Strategies for Nano-scale delivering mRNA-based vaccines: a focus on lipid nanoparticles.

mRNA delivery strategies

Numerous advantages that mRNA-based therapeutics have over traditional ones, mRNA delivery strategies have become a focal point of intense research [1].

mRNA can be used to produce normal proteins to replace mutant proteins in many genetic disorders, for cancer immunotherapy, and for prophylactic and therapeutic vaccination against pathogens. The cells of the patients are given an mRNA that encodes a desired protein or a subunit of a protein of interest. This has several advantages, including a low risk of adverse effects because the whole attenuated or killed pathogen is not injected, a low risk of toxic effects because mRNA can be produced in vitro without using a live biological system, and virtually no risk of insertional mutagenesis, unlike DNA, because mRNA is directed only to the cytoplasm and not the nucleus. Furthermore, because mRNA has a short half-life, it poses less risk, and the ability to mass-produce it in vitro means that mRNA is more cost-effective in the long run when compared to traditional methods such as whole organism or purified proteins. However, the same property of short half-life poses a challenge for effective delivery to achieve maximum effect. A variety of techniques have been used, ranging from naked mRNA injection to the use of nanoparticle-based mRNA delivery or the production of mRNA in the host via viral-based vector injection. As a result, in the following paragraphs, we will go over the various mRNA delivery methods that have been used.

Naked RNA

mRNA can be in-vitro transcribed in a cell-free system, it is free of impurities that may result from metabolite production in-vivo. This IVT transcribed mRNA can then be introduced into the host cell. One method for achieving mRNA-based protein expression is to transfect mRNA in cells and then transfer those cells into the host. For example, bone marrow cells, autologous

T cells, or autologous dendritic cells (DC) can be transfected with the desired mRNA and then reintroduced into the host. The other method is to directly inject the naked mRNA into the host [2]. In general, injections are given intramuscularly (IM), subcutaneously (SC) or intradermally (ID), or intranodally (into lymph nodes). This eliminates the potential side effects of systemic injections while providing a localized response. However, naked mRNA is inherently labile and is rapidly degraded by the host's endonucleases and exonucleases. Furthermore, passive mRNA diffusion across the plasma membrane reduces efficacy. mRNA is modified to overcome these constraints [3]. The first change is the addition of a 5' cap to the mRNA. This cap not only

protects it from exonuclease degradation, but it is also required for elongation factors to recognize it and initiate translation. Furthermore, it prevents immune recognition of the mRNA. There are three different types of 5' caps that can be introduced. cap-0 (m7G (5') pppN1pN2p), cap-1 (m7G(5')pppN1mpNp), and cap-2 (m7G(5')pppN1mpN2mp). These can be introduced via vaccinia virus-induced capping enzymes or through co-transcriptional capping. Co-transcriptional capping can be accomplished through the use

of anti-reverse cap analogs (ARCA) or other methods such as ScriptCap™ and CleanCap™. The most common clinical trial technology is the use of m7G cap analogs (GpppG), but newer cap analogs are now used to prevent them from de-capping enzymes. Boranophosphate bonds, locked nucleic acids, imidophosphate, phosphorothiolate, and phosphorothioate are examples of these. The introduction of 5' end and 3' end beta-globin untranslated regions (UTRs) can improve mRNA translation efficiency, whereas the addition of 3' end alpha-globin UTRs can improve half-life.

In some cases, the half-life of mRNA must be reduced for short-term protein expression, which can be accomplished by increasing AU in the 3' regions of the UTR. Similarly, the addition of up to 120 bp of poly-A tail improves mRNA stability and prevents de-capping, and this is typically accomplished by inserting the corresponding sequences in the DNA template for IVT mRNA.

Using alternative methods such as microneedles, electroporation, sonophoresis (using ultrasound), iontophoresis, and jet injection can improve the efficiency of naked mRNA introduction [3]. The naked mRNA can be introduced with dissolvable microneedles containing patches or arrays of sugar excipients or other water-soluble polymers that dissolve and release mRNA when introduced into the skin. These are more efficient and effectively penetrate the skin's tough stratum corneum layer.

Viral vectors

The use of viral vectors for mRNA delivery is an effective strategy because the viruses are host-specific and thus impart specificity to the mRNA delivery [4]. For example, if a virus only targets type II epithelial cells in the alveoli of the lungs, it can be used as a delivery vehicle to deliver mRNA to those cells. Furthermore, once introduced into the cells, the viral vectors synthesize the mRNA in the cells and may also replicate, increasing the effective dose of the mRNA inside the cells. In general, pathogenic viruses are modified to remove all or a portion of their structural genes related to pathogenicity and virulence, and these are replaced by the desired sequence of mRNA. Viral-like replicon particles (VRP) are virus-like self-amplifying mRNA particles that can enter the cell and produce the desired protein of interest. Many viral vectors, including Adeno associated viral vectors, RNA viruses from the Paramyxoviridae family, retroviruses, picornaviruses, flaviviruses (Kunjin virus and Yellow Fever virus), and alphaviruses, have been considered for use in the production of VRO [3]. Viruses, on the other hand, have the disadvantage of inducing cytotoxic effects and eliciting an immune response. As a result, advanced versions of the viruses have been developed to reduce the cytotoxic effects. Despite the fact

that clinical trials with HIV-based vaccines have been plagued by difficulties, induction of immune response against the VRP does not impede induction of immune response against the mRNA vaccine [5]. A chimeric virus-like particle (VLP) has recently been reported [6].

Non-viral vectors: nano-scale systems

1. Lipid-based mRNA delivery systems

mRNA is a large, negatively charged macromolecule, the electrostatic repulsion between the negatively charged phospholipid group of the plasma membrane inhibits its diffusion into cells. To combat this, lipids, lipid derivatives, and lipid-like nanoparticles (LNP) have been used with great success for the efficient delivery of mRNA. Lipid encapsulation also protects mRNA from degradation by nucleases.

There are four different types of lipid-based materials that can be used for mRNA delivery [7].

Liposomes are the most basic, consisting of cationic or ionizable lipids that form a "lipoplex" with mRNA. Lipopolyplex can be formed by combining cationic liposomes with mRNA in complex with a cationic polymer (polyp Lex) (LPR). Furthermore, lipid nanoparticles (LNP) are used for mRNA delivery, in which a core polymer is combined with a cationic liposome for the coating of DSPE-PEG to which mRNA is adsorbed. Another approach is to combine mRNA with cationic nanoemulsion (CNE), which is composed of Squalene, Cationic lipid, a hydrophilic surfactant, and a hydrophobic surfactant. Anionic or neutral lipids naturally found in the plasma membrane were used to form liposomes and mRNA complexes in one of the very first steps in this series (lipoplex). Cholesterol (Chol), Phosphatidylserine (PS), and Phosphatidylcholine (PC) are examples (PC). They did, however, demonstrate a low encapsulation efficiency [8]. Following that, cationic lipids like 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), L-a-Di-oleoyl phosphatidylethanolamine (DOPE), and N-[1-(2,3-dioleoyloxy) propyl]-N, N, N-trimethylammonium chloride were used (DOTMA). When HIV gag protein-encoding mRNA was delivered in mice, DOTAP/DOPE in a 1:1 ratio showed promising results [9]. Cationic lipids contain an alkylated quaternary ammonium group that retains its positive charge regardless of system pH. However, cationic lipids have shown toxicity issues such as hepatotoxicity in some cases, and to avoid this, lipid-like materials (lipidoids) and ionizable lipids were used [10]. Ionizable lipids have the advantage of being cationic at low pH, which helps complex negatively charges mRNA and interact with the plasma membrane, whereas at physiological pH, they are neutral and thus exhibit reduced cytotoxicity.

2. Polymer-based mRNA delivery systems

Polymer-based mRNA complexes were found to be inefficient when compared to liposome-based mRNA delivery in preliminary studies. Polydispersity is a problem with higher molecular weight polymers, so low molecular weight polymers were created [11]. However, when compared to reagents such as lipofectamine, polymers such as Diethylaminomethyl (DEAE), dextran, poly-beta-amino-esters (PBAE), and polyethyleneimine (PEI) alone failed to elicit T cell response [9]. Furthermore, the cationic nature of PEI has

been shown to complex proteins and cause cytotoxicity when administered IV. PEI was conjugated to cyclodextrin and administered intra nasally to overcome this limitation [12]. This resulted in less toxicity and increased efficiency. Toxicity can be reduced by using biodegradable polymers such as poly (lactic-co-glycolic acid) (PLGA) and PBAE [13,14]. Due to its anionic nature at physiological pH, PLGA, on the other hand, has a low efficiency. In the presence of serum proteins, PBAE has a lower efficiency, which can be improved by conjugating it with PEG [15]. Multifunctional triblock copolymers such as Dimethyl amino ethyl methacrylate (DEAMA), DEAEMA-co-n-butyl methacrylate, and poly (ethylene glycol) methacrylate have achieved higher efficiency [16]. Charge-altering releasable transporters (CART) or self-immolation polycarbonate-block-poly(a-amino) esters release mRNA after degradation at physiological pH and endosomal escape [17]. Furthermore, dendrimers such as polyamidoamine (PAMAM) modified with fatty acid chains have been shown to effectively express mRNA-based proteins in a single dose when given IM without the need for adjuvants [18]. Such dendrimer-based mRNA vaccine delivery has demonstrated excellent anti-Zika virus, H1N1 influenza, Ebola virus, and Toxoplasma infections [19,20].

3. Lipid-polymer combined systems

The liposome can be combined with mRNA-polymer polyplexes to form lipopolyplexes. These are known as LPR. Lipopolyplexes contain ternary complexes that include a cationic peptide as well as a cationic liposome containing mRNA. PEGylated histidylated polylysine (PEG-HpK)/mRNA polyplexes and L-histidine-(N, N-di-n-hexadecylamine) ethylamide (HDHE): cholesterol liposomes have been tested as melanoma vaccines [21]. Then, as a melanoma vaccine, N-methyl imidazolium lipophosphoramidate (KLN25): histamine lipophosphoramidate (MM27) liposomes (Lip100) were combined with PEG-HpK/mRNA polyplexes for MART1 melanoma antigen [22]. Carbohydrate moieties, such as mannose, can be attached to the liposomal component of the LPR and recognized by dendritic cells, resulting in an enhanced immune response [23]. They use endosome-mediated pathways, lipid nanoparticles (LNP) have shown increased efficacy for mRNA delivery [11]. Many cationic or ionizable lipid materials, as well as biodegradable lipids, have been utilized in LNPs. These include, 1,2-di-O-octadecenyl-3-trimethylammonium propane (DOTMA), N, N-Dimethyl-2,3-bis[(9Z,12Z)-octadeca-9,12-dienyloxy] propan-1-amine (DLinDMA), and N1, N3, N5-tris(3-(didodecylamino) propyl) benzene-1,3,5-tricarboxamide (TT3), Dlin-MC3-DMA (MC3, LP-01, ATX-100, lipid 5, cKK-E12 or MDI and OF-02, etc. The property of the lipid influences the delivery efficacy. Say, for example, the non-biodegradable form of OF-02 promotes mRNA expression in the liver, while the biodegradable esterified form of OF-02 promotes mRNA expression in the spleen. Similarly, cKK-E12 has a saturated fatty acid component and is less efficient, while OF-02 has an unsaturated fatty acid component with higher efficiency of mRNA delivery.

In a typical LNP, there is a lipid shell such as that of DSPE-PEG: DOTAP: DOPC, which coats a polymer such as poly (b-amino ester) (PBAE). The mRNA is not encapsulated but adsorbed over the surface [24,25]. The

diameter of this LNP is 280nm. The presence of PBAE improves interaction with mucosal cells, whereas the presence of PEG prolongs circulation time after parenteral injection. Because the lipid has a membrane disrupting property, mRNA escape from the endosome is facilitated. In recent years, there has been a lot of interest in LNP research, and TT3 LNP targeting spike protein of SARS-CoV-2 has been tested [26]. Cationic nanoemulsion (CNE) with 86nm diameter in the core has been used to deliver mRNA vaccines against HIV (gp140), RSV (fusion glycoprotein), and CMV (glycoprotein B) with promising results. Because the majority of the constituents have already been tested in clinical trials, the development of CNE is thought to be safer than that of LNP [27].

References

1. Zeng C, Zhang C, Walker PG, et al. Formulation and Delivery Technologies for mRNA Vaccines. Curr Top Microbiol Immunol. 2020 Jun 2.
2. Selmi A, Vascotto F, Kautz-Neu K, et al. Uptake of synthetic naked RNA by skin-resident dendritic cells via macropinocytosis allows antigen expression and induction of T-cell responses in mice. Cancer Immunol Immunother. 2016 Sep;65(9):1075-83.
3. Wadhwa A, Aljabbari A, Lokras A, et al. Opportunities and Challenges in the Delivery of mRNA-based Vaccines. Pharmaceutics. 2020 Jan 28;12(2).
4. Usme-Ciro JA, Campillo-Pedroza N, Almazán F, et al. Cytoplasmic RNA viruses as potential vehicles for the delivery of therapeutic small RNAs. Virol J. 2013 Jun 7;10:185.
5. Wecker M, Gilbert P, Russell N, et al. Phase I safety and immunogenicity evaluations of an alphavirus replicon HIV-1 subtype C gag vaccine in healthy HIV-1-uninfected adults. Clin Vaccine Immunol. 2012 Oct;19(10):1651-60.
6. Zhitnyuk Y, Gee P, Lung MSY, et al. Efficient mRNA delivery system utilizing chimeric VSVG-L7Ae virus-like particles. Biochem Biophys Res Commun. 2018 Nov 10;505(4):1097-1102.
7. Midoux P, Pichon C. Lipid-based mRNA vaccine delivery systems. Expert Rev Vaccines. 2015 Feb;14(2):221-34.
8. Martinon F, Krishnan S, Lenzen G, et al. Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. Eur J Immunol. 1993 Jul;23(7):1719-22.
9. Pollard C, Rejman J, De Haes W, et al. Type I IFN counteracts the induction of antigen-specific immune responses by lipid-based delivery of mRNA vaccines. Mol Ther. 2013 Jan;21(1):251-9.
10. Landesman-Milo D, Peer D. Toxicity profiling of several common RNAi-based nanomedicines: a comparative study. Drug Deliv Transl Res. 2014 Feb;4(1):96-103.
11. Kowalski PS, Rudra A, Miao L, et al. Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. Mol Ther. 2019 Apr 10;27(4):710-728.
12. Tros de Ilarduya C, Sun Y, Düzgüneş N. Gene delivery by lipoplexes and polyplexes. Eur J Pharm Sci. 2010 Jun 14;40(3):159-70.
13. Green JJ, Shi J, Chiu E, et al. Biodegradable polymeric vectors for gene delivery to human endothelial cells. Bioconjug Chem. 2006 Sep-Oct;17(5):1162-9.
14. Capasso Palmiero U, Kaczmarek JC, Fenton OS, et al. Poly(β-amino ester)-co-poly(caprolactone) Terpolymers as Nonviral Vectors for mRNA Delivery In Vitro and In Vivo. Adv Healthc Mater. 2018 Jul;7(14):e1800249.
15. Kaczmarek JC, Patel AK, Kauffman KJ, et al. Polymer-Lipid Nanoparticles for Systemic Delivery of mRNA to the Lungs. Angew Chem Int Ed Engl. 2016 Oct 24;55(44):13808-13812.
16. Cheng C, Convertine AJ, Stayton PS, et al. Multifunctional triblock copolymers for intracellular messenger RNA delivery. Biomaterials. 2012 Oct;33(28):6868-76.
17. McKinlay CJ, Vargas JR, Blake TR, et al. Charge-altering releasable transporters (CARTs) for the delivery and release of mRNA in living animals. Proc Natl Acad Sci U S A. 2017 Jan 24;114(4):E448-e456.
18. Abedi-Gaballu F, Dehghan G, Ghaffari M, et al. PAMAM dendrimers as efficient drug and gene delivery nanosystems for cancer therapy. Appl Mater Today. 2018 Sep;12:177-190.
19. Chahal JS, Fang T, Woodham AW, et al. An RNA nanoparticle vaccine against Zika virus elicits antibody and CD8+ T cell responses in a mouse model. Sci Rep. 2017 Mar 21;7(1):252.

20. Chahal JS, Khan OF, Cooper CL, et al. Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose. Proc Natl Acad Sci U S A. 2016 Jul 19;113(29):E4133-42.
21. Mockey M, Bourseau E, Chandrashekhar V, et al. mRNA-based cancer vaccine: prevention of B16 melanoma progression and metastasis by systemic injection of MART1 mRNA histidylated lipopolyplexes. Cancer Gene Ther. 2007 Sep;14(9):802-14.
22. Pichon C, Midoux P. Mannosylated and histidylated LPR technology for vaccination with tumor antigen mRNA. Methods Mol Biol. 2013;969:247-74.
23. Perche F, Benvegnu T, Berchel M, et al. Enhancement of dendritic cells transfection in vivo and of vaccination against B16F10 melanoma with mannosylated histidylated lipopolyplexes loaded with tumor antigen messenger RNA. Nanomedicine. 2011 Aug;7(4):445-53.
24. Su X, Fricke J, Kavanagh DG, et al. In vitro and in vivo mRNA delivery using lipid-enveloped pH-responsive polymer nanoparticles. Mol Pharm. 2011 Jun 6;8(3):774-87.
25. Phua KK, Staats HF, Leong KW, et al. Intranasal mRNA nanoparticle vaccination induces prophylactic and therapeutic anti-tumor immunity. Sci Rep. 2014 Jun 4;4:5128.
26. Zeng C, Hou X, Yan J, et al. Leveraging mRNA Sequences and Nanoparticles to Deliver SARS-CoV-2 Antigens In Vivo. Adv Mater. 2020 Oct;32(40):e2004452.
27. Brito LA, Chan M, Shaw CA, et al. A cationic nanoemulsion for the delivery of next-generation RNA vaccines. Mol Ther. 2014 Dec;22(12):2118-2129.