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Nano-delivery Systems for mRNA: A comprehensive review of lipid-based and polymer-based approaches

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Abstract

Messenger RNA (mRNA) has emerged as a promising therapeutic tool for the treatment of various diseases, including cancer, infectious diseases, and genetic disorders. mRNA-based therapies offer several advantages over traditional gene therapy approaches, including temporary expression, adaptability, and improved safety profiles. The development of mRNA-based therapies has undergone significant advancements, with three distinct phases identified. Phase-I involved the discovery of mRNA, in vitro synthesis, and the development of nucleic acid delivery systems. Phase-II saw the clinical trials of mRNA vaccines for infectious diseases and cancer, while Phase-III is characterized by the emergency use authorization of mRNA-based vaccines for SARS-CoV-2.

However, the delivery of mRNA remains a significant challenge due to its instability and difficulty in crossing cellular membranes. To overcome these challenges, various delivery systems have been developed, including lipid nanoparticles (LNPs), lipoplexes, and polymer-based nanoparticles.

Keywords: Messenger RNA (mRNA); mRNA -Based Therapies; Lipid Nanoparticles (LNPS) Lipoplexes; Vaccines

1. Introduction

The translation of genetic information from genes into proteins is mediated by messenger RNA (mRNA). Exogenous mRNA delivery into cells enables precise, temporary protein production. Such mRNA-mediated transfection, which expresses proteins even in non-dividing and difficult-to-transfect cells without the hazards of chromosomal integration, presents an alluring substitute for utilizing mRNA plasmid DNA (pDNA)-based gene therapy. Furthermore, mRNA can enter the cytosol and then interact with the cells translation machinery to produce the desired proteins, whereas pDNA must enter the targeted cell's nucleus [1]. In 1990, the first study of effective mRNA therapies in preclinical settings was released. Significant advancements and breakthroughs in mRNA-based therapy have also been made because of the advancements in mRNA synthesis and Intracellular delivery strategies [2]. Compared to the majority of currently available medications, the mRNA's special qualities such as its temporary nature and adaptability offer patients great promise for extensive therapeutic applications in cancer treatment, protein replacement therapies, and immunization (Hajj and Whitehead) [3]. Over the past few decades, the idea of using mRNA for preventative and therapeutic purposes has been developed. The idea that any protein can be created in situ for extended periods of time, despite the limitations of mRNA when used alone, has motivated researchers from a wide range of fields to work toward realizing its potential [4]. DNA's unpredictable onset expression kinetics, mRNA exhibits more stable and predictable protein expression kinetics. mRNA is only temporarily active, which could be useful in situations requiring temporary protein expression. Lastly, the possibility of using mRNA in therapies is further increased by the robustness and relative convenience of in vitro production [5]. mRNA has a higher safety profile since it does not integrate into the host genome, which significantly lowers the danger of mutagenesis and carcinogenesis that are typically linked to DNA [6]. However, naked mRNA is

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destabilized in vivo and readily broken down by ribonuclease (RNase) because of its single-stranded nature. Additionally, a negatively charged mRNA macromolecule finds it difficult to pass through the negatively charged host cell membrane, which leads to ineffective cell penetration. Rapid advancements in mRNA engineering technologies, such as chemical manipulation, have been made to overcome these issues [7]. In comparison to the methods established for DNA delivery, mRNA delivery schemes need significantly more enhancement and evaluation. However, several carriers and biomaterials developed for DNA and small interfering RNA (siRNA) have demonstrated a promising basis for the advancement of mRNA delivery methods. mRNA has been studied for over fifty years; however, its extensive use in medical research and the creation of innovative therapeutic approaches has been constrained by its perceived instability, susceptibility to degradation, inadequate translatability, and immunostimulatory properties [8]. The basic processes of these vaccinations will be briefly explained in this perspective, along with how drug delivery sciences enable mRNA to pass through physiological barriers and reach its target. Lipid-based nano platforms will be our main area of interest. In addition, the remaining difficulties and the need for more research will be considered [9]. Messenger RNA (mRNA) can be used as a protein replacement therapy for cystic fibrosis and lung cancer [10].

Lung cancer and cystic fibrosis can be treated with messenger RNA (mRNA), a protein replacement treatment. Since mRNA is a negatively charged macromolecule that is vulnerable to common RNase, it has recently been used to create the COVID-19 vaccine. As a result, it is very challenging for mRNA to translate functional proteins in the cytoplasm and cross the anionic cell membrane (<1/10,000 mRNAs of the initial input) [11]. mRNA was gaining traction as a potential therapeutic approach. In 1990, Jon Wolff established the foundation for the use of mRNA as a therapeutic tool for in vivo expression [12].

2. mRNA-based therapeutics: powerful and versatile tools to combat diseases.

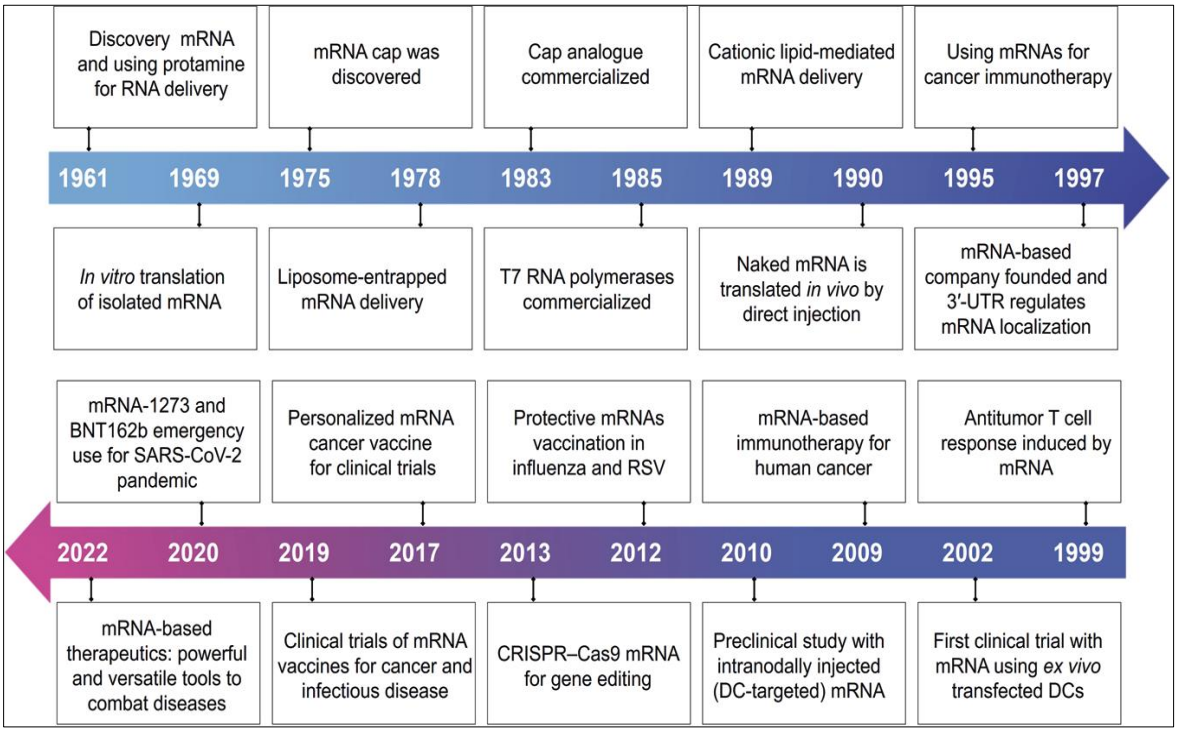

Figure 1 mRNA-based therapeutics: Powerful and versatile tools to combat diseases. [11]

Important findings and developments in mRNA-based medicines. Three phases can be distinguished in the development of mRNA-based therapies. Phase I involved the discovery of mRNA, in vitro synthesis, and the development of nucleic acid delivery systems (1961–1990). Clinical trials of mRNA vaccines for infectious diseases and cancer, mRNA-1273, and BNT162b emergency use for SARS-CoV-2 pandemic immune response are just a few examples of how mRNA-based therapeutics, a disruptive therapeutic technology, are developing into strong and adaptable tools for treating diseases (2019 to present) (Fig.1) [11].

Crucially, IVT mRNA can only be used to substitute proteins if it is given to cells that can fold the protein and cause any necessary post-translational changes. Most human proteins undergo post-translational modification in the Golgi

apparatus and endoplasmic reticulum to achieve full functioning [13]. mRNA-loaded LNP could be improved to more accurately deliver the mRNAs to a particular cell, tissue or organ, increasing its effectiveness and lowering its negative effects. Many scientists have recently been concentrating on mRNA delivery devices that are site-specific. Three factors should be considered while designing an improved mRNA carrier: the targeted cell or tissue, the delivery method, and the targeting tactics. To expand the use of mRNA therapy with high effectiveness and low systemic toxicity, this review highlights new research that use site-specific LNP for mRNA delivery [14].

3. Intracellular Trafficking of mRNA

For tiny therapeutic molecules, passive diffusion works well, but mRNA requires a vector to be efficiently absorbed by the cell. Regarding plasmid DNA, the type of NP determines the absorption and Intracellular trafficking of mRNA [15]. Some particular mRNAs contain a component called a riboswitch that binds a small molecule and regulates the expression of that mRNA in response to the concentration of the small molecule. Riboswitches have developed metabolic control mechanisms in bacteria and fold into complex structures that usually identify compounds [16].

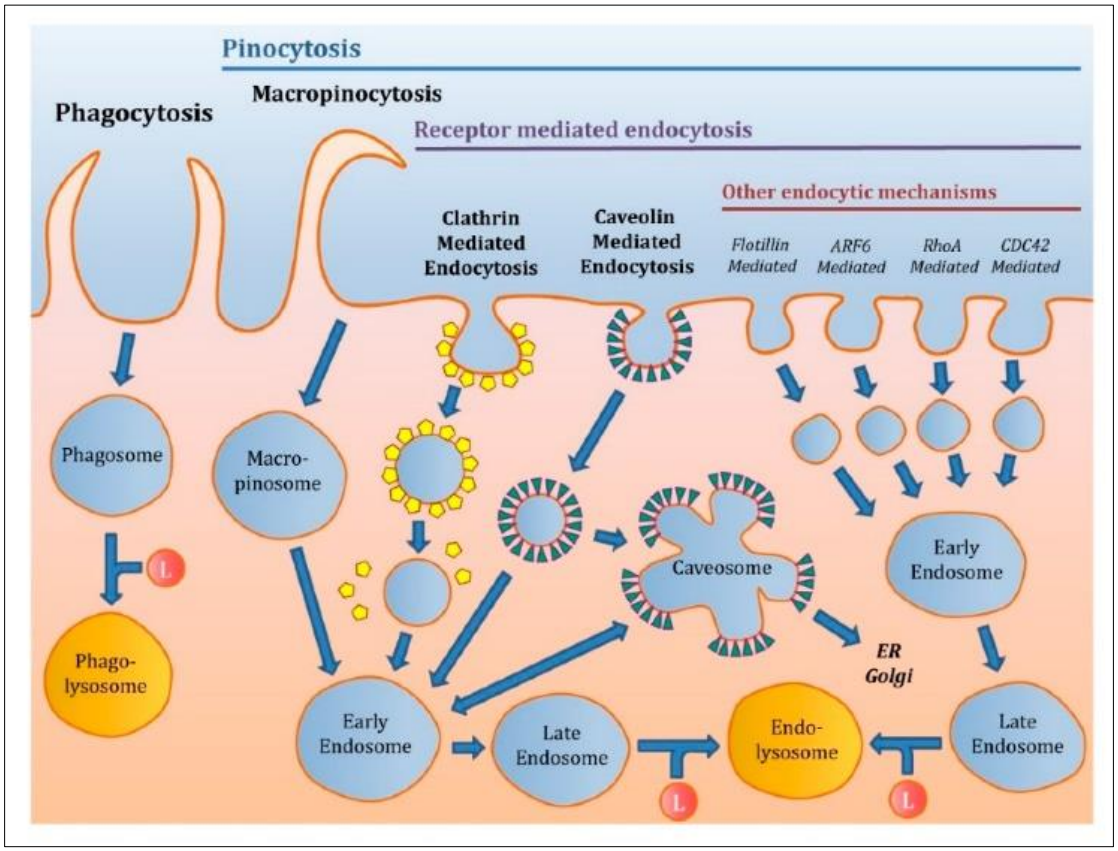

Figure 2 Principal mechanisms of endocytosis with three primary mechanisms identified for mRNA NP [15]

Phagocytosis, macropinocytosis, and endocytosis mediated by clathrin and caveolae. The clathrin-dependent pathway uses a clathrin network and is energy dependent. Clathrin-coated vesicles are formed when NP binds to the cell surface, causing membrane invagination. Once the clathrin network is eliminated, these vesicles will merge with early endosomes [15]. Several methods for incorporating nucleic acids can be employed, depending on the type of material core: covalent attachment where reactive groups can modify the NP surface, encapsulation within the material matrix, or adsorption with materials containing cationic moieties [17].

4. Enhancing mRNA Delivery through Ligand-Mediated Targeting:

The addition of targeting ligands can solve present mRNA delivery issues with polymeric nanocarriers. Hydrophilic polymeric shells, such as PEG, on nanoparticles are crucial for reducing positive charges, protecting mRNA from blood nucleases, and enhancing circulation time. Conversely, they hinder cellular absorption and limit nanocarrier internalization among targeted cells before disintegration. Effective mRNA therapeutics with targeted delivery

mechanisms and ligands are greatly desired for their precision and efficiency. According to Figure 3, ligand-receptor interactions can transfer mRNA to specific cells or tissues and increase cellular absorption through receptor-mediated endocytosis. Ligands enhance extravasation at targeted areas by interacting with endothelial cells, such as in tumor vasculatures or the brain, and modulating signaling cascades. A few ligands can enhance intracellular delivery by promoting endosomal escape, a crucial step for efficient mRNA translation after endocytosis [18].

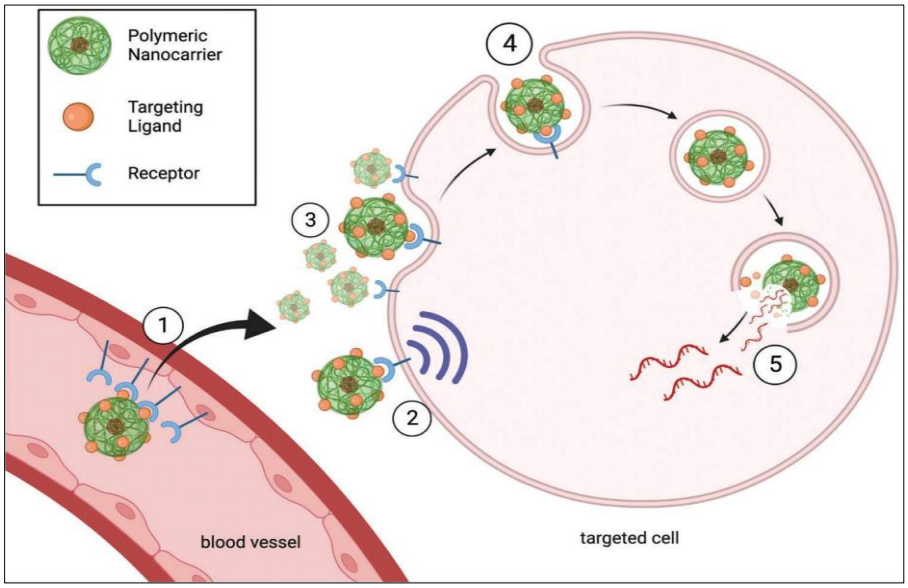

Figure 3 Targeting interactions between ligands. 1) Active ligand-mediated transport through blood vessels. 2) Ligand-mediated cell signaling 3) Ligand-mediated retention on cell surface. 4) Ligand-mediated endocytosis. 5) Endosome escape through ligand interactions [18]

5. Components and Structural Features of LNPs

In contrast to constantly positively charged lipids (liposomes), an LNP is a spherical vesicle made up of ionizable lipids that are neutral at physiological pH and positively charged (attached to RNA) at low pH. It has fewer harmful consequences [19].

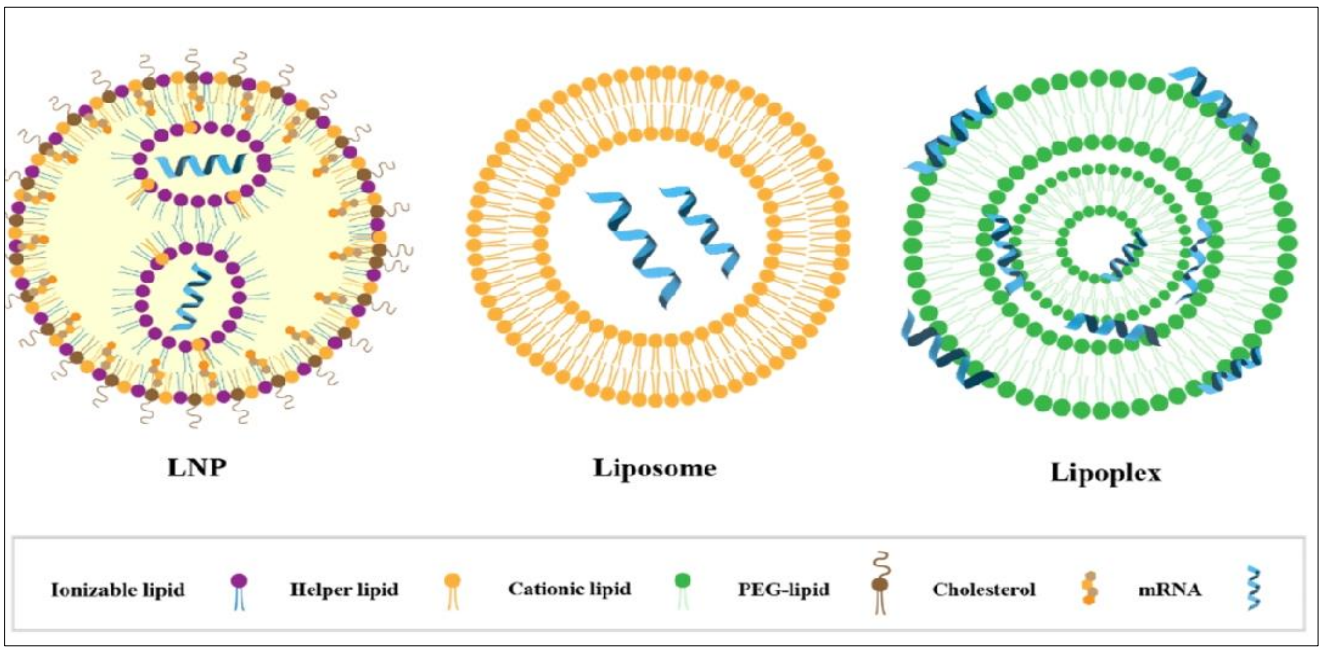

Figure 4 Schematic representations of the liposome, lipoplex, and LNP for mRNA transport [19]

5.1. Preparation of LNPs

Solid lipid nanoparticles, nanostructured lipid carriers, lipid nanocapsules, lipid–drug conjugates, polymeric lipid hybrid nanoparticle lipids, and other lipid nanoparticles can all be categorized based on their structure and drug-delivery mechanism. Various techniques, including thin film hydration, solvent injection, ultrasound-assisted, high-shear homogenization, solvent evaporation, solvent diffusion, etc., are used to manufacture different kinds of LNPs. The size, homogeneity, and encapsulation efficiency of the LNPs are all determined by the preparation process. Cost, scalability, reproducibility, and time factors should all be considered when choosing a preparation process. Table 1 lists the common laboratory techniques used today to manufacture LNPs [19].

Table 1 Characteristics of the current laboratory methods for LNP preparation

Name	Cost	Scalability	Encapsulation Efficiency	Reproducibility	Polydispersity Index
Ethanol dilution method	Low	Moderate	Moderate	Moderate	High
Manual mixing method	Low	Low	Low	Low	High
T-mix method	Low	High	High	High	Moderate
Microfluidics	High	High	High	High	Low

5.1.1. Manual Mixing Method and Ethanol Dilution Method

The most direct technique for creating LNPs now is ethanol dilution. The mRNA is dissolved in a suitable buffer solution, such as an acetate, citrate, or malate buffer, and different lipids are dissolved in organic solvents (ethanol, acetone, or isopropanol). The two phases are then quickly combined. Lipid solubility declines with ethanol phase dilution, and it eventually precipitates out of the mixture to coagulate and produce lipid nanoparticles that effectively encapsulate RNA. Lastly, the buffer's pH is corrected and any remaining ethanol is eliminated by dialysis or ultra-filtration.

An easier substitute for the ethanol dilution process is manual mixing, which involves moving the ethanol lipid mixture into an aqueous acidic mRNA solution, pipetting for 15 seconds to mix the mixture rapidly, and then letting it stand for 10 minutes. Similar to the Manual mixing and the ethanol dilution approach produce a non-homogeneous LNP with low encapsulation effectiveness and issues with inconsistent outcomes, poor particle size homogeneity, and poor reproducibility [19]. One milliliter of certified ethanol standard was mixed with nine milliliters of water to create an ethanol stock solution (1 mg/ml). Ethanol stock solution was diluted with water in the proper amounts to create ethanol working solutions [20]. Since ancient times, the fermentation of carbohydrates has created ethanol, the most prevalent volatile molecule. More over half of industrial ethanol and all beverage ethanol are still produced using the same method [21]. Water-based dilution of ethanol-containing samples is highly sensitive to pipetted dilution volumes [22]. Ethanol as a cosolvent in the preparation of aqueous extremely diluted solutions (EDS). EDS prepared in water containing 1 % ethanol. EDS prepared in a hydroalcoholic solvent, EDS_{EtOH}. EDS combined with a liquid that contains 1% ethanol called EDS_{EtOH}. [23].

5.1.2. T-Mix Method

Some investigations further refined the ethanol dilution approach by utilizing a T-mixer to combine the solution with an aqueous solution containing nucleic acids at an acidic pH to circumvent the drawbacks of the method. The ionized lipids become positively charged, attach to the negatively charged nucleic acids, and self-assemble to form complexes when these two solutions are combined. This is accomplished by diluting the lipids with ethanol, which reduces their overall solubility. To manufacture LNPs with precisely engineered tiny features within the channels that can mix the two fluids together in a controlled and repeatable manner, the T-mixer was developed[19]. In the absence of oxygen, the concentration of ethanol rises during fermentation until when it reaches 15% or more, the fermentation process stops, the zymase enzyme is blocked, and yeast becomes toxic [21]. It is necessary to calibrate an analyzer. After that, analyzers will automatically determine the ethanol content of samples following the addition of a dilution factor [22].

5.1.3. Microfluidics

Microfluidics has the potential to revolutionize the field of mRNA delivery [24]. From mRNA tumor vaccine to ligand decorating for APC targeting, pegylation for colloidal stability, or microfluidics synthesis for formulation [25]. When it comes to industrial scale-up, LNPs have several benefits over alternative carrier systems, including stability, low raw material costs, and large-scale synthesis using microfluidics or T-junction mixing [26]. Laborious procedures, such as the application of microfluidics, are required for the preparation of mRNA, particularly to stop LNP aggregation following

the combination of mRNA with cationic lipids. Furthermore, microfluidics chips also feature branching structures, vortex patterns, zigzags, etc. Lipid nanoparticle size can be regulated by microfluidics by altering the rate and the fluid injection ratio. Large-sized LNPs form under slow ethanol dilution circumstances, whereas small-sized LNPs form when ethanol is rapidly diluted with a buffer to a critical ethanol concentration. Presently, the majority of research in the literature prepared LNPs using microfluidics, and the particle sizes were typically dispersed between 20 and 200 nm. It is easy to metabolize nanoparticles that are less than 20 nm. via the kidney, the transfection effect is more affected by a larger particle size [19].

6. mRNA-Based Vaccines Nanodelivery Systems

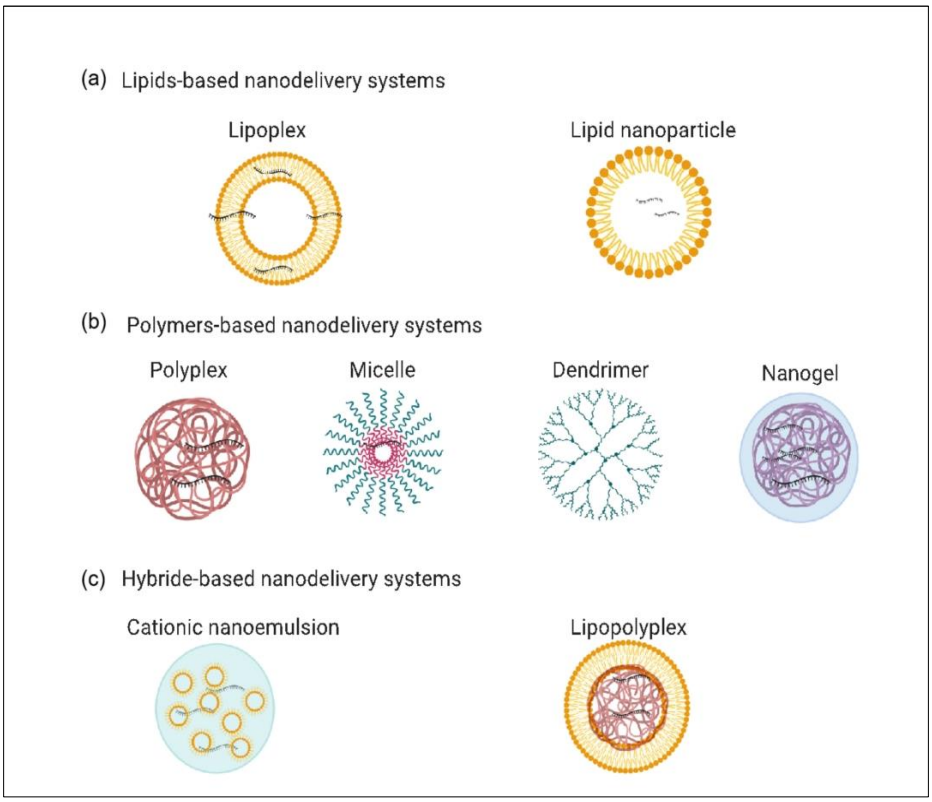

Figure 5 mRNA-based vaccine nanodelivery systems [27]

- Lipid-based nanodelivery systems, including lipoplexes and lipid nanoparticles.
- Polymer-based nanodelivery systems, including polyplexes, micelles, dendrimers, and nanogels.
- Hybrid-based nanodelivery systems, including cationic nanoemulsions and lipopolyplexes.

6.1. Lipid-Based Nanodelivery Systems

In the early to mid-1990s, detergent-dialysis techniques were created using cationic lipids that were permanently charged, like dioleoyldimethylammonium chloride (DODAC)[27]. extracellular vesicles, lipid-based nanoparticles, inorganic nanoparticles, and a new technology [28]. Nowadays, two of the most popular lipid-based nanodelivery technologies for the in vivo delivery of mRNA molecules are lipid nanoparticles and lipoplexes. A hydrophobic chain joined to a head group makes up a cationic lipid's general chemical structure. It is a hydrophobic chain that can be linear or branching, symmetric or disymmetric, modified or unmodified, and saturated or unsaturated. To improve biodegradation, the cationic head group contains a cleavable linker molecule. At least one group, such as an amine, which ionizes and transforms its negative charge into a positive one, is often held by the head group pH of physiological charge. Therefore, cationic lipids may use electrostatic interactions to form complexes with anionic compounds [29]. LNPs are lipid-based delivery vehicles that: (1) Allow nucleic acid payloads to enter the cytoplasm of cells; (2) Prevent nucleic acid payloads from being broken down or from triggering RNA-sensing systems and, consequently, innate immune responses; and (3) Act independently as adjuvants when used in vaccination [30]. Ligands can be conjugated to the surface of lipid-based structures, which typically have a core-shell configuration, to enable active targeting [31]. Lipid-based carriers, including lipoplexes and LNPs, have been extensively used for the delivery of nucleic acids. Non-viral

vectors can also be divided into polymer-based and lipid-based delivery systems [32]. With appropriate engineering [33]. Which are usually made up of several cationic lipid molecules either by themselves or in combination with neutral auxiliary lipids [34].

6.2. Lipid-Nanoparticle-Based Nanodelivery Systems

All SARS-CoV-2 mRNA vaccines that have been licensed for clinical use are administered via LNPs, which are the most clinically sophisticated mRNA delivery vehicles [35]. Patients with chronic HBV infections are presently enrolled in a Phase II trial that uses lipid nanoparticles to deliver RNAi that targets the chronic HBV virus [36]. Lipid-anchored polyethylene glycol, cholesterol, phospholipids, and cationic or ionizable cationic lipids are frequently used in the formulation of lipid nanoparticles. Through a mix of hydrophobic and attractive electrostatic interactions with mRNA, cationic lipids enable the spontaneous association of negatively charged mRNA. This creates a core that the other lipids bind with once their solubility limit is reached. Consequently, this improves the cytoplasmic endosomal release of mRNA. Incorporated is cholesterol. Phospholipids are added as a stabilizing agent to support the lipid bilayer structure [29]. Degradable lipid nanoparticles remain the most promising options for mRNA administration at this moment [37]. The chemical universe of carriers includes lipid nanoparticles and tiny cationic lipids used in lipoplexes [38]. Since more and more LNPs are now substituting sterol lipids for cholesterol to promote internal body escape, sterol lipids PE heads can enhance LNPs' inside body escape effectiveness even more [39]. To overcome these challenges, researchers have created several synthetic non-viral nanoparticles for mRNA delivery; the most clinically feasible of them are lipid nanoparticles (LNPs)[40]. Lipid nanoparticles have been extensively studied and effectively introduced into clinical settings for the administration of mRNA, siRNA medications, and small chemicals [41]. The US FDA has granted emergency use authorization (EUA) for clinical therapies against the COVID-19 virus for the first time in history to two mRNA-based vaccines created with lipid nanoparticles (LNPs) [26].

6.3. Lipoplex-Based Nanodelivery Systems

Lipoplex (LPX) administration was used to evaluate BNT113 (HPV16 E7 mRNA), an injectable cancer vaccine that effectively grows and amplifies antigen-specific effector and memory CD8+ T cells, in mice. In two HPV-positive mouse tumor models (TC-1 and C3), its treatment caused tumor regression, inhibited tumor recurrence, and demonstrated a combination effect with PD-L1 inhibitors [42]. Lipoplexes were used the same substance as a hit from a bio-active lipid screen to find lipid-like compounds that can increase mRNA lipoplex 19 transfections [30]. Lipoplex-Based nanodelivery system showed that lipoplexes delivering N1 methylpseudouridine-modified mRNA produced strong antitumor Tcell immunity with enhanced inflammatory safety. When injected subretinally[43]. Particles including 0.5% PEG encapsulating Luc-coding mRNA exhibited the maximum expression upon subretinal injection, indicating that particle size may significantly influence mRNA translation efficiency. The principal motivations for using PEGylation in lipid nanoparticles (LNPs) were to enhance particle stability and mitigate excessive serum protein binding and opsonization, which lead to accelerated clearance from the bloodstream [44]. Cholesterol-conjugated siRNAs exhibit no signs of cytotoxicity, inflammation, or immunological activation, despite the lipoplexed siRNA causing modest inflammation [45]. mRNA delivery uses a variety of lipids, both natural and synthetic. Since most lipoplex formulations cannot withstand the presence of serum and mRNA is more unstable, liposomes are not widely used, despite their intrinsic advantage of being able to replicate the makeup of the cell membrane [46]. That makes use of recombinant viruses; and non-viral delivery methods like polyplex, lipoplex, lipopolyplex, and lipid nanoparticles [47].

6.4. Polymer-Based Nanodelivery Systems

Results in thin polymer membranes enclosing aqueous or oily cores [48]. Polymer- and peptide-based systems are examples of numerous advancements in delivery methodologies that do not rely on lipid-based approaches [49]. Polyplex was first employed to transport nucleic acids using the polyethylenimine (PEI) polymer. However, because of its high surface charge density and toxicity profile, its use was restricted. Alternative techniques have been investigated by researchers, such as attaching the low molecular weight PEI to the cyclodextrin by adding polyethyleneglycol (PEG). Conjugation with cyclodextrin has been shown to be a safer and more successful method of delivering mRNA, and it can be given by a variety of ways, potentially producing various antibodies. Poly (amino esters) and other novel biodegradable polymers have shown promise in the delivery of mRNA to the lungs [24]. The resulting mRNA platform significantly reduced tumor growth and efficiently activated DCs and cytotoxic T cells. The hydrophobic agonist of TLR7/8 (Resiquimod, R848) has also been utilized extensively as a pulsation adjuvant in mRNA vaccines after being encapsulated in graphene oxide (GO) or polymer nanoparticles or modified with palmitic acid [25]. Although RNA nanoparticles are obviously superior to other nanoparticle systems in many ways, their development as medications is still lagging behind that of liposome and polymer systems [50]. Substances that exhibit buffering abilities in the pH range of 5.0 to 7.2, such as polymers containing imidazole [51]. PDP groups were transformed into SH groups by DTT treatment

prior to polymeric formation [52]. Adding credence to the potential of RNA delivery for cancer treatment aided by polymeric nanoparticles [53].

7. Conclusion

mRNA-based therapies have shown significant promise in treating various diseases, and recent advancements in mRNA engineering technologies and delivery systems have improved their efficacy and safety. LNPs have emerged as a leading delivery system for mRNA, offering improved stability, biocompatibility, and targeting capabilities. However, further research is needed to overcome the challenges associated with mRNA delivery, including instability, immunogenicity, and off-target effects. The development of novel delivery systems, such as polymer-based nanoparticles and microfluidics, may offer improved solutions for mRNA delivery. Additionally, the use of ligand-mediated targeting and ligand-functionalized LNPs may enhance the specificity and efficacy of mRNA-based therapies. Overall, mRNA-based therapies hold significant potential for the treatment of various diseases, and ongoing research and development are expected to further improve their efficacy and safety.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Uchida S, Perche F, Pichon C, Cabral H. Nanomedicine-Based Approaches for mRNA Delivery. Mol Pharm. 2020;17(10):3654-3684. doi:10.1021/acs.molpharmaceut.0c00618

[2] Li DF, Liu QS, Yang MF. Nanomaterials for mRNA-based therapeutics: Challenges and opportunities. Bioeng Transl Med. 2023;8(3):e10492. doi:10.1002/btm2.10492

[3] Karim ME, Haque ST, Al-Busaidi H. Scope and challenges of nanoparticle-based mRNA delivery in cancer treatment. Arch Pharm Res. 2022;45(12):865-893. doi:10.1007/s12272-022-01418-x

[4] Kubiatowicz LJ, Mohapatra A, Krishnan N, Fang RH, Zhang L. mRNA nanomedicine: Design and recent applications. Exploration (Beijing). 2022; 2(6):20210217. doi:10.1002/EXP.20210217

[5] Xiong Q, Lee GY, Ding J, Li W, Shi J. Biomedical applications of mRNA nanomedicine. Nano Res. 2018;11(10):5281-5309. doi:10.1007/s12274-018-2146-1

[6] Gómez-Aguado I, Rodríguez-Castejón J, Vicente-Pascual M, Rodríguez-Gascón A, Solinís MÁ, Del Pozo-Rodríguez A. Nanomedicines to Deliver mRNA: State of the Art and Future Perspectives. Nanomaterials (Basel). 2020;10(2):364. doi:10.3390/nano10020364

[7] Yuan M, Han Z, Liang Y, et al. mRNA nanodelivery systems: targeting strategies and administration routes. Biomater Res. 2023;27(1):90. doi:10.1186/s40824-023-00425-3

[8] Islam MA, Reesor EK, Xu Y, Zope HR, Zetter BR, Shi J. Biomaterials for mRNA delivery. Biomater Sci. 2015;3(12):1519-1533. doi:10.1039/c5bm00198f

[9] Wu Z, Li T. Nanoparticle-Mediated Cytoplasmic Delivery of Messenger RNA Vaccines: Challenges and Future Perspectives. Pharm Res. 2021;38(3):473-478. doi:10.1007/s11095-021-03015-x

[10] Byun MJ, Lim J, Kim SN. Advances in Nanoparticles for Effective Delivery of RNA Therapeutics. Biochip J. 2022;16(2):128-145. doi:10.1007/s13206-022-00052-5

[11] Qin S, Tang X, Chen Y. mRNA-based therapeutics: powerful and versatile tools to combat diseases. Signal Transduct Target Ther. 2022;7(1):166. doi:10.1038/s41392-022-01007-w

[12] Conde J, Langer R, Rueff J. mRNA therapy at the convergence of genetics and nanomedicine. Nat Nanotechnol. 2023;18(6):537-540. doi:10.1038/s41565-023-01347-w

[13] Hajj, Khalid A. and Kathryn A. Whitehead. Tools for translation: non-viral materials for therapeutic mRNA delivery. Nature Reviews Materials. 2017; 2: 17056. https://ui.adsabs.harvard.edu/abs/2017NatRM...217056H

[14] Xu X, Xia T. Recent Advances in Site-Specific Lipid Nanoparticles for mRNA Delivery. ACS Nanosci Au. 2023;3(3):192-203. doi:10.1021/acsnanoscienceau.2c00062

[15] Delehedde C, Even L, Midoux P, Pichon C, Perche F. Intracellular Routing and Recognition of Lipid-Based mRNA Nanoparticles. Pharmaceutics. 2021;13(7):945. doi:10.3390/pharmaceutics13070945

[16] Guo P, Haque F, Hallahan B, Reif R, Li H. Uniqueness, advantages, challenges, solutions, and perspectives in therapeutics applying RNA nanotechnology. Nucleic Acid Ther. 2012;22(4):226-245. doi:10.1089/nat.2012.0350

[17] Moraes FC, Pichon C, Letourneur D, Chaubet F. mRNA Delivery by Nanosystems: State of the Art and Perspectives. Pharmaceutics. 2021;13(11):1901. doi:10.3390/pharmaceutics13111901

[18] Yang W, Mixich L, Boonstra E, Cabral H. Polymer-Based mRNA Delivery Strategies for Advanced Therapies. Adv Healthc Mater. 2023;12(15):e2202688. doi:10.1002/adhm.202202688

[19] Yang L, Gong L, Wang P. Recent Advances in Lipid Nanoparticles for Delivery of mRNA. Pharmaceutics. 2022;14(12):2682. doi:10.3390/pharmaceutics14122682

[20] Mansur, Ahmad Rois. Determination of ethanol in foods and beverages by magnetic stirring-assisted aqueous extraction coupled with GC-FID: A validated method for halal verification. Food chemistry. 2021;366: 130526. https://doi.org/10.1016/j.foodchem.2021.130526

[21] Alzeer J, Abou Hadeed, K. Ethanol and its Halal status in food industries. Trends in Food Science & Technology. 2016; 58: 14-20. doi: 10.1016/j.tifs.2016.10.018

[22] Lacorn M, Hebler-Noll M, Wehling P, Hektor T. Determination of Ethanol in Food by Enzymatic Method Enzymatic Liquid Ethanol, Collaborative Study: Final Action 2017.07. J AOAC Int. 2023;106(2):341-347. doi:10.1093/jaoacint/qsac145

[23] Elia, Vittorio. The role of ethanol in extremely diluted solutions. Journal of Thermal Analysis and Calorimetry. 2014;116: 477-483.

[24] Fayez Al N, Nassar MS, Alshehri AA. Recent Advancement in mRNA Vaccine Development and Applications. Pharmaceutics. 2023;15(7):1972. doi:10.3390/pharmaceutics15071972

[25] Phua KK, Nair SK, Leong KW. Messenger RNA (mRNA) nanoparticle tumour vaccination. Nanoscale. 2014;6(14):7715-7729. doi:10.1039/c4nr01346h

[26] Khurana A, Allawadhi P, Khurana I. Role of nanotechnology behind the success of mRNA vaccines for COVID-19. Nano Today. 2021; 38:101142. doi:10.1016/j.nantod.2021.101142

[27] Semple SC, Leone R, Barbosa CJ, Tam YK, Lin PJC. Lipid Nanoparticle Delivery Systems to Enable mRNA-Based Therapeutics. Pharmaceutics. 2022;14(2):398. doi:10.3390/pharmaceutics14020398

[28] Holjencin C, Jakymiw A. MicroRNAs and Their Big Therapeutic Impacts: Delivery Strategies for Cancer Intervention. Cells. 2022;11(15):2332. doi:10.3390/cells11152332

[29] Alfagih IM, Aldosari B, AlQuadeib B, Almurshedi A, Alfagih MM. Nanoparticles as Adjuvants and Nanodelivery Systems for mRNA-Based Vaccines. Pharmaceutics. 2020;13(1):45. doi:10.3390/pharmaceutics13010045

[30] Kon E, Ad-El N, Hazan-Halevy I, Stotsky-Oterin L, Peer D. Targeting cancer with mRNA-lipid nanoparticles: key considerations and future prospects. Nat Rev Clin Oncol. 2023; 20(11):739-754. doi:10.1038/s41571-023-00811-9

[31] Mendes BB, Conniot J, Avital A. Nanodelivery of nucleic acids. Nat Rev Methods Primers. 2022; 2:24. doi:10.1038/s43586-022-00104-y

[32] Wadhwa A, Aljabbari A, Lokras A, Foged C, Thakur A. Opportunities and Challenges in the Delivery of mRNA-based Vaccines. Pharmaceutics. 2020;12(2):102. doi:10.3390/pharmaceutics12020102

[33] Xiao Y, Tang Z, Huang X. Emerging mRNA technologies: delivery strategies and biomedical applications. Chem Soc Rev. 2022;51(10):3828-3845. doi:10.1039/d1cs00617g

[34] Jiao L, Sun Z, Sun Z, Liu J, Deng G, Wang X. Nanotechnology-based non-viral vectors for gene delivery in cardiovascular diseases. Front Bioeng Biotechnol. 2024; 12:1349077. doi:10.3389/fbioe.2024.1349077

[35] Wang Y, Zhang R, Tang L, Yang L. Nonviral Delivery Systems of mRNA Vaccines for Cancer Gene Therapy. Pharmaceutics. 2022;14(3):512. doi:10.3390/pharmaceutics14030512

[36] Lundstrom K. Latest development on RNA-based drugs and vaccines. Future Sci OA. 2018; 4(5): FSO300. doi:10.4155/fsoa-2017-0151

[37] Simone Berger, Ulrich Lachelt, and Ernst Wagner. Dynamic carriers for therapeutic RNA delivery. PNAS 2024;121(11):e2307799120.

[38] Meng Z, O'Keeffe-Ahern J, Lyu J, Pierucci L, Zhou D, Wang W. A new developing class of gene delivery: messenger RNA-based therapeutics. Biomater Sci. 2017;5(12):2381-2392. doi:10.1039/c7bm00712d

[39] Xinyu Z, Yuanfang L,and Zehua Z.Lipid Nanoparticle-Based Delivery System A Competing Place for mRNA Vaccines.ACS Omega 2024;9[6]:6219−6234

[40] Xu Y, Golubovic A, Xu S, Pan A, Li B. Rational design and combinatorial chemistry of ionizable lipids for RNA delivery. J Mater Chem B. 2023; 11(28):6527-6539. doi:10.1039/d3tb00649b

[41] Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021; 6(12):1078-1094. doi:10.1038/s41578-021-00358-0

[42] Sun H, Zhang Y, Wang G, Yang W, Xu Y. mRNA-Based Therapeutics in Cancer Treatment. Pharmaceutics. 2023; 15(2):622. doi:10.3390/pharmaceutics15020622

[43] Zhou F, Huang L, Li S. From structural design to delivery: mRNA therapeutics for cancer immunotherapy. Exploration (Beijing). 2023;4(2):20210146. doi:10.1002/EXP.20210146

[44] Zak MM, Zangi L. Lipid Nanoparticles for Organ-Specific mRNA Therapeutic Delivery. Pharmaceutics 2021;(13): 1675.

[45] Peer D, Lieberman J. Special delivery: targeted therapy with small RNAs. Gene Ther. 2011;18(12):1127-1133. doi:10.1038/gt.2011.56

[46] Huang P, Jiang L, Pan H. An Integrated Polymeric mRNA Vaccine without Inflammation Side Effects for Cellular Immunity Mediated Cancer Therapy. Adv Mater. 2023; 35(3):e2207471. doi:10.1002/adma.202207471

[47] Baptista B, Carapito R, Laroui N, Pichon C, Sousa F. mRNA, a Revolution in Biomedicine. Pharmaceutics. 2021;13(12):2090. doi:10.3390/pharmaceutics13122090

[48] Fonseca-Santos B, Gremião MP, Chorilli M. Nanotechnology-based drug delivery systems for the treatment of Alzheimer's disease. Int J Nanomedicine. 2015; 10:4981-5003. doi:10.2147/IJN.S87148

[49] Adams BD, Parsons C, Walker L, Zhang WC, Slack FJ. Targeting noncoding RNAs in disease. J Clin Invest. 2017;127(3):761-771. doi:10.1172/JCI84424

[50] Jasinski D, Haque F, Binzel DW, Guo P. Advancement of the Emerging Field of RNA Nanotechnology. ACS Nano. 2017; 11(2):1142-1164. doi:10.1021/acsnano.6b05737

[51] Lorenz C, Fotin-Mleczek M, Roth G. Protein expression from exogenous mRNA: uptake by receptor-mediated endocytosis and trafficking via the lysosomal pathway. RNA Biol. 2011; 8(4):627-636. doi:10.4161/rna.8.4.15394

[52] Chen Q, Qi R, Chen X. A Targeted and Stable Polymeric Nanoformulation Enhances Systemic Delivery of mRNA to Tumors. Mol Ther. 2017; 25(1):92-101. doi:10.1016/j.ymthe.2016.10.006

[53] Ferdows BE, Patel DN, Chen W, Huang X, Kong N, Tao W. RNA cancer nanomedicine: nanotechnology-mediated RNA therapy. Nanoscale. 2022; 14(12):4448-4455. doi:10.1039/d1nr06991h