WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

A Review on Nanomedicine - Based Approaches for mRNA Delivery with challenges and future Directions

Ms. Sneha.M.Nikam[1]*, Ms. Swaliha.R. Mulla[2] , Mr. Deepak.J. Kare[3]

nikamsneha96@gmail.com, swalumulla29@gmail.com, karedeepak12@gmail.com
Nootan College of pharmacy,kavthemahankal,Sangli, Maharashtra,India.

ABSTRACT—

mRNA transports genetic information from DNA to the ribosomes, which are responsible for protein synthesis. The instructions for assembling amino acids into proteins are provided by mRNA, which acts as a template for protein synthesis. Because its stability, translation, and localization can be controlled by a variety of mechanisms, it is essential for controlling gene expression.mRNA holds great promise for creating a variety of treatments, including as protein replacement and immunotherapy. The method of sending mRNA molecules into cells to tell them to make particular proteins is known as mRNA delivery. This method has drawn a lot of attention lately because of its potential to treat a number of illnesses, such as infectious diseases, cancer, and genetic disorders. mRNA delivery focus on developing highly efficient and targeted systems including lipid nanoparticles and polymeric nanoparticles, to protect mRNA from degradation and enhance its delivery to target cells, leading to improved therapeutic outcomes. However, there are several obstacles to its therapeutic application, including the size of mRNA, its restricted cellular absorption, immunogenicity, and its vulnerability to enzymatic breakdown. This review examines the present status of mRNA delivery methods based on nanomedicine, emphasizing the continuous difficulties and potential paths for enhancing mRNA delivery systems to enable their successful conversion into efficient treatments.

Keywords: mRNA delivery, Nanomedicine, mRNA engineering,clinical applications, challenges,future directions.

INTRODUCTION-

The use of nanotechnology to advance healthcare is known as nanomedicine. Since targeted mRNA nanomedicines may be precisely given to particular organs or tissues to increase efficacy and prevent adverse effects, their development has emerged as a fascinating area of research in recent years. In recent years, the field of mRNA therapies has grown significantly, creating new avenues for the treatment of a number of illnesses. The effective transport of mRNA is still a significant obstacle, though. Approaches based on nanomedicine have shown promise in overcoming these challenges and improving the effectiveness of mRNA delivery. Highlighting important research and developments in the field, this article examines current developments in nanomedicine-mediated mRNA delivery. Because mRNA therapies allow for the modification of gene expression, they have enormous potential for precision medicine.mRNA therapies provide significant potential for precision medicine, facilitating the control of gene expression to cure and prevent many diseases. mRNA delivery methods based on nanomedicine have a number of benefits, such as increased stability, better cellular absorption, targeted delivery, and controlled release. However, issues such as immunogenicity, off-target effects, toxicity, and regulatory considerations must be carefully addressed to assure the safety and efficacy .Nanomedicine has become a crucial facilitator for the efficient transport of mRNA in order to overcome these obstacles. Lipid nanoparticles (LNPs), polymeric nanoparticles, and inorganic nanoparticles are examples of nanocarriers that are designed to prevent mRNA from degrading, improve cellular uptake, and guarantee targeted delivery to particular tissues or cells. These nanocarriers can be engineered to enhance mRNA stability in biological settings, make it easier for it to escape endosomes following cellular entry, and regulate mRNA release in a spatiotemporal fashion.Although mRNA-triggered immunogenic signals may be used in immunotherapy or vaccination, significant efforts have been made to decrease mRNA immunogenicity and enhance the molecule's stability through RNA architectonics or chemical modification in an attempt to increase the importance of mRNA as a therapeutic agent. Therefore, the creation of secure delivery systems that can transfer intact mRNA molecules into cells is essential to the advancement of successful therapies.In contemporary medicine, messenger RNA (mRNA) has become a ground-breaking instrument with enormous promise for a wide range of therapeutic uses, including gene therapy, protein replacement, and vaccinations. Since mRNA does not integrate into the host genome like conventional DNA-based therapies do, there is a much lower chance of genetic abnormalities. Additionally, mRNA provides a transitory expression mechanism that may be used in both dividing and non- dividing cells. This special characteristic provides mRNA a flexible platform for producing protein products without changing the genetic makeup of the host. Because of these characteristics, mRNA- based therapy has attracted a lot of interest and funding, signaling the start of a new age in medicine. Notwithstanding a number of advancements, mRNA nanomedicines
effectiveness is still lacking due to obstacles such as intracellular, endothelial, and circulatory barriers. Following local or systemic delivery, these difficulties cause buildup in the liver, which inevitably results in drug loss and decreased efficacy. Because of this, scientists are focusing on creating targeting technologies to deliver mRNA nanomedicines to certain locations. Apart from these methods, numerous other targeting mechanisms are

constantly being developed, all of which are anticipated to increase the therapeutic efficacy and delivery precision of mRNA nanomedicines, thus expanding their potential for clinical use.

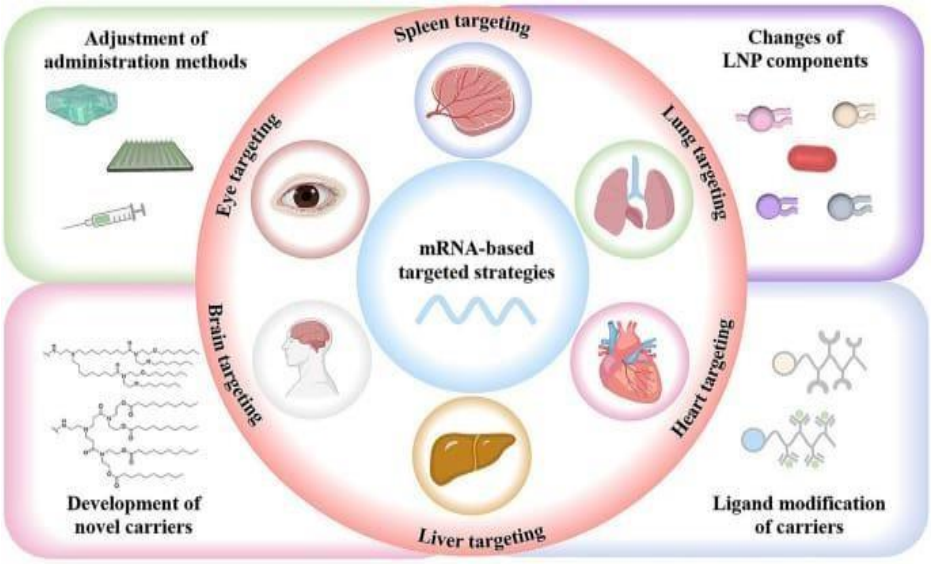


Fig1.Advancement strategies for achieving mRNA delivery

Mechanism for mRNA delivery :-

mRNA delivery systems based on nanomedicine can be built to react to different stimuli, such pH, temperature, or light, and can be tailored to target particular cells, tissues, or organs.

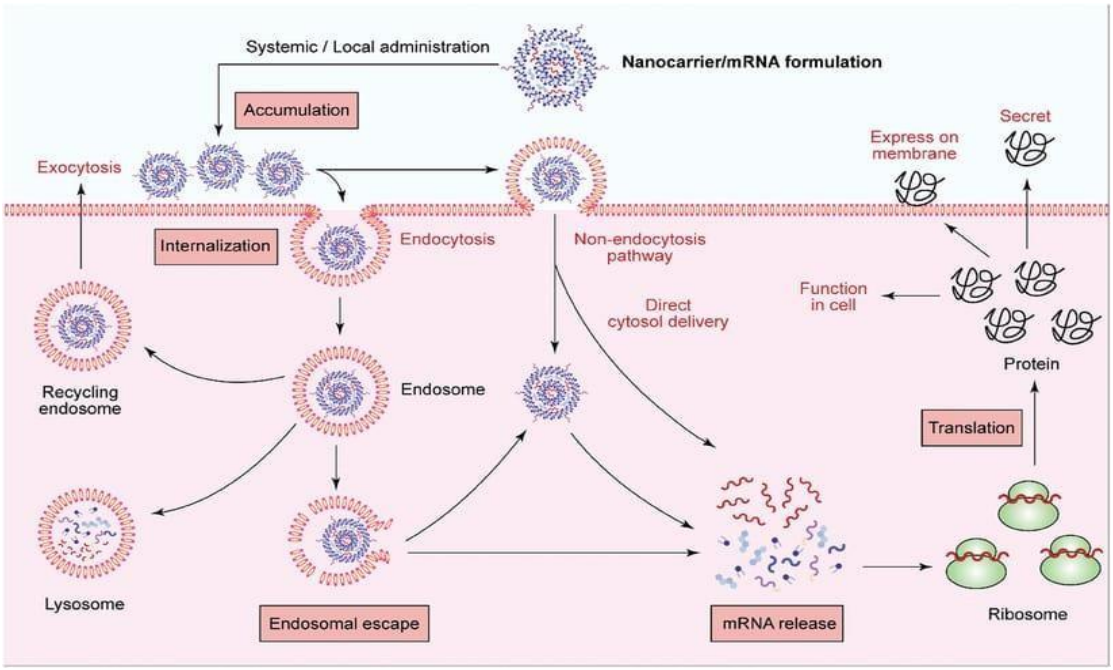


Fig2. Mechanism for mRNA delivery

mRNA molecules are complexed with nanoparticles, such as lipids, polymers, or metals. The complexation process protects the mRNA molecules from degradation and facilitates their delivery into cells.The mRNA-nanoparticle complexes are taken up by cells through various mechanisms, such as:

1. Endocytosis: Cells engulf the complexes through membrane invagination.
2. Phagocytosis: Cells engulf the comple xes through membrane extension
3. Pinocytosis: Cells take up the complexes through membrane invagination.xes through membrane extension.

The mRNA-nanoparticle complexes are released from endosomes into the cytosol.This step is critical, as endosomes can degrade the mRNA moleculesFrom the nanoparticles, the mRNA molecules are liberated.. This step can be triggered by various stimuli, such as pH, temperature, or light.

The released mRNA molecules are translated into proteins. The proteins can then perform various functions, such as enzyme activity, signaling, or immune response. The translated proteins are expressed and can perform various functions. The protein expression can be measured using various techniques, such as Western blot, ELISA, or fluorescence microscopy.

Nanocarriers system for mRNA delivery

/ approaches : --

One essential step in creating targeted delivery strategies is selecting appropriate nanocarriers. In general, the best mRNA delivery nanocarriers need to have the following properties. First and foremost, the carriers must effectively encapsulate mRNA and maintain steady circulation in vivo to shield mRNA from nuclease destruction. To promote intracellular release and cellular absorption, the mRNA carriers must secondarily target the particular organ or cell. mRNA finds it challenging to directly enter cells since it is a very delicate and negatively charged molecule. To efficiently transfer it into cells and carry out other biological tasks, a delivery system is necessary.Delivery system design, preparation, and optimization, however, are difficult tasks that call for a deep comprehension of materials science, chemistry, cell biology, and nanotechnology. Targeting other desirable organs or tissues is difficult since traditional carriers usually only passively transfer mRNA to the liver. Novel delivery methods and associated targeting technologies, including lipid-based carriers, polymer-based nanocarriers, exosome carriers, and biomimetic carriers, are being investigated by researchers in an effort to address these problems. In addition to increasing mRNA stability and delivery effectiveness, these new carriers make organ-specific targeting easier

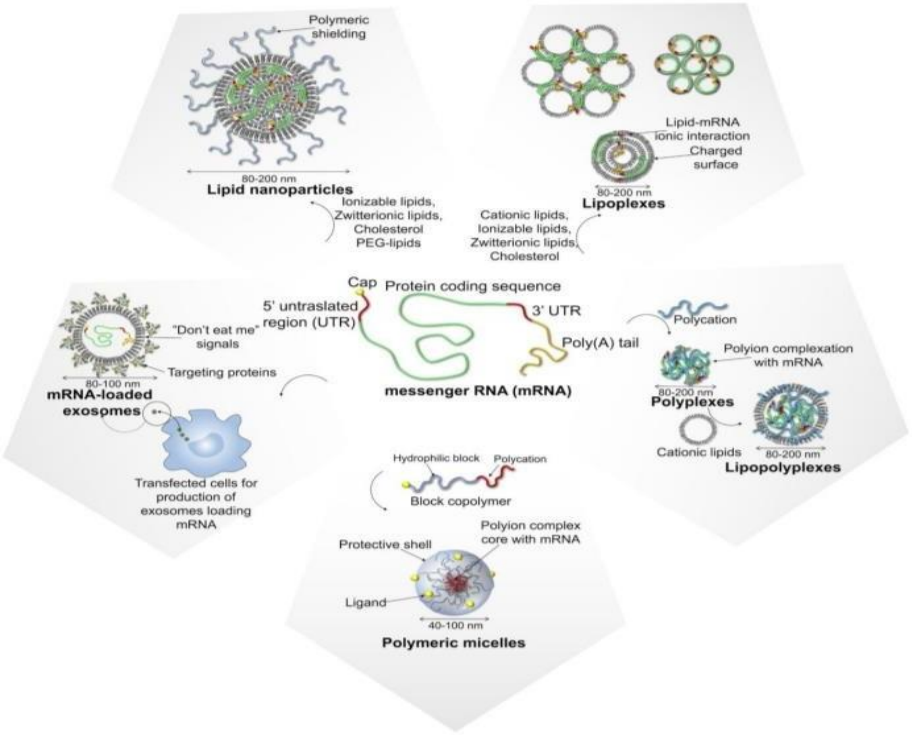


Fig3.Nanomedicine based approaches for mRNA delivery

1. **Lipid-based system-**

One of the most sophisticated methods for delivering mRNA is LNPs. Phospholipids, helper lipids (like cholesterol), ionizable lipids that encapsulate mRNA, and polyethylene glycol (PEG) for stability and circulation make up LNPs. Lipids in ethanol and mRNA in an acidic buffer (pH 3.0-4) are combined microfluidly to create LNPs. Lipids contain a PEGylated lipid to stop LNPs from aggregating, cholesterol for stabilization, an ionizable lipid (pKa < 7) that will be protonated at an acidic pH to condense and release mRNA inside the cells, and an aid lipid for endosomal escape. These lipid molecules combine to create a protective spherical nanostructure that effectively penetrates cell membranes and releases mRNA intracellularly while protecting it from destruction.LNPs improve mRNA's transport into target cells' cytoplasm and shield it from deterioration in the extracellular milieu. LNPs are absorbed by cells via endocytosis after injection. In order to destabilize the endosomal membrane and release the mRNA into the cytoplasm, where it is translated into the target protein, the acidic environment of the endosome protonates the ionizable lipids. Liposomes and other lipid-based nanoparticles are examples of LNPs, which are thought to be among the most advanced mRNA delivery mechanisms. A number of LNP platforms are leading clinical trials at the moment. They are, in fact, RNA treatment delivery devices with clinical validation.Initially, LNPs showed great promise for siRNA administration, but their use as mRNA delivery agents was more recent. A siRNA formulation in LNPs that inhibits hepatic transthyretin protein was recently approved by the FDA, indicating a major advancement in the field. Even though mRNA has a distinct structure that could affect the ability of nanoparticles to pack, the prior knowledge in siRNA formulation is helping to construct mRNA nanosystems. Liposome-based formulations are amphiphilic spherical vesicles that range in size from 20 nm to a few microns and are made up of one or more lipid bilayers encasing an aqueous core.They typically comprise a cationic lipid together with: (a) a PEG-lipid; (b) cholesterol to stabilize the lipid bilayer of the LNP; and (c) a helper lipid that supports the bilayer structure and aids in endocytosis.

PEG limits reticuloendothelial clearance, decreases protein adsorption and non-specific absorption, and provides a hydrating layer to the nanoparticle to increase colloidal stability. (d) Ionizable lipids

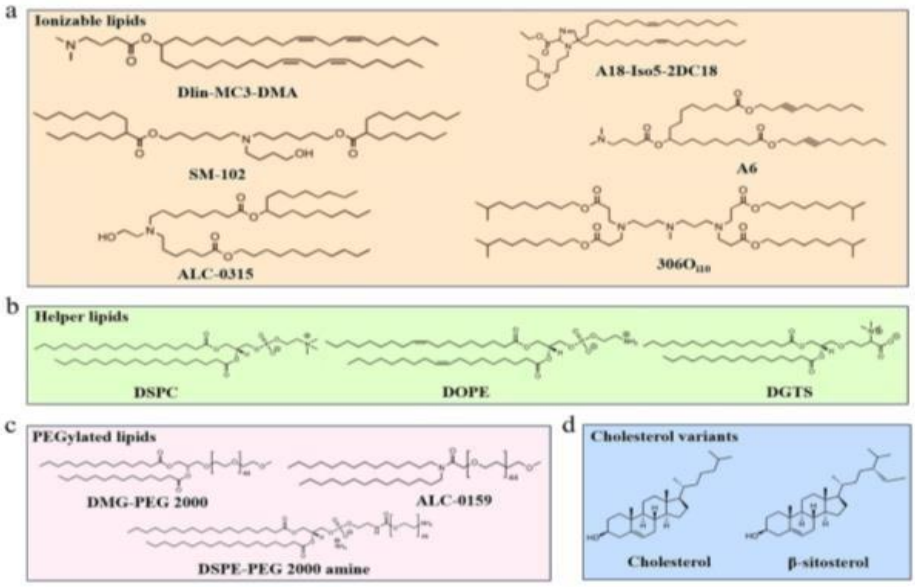


Fig4.components of LNP delivery system are as follows:a) Ionizable lipids b)helper lipid c) PEGylated-lipid d)cholesterol variants

More recently, nucleoside-modified mRNA encoding the pre-membrane and envelope glycoproteins of a Zika virus strain was encapsulated in LNPs that contained an ionizable cationic lipid, phosphatidylcholine, cholesterol, and PEG-lipid. The SAM vaccination platform is a further instance of a synthetic mRNA that is delivered using LNPs. In nanomedicine, ionizable lipids are essential, especially when it comes to delivering nucleic acids like DNA, siRNA, and mRNA. They can be made to target particular cells or tissues, which increases the specificity of nucleic acid delivery; they can also decrease toxicity and enhance the distribution of nucleic acids to cells, boosting their therapeutic efficiency.Ionizable lipids are able to alter their charge in reaction to pH variations. They are usually neutral or anionic (negatively charged) at physiological pH and cationic (positively charged) at acidic pH. Via electrostatic interactions, ionizable lipids form complexes with nucleic acids, including mRNA. A complex is created when the negatively charged nucleic acid and the cationic lipid bond. Cells can absorb the nanoparticle that is created by the lipid- nucleic acid complex. Through a process called endocytosis, in which cells engulf foreign particles, the nanoparticles are absorbed by the cells.The ionizable lipids help the nanoparticles get out of endosomes, which are cell compartments that are membrane-bound. The endosome's acidic environment causes the lipids to get protonated, which alters their structure and damages the endosomal membrane. The lipid nanoparticle releases the nucleic acid into the cytosol, where it can carry out its medicinal action.

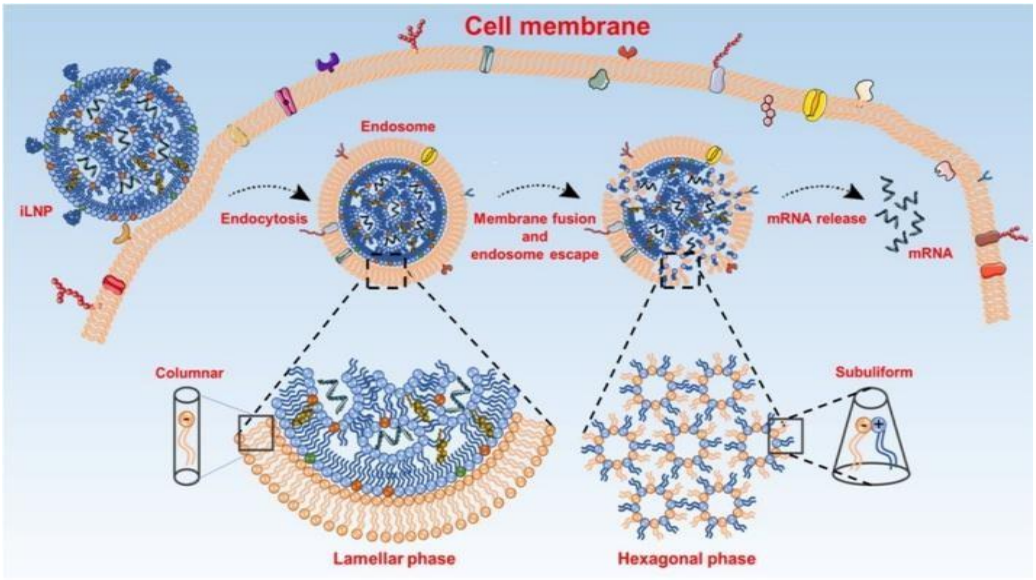


Fig5.Ionizable lipid Nanoparticles for mRNA delivery

As essential parts of LNPs, helper lipids primarily affect the nanoparticles' fluidity, structural stability, and physical integrity. By interacting with other lipid components, these helper lipids modify LNP properties, which impacts drug delivery effectiveness. Cholesterol has a superior ability to fuse

membranes, whereas phospholipids have strong bilayer forming properties and a high phase transition temperature. Both guarantee the structural stability of LNPs, control the effectiveness of transfection, encourage endosomal escape, and facilitate intracellular mRNA absorption. 1,2- distearoyl-sn-glycero-3-phosphocholine (DSPC) and 1,2-dioleoyl-sn-glycero-3- phosphoe-thanolamine (DOPE) are examples of common phospholipids.PEG-lipids, which are found on the surface of LNPs, are the least abundant component. Enhancing hydrophilicity, regulating LNP size, limiting LNP aggregation to preserve stability, inhibiting rapid removal of LNPs, and extending the circulation half-life of LNPs in blood are all significant functions of PEG-lipids. To avoid causing possible anti-PEG antibodies and lowering transfection efficiency, PEG's quantity and structure must be carefully controlled.Another kind of LNP used to carry mRNA for immunization is called a nanostructured lipid carrier (NLC). NLCs are colloidal structures made up of an unstructured lipid matrix and a core that contains a mixture of liquid and solid lipids. NLCs have a significant benefit over other lipid systems, such as emulsions, which need large amounts of surfactants and cosurfactants, in that they are less hazardous. In comparison to other systems, NLCs are also inexpensive and simple to produce and sterilize. In particular, it is challenging to sterilize liposomal formulations because phospholipids are sensitive to heat and radiation, their synthesis is expensive, and achieving batch- to-batch repeatability and large-scale manufacturing is challenging and costly.NLCs have been researched primarily for improving the oral bioavailability of poorly soluble medications, as they are superior than nucleic acid delivery.

polymer based carriers –The versatility and adaptability of polymer carriers make
them an important area of study in mRNA distribution, in addition to lipid carriers. In nanomedicine, polymer-based carriers—in particular, nanoparticles—are used to transfer mRNA by encasing or complexing the mRNA with a polymer, preventing its breakdown, and promoting intracellular delivery and cellular uptake. These carriers are designed to boost mRNA stability, target particular tissues or cells, and facilitate effective mRNA release inside the cell.Using polymer-mRNA complexes (polyplexes), especially PEI (polyethylenimine), was one of the first methods for delivering nucleic acids. The "proton-sponge" effect of PEI's high cationic density permits RNA complexation and endosomal escape, but it also causes cytotoxicity and modifies cellular homeostasis. When low molecular weight PEI conjugates with lipids, the transfection effectiveness is maintained but the cytotoxicity is reduced. Another popular DDS is polymers. Biodegradable, amphiphilic block copolymers self-assemble to form polymers. Polymers offer enormous potential in mRNA delivery because their physicochemical characteristics—such as their size, structure, and function—can be readily altered, and their formula components influence their efficacy, stability, and capacity to target certain organs.
Polymer the first mRNA-carrying polymer nanoparticles (NPs) were created because negatively charged mRNA is likely to mix with positively charged polymers by straightforward electrostatic contact and be carried into the cell.made of cationic polymers that contained amino groups like polyethyleneimine (PEI). However, cationicpolymers have non-negligible cytotoxicity due to their positive charge, which limits their clinical development, just like cationic lipids. The creation of biodegradable and functional polymers is currently a focus area to solve toxicity concerns.Poly(2- (dimethylamino)ethyl methacrylate) (PDMAEMA), polyester, poly(lactic-co- glycolic acid) (PLGA), poly(lactic acid) (PLA), poly(β-amino ester) (PBAE), and poly(amine-co-ester) (PACE) are a few examples of these polymers. Although polymers are not as commonly used in clinical practice as LNPs for mRNA delivery, their active chemical properties offer additional material characteristics, such as endosome escape ability and cell and tissue tropism (e.g., for lungs), which are advantageous for organ-specific delivery and increase mRNA translation efficiency. The use of polymer nanoparticles has been actively researched for pDNA transport, however few studies have addressed their usage for mRNA.The ability to change the chemical characteristics of polymeric systems to match the active ingredient is one of their main advantages. Polyplexes are created when nucleic acids and cationic polymers bond together. A variety of cationic polymers, including as polyethyleneimine (PEI), polyacrylates, poly(β-amino esters) (PBAEs), and poly(aspartamides) (PAsp), have been investigated for mRNA complexation. For mRNA-based treatments, polymeric systems are not as clinically developed as lipidic systems, even though there are a large range of polymeric materials accessible.

One of the earliest polymers used to transfer nucleic acids was PEI, which has a lot of amine groups in its structure that give it a positive charge. PEI can exhibit both a branching and a linear shape. Whereas branched PEI comprises primary and secondary groups along with a few tertiary amines, linear PEI has secondary amino groups that are largely protonated at physiological pH. A great affinity for nucleic acids, including mRNA, is caused by the presence of amino groups, and cationic charges make it easier for the polyplexes to interface with the cell membrane and enter the target cell. To increase PEI's transfection effectiveness and biocompatibility, several compounds have been created.For instance, the linear PEI jetPEI®, which is marketed as an in vivo transfection reagent for mice, was initially tested for pDNA and siRNA transfection before being tested for mRNA delivery. More recently, the expression of the protein in the lungs was shown in mice administered IVT mRNA with jetPEI® via direct myocardial injection. By adding amines and acrylates through a Michael- type reaction, PBAEs—biodegradable and pH-responsive copolymers—are created. The negative charge of nucleic acids can interact electrostatically with the tertiary amine of its structure. PBAEs can be delivered in a wide range of ways since they work well with other polymers including PEG, poly(lactic acid) (PLA), and poly(ε-caprolactone) (PCL).When PBAEs made with PEG-lipid are used, serum stability of mRNA following intravenous injection is improved. Luciferase mRNA was recently evenly dispersed throughout the lung, and 24 hours after hyperbranched PBAEs transported IVT mRNA into the lungs, protein expression continued. In order to create PAsp, DL-aspartic acid is polymerized in an orthophosporic acid medium, and a nucleophilic amine is then added . Both the buffering ability and cationic charge of each PAsp construct are influenced by the length of the amynoethylene side chain. PAsp(DET), for instance, demonstrates endosomal scape capability and biodegradability once 1,2-diaminoethane is added to its side chain.For various nucleic acid payloads, an odd-even effect of the amynoethylene repeating units has been reported. While an odd-numbered unit of PAsp steadily raised mRNA expression and improved resistance to, PAsp with even- numbered amynoethylene units demonstrated greater transfection with pDNA. For example, when IVT mRNA complexed with PEG-PAsp (DET) was administered intranasally to mice to deliver brain-derived neurotrophic factor (BDNF), the animals's nasal tissues showed protein expression for almost two days. PEG-PAsp polyplexes have demonstrated improved stability and decreased cytotoxicity.Additionally, Bcl-2 IVT mRNA has been complexed using PEG- PAsp(DET), which is superior to pDNA in lowering apoptosis in the liver of animals with fulminant hepatitis. It has been documented that IVT mRNA can be transfected with a different type of polyplex in addition to the most popular polymers covered above. The stability of mRNA was enhanced by nanoparticles made of IVT mRNA complexed with the peptide protamine

and encased in PCL layers. Luciferase-encoding mRNA was successfully delivered using chitosan, a biocompatible cationic glycopolymer with amines, which was prepared as

chitosan/hyaluronic acid nanoparticles.The ability of self-immolative polycarbonate- block-poly(α-amino)esters, also referred to as charge-altering releasable transporters (CARTs), to decrease chelative electrostatic anion-binding ability with the cationic polymer and promote endosomal escape has shown promise in delivering mRNA. Through intramuscular, intratumoral, and intravenous administration, CARTs are efficient for delivering mRNA in vivo with low toxicity in a variety of cell lines and animal models. In a mouse model, these CARTs have demonstrated a robust antigen-specific immune response against viral epitopes transcribed by mRNA. Tissue-selective for mRNA distribution are further biodegradable ionizable amino polyesters (APEs), which are produced by ring opening polymerization of lactones with tertiary amino-alcohols.

1. Exosomes carriers

Cells secrete exosomes, which are extracellular vesicles that range in size from 30 to 150 nm and are involved in molecular communication between cells. For the following reasons, exosomes are regarded as the best drug delivery method: First, exosomes' inherent biocompatibility allows them to effectively bond with the body's target cells, preventing the immunological a Techniques for loading cargo into exosomes mainly include pre-loading and post-loading approaches. Reproduced with permission from Ref. [8]. Copyright 2022, MDPI. b Under the action of ultrasound, the modifed exosomes can be selectively transported to adipocytes for the treatment of obesity. c Compared with the untreated group, HE staining showed signifcant browning in the

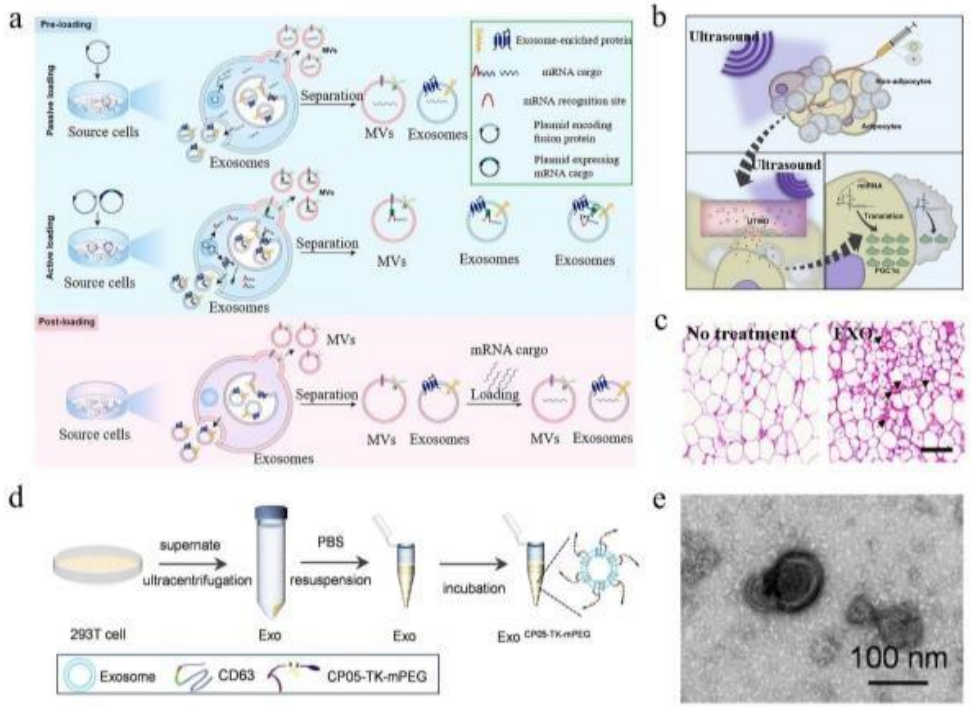


Fig6. Exosome carriers and related targeting strategies.

exosome-treated group. (b, c) Reproduced with permission from Ref. [9]. Copyright 2020, Cell Press. d Prepara-tion method of CP05-TK-mPEG-modifed exosomes. e Transmission electron microscopy (TEM) image of CP05-TK-mPEG-modifed exosomes. (d, e) Reproduced with permission from Ref. [10]. Copyright 2021, Springer Nature rejection reactions that conventional drug delivery methods may trigger. Second, exosomes are very stable and long-lived in the body, which is advantageous for prolonged medication release

Additionally, exosomes have the extraordinary capacity to penetrate a variety of biological barriers, including the blood- brain barrier (BBB), which is essential for treating tumors and other difficult-to-reach illness locations like the nervous system.Modified exosome-based carriers, in particular, can increase medication concentration at the target site by achieving targeted distribution to extrahepatic tissues. Pre-loading and post-loading techniques are the two main categories of mRNA

packaging techniques for exosomes, as seen in Fig. 7a. Using either viral or non-viral vectors, plasmids expressing the desired mRNA are transfected into cells that secrete exosomes for the pre-loading procedure. These mRNA are naturally encapsulated within the exosomes that cells produce and discharge. When compared to non- transfected groups, mRNA expression levels increased by more than 100 times in cells transfected with DNA expressing low- density lipoprotein receptors.The exosomes that were secreted likewise showed comparable increases. By coincubating exosomes with the targeted mRNA under the right circumstances, post-loading techniques—also referred to as exogenous loading techniques—allow the mRNA to be spontaneously absorbed by the exosomes. Despite being straightforward, this approach is not very effective. Furthermore, developments in the field have brought about methods like electroporation and commercial chemicals that temporarily alter the permeability of exosome membranes, improving the efficiency of mRNA loading. In the field of vesicle research, engineering exosomes for targeted distribution is an important avenue. The ability of exosomes to target particular cells or tissues is enhanced by altering their surface.For example, a study that engineered the exosome membrane protein Lamp2b using the glycoprotein from the rabies virus showed that the altered exosomes could effectively deliver cargo to ischemic regions of the brain. A peptide sequence with adipose-targeting capabilities (CKGGRAKDC) was added to the N- terminus of the exosome membrane protein Lamp2b in

a work that paralleled other investigations. This alteration enhanced the delivery effectiveness and expression levels of therapeutic mRNA in addition to enabling exosomes to more effectively identify and target adipose tissue. Furthermore, Wang and colleagues modified the exosome surface using a high-affinity anti-HER2 scFv antibody, which significantly enhanced exosome accumulation in target cells and provided a new strategy for targeted breast cancer treatment.Using ultrasonic help or inserting miRNA sequences are viable solutions to reduce off-target effects. According to reports, liver-specific miRNA- 122 can detect the hepatitis C virus's internal ribosome entry site (IRES), starting mRNA translation in particular organs. Based on this finding, Sun et al. conducted a significant study in which they replaced the liver-specific IRES with a fat-specific IRES and encapsulated PGC1α cargo associated with exosome energy metabolism. The ability of the modified exosomes to be efficiently transferred to adipose tissue and carry out PGC1α mRNA translation was confirmed with the aid of ultrasound, further encouraging browning in mice fed a high-fat diet (Fig. 7b, c). Interestingly, Guo et al. combined active targeting and ultrasound technology to create a sophisticated exosome-based delivery platform.First, they created CP05-TK-mPEG, a multipurpose stealth coating (Fig. 7d). While the PEG component shields the carrier from phagocytosis and extends its circulation time in vivo, the peptide component CP05 (CRHSQMTVTSRL) can interact with the exosome surface marker CD63. The CP05- TK-mPEG-engineered exosomes had a roughly spherical form and were around 100 nm in diameter, as seen in Fig. 7e. The
Chlorin e6 (Ce6) fused in exosomes quickly generated reactive oxygen species when exposed to ultrasonic action, which destroyed thioketals and released the active component bone morphogenetic protein 7 mRNA. According to the findings, the smart delivery platform provided a fresh approach to the creation of treatments for obesity, and the targeted techniques used might be applicable to all situations.

3) Other carriers-

Targeting specificity, biocompatibility, and transfection efficiency of mRNA carriers are anticipated to be improved by carefully controlling a variety of physical and chemical interactions between nanomedicines and organisms. .

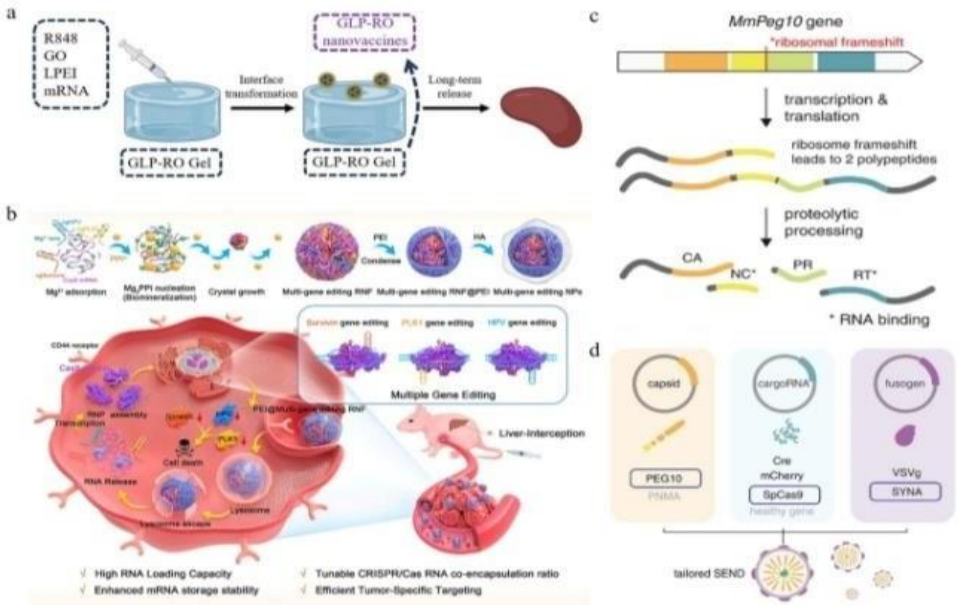


Fig9. Other nanocarriers and related targeting strategies.

a A mixed carrier based on GO, PEI, and R484 for targeted mRNA delivery. Reproduced with permission from Ref. [11]. Copyright 2021, American Chemical Society. b Preparation and working mechanism of a Mg2+-based biomimetic carrier. Reproduced with permission from Ref. [12]. Copyright 2023, American Chemical Society. c Four structural domains of the PEG10 gene. d Composition of the SEND delivery platform, including cargo RNA, fusogen structure, and PEG10.

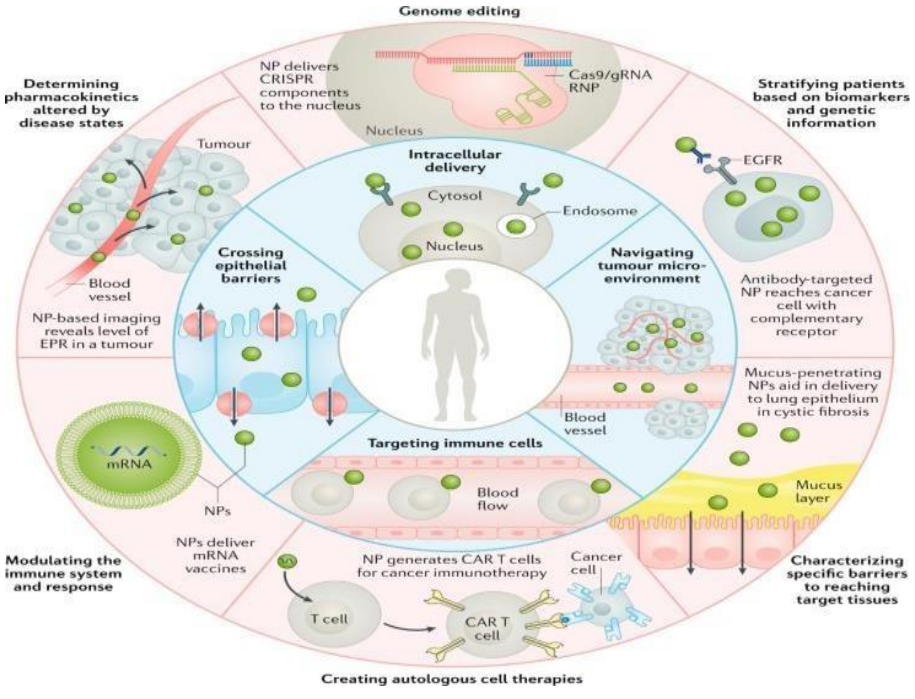
(c, d) Reproduced with permission from Ref. [13]. Copyright 2021, American Association for the Advancement of Science This complex regulatory approach raises the bar for precision medicine by providing countless options for mRNA carrier design. An expanding variety of innovative nanocarriers, such as inorganic nanoparticles, hybrid carriers, biomimetic systems, virus-like particles, etc., are continuously emerging as a result of a deeper understanding of carrier design concepts. Even though the content, structure, and design of these new carriers vary, they all effectively overcome biological barriers and play crucial roles in targeted therapy.These developments offer fresh possibilities for the treatment of complicated illnesses in addition to quickening the development of mRNA therapy. According to reports, graphene oxide (GO) has special benefits for drug delivery. Its large surface area facilitates effective drug loading, and the many functional groups allow drug molecules to attach by electrostatic, covalent, or physical adsorption. GO's potential for application in precision medication administration is further enhanced by its outstanding biocompatibility and tunability. Ovalbumin (OVA) mRNA was encapsulated in a study using the TLR7/8 agonist resiquimod (R848), the low molecular weight PEI, and the inorganic substance GO, as illustrated in Fig. 8a.When administered subcutaneously, the resultant mixture GLP- RO activated immune cells by targeting lymph nodes and showed potent anti-tumor properties in a B16-OVA melanoma model Remarkably, these nanomedicines remained effective for at least 30 days with no

discernible adverse effects. Similarly, mRNA delivery to other organs is made possible by the combination of lipid molecules and polymer carriers. Andretto and his associates coated the surface of cationic lipid-mRNA complexes with doped hyaluronic acid. This negatively charged coating improved stability and stimulated mRNA translation in the spleen by fine- tuning the nanoparticles' physicochemical characteristics.Another study used a self- assembly approach to create nanomedicines by combining mRNA, PLGA polymers, and cationic lipid-like substances. With incredibly minimal cytotoxicity, the hybrid carrier was confirmed to help transfer mRNA to prostate cancer cells that are functionally deficient. After being administered intravenously in a PC3 xenograft tumor model, the mRNA nanomedicines demonstrated excellent therapeutic results and high accumulation levels in mice tumors. By imitating the composition and operation of biological systems, biomimetic nanocarriers show enormous promise for mRNA delivery. In order to effectively avoid immune system detection and clearance during mRNA distribution, these biomimetic nano-carriers can typically mimic natural structures like cell membranes.Macrophages were pre- transfected with plasmids encoding tumor necrosis factor (TNF-α) receptors in an arthritis therapy research. The inner PLGA- mRNA complex was subsequently encapsulated in the macrophages' cell membranes, which were obtained by ultracentrifugation. Research demonstrated that following intravenous treatment, the resultant biomimetic carriers may rapidly target the inflammatory areas of arthritis, release therapeutic mRNA, and block the expression of markers linked to inflammation. In a related investigation, cell membranes with high hemagglutinin expression were created using genetic engineering technology.The PLGA-mRNA complexes were enclosed in the pre-made cell membrane to create biomimetic nanoparticles. It was confirmed that the cell membrane-coated nanocarriers showed greater expression levels than the non- coated group, regardless of whether they were supplied locally or systemically. Liang and his associates developed a Mg2+-based biomimetic system for precise CRISPR/Cas9 RNA delivery, which was motivated by natural biomineralization (Fig. 8b) [149]. In order to create crystal nuclei, the metal cation Mg2+ first coupled with RNA. PEI and HA functioned as exterior coverings that wrapped around the Mg2+-RNA core after the crystals formed, modifying the carrier's chemical and physical characteristics. Subsequent research revealed that the biomimic delivery method could precisely modify genes at tumor sites while being stored at 4 °C for at least a month.Given these characteristics, the creation of biomimetic nanocarriers offers a viable option for targeted mRNA delivery, which is crucial for the advancement of mRNA-based precision medicine. Drug delivery research has shown a great deal of interest in virus-like particles (VLPs), which resemble parent viruses in size and form but do not have transfective genomes. VLPs use their innate cell invasion mechanisms to transport mRNA to specific cells while effectively shielding it from biological obstacles that would otherwise degrade it. A study that was published in Science in 2021
created the SEND VLP delivery system, which is based on the mammalian retroviral- like protein PEG10.The key components of the SEND delivery platform were cargo RNA, a fusogen, and PEG10 with four structural domains, which had modular and adjustable features, as seen in Figs. 8c, d. Gene editing tools were successfully supplied to human cells via the SEND platform. Li et alAdditionally, this study demonstrated that PEG10-based VLPs can be employed to treat eye diseases. Remarkably, a significant amount of PEG10-based VLPs were administered to retinal pigment epithelial cells via the retina, leading to elevated levels of mRNA expression. Also, because PEG10 was an endogenous human protein, the VLPs showed less immunogenicity than other conventional lentiviral vectors.Using five plasmids, including pMD.2G, pRSV-REV, pMDlg/pRRE-D64V, pCMV-spike-6× MS2, and pMS2M-PH-gagpol-D64V, Yin et al. created VLP carriers that were targeted to dendritic cells. mRNA vaccines based on VLPs provided mice with longer-lasting protection and greater cellular and humoral immunity than LNP formulations at the same dose. The VLP carriers were also expanded for use in vaccines against the herpes simplex virus, which similarly produce excellent antiviral effects. Nearly any functional protein or peptide in the human body may now be produced because to recent advancements in molecular sciences and nanotechnology, including mRNA as a therapeutic agent.

mRNA Engineering:-

mRNA modification by approaches that enhance the stability and promote its function have been considered for improving mRNA biological activity as well as reducing immunogenicity issues. these modification methods do not affect the main structural features of mRNA, i.e. being a negatively charged macromolecule, which provides opportunities for developing orthogonal cooperative approaches in combination with nanomedicine with potential for synergistic enhancement of mRNA function.mRNA engineering refers to the design, optimization, and modification of messenger RNA (mRNA) molecules to achieve specific therapeutic or research goals. Here are some key aspects of mRNA engineering

Fig7.Engineering precision nanoparticles for drug delivery



1. Sequence Optimization- Designing mRNA sequences to optimize translation efficiency, stability, and specificity. Using computational tools and machine learning algorithms to predict and optimize mRNA sequence.
2. Codon Optimization- Optimizing codon usage to improve translation efficiency and reduce mistranslation. Using codon optimization algorithms to design mRNA sequences with optimal codon usage.
3. RNA Modification- Introducing chemical modifications to mRNA molecules to improve stability, reduce immunogenicity, and enhance translation. Using techniques such as pseudouridylation, 2'-O-methylation, and 5'-capping to modify mRNA molecules.
4. mRNA Stability Engineering- Designing mRNA molecules with improved stability to prolong their half-life and enhance their therapeutic efficacy.Using techniques such as RNA stabilization elements, antisense oligonucleotides, and RNA-binding proteins to improve mRNA stability.
5. Targeted mRNA Delivery- Designing mRNA molecules with targeting elements to deliver them to specific cells, tissues, or organs.Using techniques such as aptamers, antibodies, and peptides to target mRNA molecules to specific cells or tissues.
6. mRNA Vaccine Development-Designing mRNA molecules as vaccines to prevent infectious diseases.Using techniques such as mRNA encapsulation, adjuvantation, and immunization to develop mRNA vaccines.
7. Gene Editing-Designing mRNA molecules to deliver gene-editing enzymes, such as CRISPR-Cas9, to specific cells or tissues. Using techniques such as mRNA- mediated gene editing and RNA-guided gene editing to modify genes.
8. Cancer Therapy-Designing mRNA molecules to deliver cancer-specific antigens or immunomodulatory molecules to stimulate anti-tumor immune responses.Using techniques such as mRNA- based cancer vaccines and mRNA-mediated.

Clinical applications-

mRNA delivery using a nanomedicine-based strategy Use of exosomes for mRNA delivery applications A great deal of optimism against a number of incurable diseases was sparked by the therapeutic use of mRNA. Recent developments in molecular sciences and nanotechnology have enabled the production of almost any functional protein or peptide in the human body, including mRNA as a therapeutic agent. Because of their biocompatibility, low immunogenicity, toxicity, and innate capacity to traverse the blood-brain barrier, exosomes have emerged as a promising nanocarrier for mRNA-based medication delivery, according to recent studies.Exosomes with mRNA medications can be targeted by surface engineering or passively moved throughout the body. Target cells can internalize exosomes primarily through receptor-mediated uptake, membrane fusion, or endocytosis.Additionally, in diseases like glioblastoma, breast cancer, COVID, Parkinson's disease, leukemia, kidney disease, and infectious diseases, exosomes made from particular cell lines—like dendritic cells, natural killer cells, or stem cells—can encapsulate mRNA to produce proteins that alter the expression of disease- related proteins. Using microneedles to deliver mRNA-loaded exosomes intradermally has demonstrated promise in recent years for effectively secreting particular proteins.

CNS disorders-

Exosomes were generated from the HEK- 293 cell line and utilized as possible delivery vehicles for RNA medications to treat Parkinson's disease. The brain was the specific focus of these exosomes laden with catalase mRNA. Catalase's selective administration to the brain prevented oxidative damage to neurons, which is the primary cause of neuronal cell death.
According to the study's findings, Parkinson's disease neuroinflammation caused by 6-OHDA has decreased.

Cancer diseases-

HEK-293 and dendritic cells were used to produce exosomes from cancer research that were loaded with mRNA encoding the HChrR6 enzyme.In a mouse model, the intended exosomes showed selective death and nearly total suppression of breast cancer when they were administered to HER2+ human breast cancer cells. By converting the prodrug CNOB into the active drug 9-p amino-6-chloro-5H-benzo[a]phenoxazine-5- one (MCHB) in tumors, HchrR6 was able to destroy the target.In vitro transcribed (IVT) HChrR6 mRNA-exosomes were created by Forterre et al. and were shown to be selectively absorbed by HER2 cancer cells with negligible bone marrow impairment and no harm to other cells. CB1954, a prodrug that was converted into an active drug, showed better suppression of HER2 breast cancer cells. In addition to breast cancer, exosome-based mRNA delivery has shown therapeutic promise in leukemia. The Cas9 mRNA system was created to treat acute myeloid leukemia (ALL) using exosomes obtained from red blood cells. The expression of two well-known oncogenic miRNAs in leukemia, miR-125a and miR-125b, was significantly reduced with targeted administration of Cas9 RNA.Yang and colleagues altered exosomes produced from CD47 by adding a glioma- targeting peptide to the N-terminus. Because of this alteration, glioma cells were able to selectively absorb the cargo of the tumor suppression gene, phosphatase, and tensin homolog (PTEN). Consequently, there was an effective inhibition of tumor cell proliferation. Mizrak et al. created genetically altered exosomes from HEK-293 cells that contained uridine phosphoribosyl transferase (UPRT) and suicide cytosine deaminase (CD) mRNA/protein to treat schwannomas, which are cancers of the nerve sheath.According to the study, CD changed the prodrug (5-FC) into its active form (5-FU), which in turn prevented DNA synthesis and caused cancer cells to undergo apoptosis. Infectious illnesses Popowski et al. used lung-derived exosomes for mRNA and protein cargos to create an inhaled vaccination. According to their findings, Lung-Exo demonstrated significant mRNA translation, protein production, and enhanced cargo retention in bronchioles and parenchyma, indicating improved therapeutic efficacy against the coronavirus that causes severe acute respiratory syndrome (SARS-COV-2). An exosome- derived vaccine containing mRNA encoding the immunogenic SARS-CoV-2 virus's N and S spike proteins was developed, according to another study.Compared to the current SARS-CoV-2 vaccine, the developed vaccine demonstrated enhanced and sustained humoral and cellular protection against the viral N and S proteins with few adverse effects.

Metabolic disorders-

Disorders of metabolism In order to cure obesity, smart Bmp7 mRNA was delivered locally utilizing decorated exosomes.
According to the study's findings, localized Bmp7 administration with US assistance effectively browned OAT and showed promise as an anti-obesity treatment. To cure obesity, Zhao et al. created exosomes with adipose-derived stem cells (ADSC). The smart exosomes enhanced energy expenditure, decreased WAT inflammation, and increased resistance to the evolution of obesity.

CNS diseases-
NGF-mRNA-loaded exosomes functionalized with RVG were produced for cardiac diseases.When administered systemically, the system decreased inflammation and increased cell survival, demonstrating its encouraging potential as a stroke treatment. It has also been shown that this technique works well for CNS conditions. Possible uses for exosome- mediated mRNA delivery

Additional uses –

- Wound Healing: To aid in wound healing, nanoparticles can transport growth hormones, antibiotics, or other medicinal substances.
- Tissue Engineering: To encourage tissue regeneration, nanoparticles can transport growth factors, cells, or other medicinal substances.
- Neurological illnesses: Therapeutic medicines can be delivered via nanoparticles across the blood-brain barrier to treat neurological illnesses.

Challenges-
Difficulties with mRNA Delivery The actual use of mRNA therapies is hampered by a number of issues, despite the apparent advantages. The intrinsic instability of mRNA is one significant obstacle. mRNA molecules' effectiveness and shelf life may be restricted due to their susceptibility to ribonuclease and environmental degradation.Moreover, the size of mRNA molecules hinders their cellular absorption. In order to be translated into protein in the cytoplasm, the mRNA must pass through the cell membrane and get out of the endosomes. There are many obstacles in this process that could prevent a successful delivery.The immunogenicity of mRNA is another important concern. Although mRNA is not intrinsically dangerous, its presence in the body might cause immunological reactions, which may result in adverse effects and decreased effectiveness. Although they have made progress, researchers are still working to alter mRNA to lessen its immunogenicity.

1. Problem: mRNA is prone to nuclease degradation and thus intrinsically unstable. The role of nanomedicine is to preserve mRNA from enzymatic degradation by using protective carriers such as lipid nanoparticles (LNPs)
2. Effectively delivering the nanoparticles to the target cells is a challenge, particularly when navigating intricate biological barriers like the blood-brain or mucosal barriers. The role of nanomedicine is to enhance uptake through endocytosis by optimizing the size, charge, and ligands of nanoparticles
3. Challenge: mRNA must get out of endosomes and into the cytoplasm once it enters cells to avoid degradation. The function of nanomedicine is to encourage endosomal egress by creating nanoparticles with pH-responsive or membrane-disruptive components.Delivering mRNA to particular organs (such as the liver, tumor locations, and immune cells) while avoiding off-target effects is a challenge. The function of nanomedicine is to guide nanoparticles to particular cell types by altering their surfaces with targeting ligands or antibodies.
4. Problem: delivery vehicles and mRNA may cause unintended immunological reactions. The role of nanomedicine is to lessen immunogenicity by using modified
nucleosides (such as pseudouridine) in mRNA and biocompatible nanoparticle materials.
5. The difficulty lies in mass-producing reliable, superior nanoparticles. The role of nanomedicine: Reproducibility is being enhanced by developments in microfluidic technologies and uniform formulations.
6. The challenge is to ensure low toxicity, long-term safety, and to overcome regulatory barriers for clinical application. Nanomedicine Role: To confirm the pharmacokinetics and biocompatibility of novel formulations, preclinical and clinical research are required.

Future perspectives-

There is potential for revolutionary developments in personalized medicine from the ongoing research on mRNA delivery via nanoparticles. Multifunctional nanoparticle design, delivery kinetics optimization, and the investigation of new materials for increased safety and effectiveness are possible future advancements. These days, mRNA therapies are progressively approaching clinical use. One of the most cutting-edge areas is cancer vaccination; the first clinical trial reports date back to 2002. By avoiding immune tolerance mechanisms, neoantigens—which are expressed exclusively in cancer cells—can be targeted to increase the effectiveness of vaccines.More improvements in treatment outcomes are anticipated as mRNA vaccination technologies advance, including RNA design, administration methods, and transport systems. The approach to treating cancer could be completely changed by such tailored therapy.
Since protein expression from mRNA can start as soon as a few hours after transfection, acute disorders are a prospective target for mRNA treatments. For antibody-based therapy, mRNA might offer a practical and affordable alternative. Compared to protein administration, mRNA distribution makes it simpler to introduce intracellular and membrane proteins, increasing the pool of potential therapeutic protein candidates for the treatment of disease.
Enhancing Delivery Efficiency: To increase the effectiveness and specificity of mRNA delivery methods based on nanomedicine, more study is required. Converting to Clinical Practice: The goal of ongoing research is to explore the therapeutic potential of these promising techniques in a variety of disorders and bring them to clinical reality.

Conclusion:-

Numerous efforts were made to address the issues with mRNA therapies, including their immunogenicity, short protein expression lifetime, quick enzymatic breakdown, and restricted cellular absorption. In order to facilitate mRNA distribution and reduce safety concerns, the methodologies are divided into two orthogonal approaches: mRNA designing and nanomedicines Numerous therapeutic uses of mRNA, such as cancer treatment, protein replacement therapy, vaccines, and cell reprogramming in preclinical and clinical trials, were made possible by these technological advancements. In addition to promoting novel therapeutic approaches, mRNAnanomedicines will address a number of medical issues that cannot be resolved with traditional methods by improving the delivery mechanism and carefully choosing the target disorders.

REFERENCE:-

1. Satoshi Uchida, Federico Perche, Chantal Pichon, Haoracio Cabral Nanomedicine - Based Approaches for mRNA Delivery. Molecular Pharmaceutics, 2020,

2. Wang1,2, Lijun Cai2, Ning Li2, Zhiqiang Luo2, Haozhen Ren1,3 , Bing Zhang1, Yuanjin Zhao1,2 Nano-Micro Lett. (2025) 17:155

3. Itziar Gómez-Aguado , Julen Rodríguez- Castejón , Mónica Vicente-Pascua Alicia Rodríguez-Gascón , María Ángeles Solinís and Ana del Pozo-Rodríguez Nanomedicines to Deliver mRNA: State of the Artand Future Perspectives

4. Ms. Shilpa Amit Shinde, Dr. Madhu B. Krishnegowda, Pallavi Ganesh Borse, Omkar Santosh Sonawane, Mayuri Macchindra LandaveNanomedicine-Based Approaches for mRNA Delivery: Challenges and ProspectIJIRT | Volume 11 Issue 3

5. Shubham Singh, Dr. Arun Patel, Shailendra Patel, Vijay Pratap Ahirwar NANOPARTICLE-BASED

APPROACHES FOR mRNA

DELIVERY(IJRD)Volume: 9 | Issue: 2 |

6. mRNA nanodelivery systems: targeting strategiesMujie Yuan1†, Zeyu Han1†, Yan Liang2, Yong Sun2, Bin He3, Wantao Chen4 and Fan Li1

7. Nanomedicine based approach on mRNA deliveryPawan Hadole*, Nikam H. M., Avinash Gite, Pratik Kamble, Umesh Jadhav

8. Z. Li, Z. Liu, J. Wu, B. Li, Cell-derived vesicles for mRNA delivery. Pharmaceutics 14,2699(2022).https://doi.org/10.3390/pharmaceutics14122699

W. Sun, C. Xing, L. Zhao, P. Zhao, G. Yang et al., Ultra-sound assisted exosomal delivery of tissue responsive mRNA for enhanced efcacy and minimized of-target efects. Mol. Ther. Nucleic Acids 20, 558– 567(2020).https://doi.org/10.1016/j.omtn.20 20.03.016

9. Y. Guo, Z. Wan, P. Zhao, M. Wei, Y. Liu et al., Ultra-sound triggered topical delivery of Bmp7 mRNA for white fat browning induction via engineered smart exosomes. J. Nanobiotechnol. 19, 402

(2021). https://doi.org/10.1186/s12951-021-

01145-3

10. Y. Yin, X. Li, H. Ma, J. Zhang, D. Yu

et al., In situ transforming RNA nanovaccines from polyethylenimine functionalized graphene oxide hydrogel for durable cancer immunotherapy. Nano Lett. 21,22242231(2021).https://doi.org/10.1021/ acs.nanolett.0c05039

11. Y. Liang, J. Zhang, C. Xu, J. Wang, W. Han et al., Biomimetic mineralized CRISPR/cas RNA nanoparticles for efcient tumor-specifc multiplex gene editing. ACS Nano 17, 15025–15043 (2023).

https://doi.org/10.1021/acsnano.3c04116

12. M. Segel, B. Lash, J. Song, A. Ladha, C.C. Liu et al., Mammalian retrovirus-like protein PEG10 packages its own mRNA and can be pseudotyped for mRNA delivery. Science 373, 882–889 (2021).

https://doi.org/10.1126/science.abg6155

13. Fig7fromhttps://images.app.goo.gl/BusV xfy3rwUrGSfCA

14. fig2https://www.google.com/imgres?img url=https%3A%2F%2Fadvanced. onlinelibrary.wiley.com%2Fcms%2Fasset% 2F529c72bf-8a30-4071-8758-

2542c0d7899d%2Fadma202307822-

fig0005m.jpg&tbnid=qTEQghH5 c_7atM&vet=1&imgrefurl=https%3A%2F% 2Fonlinelibrary.wiley.com%2Fdoi%2Ffull% 2F10.1002%2Fadma.202307822&docid=