exosome release. *ACS Nano*. 2019;13(9):10279–10293. doi:10.1021/acsnano.9b03656
53. Wu J, Kuang L, Chen C, et al. miR-100-5p-abundant exosomes derived from infrapatellar fat pad MSCs protect articular cartilage and ameliorate gait abnormalities via inhibition of mTOR in osteoarthritis. *Biomaterials*. 2019;206:87–100. doi:10.1016/j.biomaterials.2019.03.022
54. Kim YG, Choi J, Kim K. Mesenchymal stem cell-derived exosomes for effective cartilage tissue repair and treatment of osteoarthritis. *Biotechnol J*. 2020;15(12):e2000082. doi:10.1002/biot.202000082
55. Sun Z, Yang S, Zhou Q, et al. Emerging role of exosome-derived long non-coding RNAs in tumor microenvironment. *Mol Cancer*. 2018;17 (1):82. doi:10.1186/s12943-018-0831-z
56. Urbich C, Kuehbacher A, Dimmeler S. Role of microRNAs in vascular diseases, inflammation, and angiogenesis. *Cardiovasc Res*. 2008;79 (4):581–588. doi:10.1093/cvr/cvn156
57. Bang C, Batkai S, Dangwal S, et al. Cardiac fibroblast-derived microRNA passenger strand-enriched exosomes mediate cardiomyocyte hypertrophy. *J Clin Invest*. 2014;124(5):2136–2146. doi:10.1172/JCI70577
58. Li D, Wang Y, Jin X, et al. NK cell-derived exosomes carry miR-207 and alleviate depression-like symptoms in mice. *J Neuroinflammation*. 2020;17(1):126. doi:10.1186/s12974-020-01787-4
59. Jiang R, Tang J, Chen Y, et al. The long noncoding RNA lnc-EGFR stimulates T-regulatory cells differentiation thus promoting hepatocellular carcinoma immune evasion. *Nat Commun*. 2017;8:15129. doi:10.1038/ncomms15129
60. Kou N, Liu S, Li X, et al. H19 facilitates tongue squamous cell carcinoma migration and invasion via sponging miR-let-7. *Oncol Res*. 2019;27 (2):173–182. doi:10.3727/096504018X15202945197589
61. Li L, Li W, Chen N, et al. FLI1 exonic circular RNAs as a novel oncogenic driver to promote tumor metastasis in small cell lung cancer. *Clin Cancer Res*. 2019;25(4):1302–1317. doi:10.1158/1078-0432.CCR-18-1447
62. Maji S, Chaudhary P, Akopova I, et al. Exosomal Annexin II promotes angiogenesis and breast cancer metastasis. *Mol Cancer Res*. 2017;15 (1):93–105. doi:10.1158/1541-7786.MCR-16-0163
63. Tiedemann K, Sadvakassova G, Mikolajewicz N, et al. Exosomal release of L-Plastin by breast cancer cells facilitates metastatic bone osteolysis. *Transl Oncol*. 2019;12(3):462–474. doi:10.1016/j.tranon.2018.11.014
64. Zhang S, Dong Y, Wang Y, et al. Selective encapsulation of therapeutic mRNA in engineered extracellular vesicles by DNA aptamer. *Nano Lett*. 2021;21(20):8563–8570. doi:10.1021/acs.nanolett.1c01817
65. Li W, Li C, Zhou T, et al. Role of exosomal proteins in cancer diagnosis. *Mol Cancer*. 2017;16(1):145. doi:10.1186/s12943-017-0706-8
66. Keryer-Bibens C, Pioche-Durieu C, Villemant C, et al. Exosomes released by EBV-infected nasopharyngeal carcinoma cells convey the viral latent membrane protein 1 and the immunomodulatory protein galectin 9. *BMC Cancer*. 2006;6:283. doi:10.1186/1471-2407-6-283
67. Jiang L, Dong H, Cao H, et al. Exosomes in pathogenesis, diagnosis, and treatment of Alzheimer's disease. *Med Sci Monit*. 2019;25:3329–3335. doi:10.12659/MSM.914027
68. Yang F, Liao X, Tian Y, et al. Exosome separation using microfluidic systems: size-based, immunoaffinity-based and dynamic methodologies. *Biotechnol J*. 2017;12(4):1600699. doi:10.1002/biot.201600699
69. Konoshenko MY, Lekchnov EA, Vlassov AV, et al. Isolation of extracellular vesicles: general methodologies and latest trends. *Biomed Res Int*. 2018;2018:8545347. doi:10.1155/2018/8545347
70. Muñoz-Domínguez N, Roura S, Prat-Vidal C, et al. Wharton's jelly mesenchymal stromal cells and derived extracellular vesicles as post-myocardial infarction therapeutic toolkit: an experienced view. *Pharmaceutics*. 2021;13(9):1336. doi:10.3390/pharmaceutics13091336
71. Liu L, Jin X, Hu C-F, et al. Exosomes derived from mesenchymal stem cells rescue myocardial ischaemia/reperfusion injury by inducing cardiomyocyte autophagy via AMPK and akt pathways. *Cell Physiol Biochem*. 2017;43(1):52–68. doi:10.1159/000480317
72. Zhuo CJ, Hou W-H, Jiang D-G, et al. Circular RNAs in early brain development and their influence and clinical significance in neuropsychiatric disorders. *Neural Regen Res*. 2020;15(5):817–823. doi:10.4103/1673-5374.268969
73. Hofmann L, Ludwig S, Vahl JM, et al. The emerging role of exosomes in diagnosis, prognosis, and therapy in head and neck cancer. *Int J Mol Sci*. 2020;21(11):4072. doi:10.3390/ijms21114072
74. Momen-Heravi F, Bala S. Emerging role of non-coding RNA in oral cancer. *Cell Signal*. 2018;42:134–143. doi:10.1016/j.cellsig.2017.10.009
75. Stephenson KA, Pandey S, Lubbe DE, et al. Use of surgical sealant in the prevention of pharyngocutaneous fistula after total laryngectomy. *Head Neck*. 2018;40(12):2606–2611. doi:10.1002/hed.25334

76. van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. *Nat Rev Mol Cell Biol*. 2018;19(4):213–228. doi:10.1038/nrm.2017.125
77. Wang WU, Wei XL, Su JP. The application status of the linear stapler device in the total laryngectomy. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*. 2017;31(1):78–81. doi:10.13201/j.issn.1001-1781.2017.01.022
78. Yin S, Ji C, Wu P, et al. Human umbilical cord mesenchymal stem cells and exosomes: bioactive ways of tissue injury repair. *Am J Transl Res*. 2019;11(3):1230–1240.
79. Huang J, Zhang J, Xiong J, et al. Stem cell-derived nanovesicles: a novel cell-free therapy for wound healing. *Stem Cells Int*. 2021;2021:1285087. doi:10.1155/2021/1285087
80. Macías M, Rebmann V, Mateos B, et al. Comparison of six commercial serum exosome isolation methods suitable for clinical laboratories. Effect in cytokine analysis. *Clin Chem Lab Med*. 2019;57(10):1539–1545. doi:10.1515/cclm-2018-1297
81. Wang C, Wang M, Xu T, et al. Engineering bioactive self-healing antibacterial exosomes hydrogel for promoting chronic diabetic wound healing and complete skin regeneration. *Theranostics*. 2019;9(1):65–76. doi:10.7150/thno.29766
82. Qi X, Zhang J, Yuan H, et al. Exosomes secreted by human-induced pluripotent stem cell-derived mesenchymal stem cells repair critical-sized bone defects through enhanced angiogenesis and osteogenesis in osteoporotic rats. *Int J Biol Sci*. 2016;12(7):836–849. doi:10.7150/ijbs.14809
83. Li L, Zhang Y, Mu J, et al. Transplantation of human mesenchymal stem-cell-derived exosomes immobilized in an adhesive hydrogel for effective treatment of spinal cord injury. *Nano Lett*. 2020;20(6):4298–4305. doi:10.1021/acs.nanolett.0c00929
84. Chew JRJ, Chuah SJ, Teo KYW, et al. Mesenchymal stem cell exosomes enhance periodontal ligament cell functions and promote periodontal regeneration. *Acta Biomater*. 2019;89:252–264. doi:10.1016/j.actbio.2019.03.021
85. Turner JG, White LR, Estrela P, et al. Hydrogel-forming microneedles: current advancements and future trends. *Macromol Biosci*. 2021;21(2):e2000307. doi:10.1002/mabi.202000307
86. Nooshabadi VT, Khanmohamadi M, Valipour E, et al. Impact of exosome-loaded chitosan hydrogel in wound repair and layered dermal reconstitution in mice animal model. *J Biomed Mater Res A*. 2020;108(11):2138–2149. doi:10.1002/jbm.a.36959
87. Sun D, Zhuang X, Xiang X, et al. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Mol Ther*. 2010;18(9):1606–1614.
88. Wang L, Wang J, Zhou X, et al. A new self-healing hydrogel containing hucMSC-Derived exosomes promotes bone regeneration. *Front Bioeng Biotechnol*. 2020;8:564731. doi:10.3389/fbioe.2020.564731
89. Gong C, Tian J, Wang Z, et al. Functional exosome-mediated co-delivery of doxorubicin and hydrophobically modified microRNA 159 for triple-negative breast cancer therapy. *J Nanobiotechnology*. 2019;17(1):93. doi:10.1186/s12951-019-0526-7
90. Thakur A, Sidu RK, Zou H, et al. Inhibition of glioma cells' proliferation by doxorubicin-loaded exosomes via microfluidics. *Int J Nanomedicine*. 2020;15:8331–8343. doi:10.2147/IJN.S263956
91. Zhang C, Song J, Lou L, et al. Doxorubicin-loaded nanoparticle coated with endothelial cells-derived exosomes for immunogenic chemotherapy of glioblastoma. *Bioeng Transl Med*. 2021;6(3):e10203. doi:10.1002/btm2.10203
92. Li S, Wu Y, Ding F, et al. Engineering macrophage-derived exosomes for targeted chemotherapy of triple-negative breast cancer. *Nanoscale*. 2020;12(19):10854–10862. doi:10.1039/D0NR00523A
93. Yong T, Zhang X, Bie N, et al. Tumor exosome-based nanoparticles are efficient drug carriers for chemotherapy. *Nat Commun*. 2019;10(1):3838. doi:10.1038/s41467-019-11718-4
94. Yan F, Zhong Z, Wang Y, et al. Exosome-based biomimetic nanoparticles targeted to inflamed joints for enhanced treatment of rheumatoid arthritis. *J Nanobiotechnology*. 2020;18(1):115. doi:10.1186/s12951-020-00675-6
95. Tran T-H, Mattheolabakis G, Aldawsari H, et al. Exosomes as nanocarriers for immunotherapy of cancer and inflammatory diseases. *Clin Immunol*. 2015;160(1):46–58. doi:10.1016/j.clim.2015.03.021
96. Villarreal-Ponce A, Tiruneh MW, Lee J, et al. Keratinocyte-macrophage crosstalk by the Nrf2/Ccl2/EGF signaling axis orchestrates tissue repair. *Cell Rep*. 2020;33(8):108417. doi:10.1016/j.celrep.2020.108417
97. Zeng T, Wang X, Wang W, et al. Endothelial cell-derived small extracellular vesicles suppress cutaneous wound healing through regulating fibroblasts autophagy. *Clin Sci (Lond)*. 2019;133(9). doi:10.1042/CS20190008
98. Okonkwo UA, DiPietro LA. Diabetes and wound angiogenesis. *Int J Mol Sci*. 2017;18(7):1419. doi:10.3390/ijms18071419
99. Lassig AAD, Lindgren BR, Itabiyi R, et al. Excessive inflammation portends complications: wound cytokines and head and neck surgery outcomes. *Laryngoscope*. 2019;129(7):E238–e246. doi:10.1002/lary.27796
100. Hosseini Mansoub N. The role of keratinocyte function on the defected diabetic wound healing. *Int J Burns Trauma*. 2021;11(6):430–441.
101. Zhang F, Liu Y, Wang S, et al. Interleukin-25-Mediated-IL-17RB upregulation promotes cutaneous wound healing in diabetic mice by improving endothelial cell functions. *Front Immunol*. 2022;13:809755. doi:10.3389/fimmu.2022.809755
102. Blanchard N, Lankar D, Faure F, et al. TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. *J Immunol*. 2002;168(7):3235–3241. doi:10.4049/jimmunol.168.7.3235
103. Torralba D, Baixauli F, Villarroya-Beltri C, et al. Priming of dendritic cells by DNA-containing extracellular vesicles from activated T cells through antigen-driven contacts. *Nat Commun*. 2018;9(1):2658. doi:10.1038/s41467-018-05077-9
104. Li L, Jay SM, Wang Y, et al. IL-12 stimulates CTLs to secrete exosomes capable of activating bystander CD8+ T cells. *Sci Rep*. 2017;7(1):13365. doi:10.1038/s41598-017-14000-z
105. Veerman RE, Güçlüler Akpinar G, Eldh M, et al. Immune cell-derived extracellular vesicles - functions and therapeutic applications. *Trends Mol Med*. 2019;25(5):382–394. doi:10.1016/j.molmed.2019.02.003
106. Li F, Wang Y, Lin L, et al. Mast cell-derived exosomes promote Th2 cell differentiation via OX40L-OX40 Ligation. *J Immunol Res*. 2016;2016:3623898. doi:10.1155/2016/3623898
107. Skokos D, Botros HG, Demeure C, et al. Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. *J Immunol*. 2003;170(6):3037–3045. doi:10.4049/jimmunol.170.6.3037
108. Li X, Xie X, Lian W, et al. Exosomes from adipose-derived stem cells overexpressing Nrf2 accelerate cutaneous wound healing by promoting vascularization in a diabetic foot ulcer rat model. *Exp Mol Med*. 2018;50(4):1–14.
109. Zhang J, Guan J, Niu X, et al. Exosomes released from human induced pluripotent stem cells-derived MSCs facilitate cutaneous wound healing by promoting collagen synthesis and angiogenesis. *J Transl Med*. 2015;13(1):49.

110. Narauskaitė D, Vydmantaitė G, Rusteikaitė J, et al. Extracellular vesicles in skin wound healing. *Pharmaceuticals*. 2021;14(8):811. doi:10.3390/ph14080811
111. Ha DH, Kim H-K, Lee J, et al. Mesenchymal stem/stromal cell-derived exosomes for immunomodulatory therapeutics and skin regeneration. *Cells*. 2020;9(5):1157. doi:10.3390/cells9051157
112. Dalirfardouei R, Jamialahmadi K, Jafarian AH, et al. Promising effects of exosomes isolated from menstrual blood-derived mesenchymal stem cell on wound-healing process in diabetic mouse model. *J Tissue Eng Regen Med*. 2019;13(4):555–568. doi:10.1002/term.2799
113. Ti D, Hao H, Tong C, et al. LPS-preconditioned mesenchymal stromal cells modify macrophage polarization for resolution of chronic inflammation via exosome-shuttled let-7b. *J Transl Med*. 2015;13(1):308. doi:10.1186/s12967-015-0642-6
114. Zhao J, Li X, Hu J, et al. Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. *Cardiovasc Res*. 2019;115(7):1205–1216. doi:10.1093/cvr/cvz040
115. Gupta D, Wiklander OPB, Görgens A, et al. Amelioration of systemic inflammation via the display of two different decoy protein receptors on extracellular vesicles. *Nat Biomed Eng*. 2021;5(9):1084–1098. doi:10.1038/s41551-021-00792-z
116. Schneider E, Winzer R, Rissiek A, et al. CD73-mediated adenosine production by CD8 T cell-derived extracellular vesicles constitutes an intrinsic mechanism of immune suppression. *Nat Commun*. 2021;12(1):5911. doi:10.1038/s41467-021-26134-w
117. An Y, Lin S, Tan X, et al. Exosomes from adipose-derived stem cells and application to skin wound healing. *Cell Prolif*. 2021;54(3):e12993. doi:10.1111/cpr.12993
118. Hu Y, Rao -S-S, Wang Z-X, et al. Exosomes from human umbilical cord blood accelerate cutaneous wound healing through miR-21-3p-mediated promotion of angiogenesis and fibroblast function. *Theranostics*. 2018;8(1):169–184. doi:10.7150/thno.21234
119. Chen B, Sun Y, Zhang J, et al. Human embryonic stem cell-derived exosomes promote pressure ulcer healing in aged mice by rejuvenating senescent endothelial cells. *Stem Cell Res Ther*. 2019;10(1):142. doi:10.1186/s13287-019-1253-6
120. Liu W, Long Q, Zhang W, et al. miRNA-221-3p derived from M2-polarized tumor-associated macrophage exosomes aggravates the growth and metastasis of osteosarcoma through SOCS3/JAK2/STAT3 axis. *Aging*. 2021;13(15):19760–19775. doi:10.18632/aging.203388
121. Bai Y, Yan XL, Ren J, et al. Adipose mesenchymal stem cell-derived exosomes stimulated by hydrogen peroxide enhanced skin flap recovery in ischemia-reperfusion injury. *Biochem Biophys Res Commun*. 2018;500(2):310–317. doi:10.1016/j.bbrc.2018.04.065
122. Wu D, Chang X, Tian J, et al. Bone mesenchymal stem cells stimulation by magnetic nanoparticles and a static magnetic field: release of exosomal miR-1260a improves osteogenesis and angiogenesis. *J Nanobiotechnology*. 2021;19(1):209. doi:10.1186/s12951-021-00958-6
123. Qian L, Pi L, Fang B-R, et al. Adipose mesenchymal stem cell-derived exosomes accelerate skin wound healing via the lncRNA H19/miR-19b/SOX9 axis. *Lab Invest*. 2021;101(9):1254–1266. doi:10.1038/s41374-021-00611-8
124. Hu L, Wang J, Zhou X, et al. Exosomes derived from human adipose mensenchymal stem cells accelerates cutaneous wound healing via optimizing the characteristics of fibroblasts. *Sci Rep*. 2016;6(1):32993.
125. Ma T, Fu B, Yang X, et al. Adipose mesenchymal stem cell-derived exosomes promote cell proliferation, migration, and inhibit cell apoptosis via Wnt/β-catenin signaling in cutaneous wound healing. *J Cell Biochem*. 2019;120(6):10847–10854. doi:10.1002/jcb.28376
126. Zhang B, Wang M, Gong A, et al. HucMSC-exosome mediated-Wnt4 signaling is required for cutaneous wound healing. *Stem Cells*. 2015;33(7):2158–2168. doi:10.1002/stem.1771
127. Chen K, Yu T, Wang X. Inhibition of circulating exosomal miRNA-20b-5p accelerates diabetic wound repair. *Int J Nanomedicine*. 2021;16:371–381. doi:10.2147/IJN.S287875