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ADVANCEMENTS IN NANOPARTICLE-BASED RNA THERAPEUTICS AND RNA DELIVERY SYSTEMS

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Indeed, because of their potential to overcome some of the limitations of synthetic polymer-based nanoparticles, the usage of RNA-based nanoparticles has attracted substantial interest in recent years. Because RNA is a naturally occurring molecule that can be easily synthesized and manipulated to attain certain features, it is an appealing platform for the generation of nanoparticles. Drug delivery, gene therapy, and imaging have all been investigated using RNA-based nanoparticles. One of its advantages is RNA-based nanoparticles' biocompatibility, making them less likely to produce toxicity or unfavorable effects in biological systems. Furthermore, RNA-based nanoparticles can be made to be very selective, allowing therapeutic medicines to be delivered to specific cells or tissues. This selectivity is achieved by creating RNA molecules that recognize and bind to specific targets such as cancer cells or virus particles. Because RNA-based nanoparticles are biodegradable and do not persist in the environment, they have the potential to be more environmentally friendly than synthetic polymer-based nanoparticles. As a result, the usage of RNA-based nanoparticles may lessen the potential for negative effects on aquatic and other habitats. Overall, the development of RNA-based nanoparticles has opened up new possibilities for medicinal delivery and other applications. While there are still obstacles to overcome, such as optimizing their stability and efficacy, the potential benefits of these nanoparticles make them an intriguing area of research in nanotechnology.

Keywords: *RNA-based nanoparticles, drug delivery, gene therapy, drug development*

INTRODUCTION

RNA, or Ribonucleic acid, is essential in many biological activities. It is divided into two types: coding RNA, which is responsible for protein translation, and non-coding RNA, which controls biological processes. Non-coding RNA is further subdivided into housekeeping ncRNAs like transfer RNA and ribosomal RNA and regulatory (non-coding RNA) ncRNAs like small interfering RNA and microRNA. Natural RNAs can cause disease when they malfunction or become mis-regulated, making them interesting therapeutic targets[1].

For disease treatment, oligonucleotides like patisiran Small interfering RNA (siRNA) and small compounds like risdiplam can target RNA. Artificial RNAs, in addition to natural RNAs, have been produced for medicinal purposes[2].

With the approval of various RNA medications, including antisense oligonucleotide, siRNA, and mRNA vaccination, RNA drugs have become a key milestone in drug development. Along with small-molecule medications and protein-based therapeutics, they have become the third pillar in drug development[3].

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RNA is a polymer made up of four nucleotides that contain four nitrogenous bases: adenine (A), guanine (G), cytosine (C), and uracil (U). These bases in RNA can form various secondary and tertiary structures because of the canonical Watson-Crick base pairs (A-U, G-C) and non-canonical base pairs (such as G-U). This diversity in base pairing enables RNA molecules to form a broad range of secondary and tertiary structures[4].

Watson-Crick base-pairing determines the secondary structure of RNA and can be predicted using nearest neighbor parameters. The tertiary structure, on the other hand, contains long-range connections between secondary motifs, whilst the quaternary structure refers to higher-order designs generated by RNA-RNA interactions or RNA interactions with other molecules such as proteins. For RNA's functional flexibility, its exact structure and dynamics are critical. In solution, RNA molecules can assume a variety of conformations, and their distribution can shift in response to environmental cues[5].

RNA molecules, including siRNA (small interfering RNA), miRNA (microRNA), and mRNA (messenger RNA), have gained significant attention as potential therapeutic agents due to their ability to modulate gene expression and treat various diseases. Here's a brief about these molecules and their therapeutic applications:

siRNA (small interfering RNA)

siRNA is a double-stranded RNA molecule typically 20-25 nucleotides in length. It can specifically target and degrade complementary messenger RNA (mRNA) molecules, effectively silencing the expression of the corresponding gene. siRNA exerts its therapeutic effect by downregulating the production of disease-causing proteins. siRNA-based therapies have shown promise in the treatment of various diseases, including viral infections, cancer, and genetic disorders. For example, in viral infections, siRNA can be designed to target viral genes, inhibiting viral replication. In cancer, siRNA can be used to silence oncogenes or target specific genes involved in tumor growth, leading to tumor regression. Additionally, siRNA has the potential to treat genetic disorders by targeting disease-causing mutations or correcting abnormal gene expression[6].

miRNA (microRNA)

miRNAs are small, single-stranded RNA molecules approximately 22 nucleotides in length. They play a crucial role in post-transcriptional gene regulation by binding to specific mRNA molecules, resulting in their degradation or translational repression. miRNAs are involved in the regulation of numerous cellular processes, including development, differentiation, and disease pathways. miRNA-based therapeutics aim to exploit the ability of miRNAs to modulate gene expression. By targeting specific miRNAs, it is possible to restore their normal expression levels or inhibit the overexpression of disease-associated miRNAs. miRNA-based therapies have shown potential in various diseases, including cancer, cardiovascular disorders, neurodegenerative diseases, and viral infections[7].

mRNA (messenger RNA)

mRNA is a single-stranded RNA molecule that carries genetic information from DNA to the ribosomes, where it serves as a template for protein synthesis. In recent years, mRNA-based therapeutics have emerged as a breakthrough technology, particularly with the development of mRNA vaccines against COVID-19. mRNA therapies involve the direct administration of modified mRNA molecules encoding therapeutic proteins or antigens. Once inside the target cells, the mRNA is translated into the corresponding protein, leading to the desired therapeutic effect. mRNA-based approaches have shown promise in various areas, including protein replacement therapy, cancer immunotherapy, and regenerative medicine[8].

RNA dynamics are the movements that occur across a wide range of timescales, such as secondary structure transitions, changes in tertiary interactions, and minute "jittering" motions. These dynamics are intimately related to the biological functions of RNA, including as catalysis in ribozymes, translation in the ribosome, and gene control in riboswitches[9].

Stability of RNA molecules

Indeed, one of the major challenges in utilizing RNA molecules as therapeutic agents

is their inherent instability in plasma or biological fluids. RNA is susceptible to degradation by various nucleases present in these environments, which limits their therapeutic potential. To overcome this challenge, chemical modifications of RNA molecules are often employed to enhance their stability. Here's a detailed explanation of the need for chemical modifications and their impact on RNA stability[10].

Enhancing stability

Unmodified RNA molecules are rapidly degraded by endogenous nucleases, resulting in a short half-life. To prolong their stability and therapeutic efficacy, chemical modifications are introduced to protect RNA molecules from enzymatic degradation. These modifications can enhance resistance to nucleases and improve RNA stability in biological fluids.

Types of chemical modifications

Various chemical modifications can be incorporated into RNA molecules to enhance stability. Some commonly employed modifications include:

a) 2'-O-methyl (2'-OMe) modification: This modification involves the addition of a methyl group to the 2'-carbon of the ribose sugar. It enhances RNA stability by reducing susceptibility to nuclease-mediated degradation[11] .
b) Phosphorothioate (PS) modification: In this modification, one of the non-bridging oxygen atoms in the phosphate backbone is replaced with a sulfur atom. Phosphorothioate modification improves RNA stability by rendering it resistant to nuclease cleavage.
c) Locked nucleic acid (LNA) modification: LNA modification involves the introduction of a bridging oxygen atom between the 2'-carbon and 4'-carbon, effectively "locking" the ribose in a conformation that enhances RNA stability and binding affinity.
d) 5-methylcytidine (m5C) modification: This modification involves the addition of a methyl group to the 5-carbon of the cytidine base. m5C modification enhances RNA stability and can also improve translation efficiency[11] .

Impact on RNA stability

Chemical modifications significantly improve the stability of RNA molecules, allowing them to persist in plasma or biological fluids for longer periods. These modifications can protect RNA from enzymatic degradation and maintain their therapeutic efficacy. By increasing stability, modified RNA molecules have a better chance of reaching target cells or tissues and exerting their intended therapeutic effects. Furthermore, chemical modifications can also enhance other desirable properties of RNA molecules, such as improved target specificity, reduced off-target effects, and enhanced cellular uptake[11].

RNA nanotechnology is a branch of research that focuses on nanoparticles made primarily of RNA[12]. This field is concerned with the creation of RNA nanoparticles of varied complexity and the investigation of their biological uses[13]. RNA nanoparticles can be made using naturally occurring RNA motifs or from scratch using computer modelling. RNA nanoparticles can be created using a variety of techniques, including one-pot assembly, rolling circle transcription (RCT), and RNA origami[14].

Because of their deformability and small size (10-40 nm), RNA is useful for avoiding nonspecific diffusion and access into organs, allowing them to permeate leaky blood arteries and aggregate at tumor sites while bypassing renal filtration and decreasing toxicity[15]. This advantageous biodistribution of RNA nanoparticles can be improved further by adding ligands or aptamers for active cancer-targeting[16].

Aside from their deformability, RNA nanotechnology has a number of other advantages for therapeutic applications. RNA has a negative charge by nature, which decreases its interaction with negatively charged cell membranes without requiring the nanoparticles to be polymer-coated. This feature aids in the prevention of interactions with healthy organs. RNA nanoparticles may self-assemble with great stability from shorter oligonucleotides and are highly programmable in terms of form, size, stability, and stoichiometry[13].

Because RNA nanoparticles are highly hydrophilic, they can help increase the aqueous solubility of hydrophobic medicines. This property has the potential to remove the

requirement for poisonous formulations[17]. Because RNA is multivalent, it can be multi-functionalized and synthesized with a specific structure and stoichiometry. This feature is useful for attaining combination therapy, which can aid in the treatment of drug resistance [18].

RNA nanoparticles are frequently synthesized with targeting ligands, which can boost local nanoparticle concentrations and allow targeted cell entrance via receptor-mediated endocytosis[19]. RNA nanoparticles, when combined with extracellular vesicles, have the potential to improve the transport efficiency of therapeutic oligos such as siRNA by targeting delivery and avoiding endosome trapping[20].

For the treatment of various diseases, RNA nanoparticles have been used to deliver both small molecule medications, such as doxorubicin (DOX) and paclitaxel (PTX), and oligonucleotide therapeutics, such as siRNA and anti-miRNA (microRNA)[21]. Furthermore, the immunogenicity of RNA nanoparticles is determined by their size, shape, and sequence, allowing for a customizable immune response. Furthermore, the lack of proteins minimizes worries about antibody induction and allows for the repeated delivery of intravenous injections without toxicity[22]. RNA nanoparticles have shown promise in the treatment of cancers such as breast, prostate, and colorectal cancer, as well as other disorders such as those affecting the central nervous system (CNS) and the eye[4].

Definition of RNA nanotechnology

RNA nanotechnology is a recent field that differs from standard RNA biology. The study of the design, manufacturing, and application of RNA nanoparticles at the nanoscale scale that are largely constituted of RNA by bottom-up self-assembly is characterized as RNA nanotechnology[23]. Scaffolds, targeting ligands, regulatory moieties, and therapeutic modules can all be found in the primary framework of RNA nanoparticles. In contrast to traditional RNA research, which focuses on intra-RNA interactions and 2D/3D structure-function correlations, this discipline focuses on inter-RNA interactions and quaternary interactions of tiny RNA motifs. Nonetheless, the breadth of knowledge garnered from traditional RNA biology research has provided a good foundation for RNA nanotechnology. Since the notion of RNA nanoarchitectures was introduced in 1996 and 1998 by RNA tectonics and reengineered pRNA (packaging RNA) molecules, respectively[24]. Over the last decade, RNA nanotechnology has expanded quickly as a platform with considerable applicational investigations in nanomedicine [25].

Techniques for constructing RNA nanoparticles

Nanoparticles require programmable, addressable, and predictable building blocks. RNA building blocks can self-assemble to generate bigger two-, three-, and four-dimensional (2, 3, 4D) structures, making it a popular bottom-up technique. This strategy is critical for properly integrating biological procedures and biomacromolecules into nanotechnology[26].

(1)Construction of RNA Nanoparticle via Hand−Hand Interactions.

The design principles are illustrated by the structural characteristics of pRNA from bacteriophage phi29[27]. pRNA can form dimers, trimers, tetramers, pentamers, hexamers, and heptamers using "hand-in-hand" interactions through interlocking loops[28].

(2)Construction of RNA Nanoparticle via Foot-to-Foot

pRNA molecules are linked together through single-stranded palindrome sequences that facilitate self-assembly via "foot-to-foot" interactions[29].

(3)Rational Design Utilizing Stable Natural RNA Motifs

RNA nanoparticles can be constructed using motifs such as kissing loops, dovetails, pseudoknots, kink turns, and multiway junctions[30].

(4)Construction of RNA Nanoparticle via Extension of Robust

Motif pRNA-3WJ. The 3WJ's robustness causes the folding of pRNA modules connected to each vertex[31].

(5)Construction of RNA Nanoparticle via Tectonics

The expanding understanding of RNA folding and the accessibility of databases, such

as the nucleic acid database (NAD) and the RNA junction database, have strengthened the potential of tectonics. With a vast number of motifs available, there are numerous potential tectoRNAs that can be utilized. Examples of these motifs include tectoRNA squares and cubes, which are designed from Transfer RNA (tRNA), and nanoprisms, which are designed from pRNA[32].

(6)Construction of RNA Nanoparticles via Computational Design

Several software programs have been developed over the years, such as NanoTiler, Assemble2, RNA2D3D, INFO-RNA, and NUPACK, which are useful to design de novo RNA nanoparticles. Examples are a six membered RNA nanoring and an RNA nanocube generated using Nanotiler. Long sequences as well as individual monomer units that contain internal structures can be computationally designed and experimentally assembled in a one-pot manner including co-transcriptional assembly[12].

Advantages of using RNA nanoparticles for pharmaceutical applications

Scientists throughout the world are becoming more interested in RNA nanotechnology, which holds enormous promise for therapies, especially after overcoming substantial obstacles in the field. Although the use of RNA for treatments is still in its early phases, it is clear that RNA nanotechnology has a number of advantages.

1. One advantage is that RNA nanoparticles are nano-sized and have a branching, ratchet form, allowing for passive tumor targeting and penetration of tiny cavities, allowing for enhanced permeability and retention (EPR) effects, and possesses favorable biodistribution[33].
2. RNA nanoparticles with controlled size, structure, and stoichiometry can be created.
3. The polyanionic structure of RNA prevents it from crossing negatively charged cell membranes inadvertently[34].
4. Under typical physiological conditions, RNA nanoparticles are very water-soluble and do not clump[35].
5. Because they are made of "polynucleic acid," a biocompatible substance, RNA nanoparticles are less immunogenic than other heterogeneous nanoparticles. Unpredictable adverse effects caused by heterogeneous particles are thus avoided. Because minimal stimulation of antibody formation is required, chronic disorders can be treated repeatedly[36].
6. Because RNA nanoparticles are multivalent, they can be conjugated with targeting, therapeutic, and imaging modules all in one nano construct to achieve synergistic or increased effects without crosslinking[31].

Challenges and perspectives

Certain problems must be overcome in order to employ RNA nanotechnology in medical and nanotechnological sectors. The first challenge is understanding the correct overall folding of RNA constructs in order to ensure the efficiency of the RNA nanoparticles created. As a result, computational approaches for analyzing RNA folding and structure are critical for evaluating innovative structural concepts[37].

Despite the proliferation of online resources, predicting RNA folding or structure for nanoparticle assembly remains a considerable challenge. This is due to the assembly involving tertiary/quaternary interactions, non-standard base pairing, and inter-molecular contacts, all of which necessitate more complex RNA folding tools. Furthermore, the laws governing RNA nanoparticle assembly are still unknown. Previously, computational approaches for RNA folding focused exclusively on 2D structure, and current RNA 2D prediction programs can only reliably predict about 70% of the folding[38].

The evolution of RNA nanotechnology necessitates the development of algorithms capable of forecasting 3D and 4D structures, which are currently unknown. Although there has been some positive success in this subject, it is still in its early phases and requires additional joint efforts[39].

The second barrier is assuring therapeutic RNA nanoparticle stability. While problems

about RNA stability in serum and in vivo have been addressed, challenges with metabolism and clearance suggest that the most stable RNA may not be the most effective therapeutic option in vivo. It is critical to have an ideal retention time in the pharmaceutical industry. To overcome this obstacle, the concentration and location of chemically modified stable nucleotides inserted into the RNA may need to be changed[40].

The third challenge involved in the ligand-mediated endocytosis for targeted delivery of siRNA is the issue of endosome escape. To address this challenge, RNA nanoparticles can leverage their multivalent properties to deliver therapeutic molecules in a complex with agents that facilitate endosome disruption and trigger the release of the therapeutic molecules from the endosome. Various methods can be employed to assist with endosome escape, including the use of acid-responsive chemical functional groups such as acid-cleavable linkers like acetal, hydrazone, and maleic amides, or acid protonating groups such as amino esters, imidazole, and sulfonamide[41].

Finally, despite great attempts to address the obstacles associated with the low yield and high cost of large-scale RNA production, there are still issues to be addressed. While the bacterial fermentation approach for industrial-scale RNA synthesis shows promise, its use of the tRNA scaffold, including its promoter, for expressing neatly folded RNA motifs is currently limited. More research is needed to improve the in vivo RNA expression vector and to develop host cells that are resistant to exonucleases and endonucleases or that can absorb modified nucleotides to generate RNase-resistant RNAs. However, given the history of quickly dropping DNA synthesis costs, it is projected that the cost of RNA synthesis will gradually reduce as industrial-scale RNA manufacturing technologies develop[42].

Advancement in RNA Delivery Systems

Lipid Nanoparticle

The utilization of nano-scale carriers as an effective means of intracellular transportation has gained significant interest. These tiny particles have the ability to enclose and safeguard their cargo against harsh intracellular conditions, thus preventing degradation. Additionally, chemical modifications can be made to the nanoparticles, enabling them to facilitate the intracellular approach, including cellular uptake, endosomal escape, and localization. Historically, lipid and lipid-like substances have been considered cationic under physiological circumstances, particularly for delivering nucleic acids[43]. The proton sponge effect is regarded as a fundamental idea that may aid in endosomal escape, while its validity is debatable[44].

This behavior can be interpreted as the result of lipoplex components driving buffering capacity. H-ATPase acidifies the aspH state during endosomal maturation. Cationic lipid components are protonated during activity to induce excess H^+ and Cl^- inflow and water molecules inside the endosome. The overall osmotic pressure causes endosomal enlargement or rupture, allowing the laden cargo (RNAs) to be released into the cytoplasm. It could also be used to illustrate the polymer-based intracellular delivery principle. There have also been various attempts to create modular LNPs that are exclusively cationic in acidic circumstances. The nanoparticle's unique behaviour can be accomplished by administering ionizable lipid molecules with sufficiently low pKa such that the lipid is not protonated[45.] Because of their ionic modulatory characteristics, the nanoparticles are thought to improve intracellular distribution and promote endosomal escape[46]. Many lipid-like nanocarriers, also known as lipidoids, have been created and tested for their capacity to transport RNA. Comparisons between their delivery efficacy and that of existing lipofection agents were regularly done[47].

Several lipid-like nanocarriers, or lipidoids, have been produced and tested for their ability to carry RNA. Furthermore, numerous biochemical functionalization have been investigated to improve the efficacy of lipid nanoparticles (LNPs). One such strategy was the introduction of a disulfide link, which allowed lipid structures to be cleaved and broken down within the reducing environment of the cell cytoplasm. The addition of bio-reducible moieties enhanced the intracellular breakdown of particle architectures, resulting in very effective siRNA transport to cultivated cells[48].

Significant progress has been achieved in the utilization of LNPs for in vivo RNA delivery, and several ways to improve their performance have been investigated. Exosomes, which are naturally occurring nanovesicles generated from cells and range in size from 30 to 150 nm, are one such strategy. Exosomes are recognized to play a role in intercellular communication, and various attempts to use them as an intracellular delivery method have proven effective. Various efforts have also been made to alter exosomes, if necessary, for use in intracellular gene delivery under cellular circumstances[49].

Exosomes have also been used in a number of in vivo experiments, most notably for transporting drugs to the brain over the blood-brain barrier (BBB). Exosomes were obtained from certain host cells and manipulated chemically or biologically as needed in these investigations. One modification method for targeting the brain is to combine exosomes with neural-specific peptide moieties[50]. These altered exosomes have the potential to treat a variety of neuronal or neurodegenerative illnesses, including Parkinson's disease[51].

Several difficulties remain unanswered despite the numerous applications of LNPs. One of the most serious concerns is the poor colloidal stability of liposomes. Liposomes have a tendency to fuse with one another when stored for a lengthy period of time, resulting in changes to their original structure such as enlargement or breaking, which can induce molecular leakage and diminish delivery efficiency. Furthermore, the lipid moieties are vulnerable to oxidative processes, which might affect the liposome bilayer's permeability. Other obstacles include poor batch-to-batch reproducibility due to heterogeneous size distribution, relatively low loading capacity, and mass production difficulties. Furthermore, to avoid microbiological contamination, the final sterilization technique must be carefully studied[52].

Polymeric Nanoparticle

Cationic polymers have been intensively researched as potential RNA delivery vehicles. One of the aspects that makes them ideal for use in intracellular delivery systems is their chemical flexibility, which allows for diverse functionalization[53].

The adsorption of RNA molecules onto polymer nanoparticles is primarily driven by electrostatic contact. Because of the positively charged composition of the nanoparticle and the negatively charged nucleic acid, such condensation is expected. Amine functional groups are often used to add cationic characteristics to polymers. The inclusion of amine functional groups improves RNA loading onto nanoparticles and influences the intracellular behavior of the nanoparticle complex following uptake[54].

Natural and manmade polymers have been employed to provide efficient intracellular RNA delivery carriers in a variety of applications. Chitosan nanoparticles, a type of natural polymer, have been used as delivery vehicles for self-amplifying replicon RNA to induce high antigen load in dendritic cells[55].

This nanocomplex was shown to trigger influenza virus hemagglutinin translation within dendritic cells, culminating in the development of an immunological response. The use of synthetic polymers, particularly those with cationic characteristics, for intracellular RNA transport has also been investigated. Polypeptide-derived nanoparticles, such as poly-L-lysine, have been studied for their ability and effectiveness in RNA transport. A wide range of polymers with amine-functional groups have been investigated for their ability to transport RNA therapeutically. Polyethyleneimine (PEI) nanoparticles have been employed widely in RNA delivery systems for a long time, ranging from the usage of PEI nanoparticles alone to the production of hybrid nanoparticles with unique properties derived from a polymer cocktail[56].

Poly (-amino ester) (PBAE) has been found as another possible intracellular RNA delivery carrier in addition to PEI. This is owing to its electrostatic interaction with RNA and bio-reducibility[57].

Polyamidoamine (PAA) has also piqued the interest of researchers due to its capacity to undergo a variety of chemical functionalization on the polymer backbone. Because of this property, PAA has been used as a nanocarrier for RNA with various modifications, including

the addition of disulfide groups for the delivery of short hairpin RNA[58].

Poly (glycoamidoamine) (PGAA) has been employed in a similar way for siRNA delivery, with branching functionalization using PGAA brush nanoparticles to improve in vivo siRNA distribution. Poly (L-glycolic acid) (PLGA) is another polymer that has been recommended for this purpose. PLGA, like other polymers, is a viable coating material that can impart a variety of beneficial qualities to aid in vivo delivery[59]. Dendrimers are distinct from other types of polymers due to their unique repeated branch-like topology. Their symmetrical and branching ionizable properties are thought to improve targeted RNA delivery to the lungs[60].

Several methodologies have been developed that focus on the response qualities of stimuli in order to improve structural stability or delivery efficiency. One example is the usage of pH-responsive polymer nanoparticles, which can take advantage of specific polymer properties. For example, poly(allylamine) (PAA) can be engineered to have high stability within a restricted pH range (from 7 to 9), allowing for rapid cargo release under certain pH circumstances (i.e., pH below 7 or above 9). Furthermore, with the co-delivery of two siRNAs and an anticancer medication after intravenous injection, PAA has been demonstrated to have a considerable inhibitory effect on breast cancer metastasis. Numerous case studies have been carried out in order to uncover important characteristics that promote intracellular delivery[61].

It is significantly more difficult to achieve selective targeting of RNA to target endothelium cells without causing damage to other organs, such as the liver. A more complicated technique is necessary to overcome this issue. One method involves the hybridization of polymers with lipid molecules, which allows for the selective transport of RNA to the lungs[62]. Strategies incorporating hybrid polymer nanoparticles was also applied to avoid rapid clearance. were taken into account, including a specific chemical modification[63].

Silica Nanoparticle

Silica nanoparticles have demonstrated numerous advantages for use as intracellular delivery vehicles, including reduced cytotoxicity. Its inertness in the presence of degradation-promoting agents such as bile salts or lipase under harsh physiological circumstances. Traditional research on silica nanoparticles concentrated on the function offered by its density and size, as an enhancer to enable intracellular gene delivery by enhancing penetration efficiency through the cellular barrier[64].

Recent research has revealed that positively charging the surface of solid silicon nanoparticles (SSNPs) can considerably improve their binding affinity with nucleic acid cargos while also protecting the loaded materials. With the right surface changes, silica nanoparticles have the potential to be intracellular delivery vehicles for a wide spectrum of cargos[65].

In recent years, substantial research emphasis has been focused on mesoporous silica nanoparticles (MSNs), which are porous silica particles. These particles have a huge surface area (about 800 m^2 g^1), consistent and customizable pore diameters, the ability to integrate diverse guest molecules, and low immunogenicity. As a result of their great potency and potential for a wide range of applications, MSNs have sparked tremendous attention[66]. In addition to facilitating intracellular delivery to numerous cell types, MSN may also carry relatively big macromolecules such as DNA, RNA, and proteins within its porous structure. Furthermore, research has revealed that MSNs can be engineered with precise surface chemistry, allowing the development of a stimulus-responsive system[67].

Numerous studies have established the applicability of MSN as biomolecule delivery vehicles. Given these benefits, researchers have investigated numerous techniques for exploiting MSNs to facilitate the delivery of siRNA, miRNA, and antisense-miRNA oligomers. To improve siRNA loading capacity and cellular uptake efficiency, one commonly utilized technique involves altering the surface of MSNs using a cationic polymer[68].

Surface functionalization is one example of this method, which allows for the loading of diverse cargos both on the particle surface and inside the pores. This method has been shown

to be effective against multidrug-resistant cancer cells[69].

Reversible stimuli-responsive polymer functionalization has also been achieved through the introduction of functional groups such as disulfide bonds, in addition to irreversible polymer functionalization. When siRNA-laden particles containing disulfide bonds are exposed to the intracellular reducing environment, the disulfide bonds rupture, allowing the loaded cargo to be released[70].

Although cationic modification is the most common method, other approaches of modifying the surface of MSNs have been tried, such as utilizing peptide nucleic acid (PNA), an artificial nucleic acid with a peptide backbone. When PNAs were placed on the MSN surface, they improved cell permeability and inhibited the PNA-targeted miRNA sequences[71].

MSN polymerization is not confined to cationic polymers. Polyethylene glycol (PEG), for example, can be used to disperse nanoparticles in aqueous solutions and improve RNA delivery efficiency. PEG modification has been mixed with a variety of cationic polymers or copolymers in a number of investigations[72].

Because of the biocompatibility and plasticity of silica's surface chemistry, it is possible to incorporate diverse core materials to form a core-shell shaped nanoparticle with unique functionality. Magnetic nanoparticles (MNP), for example, can be employed as a core material in order to make the final particle responsive to external magnetic stimuli [73].

By introducing diverse targeting ligand moieties, proper surface modification of MSNs can permit targeted intracellular delivery[74].

MSN pore sizes are typically around 2-5 nm, limiting the sorts of cargo that can be loaded to tiny molecules. To circumvent this constraint, a new synthetic approach for creating MSNs with ultra-large holes (about 23 nm) has been created. Through electrostatic contact, siRNA can be easily loaded into the ultra-pore structure due to amine functionalization creating a positively charged inner surface. The resulting particle combination is extremely stable and requires no additional polymeric modification or capping[75].

Furthermore, in a transgenic mouse model, MSNs with ultra-large holes were successfully employed for in vivo systemic distribution of siRNAs by intravenous injection to prevent and treat hepatocellular cancer[76].

MSNs have also been modified with functional peptides to enhance the delivery of double-stranded siRNA and single-stranded miRNA. Another silica nanoparticle-based delivery technique is the use of dual-pore silica nanoparticles (DpSN), which feature two types of pores with distinct sizes and surface functionalities. One of the big pores is utilized to store siRNA in a stable manner, while the other is used to encapsulate a chemical therapeutic molecule. These holes are capped with a stimuli-responsive linker, allowing the loaded contents to be released selectively only within the intracellular environment[77].

One of the general concerns with nanoparticle application in vivo is the risk of accumulating in the human body. However, multiple studies have demonstrated that siloxane exposed on the surface of MSNs can degrade in three processes under physiological conditions: hydration, hydrolysis, and ionic exchange. MSN biodegradability is affected by a variety of parameters including as size, shape, surface area, pore size, and surface functional group. It is expected that the range and effectiveness of MSNs as medication delivery vehicles in vivo will continue to expand in the near future.

Gold nanoparticles

Among the many candidates, gold nanoparticles (AuNPs) have been suggested as a potential RNA delivery carrier[78]. Surface functionalization of AuNPs is a straightforward procedure that relies on a strong sulfur-Au link. AuNPs have various unique features when modified with polyvalent nucleic acid, known as a spherical nucleic acid (SNA), including resistance to nuclease degradation and high cellular absorption efficiency[79].

Because of these features, SNA is a good candidate for use as an RNA therapeutic agent. A thiol group must be inserted into the RNA to associate siRNA with the AuNP core. AuNPs can also be given through the skin, implying that they could be used in gene therapy to treat cutaneous tumors, skin inflammation, and other skin-related disorders[80]. Furthermore, studies

have shown that covering AuNPs with polycationic polymers can improve siRNA transport into cells[81].

Incorporating SNA that mimics tumor-suppressor miRNA has been proven to inhibit cancer cell proliferation and migration. However, tweaking SNA to imitate oncogenic miRNA has the opposite effect and can stimulate cancer cell proliferation [82]. These findings imply that employing SNA instead than miRNA as a cancer therapeutic technique could be studied. Because of their features, such as the capacity to create heat, elicit cellular mechanotransduction, and enhance magnetic resonance imaging (MRI), MNPs, in addition to AuNPs, are promising candidates for building therapeutic and diagnostic systems[83].

FDA approved RNA-NP therapy

In the United States, drugs must receive FDA approval before they can be marketed and sold. Various factors are considered during regulatory review for FDA approval, including not only the physiochemical properties of RNA-NPs, such as charge, size, and reaction to environmental factors such as pH, salt concentration, and temperature, but also manufacturing processes and controls. RNA-NPs must be tested for stability, sensitivity, and purity/quality during synthesis and storage[84].

Clinical trials must be conducted to monitor the pharmacokinetics/ pharmacodynamics, dosage and dose frequency, administration route, immunogenicity, bioavailability, biodistribution, biodegradation, and elimination pathway of RNA-NPs, and the results must be submitted to the FDA for review. The drug's safety and efficacy may differ based on the RNA-NP's target illness. However, it is critical that the medication has a strong therapeutic benefit while causing minimum unfavourable side effects. Furthermore, the FDA must be informed on the environmental impact of RNA-NP, particularly its manufacturing method[84].

The small number of FDA-approved RNA-NPs can be attributed, at least in part, to the lengthy preclinical and clinical evaluations required before they can be approved for public use, as well as the variety in acceptable efficacy and safety characteristics. For example, in the event of uncommon disorders with no alternative therapy, the FDA may consider decreased drug efficacy acceptable. Onpattro® (patisiran), developed by Alnylam Pharmaceuticals Inc. for the treatment of polyneuropathy associated with autosomal dominant hereditary transthyretin amyloidosis (hATTR), is an example of an FDA-approved RNA-NP. This kind of amyloidosis is distinguished by aberrant amyloid protein deposition in the liver, resulting in decreased organ function. Onpattro® is a LPNs encapsulated siRNA that targets transthyretin (TTR), a protein generated by the liver[85]. SiRNA is a double-stranded, 20-25 nucleotide molecule that binds to and inhibits a specific mRNA. The specificity of siRNA provides therapeutic benefits by reducing non-target binding and the undesirable side effects that occur. This specialization, however, restricts its adaptability[86].

Onpattro® liposomes have a diameter of 60 to 100 nm and are made of DLin-MC3-DMA (MC3), cholesterol, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and PEG. Following intravenous (IV) treatment, Onpattro® NPs that bind to plasma proteins then taken up by hepatocytes through LDL receptors. The integration of pH-sensitive ionizable lipid MC3 is one of the primary aspects contributing to Onpattro®'s therapeutic success. The pKa of MC3 is around 6.5, which is the same as the pH of the late endosome/lysosome, allowing the liposome to fuse with the endosomal membrane and release therapeutic siRNA into the cytoplasm upon uptake[87].

Despite its capacity to decrease TTR expression by more than 80%, Onpattro® must be provided through intravenous infusion every three weeks, which limits the convenience and widespread use of this RNA-NP treatment. The two most recent RNA-NP therapies to gain approval/authorization and widespread attention are the RNA-NP vaccines developed by Pfizer-BioNTech and Moderna Therapeutics Inc. in 2020, which aim to prevent severe coronavirus disease 2019 (COVID-19) infection and hospitalization caused by SARS-CoV-2[88].

mRNA vaccines make use of mRNA, a single-stranded molecule that is complementary to a single DNA strand. This mRNA is rapidly

and transiently translated into a protein in the cytoplasm, and it is immediately degraded, frequently within minutes. This ensures that long-term protein expression and any side effects are kept to a minimum. mRNA vaccines induce a robust immune response that confers long-term protection against the targeted virus by transiently causing cells to express a specific antigen[89].

Moderna had previously developed cytomegalovirus (CMV) mRNA vaccines, but Pfizer's COVID-19 vaccine is the company's first mRNA-based vaccination. Both COVID-19 vaccines completed clinical trials from phase I through phase III in less than seven months and acquired FDA emergency use authorization (EUA) in 2020[90].

The Pfizer-BioNTech vaccine received full FDA approval in 2021. Furthermore, the EMA (European Medicines Agency) approved the Pfizer-BioNTech and Moderna vaccines for release in 2020 and 2021, respectively. The liposome formulations of both COVID-19 vaccines contain PEG and cholesterol, with Moderna using the ionizable lipid sphingomyelin-102 (SM-102)[91].

Despite constraints, three RNA-NPs have met the necessary conditions and acquired FDA approval for public use in the United States. An RNA-NP was approved by both the FDA and the EMA in 2018, making it the first RNA-NP to be approved by either organization.

The 4.3 kb long mRNA BNT162b2 is used in the Pfizer-BioNTech vaccine. This mRNA encodes the full-length prefusion SARS-CoV-2 membrane-bound spike protein, which is stabilized by two prolines in the C-terminal furin cleavage segment, which is responsible for proteolytic cleavage during viral protein assembly. These prolines both prevent and encode viral fusion machinery[92].

During phase III clinical trials, the antigen in the Pfizer-BioNTech vaccine was able to elicit antibody formation against SARS-CoV-2 as well as a robust Th-1 T-cell response, resulting in 94% effectiveness in avoiding infection. Similarly, the Moderna vaccine uses mRNA-1273, which encodes the transmembrane-anchored prefusion spike protein with a natural furin cleavage site stabilized by two prolines. The Moderna vaccine evoked a high Th-1 T-cell response and demonstrated 94.5% effectiveness in intermediate phase III trials. However, the Moderna vaccination caused more side effects than the Pfizer-BioNTech vaccine, including weariness (9.7% vs. 3.8%) and headaches (4.5% vs. 2%)[92]. While both COVID-19 vaccines are highly effective, one of their primary drawbacks is temperature instability, with both vaccines requiring storage at 20-(Moderna) or 80 -(Pfizer-BioNTech), a characteristic that is especially problematic when administering immunizations to underserved regions.

Conclusion

RNA nanotechnology is rapidly expanding, despite its late start compared to other nano-delivery technologies. This review highlights the numerous advantages of RNA nanoparticles in biomedicine. As drug carriers, RNA nanoparticles have demonstrated immunologically inert behavior and can be manipulated to exhibit controlled immunostimulant. RNA nanoparticles possess several advantageous physicochemical properties over other nanomaterials, including their precise programmability. Consequently, RNA nanoparticles can be rationally designed, optimized, and constructed for specialized in vivo applications. As a biocompatible nanomaterial, RNA nanoparticles exhibit favorable tumor targeting proficiency, which has been observed in various pre-clinical cancer models. Extensive research has been conducted to understand the safety, immunological, and pharmacological profiles of RNA nanoparticles, paving the way for clinical trials. The future of RNA nanotechnology in cancer therapy appears to be promising.

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التطورات في علاجات الحمض النووي الريبي (RNA) القائمة على الجسيمات النانوية وأنظمة توصيل الحمض النووي الريبي (RNA)

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في الواقع ، نظرًا لقدرتها على التغلب على بعض قيود الجسيمات النانوية الاصطناعية القائمة على البوليمر ، فقد جذب استخدام الجسيمات النانوية القائمة على الحمض النووي الريبي اهتمامًا كبيرًا في السنوات الأخيرة. نظرًا لأن الحمض النووي الريبي هو جزيء يحدث بشكل طبيعي ويمكن تصنيعه والتلاعب به بسهولة لتحقيق ميزات معينة ، فهو منصة جذابة لتوليد الجسيمات النانوية. تم التحقيق في توصيل الأدوية والعلاج الجيني والتصوير باستخدام الجسيمات النانوية القائمة على الحمض النووي الريبي. تتمثل إحدى مزاياها في التوافق الحيوي للجسيمات النانوية القائمة على الحمض النووي الريبي ، مما يجعلها أقل احتمالية لإنتاج السمية أو التأثيرات غير المواتية في النظم البيولوجية. علاوة على ذلك ، يمكن جعل الجسيمات النانوية القائمة على الحمض النووي الريبي انتقائية للغاية ، مما يسمح بإيصال الأدوية العلاجية إلى خلايا أو أنسجة معينة. يتم تحقيق هذه الانتقائية من خلال إنشاء جزيئات الحمض النووي الريبي التي تتعرف على أهداف محددة وترتبط بها مثل الخلايا السرطانية أو جزيئات الفيروس. نظرًا لأن الجسيمات النانوية القائمة على الحمض النووي الريبي قابلة للتحلل الحيوي ولا تستمر في البيئة ، فمن المحتمل أن تكون صديقة للبيئة أكثر من الجسيمات النانوية الاصطناعية القائمة على البوليمر. نتيجة لذلك ، قد يقلل استخدام الجسيمات النانوية القائمة على الحمض النووي الريبي من احتمالية حدوث تأثيرات سلبية على الموائل المائية وغيرها. بشكل عام ، فتح تطوير الجسيمات النانوية القائمة على الحمض النووي الريبي إمكانيات جديدة لتقديم الأدوية والتطبيقات الأخرى. في حين لا تزال هناك عقبات يجب التغلب عليها ، مثل تحسين استقرارها وفعاليتها ، فإن الفوائد المحتملة لهذه الجسيمات النانوية تجعلها مجالًا مثيرًا للبحث في مجال تكنولوجيا النانو.