Comparative Analysis of RNAs' Delivery Systems in diabetic wound healing: Mechanisms, Applications, and Future Prospects

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Abstract: The role of RNA in the treatment of the diabetic foot has received increasing attention as RNA therapy has been used in a variety of ways. This article explores various delivery methods for different types of RNA, with a focus on their mechanisms, efficiency, and potential applications. Discussed encapsulation, adsorption, layer by layer assembly, covalent binding, and targeting strategies involving MiRNA, SiRNA, mRNA, and LncRNA. Analyze the advantages and disadvantages of each method in targeted delivery and release of RNA, and point out their potential in clinical applications for diabetic wound.

1 Introduction

Diabetes is a chronic illness that impacts a significant portion of the global population. Over 440 million individuals worldwide are being impacted by the diabetes pandemic. The Asia-Pacific area is home to the biggest number of persons with diabetes, and the prevalence of diabetes in the region has risen substantially over the last few decades [1, 2]. With 1.4 billion people living there, China presently has the highest rate of diabetes patients worldwide. The social toll that diabetes and its numerous complications take on is also very significant [3].

Due to various comorbidities and pathophysiological changes, diabetic patients suffer from poor wound healing, prolonged inflammation, and slow epithelialization. Remarkably, diabetic foot ulcers (DFUs), localized ulcers on the lower extremities, occur in 15% of persons with type 2 diabetes. The most serious type of diabetic wounds, DFUs can result in lower limb amputation or even death [4]. The prognosis of chronic diabetic wounds remains poor despite different approaches such as tight glycemic control and careful wound care, hence there is an urgent need to find new effective interventions to promote chronic diabetic wound healing [5].

Research has demonstrated the significant significance that non-coding RNAs, including siRNAs, lncRNAs, and miRNAs, play in a variety of biological processes [6]. Additionally, each of them is crucial to the healing of diabetic wounds [7, 8]. For instance, through controlling macrophage polarization, lncRNA GAS5 can influence diabetic wound healing; overexpression of miR-203 in DFU tissues may impede the EMT process and postpone wound repair [9, 10]. In terms of diabetic wound healing, in the case of encoding ribonucleic acid (mRNA), some of the proteins produced by its translation (e.g., HIFA) have been shown to have a crucial role [11].

RNAs are presently being delivered to targets in a variety of domains in order to treat illnesses and carry out their pathophysiological effects [12]. To date, there have been relatively few studies on RNA delivery in specific diabetic wound healing. Consequently, the mechanisms of transport that could allow four RNAs to be delivered to wounds and aid in the promotion of wound healing are outlined in this review. They both hold the promise of being applied to RNA transport associated with diabetic wound healing.

2 Delivery of MiRNA

MicroRNAs are RNA molecules of about 21 to 23 nucleotides in length that are widely found in eukaryotes and can regulate the expression of other genes. MiRNAs play an important part in regulating the expression of most genes by specifically binding to target messenger ribonucleic acids (mRNAs), thereby promoting or inhibiting post-transcriptional gene expression.

2.1 Encapsulation

Encapsulation is achieved by encapsulating miRNA in nanoparticles, which not only protects miRNA from being easily broken down during transportation within the human body, but also ensures that it can be released when transported to a specific location. Common materials used for packaging include lipids, polymers, and inorganic nanoparticles such as gold. Lipid nanoparticles can fuse well with the cell membrane and release miRNA into the cytoplasm. Polymers, such as poly (lactic acid glycolic acid) copolymer (PLGA), can

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gradually decompose over time to achieve the function of sustained-release miRNA [13].

2.2 Adsorption

We noticed that some miRNAs have a negative charge on their surface, so we can use positively charged nanoparticles to adsorb miRNAs onto their surface. This method is simpler to operate than encapsulation. Usually, we use positively charged liposomes or polymers to adsorb miRNA, but some materials may cause early field damage to the cell membrane. Therefore, in design, we should adjust the properties as much as possible while ensuring the charge to reduce its toxicity, in order to achieve normal binding and transfer.

2.3 Layer-by-Layer (LbL) Assembly

LbL is a relatively advanced method that also focuses on transferring and regulating miRNAs through charges. By alternating miRNA with positively charged polymers, a multi-layered structure is formed that protects miRNA and regulates its release.

LbL achieves the loading and release of miRNA by changing the composition and thickness of each layer. Its advantage lies in the ability to use different materials to enhance applicability in different environments, such as using different materials and nanoparticles for different pH values. However, the process of LbL assembly, delivery, and release of miRNA may require a long time, and careful regulation is needed at all times to ensure its correctness. Currently, it does not have the ability for large-scale production [14].

2.4 Covalent Bnding

Covalent binding establishes covalent bonds between nanoparticles and miRNA to achieve stronger binding between miRNA and nanoparticles. MiRNAs with thiol groups can be chemically linked to gold nanoparticles using gold thiol, forming a stable complex and releasing miRNA when pH changes. This method can bind miRNA more stably and for a longer period of time, but it is important to ensure that the miRNA is released correctly and remains effective.

2.5 Target strategies

In addition to the physical methods of introducing miRNA into cells, how we target delivery is crucial for success. Targeting involves altering nanoparticles with special molecules (such as peptides, antibodies, or small chemicals) that attach to specific receptors on target cells.

The reason why targeting is effective is because these special molecules interact with receptors, which are typically present in higher quantities on disease cells such as cancer cells. For example, peptides focused on integrins or folate receptors can guide nanoparticles to cancer cells, ensuring miRNA reaches the correct location and reducing unintended effects. The use of dual targeting methods, combined with different targeting molecules or adding responsive features (such as coatings that respond to pH changes), can improve accuracy and effectiveness [15].

There is a growing need for safe and effective methods of wound healing. Emerging therapeutic options already incorporate the need to address key signaling pathway defects, cellular dysfunction, and tissue regeneration deficits associated with chronic wounds. However, most of these treatments are experimental or in very early stages of development. The use of miRNAs offers an attractive proposal for the development of wound healing gene therapies. miRNA-based therapies show unique advantages because by modulating a single miRNA, a set of functionally relevant genes in the pathway can be targeted, which is very effective compared to traditional drug therapies or DNA therapies.

3 Delivery of SiRNA

3.1 Introduction to siRNA delivery

RNA interference (RNAi) is a new technology developed in recent years, which can block the expression of specific genes and has great potential in gene therapy. The process begins with the entry of endogenous or exogenous double-stranded RNA into the cell, which is processed by Dicer's enzyme to become a short-stranded RNA of 21-23 nucleotides, namely siRNA [16, 17]. SiRNA plays a pivotal role in the process of RNA interference. After entering the cell, it forms RNA-induced silencing complex (RISC) with proteins in the cell. Then siRNA is unspun, and the siRNA sequence on the RISC complex is used as a guide to find and bind mRNA with specific sequence. The mRNA was enzymatically cut. The mRNA fragments after digestion are nonspecifically degraded by nucleases in the cytoplasm, resulting in the failure of expression of specific proteins and gene silencing. The RISC complex can be recycled to continue enzyme-cutting other targeted mRNA molecules, so that siRNA can amplify the inhibition of mRNA expression. At present, it is an effective method to specifically inhibit gene expression [18].

Also, siRNA has great potential for treatment. It plays a therapeutic role by interacting with mRNA rather than proteins, reducing harmful proteins that may be produced before synthesis. Another advantage of siRNA as a therapeutic drug is that it can achieve gene silencing for many proteins in treating diseases. The targets of conventional chemical drugs are limited to specific enzymes, receptors and ion channels. However, siRNA drugs can target any susceptible mRNA, regardless of the location of the transcription protein in the cell. In addition, only a small amount of siRNA is needed to achieve gene silencing. Also, siRNA has a high degree of sequence specificity, and any base mismatch will lead to the loss of RNAi effect, so its targeting is strong. And the siRNA is relatively stable, especially the siRNA with 3' -end overhanging TT base, which can be stable in the cell for 3 to 4 days, and the half-life is much longer than that of antisense oligonucleotides [19].

3.2 Overview of siRNA delivery modes

There are many ways to deliver siRNA. At present, there are two common ways: one is direct delivery through chemical modification, and the other is carrier transportation. The following will separately expound the two transmission modes, and the carrier transport mode is the main one.

3.2.1 Direct delivery of chemically modified siRNA

There are many modification methods of siRNA double helix: ribose modification, phosphoric acid modification, base modification, synaptic and terminal modification, and double helix modification. The chemical modification can improve plasma stability of siRNA, increase high potency, regulate immune activity and reduce off-target effect. Dar et al developed a dedicated databse of chemically modified siRNAs containing 4,894 chemically modified siRNA sequences [20]. While siRNA is rich in importance as a therapeutic tool, it has little harm that needs to be eliminated before it can be used as a drug. SiRNA has properties that stimulate innate immunity and can also show nonspecific binding. Furthermore, the stability of the double-stranded body inside the serum is also affected, and the negatively charged RNA has difficulty crossing the cell membrane, so the pharmacokinetic characteristics are affected [21].

To overcome all these shortcomings, a large number of chemical modifications were made by changing the sugar portion of the siRNA, the skeleton phosphodiester, the nuclear base, and the terminal and coupling groups. These modifications resulted in the improvement of the differences described above. Therefore, compared with unmodified siRNAs, modified siRNAs have a wide range of applications in the therapeutic field. In addition to traditional chemical modification methods, by using the experimental structure information of siRNA-PAZ domain, rational drug design methods have been well applied [21]. The structures are shown in Figure 1.

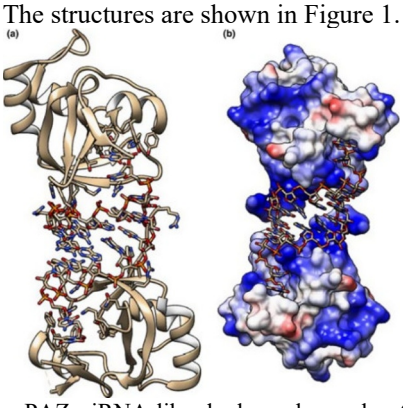

Figure 1. The PAZ–siRNA-like duplex subcomplex (the PAZ domain crystal structure of human Ago2.) (PDB entry 1SI2). (a) The two PAZ domains entire complex bound to each end of an siRNA-like duplex. RNA and Protein are presented in stick and ribbon representations; respectively. (b) The PAZ domain surface view is shown with the colouring option of the columbic surface (blue equals to positive potential, white equals to neutral, red equals to negative potential) and the RNA is shown in stick representation [21]. Copyright 2017, Chemical Biology & Drug Design.

3.2.2 SiRNA delivered by carrier

Although RNA delivered directly by chemical modification has many advantages, naked siRNA is easily degraded by ribozyme (RNase) in vivo, has a short half-life, and has a low transfection efficiency. Therefore, stability is crucial for the inhibition of siRNA [22]. Viral vector is the earliest vector used to deliver siRNA in vivo. Due to some toxic side effects and potential immunogenicity and tumorigenicity of viral vector in gene therapy experiments, viral vector is no longer the preferred delivery method of siRNA. A good delivery vector can improve the stability and targeting of siRNA [18].

In order to find a good adsorbent, Piul S. Rabbani et al. optimized a novel liposome and protein hybrid nanoparticle drug delivery system for topical healing of diabetic wounds in the presence of severe oxidative stress. They used cationic lipid nanoparticles (CLN) consisting of 1,2-dioleyl-3-trimethylammonium propane (DOTAP) and the edge activator sodium cholate (NaChol), with cationic engineered pressurized spirochetal proteins (CSPs) added proportionally to produce stable lipoprotein complex (LPP) nanoparticles [23]. This method has the best siRNA complex, minimal cytotoxicity and higher transfection efficiency. This approach is safe and efficient and will translate into clinical use. The images and data are shown in Figure 2.

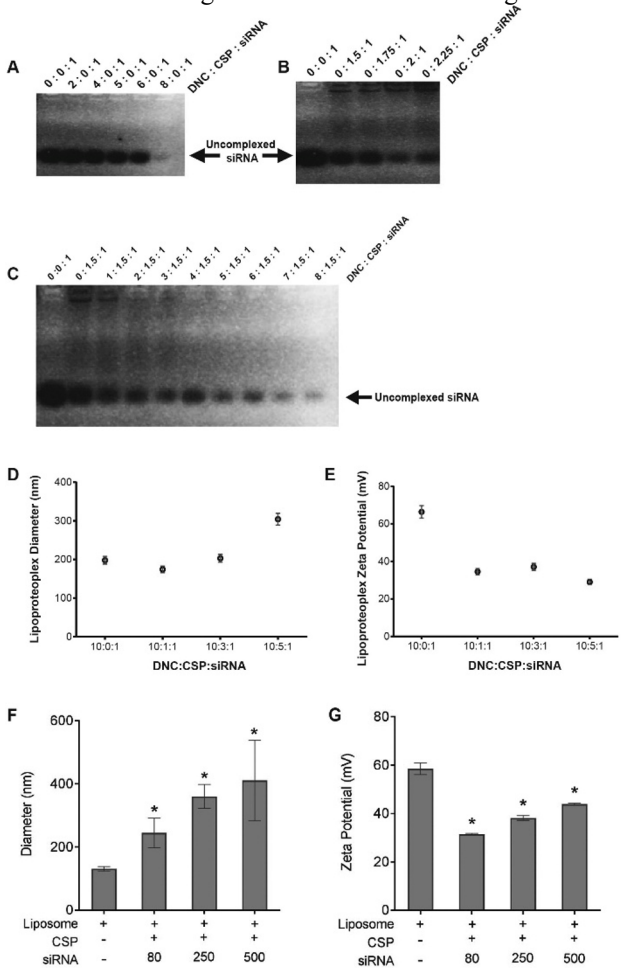

Figure 2. SiRNA complexes with LPP to form the stable nanoparticle. Electrophoretic mobility changes were analyzed

to investigate the impact of increasing the DNC-to-CSP(w/w) weight ratio while maintaining a constant nuclear weight of siRNA for all samples (A). Additionally, the effect of increasing the DNC-to-CSP(w/w) weight ratio was examined with a constant siRNA weight across all samples (B). Electrophoretic mobility changes were also analyzed to evaluate increases in the DNC-to-CSP-to-siRNA(w/w/w) weight ratio when both CSP and siRNA weights were held constant for samples (C). The vertical distance measurement diameter (D) and surface charge zeta potential measurement (E) were conducted for samples with varying DNC-to-CSP-to-siRNA(w/w/w) weight ratios. Furthermore, LPP diameter (F) and zeta potential measurements (G) were performed as siRNA loading increased [23]. Copyright 2017, Biomaterials.

Another case of an excellent dilivery is that John R. Martin et al explored siNP delivery from the scaffold chemistry of a novel polythioacetal polyurethane (PTK-UR) with a cell-mediated (i.e., non-hydrolytic) degradation mechanism which is driven by reactive oxygen species (ROS) [24, 25]. The PTK-UR chemical can achieve a better degradation rate and cell infiltration rate compared to the ester-based PEUR material, while inhibiting wound contraction more effectively [26]. In the paper, they investigated the use of PTK-UR stents for local delivery of siNPs to knock down PHD2 in vivo to promote cell proliferation, angiogenesis and new tissue formation rate in diabetic resected skin wounds. As a novel scaffold, it promotes wound tissue regeneration and has good biocompatibility. As a new type of stent, it can promote the regeneration of wound tissue and has good biocompatibility [24, 25].

Also for the first time, Huan Lei and Daidi Fan prepared TA-SiRNA nanogels based on self-assembled interactions between tannic acid (TA) and short interfering RNA (siRNA) [27]. This kind of high efficient and biodegradable nanogel is crosslinked with polyvinyl alcohol (PVA), human-like collagen (HLC), TA and borax to prepare an adaptive, electrically conductive PHTB (TA-SiRNA) hydrogel. Under the action of high concentrations of reactive oxygen species (ROS), the boronic acid bonds in the hydrogel in response to ROS were oxidized and destroyed, and TA-siRNA nanogels were released into cells, thereby reducing the expression of MMP-9. The advantage of using this hydrogel is that it has adaptive conductivity, which can enhance the effective conduction between cells, and it is very applicable [27]. The mechanism and data are shown in Figure 3 and Figure 4.

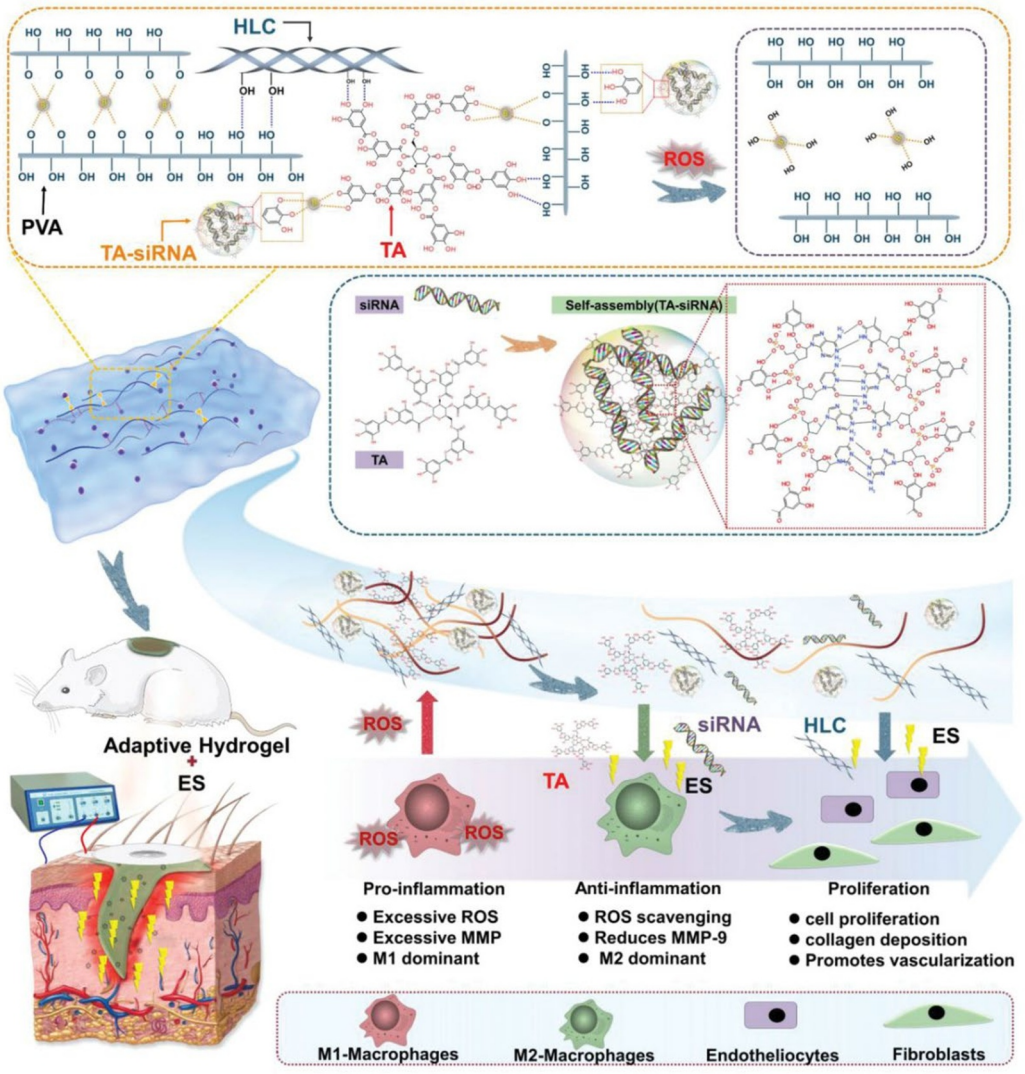

Figure 3. Using the combination of ES therapy and adaptive, conductive PHTB(TA-siRNA) hydrogels to repair the diabetic chronic wounds [27]. Copyright 2022, Advanced Science (Weinh).

In addition, Exosomes are membranous vesicles that are released into the extracellular matrix after the cellular multivesicular body (MVB) fuses with the cell membrane and can transport many substances. Generally, exosomes have a particle size between 30 and 150nm and can contain siRNA inside, which is a container for comparison [28]. Exosomes have excellent specificity, targeting and stability.

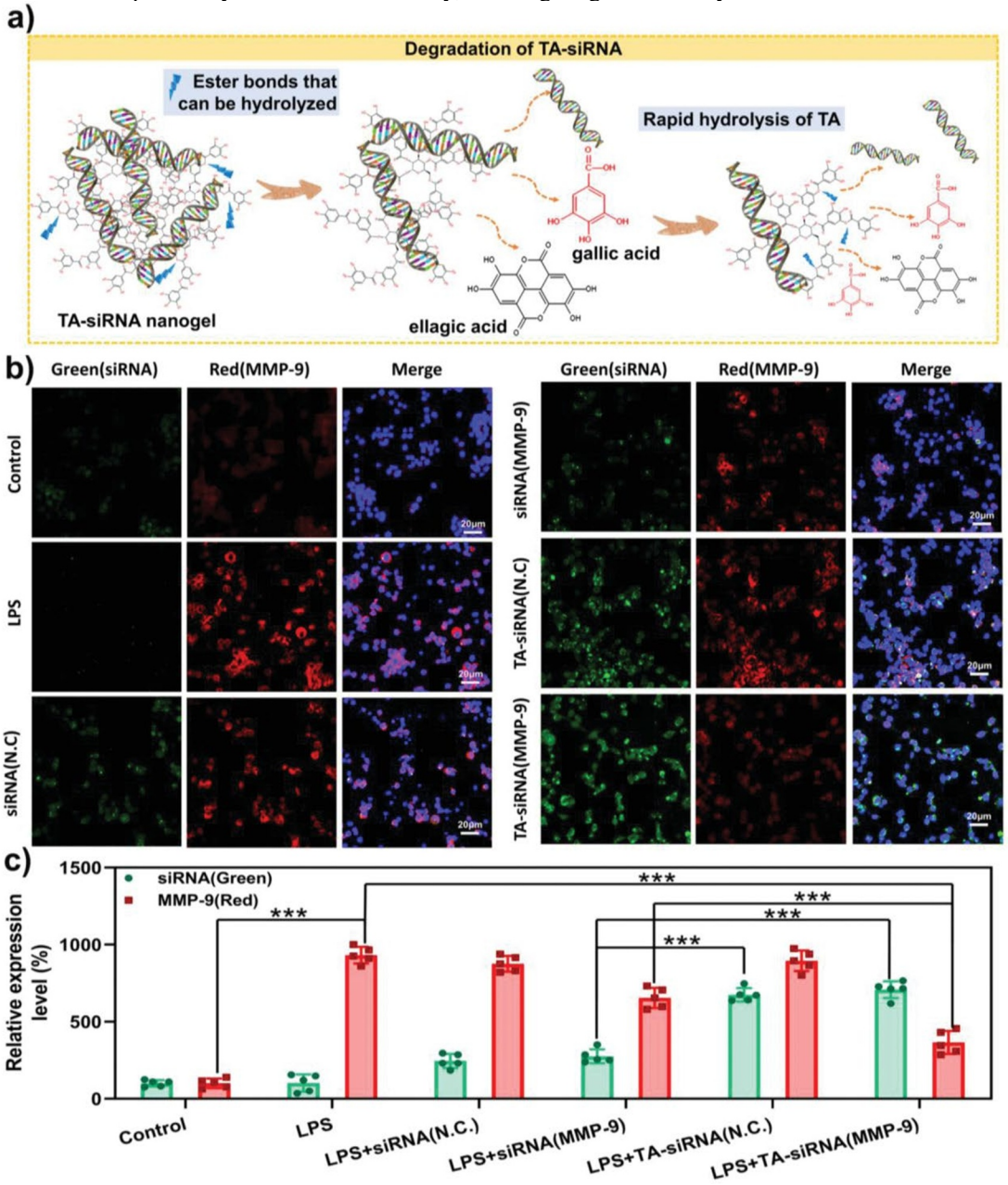

Figure 4. a) The mechanism of the potential release of siRNA from TA-siRNA nanogels. Through the TA-siRNA nanogels degradation, siRNA is progressively released, primarily due to the hydrolysis of the TA structure (as indicated by the blue arrow points: the ester bond in the TA structure can be hydrolyzed). b) Immunofluorescent staining was employed to evaluate the gene-silencing efficiency of TA-siRNA nanogels in RAW264.7 cells. The MMP-9 was visualized using a red immunofluorescent stain, while siRNA was labeled with 5-FAM. The nucleus of the cells was stained with DAPI (blue). The scale bar indicates a length of 20 μm. c) The quantitative analyses of the levels of MMP-9 and siRNA in the RAW264.7 cells on the basis of the immunofluorescence staining. The levels of MMP-9 and siRNA in the cells in the negative control group were set to 100% (***: $p < 0.001$, $n = 5$)[27]. Copyright 2022, Advanced Science (Weinh).

These are some typical ways of transporting siRNA by carrier. Compared with directly modified delivery, the use of vector delivery can better improve transfection efficiency, reduce immunogenicity, extend the survival time of siRNA and overcome the restriction of biological barriers. In particular, the use of non-viral delivery vectors, such as silicone grease vectors, can maximize transfection efficiency. This vector combines a customized surface lipid with PH-dependent silicon/RNA bond dissolution to ensure that siRNA can be efficiently taken up by cells. Silicone lipid carriers, through their positive charge and high ζ potential, electrically bind and condense RNA, preventing premature RNA dissociation, and reduce dependence on

potentially toxic cationic lipids, thereby reducing immunogenicity. In addition, a large number of tightly condensed RNAs are statically bonded to the silicon matrix, protecting them from hydrolysis and further reducing the immune response. Moreover, the structural integrity of the silicon matrix prevents physical collapse of the particles, enables freeze-drying while maintaining RNA integrity, and reduces the need for pegylation. This step is the key to avoiding the need for ultra-cold supply chain storage, thereby extending the systemic survival time of siRNA [29]. Modern medicine is gradually paying more attention to non-viral delivery methods, and researchers are striving for the safety, accuracy and rapidity of delivery in wound healing.

3.2.3 Prospects for delivery of siRNA

In the past decade, there has been a global upsurge in research on siRNA to treat wounds. The clinical application of small interfering RNA has been reported in the scientific community [30]. However, there are still many problems to be solved and improved in the application of siRNA in clinical therapy: the route of administration, the relationship between aging and dose-effect, the reduction of non-specific reactions, and the therapeutic mechanism based on siRNA drugs also need to be further studied [19].

4 Delivery of mRNA

4.1 Introduction to mRNA delivery

MRNA therapy is a crucial therapeutic tool that can target and intervene with all of a cell's genetic material. It can be used to carry out treatments including gene therapy, cell therapy, cancer therapy, protein replacement therapy, vaccination, and more [31]. Theoretically, utilizing the applicable mRNA base editing technology, all of the mRNAs corresponding to proteins that are important for diabetic wound healing can be modified and subsequently transported through the proper transport modes.

4.2 Overview of mRNA delivery modes

Lipid nanoparticles, or LNPs, are one of the most researched and discussed ways recently. They have been around for a while and are presently undergoing clinical trials [32]. Lipid nanoparticle-mRNA vaccines are currently being employed to treat COVID-19, the coronavirus illness [33]. They are particularly effective in their delivery, biocompatible, and biodegradable. Exogenous mRNA has been demonstrated to be delivered to host cells via lipids [34].

Three structural domains make up lipids, which are amphiphilic molecules: a hydrophobic tail area, a polar head group, and a connector between the two structural domains. To transport mRNA, cationic lipids, ionizable lipids, and other lipid types have been studied. There are four common components presented in LNP formulations: (1) cationic or ionizable lipids or polymeric materials made of tertiary or quaternary amines to encapsulate polyanionic mRNAs; (2) lipids like those in cell membranes; (3) cholesterol lipid bilayers used to stabilize LNPs; (4) lipids derived from PEG to give nanoparticles a hydration layer, enhance colloidal stability, and decrease protein uptake [35].

To work in vivo, Lipid nanoparticle-mRNA compositions must get past several extracellular and intracellular barriers [36]. To facilitate translation, the mRNA molecules must first be shielded from nuclease degradation in physiological fluids; then, the lipid nanoparticle-mRNA system must reach the target tissues and the target cells internalize it; and last, the mRNA molecules must flee endosomes to reach the cytoplasm [37]. The mechanism can be shown in Figure 5.

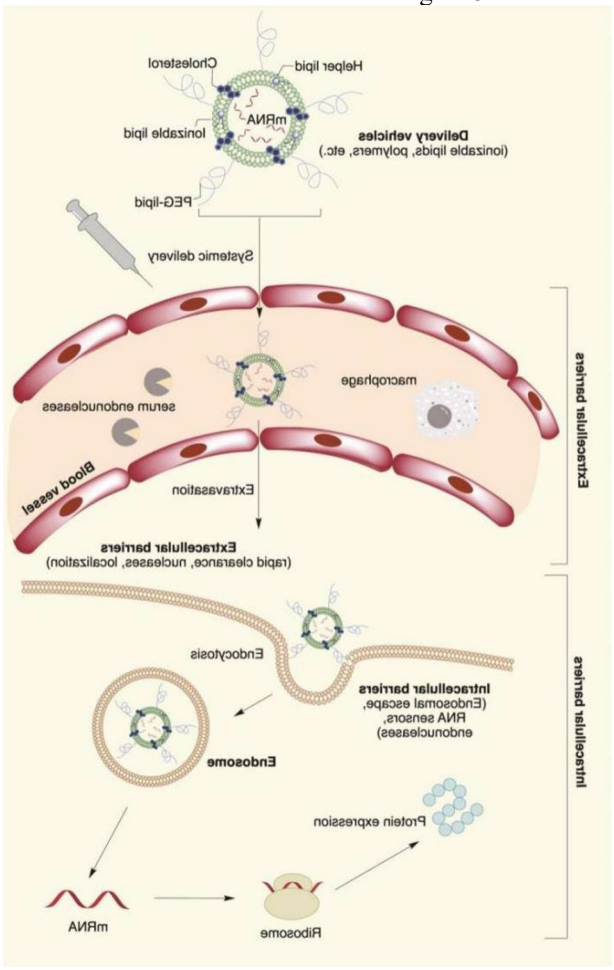

Figure 5. Extra- and Intracellular Barriers Schematic Representation for mRNA Delivery [37]. Copyright 2019, Molecular Therapy.

Lipid nanoparticle-mRNA formulations created by fast mixing display stable nanostructures [36], where the mRNA molecules can be contained in the inner core by electrostatic interactions with lipids, despite the fact that the mRNA delivery mechanism of LNPs is not entirely understood [38, 39]. This structural characteristic improves the nanoparticles' stability in physiological fluids and shields the mRNA molecules from nuclease destruction [40]. Lipid nanoparticles can be swallowed by a range of methods once they reach the target cells, such as

endocytosis mediated by kavicells and clamp-like proteins and macrophages [41-43].

In terms of targeting, it is possible to assess the targeting of lipids for diabetic wounds by adjusting the formulation's lipid content and composition. It has been demonstrated that the lead TS LNP effectively delivers IL4 mRNA to the wound site, modifying the milieu of wound inflammation and promoting diabetic wound healing. With great promise for clinical translation, a single dosage of the TS-IL4 LNP-mRNA formulation provides a convenient, safe, and effective way to speed up diabetic wound healing [44]. Intradermal treatment of diabetic skin wound mouse models with VEGFA mRNA-LNPs resulted in almost complete healing of the wounds treated with VEGFA mRNA-LNPs, and LNPs are also an efficient transport pathway for VEGFA mRNA [45]. The mechanism can be shown in Figure 6.

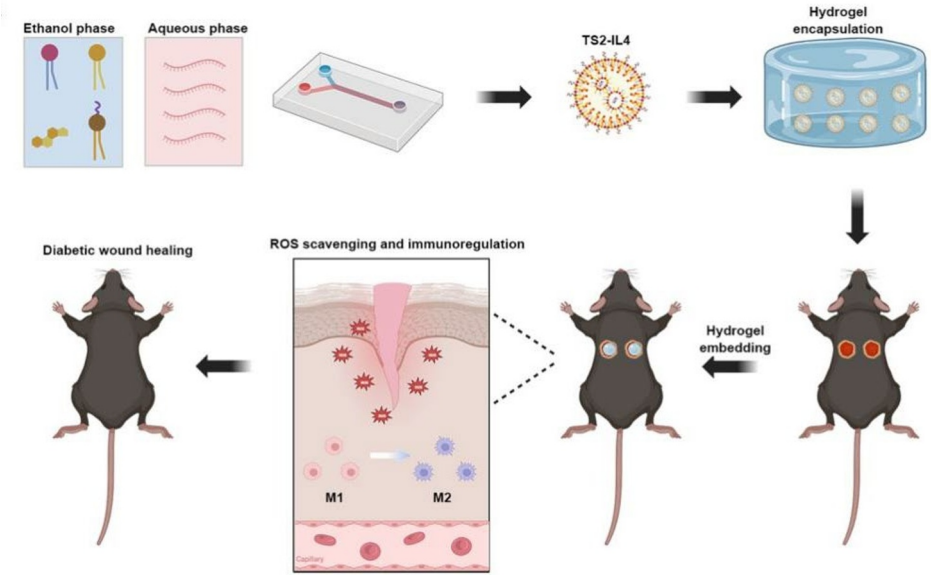

Figure 6. Trisulfide-derived ionizable lipids for the treatment of diabetic wounds: Illustration of synthetic TS2-IL4 LNP-mRNA loaded into a hydrogel accelerates diabetic wound healing by scavenging ROS and modulating the phenotype of macrophages at the wound site [44]. Copyright 2024, Proceedings of the National Academy of Sciences of the United States of America.

Numerous alternative methods of mRNA transport exist, and different polymeric materials, like cell-penetrating peptides and aliphatic chain-modified low molecular weight polyethylene amines (PEIs), have been utilized for mRNA delivery, but none of these have shown promise in the treatment of diabetic wounds [46, 47]. However, it has also been demonstrated that some of the common transport mechanisms for other forms of RNA, like extracellular vesicle-mediated distribution, can also be used to carry mRNAs during skin restoration [48].

5 Delivery of LncRNA

LncRNA, full name Long non-coding RNAs, which defined as RNAs that are longer than 200nt but do not have protein-coding capabilities, have attracted increasing attention due to their powerful biological functions. Large intergenic non-coding (lnc)RNAs of more than 1000 nt were found in the mammalian genome to be markedly conserved in different mammals, and thus their functional and gene expression patterns suggest that these lncRNAs are involved in and contribute to a variety of biological processes, including immune surveillance, cell-cycle regulation, and embryonic pluripotency of stem cell [49]. The studies recently have also shown that lncRNAs are also participated in many vital regulatory processes, including chromatin modification, genomic imprinting and X chromosome silencing, nuclear transport, transcriptional activation and interference, etc [50-53]. Until now, it has not been possible to deduce their function based on their structure or sequence alone. According to where lncRNAs locating on the genome with respect to protein-coding genes, they can be separated into five classes: sense, antisense, intergenic, intronic, and bidirectional. Different amounts of lncRNAs have been found in different human organ systems due to the binding of lncRNAs to relevant regions of proteins or target RNA and other mechanisms that play important biological functions. Because of their powerful capabilities, lncRNAs have emerged as potential new targets, affecting the treatment of diseases in many ways.

Due to the powerful function of lncRNAs, the dilivery systems should be precise and effective. Here we find two typical methods of dilivering amd each method shows significant biological characteristics.

5.1 Extracellular Vesicles and EMNVs

Extracellular vesicles (EVs) are naturally secreted nanoparticles by cells that regulate intercellular communication by transferring proteins, lipids, and accounting (including LncRNA). Due to its protective lipid bilayer, it is considered a good carrier for transporting LncRNA.

Researchers have developed extracellular vesicle mimicking nanovesicles (EMNVs) to simulate the excellent properties of EVs EMNV produces vesicles

with controllable size, composition, and surface properties through ultrasonic treatment and other methods A study by Tao et al shows that EMNVs, as the delivery vector of lncRNA-H19, have the effect of specifically increasing the expression of L rural RNA-H19 at the wound site in the treatment of diabetes wounds [54].

5.2 ELECTS

If transported by lentiviral vectors, the resulting lncRNAs may terminate improperly, and an additional ~2 kb fragment is attached to the end of 3′-. As a result, the secondary structure was altered, RNA-protein interactions were blocked, and the function of some lncRNAs was impaired, suggesting that lentiviral vectors were not suitable delivery systems for lncRNAs. In view of this problem, Yin Zhang et al developed a new way to use ELECTS for transport [55]. By inserting a termination signal after the sequence of lncRNA, ELECTS produces transcripts that do not contain the 3′-fanking sequence and retain the natural features and functions of lncRNA, which is not possible by using lentiviral vectors. For stable expression and gene therapy, it is an ideal system. Unlike retroviruses, it does not require transcription, which highly promotes the integration of the plasmid into the host genome [56]. Also, ELECTS expresses LncRNAs without flanking sequences and reserves nearly the same length as the endogenous one. So ELECTS provides an efficient and safe method for lncRNA expressing. The mechanism can be shown in Figure 7.

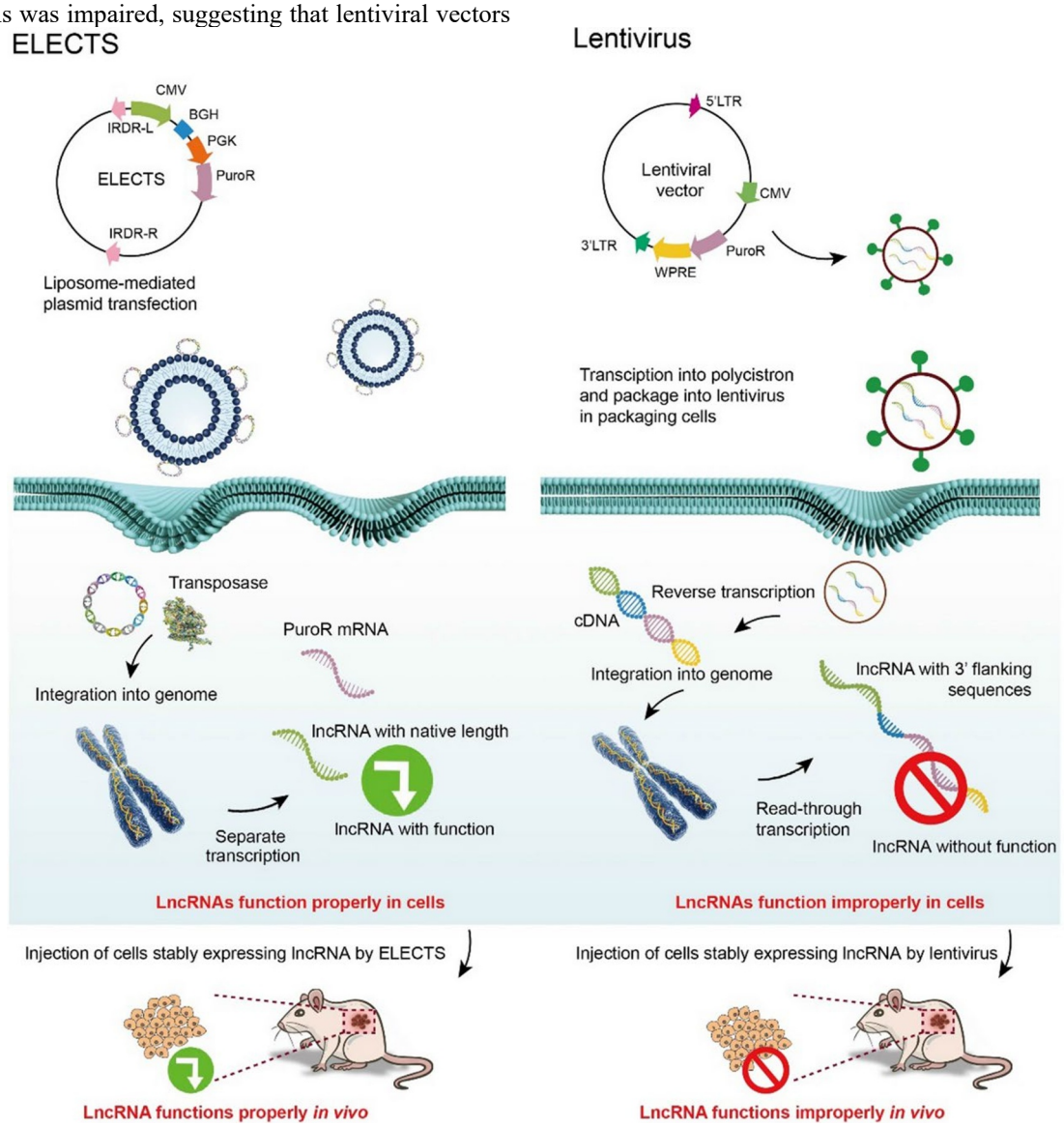

Figure 7. The schematic diagram of lentiviral vector and ELECTS exogenous expression mechanism. After being loaded with liposome nanoparticles, the ELECTS non-viral vector directly delivered into destination cells. The lentiviral vector should be packaged in 293 T cells and then the destination cells are infected by using the viral nanoparticles. LncRNAs can function properly after being expressed by ELECTS both in vitro and in vivo while the lncRNAs which are expressed by lentiviral vectors may not function properly [55]. Copyright 2021, Journal of Nanobiotechnology.

Although the future is bright, gene therapy targeting lncRNAs is still an emerging strategy and concept compared to traditional proteins and drug targets, so there are still some concerns about its possible adverse

effects. The biggest risk is the lack of basic research on the potential effects and function of medicable lncRNAs. Therefore, there may be inappropriate pathological effects and unexpected risks in the clinical application of lncRNA-targeted drugs. To be specific, off-target effects can lead to adverse reactions. Delivery systems and highly specific targeting methods need to be improved to ensure that only selected lncRNAs are affected. Due to the lack of sufficient clinical trial data, the safety and effectiveness of lncRNA drugs uesd in humans are still indeterminate.

LncRNA-targeted drug design presents numerous challenges and opportunities for the pharmaceutical industry. The Current gene therapy is the "third generation" of therapeutic drugs after chemical small molecule drugs and biological macromolecule drugs, which aims to treat diseases by artificially controlling gene expression. Such therapies hold promise for modulating disease at the genetic level and are able to overcome the limitations of incompatible proteins. Judging from the clinical breakthroughs in the cumulative recruitment of miRNA clinical trials and the targeted mRNA drugs, it can be imagined that the targeted lncRNAs may play a key role in gene therapy in the near future, providing new choices for precision medicine [57].

6 Conclusion

In conclusion, this review article emphasizes the important role of four different RNAs, including miRNA, siRNA, mRNA and LncRNA, in the complex process of wound healing in diabetes. Each RNA has a unique mechanism of action and purpose that distinguishes it from other RNAs, and investigating the targeting mode of delivery of different types of RNAs can help them get more stability and targeting in clinical applications.

MiRNA has the ability to regulate gene expression. Packaging, adsorption, layer by layer encapsulation, covalent binding, and targeting strategies can achieve precise delivery of miRNAs to the wound site while protecting them from degradation. To achieve regulation of validation and cell proliferation, further promoting wound healing.

SiRNA is known for its ability to interfere with RNA and silence gene expression, and can be used to downregulate gene expression that is detrimental to wound healing to promote wound healing. Carriers such as chemical modifications, liposomes, and nanoparticles have shown great potential in enhancing stability, reducing immunogenicity, and ensuring the silencing of relevant genes at the wound site when delivering siRNA.

MRNA, as a necessary template for protein synthesis, can directly affect the proteins required for wound healing through its regulation. When delivering mRNA, the commonly used carrier is lipid nanoparticles. It can effectively stabilize mRNA deficiency and promote its uptake by target cells. This method has been applied to a certain extent in reality.

LncRNA is a relatively long non coding RNA with regulatory functions at the transcriptional and post-transcriptional levels. Researches on the delivery of LncRNA is still in its early stages, such as extracellular vesicles and ELECTS systems. Although these methods can deliver LncRNA stably and specifically, their clinical applications still need further validation.

Most of the above modes of RNA transportation have relevant experiments to prove their promotional effects on the healing of diabetic foot, which lays a solid foundation for possible clinical experiments and clinical applications in the future. Each type of RNA has its own unique characteristics in the treatment, so it is crucial to continue to study the delivery system of different RNA and how to study its clinical application, which will also change the treatment of diabetes wounds.

Acknowledgments

Yijun Gong and Liaojia Guo contributed equally to this work and should be considered co-first authors.

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