CELL & GENE THERAPY INSIGHTS

GENE DELIVERY/GENE EDITING
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SPOTLIGHT

Process & analytical insights for GMP manufacturing of mRNA lipid nanoparticles

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The successful development and rapid deployment of the messenger RNA (mRNA) vaccines against SARS-CoV-2 virus during the COVID-19 pandemic has catalyzed the industry to look even more closely at the technology beyond their potential use for novel vaccines to enable breakthrough treatments for cancer, rare diseases and more. Indeed, the mRNA and lipid nanoparticles (LNP) technologies that underpin the COVID-19 vaccines have far-reaching potential to transform modern medicine. However, as a relatively new technology, there remain barriers to successful industrialized manufacture of LNP-encapsulated mRNAs (mRNA–LNPs).

The manufacturing of the mRNA–LNP drug product can be broken down into five key steps (Figure 1): DNA template manufacturing, mRNA drug substance synthesis and purification, mRNA–LNP formulation and purification, fill/finish operations, and analytical testing. This article will first examine each step and discuss challenges and opportunities pertaining to the process itself and for the manufacturing facilities.

Cell & Gene Therapy Insights 2022; 8(4), 621–635

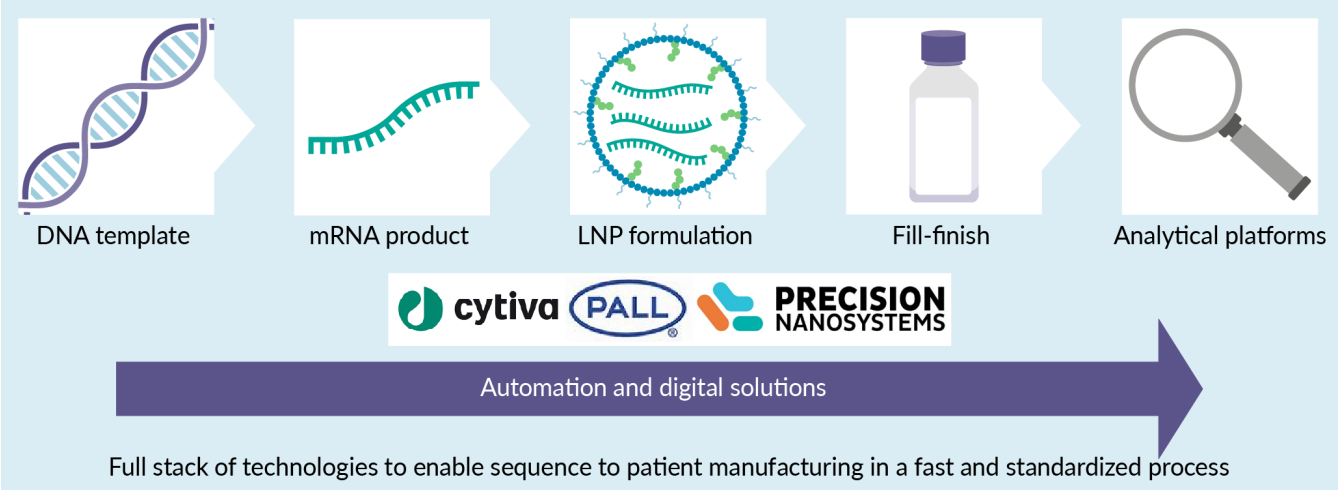
DOI: 10.18609/cgti.2022.095

INTRODUCTION

The past decade has seen an extraordinary leap in our knowledge of the human genome and its role in health and disease, which has driven exciting advancements in precision genomic medicines that are now poised to revolutionize ways to improve human health. The wealth of sequencing and bioinformatics data has provided deep insight into genomic and epigenetic factors that contribute to the underlying molecular causes of diseases. Combined with decades of research in drug development and manufacturing, this perfect storm of innovation has made it possible for pharmaceutical innovators to develop novel nucleic acid-based

FIGURE 1

mRNA–LNP therapeutic production process global overview.



drugs that can act upon the genetic instructions defining the disease itself.

Among genomic medicine modalities, RNA-based therapeutics comprise a rapidly expanding category of drugs accelerated even further by the clinical success of the mRNA COVID-19 vaccines to combat the SARS-CoV-2 virus. The mRNA and LNP delivery technology that forms the foundation of these vaccines has gained tremendous attention recently but is built upon years of research and groundwork by dedicated scientists [1–3]. mRNA as the technological basis of therapeutics and vaccines offers a great flexibility with respect to production and application. The mRNA backbone's physicochemical characteristics are unaffected by changes in the encoded antigen, allowing for the establishment of product-agnostic manufacturing platforms that can be standardized and easily adapted to new sequences [4] – a game-changing technology well-suited for rapid pandemic responses.

Additionally, while the recent focus has been their use for vaccines, RNA-based therapeutics have immense potential for similar approaches in the fight against cancer and other diseases. Researchers are investigating the therapeutic potential of mRNA beyond infectious diseases, including replacement therapy for genetic deficiency, as cancer vaccines, or as adjuvants for cancer drugs [5].

Data from early phase clinical trials shows dosage levels of mRNA–LNP therapeutic drug products ranging from single to double digit milligrams per injection [6–8], two to three logs higher than the COVID-19 vaccines. The lack of long-term clinical and real-world experience warrants caution and due diligence to assess the safety/toxicity of such high doses of mRNA as therapeutics. Next-generation mRNA vaccine designs that utilize self-amplifying RNA (saRNA) or circular RNA (circRNA) molecular formats could decrease the required dosage levels or elicit a higher and more durable antigen expression. This may improve both the performance and cost of existing prophylactic mRNA vaccines and may prove useful to ease dosage requirements for other therapies. Researchers are also exploring other classes of RNA molecules such as small interfering RNA (siRNA) and microRNA (miRNA) in silencing or regulating gene expression or acting as guide RNAs as therapeutic options for a variety of diseases (reviewed in [9, 10]).

To meet the increased demand for mRNA vaccines and other RNA-based therapeutics, with the potential to range all the way from pandemic response to truly personalized treatments, cost-effective manufacturing processes at the right scales coupled with well-defined product characterization will be needed to bring forward promising new solutions to treat and cure diseases [11].

DOI: 10.18609/cgti.2022.095

DELIVERY METHODS FOR GENOMIC MATERIAL INTO HUMANS

The goal of genomic medicines is to deliver genetic information to a target cell, either to replace a defective function (monogenic disease), or to introduce an additional function to treat (as in cancer) or to prevent (as in a vaccine) disease. Gene delivery vehicles for the introduction of such genetic material (i.e., DNA, RNA, oligonucleotides) are called vectors. There are broadly two categories of vectors: viral and non-viral. The choice of delivery method depends on the intended application, technology, target tissue, and indication, but there is certainly a place for both in the genomics toolkit, each with their advantages and disadvantages (reviewed in [12]).

Viral vectors

Viruses have an innate ability to invade cells and can efficiently transduce specific cell types and tissues *in vivo*. Contemporary viral vectors are based on retroviruses (lentivirus), adenoviruses (Ads) or adeno-associated viruses (AAVs) modified to disable their replication capability. As pathogens, viruses are naturally immunogenic and can still induce significant immune responses, which not only reduce vector penetration and treatment efficacy, but can also have severe adverse health consequences. Researchers have taken steps to reduce the immunogenicity of viral vectors. Engineering viral vector capsid proteins to make them 'invisible' to the human immune system or incorporating 'suicide genes' are potential methods to alleviate this risk [13].

Non-viral delivery methods

Traditional non-viral delivery methods rely on physical methods like electroporation, passive, and ballistic delivery. The concept relies on delivering naked DNA or RNA using high voltage electroporation to increase the permeability of cell membranes to promote the entry of genetic material into the cell, but these methods are limited to *ex vivo* usage. *In vivo* delivery of RNA is particularly challenging since naked RNA is quickly degraded by extracellular RNases and is not internalized into cells efficiently (reviewed in [14]). A great deal of work has been put towards developing transfection reagents that can protect RNA from degradation and facilitate its cellular uptake. A prime example are the LNPs, which were utilized for the COVID-19 vaccines. The LNP encapsulates the mRNA within a protective shell, protecting it from nuclease degradation. The lipid composition of the LNP facilitates entry into the target cells by endocytosis where RNA is released into the cytosol via endosomal escape mechanism (reviewed in [15]).

LNPs have demonstrated a promising record of safety and tolerability for repeat treatment. Billions of doses of the mRNA–LNPs have been administered during the COVID-19 pandemic with mostly brief and mild adverse events reported after two doses [8]. Additionally, clinical trial data for Onpattro™, an siRNA-LNP drug, reported comparable reactions with the placebo from repeated infusions every 3 weeks for 18 months [16]. Being non-viral, the risk of genome integration is also low. This is attractive for genome editing applications, particularly since LNPs also can package multiple RNA payloads within the same formulation, allowing for Cas9 mRNA and single guide RNAs (sgRNA) to be packaged together [17]. Co-formulation of multiple RNAs reduces the pharmacokinetic and regulatory complexity of such drugs. LNPs can package and deliver large payloads such as 11 kb single-strand self-amplifying RNA, which promises to increase RNA potency by orders of magnitude. LNPs can encapsulate RNA that encodes for any protein antigen with minimal change to chemical characteristics, easing the burden of multi-product manufacturing. This platform technology provides opportunities for lipid raw materials to be pre-purchased, while common equipment and analytical methods can be used to produce RNA drugs

across vastly different indications, thus shortening the drug development duration.

As a relatively new technology, innovative solutions, expertise, and out-of-the-box thinking will need to coalesce to address challenges and bottlenecks in manufacturing to truly realize the transformative potential of this disruptive technology. Below, we summarize the process for manufacturing an mRNA drug product and examine important factors and considerations for both process and analytical development.

PROCESS & GMP MANUFACTURING

1. Process overview

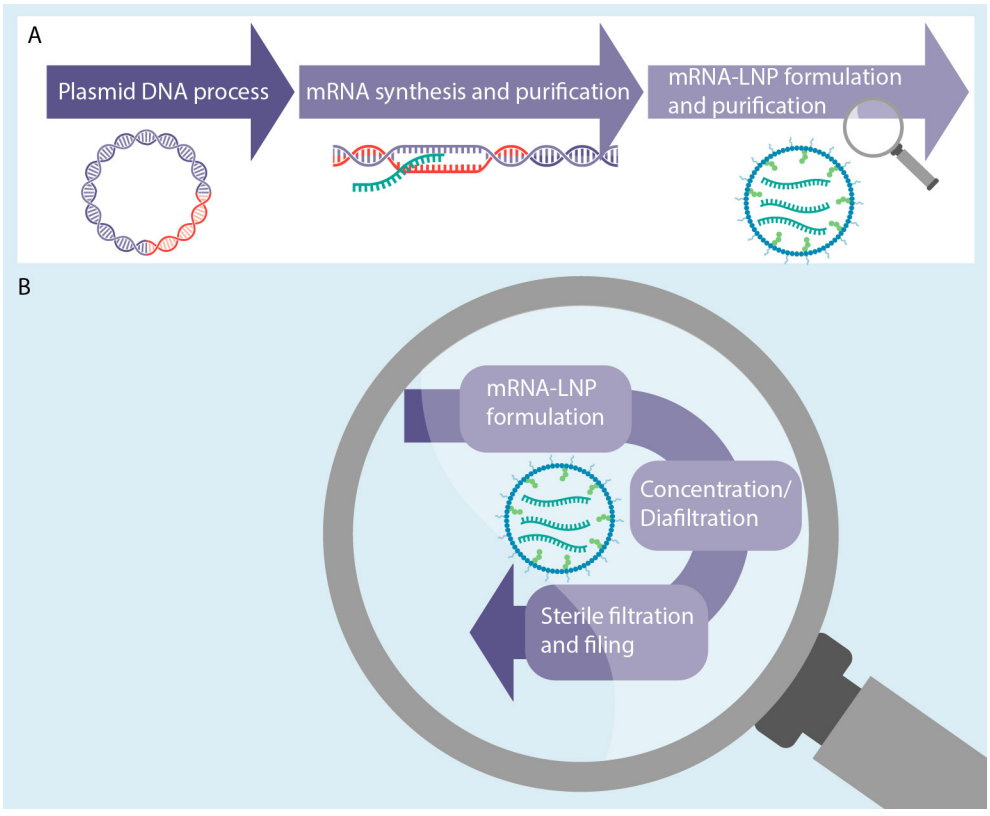
The manufacturing process of mRNA–LNPs is composed of three different key sub-processes: plasmid manufacturing, mRNA synthesis and purification and mRNA–LNP formation and purification (Figure 2 A & B).

1. **Plasmid (pDNA) manufacturing:** Production of pDNA is a microbial process utilizing *E. coli* fermentation. Cell lysis is required to release the intracellular pDNA, which is followed by a series of downstream purification (DSP) steps to remove impurities and host cell contaminants (e.g., endotoxin) to achieve high purity and quality [18]. This is especially important because these attributes can impact the overall yield of the following mRNA cell-free synthesis step.

2. **Cell-free mRNA synthesis:** Frequently known as the *in vitro* Transcription (IVT), this step relies on a

FIGURE 2

mRNA–LNP manufacturing process sequence from pDNA production to mRNA–LNP bulk filling.



A: Overall mRNA–LNP process sequence; **B:** Detailed process steps of the mRNA–LNP formation and purification process.

DOI: 10.18609/cgti.2022.095

series of enzymatic reactions. First, the pDNA needs to be linearized to act as a template for mRNA production and capping. After mRNA synthesis, purification usually includes tangential flow filtration (TFF) steps using either flat sheets or hollow fibers. A capture step follows TFF, using resin or membrane chromatography in either bind/elute or flow-through mode. Formulation (concentration/diafiltration with TFF) and finally, sterile filtration (0.2 µm) prepares the bulk drug substance, which can then move to LNP encapsulation [19].

3. **mRNA–LNP formation:**
This process usually consists of three steps: rapid mixing of the mRNA and lipid solutions to create encapsulated mRNA–LNPs, a concentration/diafiltration TFF step to remove residual solvents and concentrate the mRNA–LNPs drug product in the desired buffer formulation, followed by a final 0.2 µm sterile filtration. The product can also be further formulated as required and processed through filling operations [19].

2. Important process considerations

Despite the success and large-scale production of mRNA vaccines for COVID-19, mRNA technology is not yet mature and there is no single, standardized manufacturing workflow. Many of the technologies used are designed for other processes (e.g., monoclonal antibody production), which can be a challenge to reconcile with the unique requirements of mRNA–LNPs manufacturing [20]. However, it is promising to see that there are initiatives amongst the solution providers to develop tailored, scale-appropriate products specifically for mRNA therapeutics production. The absence of standardized protocols means manufacturers must develop and optimize their process, leading to a considerable number of process variations in both upstream and downstream processes. There are many variables and decisions throughout the production workflow that will greatly impact the equipment selection, setup, batch cost, and throughput. In this article we discuss a few of the more challenging steps, but optimization is key to the whole process, from IVT to final drug product.

Capping strategy

The choice of the mRNA capping strategies is one of the key process decisions. The cap is a methylated guanosine at the 5′ end of the sequence, is essential for mRNA maturation, and allows the ribosome to recognize the mRNA for efficient protein translation. The cap also stabilizes mRNA by protecting it from nuclease digestion. The cap can be added in two ways, either co-transcriptionally, or enzymatically as a separate reaction from the IVT. Co-transcriptional capping is less expensive and faster than enzymatic capping since it occurs during the IVT step, in the same reactor mix. However, capping efficiency and yield are typically lower and can lead to the formation of non-capped impurities or cap analogs incorporated in the wrong orientation.

Enzymatic capping is achieved in a separate reaction after mRNA purification from the IVT mixture. This reaction usually uses a vaccinia virus-capping enzyme to add the capping structure to the mRNA. While enzymatic capping has a very high capping efficiency, it is more expensive and requires an extra unit operation. This results in a longer process, which can decrease the total process yield, increase the consumables used and therefore impact the overall process cost.

mRNA–LNP formulation

The formulation method to create mRNA–LNP molecules involves mixing lipids dissolved in an organic solvent with RNA in an acidic buffer to induce spontaneous self-assembly. This is governed by complex intermolecular interactions between the RNA and four different lipids species. Since physical properties of the LNPs such as size and morphology are intricately tied to their biodistribution and

function, fine control over both the chemistry and the mixing environment in which self-assembly occurs is vital to ensuring the uniformity and quality of the particles [21]. Highly specialized expertise is required to design and optimize the right combination and proportions of lipid species, buffers, and solvents, to effectively deliver the RNA drug substance for a defined therapy. A reproducible method for mixing RNA and lipids is also necessary to ensure a uniform population of particles and batch-to-batch consistency. Additionally, a scalable process is desired to minimize process redevelopment when translating from bench to clinic. Both T-junction and microfluidic mixing have been reported extensively for LNP production. T-junction mixing is a continuous process suitable for large scale production, with typical flow rates of 40–60 mL/min. However high flow rates and high minimum volumes make small scale production for formulation screening and development more challenging. Emerging technologies based on microfluidic mixers offer access to non-turbulent, well-controlled mixing environments and ensure the scalability from lab to manufacturing scale. The staggered herringbone mixer (SHM) has been used extensively for preclinical development (reviewed in [22]). With flow rates on the order of 10's of mL/min SHM is well suited for preclinical scale production. Multiple mixers have been arrayed in parallel to increase throughput for larger scale production [23]. Recently, Precision NanoSystems have developed a toroidal microfluidic mixer that is scalable from tens of mL/min to hundreds mL/min to enable scalable manufacturing from RNA screening [17] to clinically relevant scales [22, 24]. Ensuring that LNPs can be manufactured at bench scale and scaled up to commercial scale, at high quality and yield, will be crucial to support the industry as it looks to translate mRNA medicines beyond vaccines to more advanced therapies.

Formulation considerations

Decisions made during the final TFF formulation and sterile filtration steps may also impact production outcomes, since the encapsulated mRNA–LNP intermediates are shear sensitive. The choice of TFF consumables such as the selection of hollow fiber or flat sheet cassettes, the molecular weight cut off threshold and the sterile filter membrane type needs to be carefully evaluated together with the processing conditions of each of operation to maximize process efficiency while minimizing impacts to product quality (i.e., LNPs size and average size distribution). The time it takes to execute the process may need to be balanced by the stability of the product or examined in the context of overall facilities usage. Ultimately, good process knowledge and planning for future demands early in process development can mitigate risk and enables cost and time-efficient decisions.

Capital equipment

Today, many capital investments are made while products are still in the early-stage process development. As mRNA technologies are still evolving, the key for mRNA manufacturers is to build in flexibility with modularized single-use equipment. This can mitigate risk and enable rapid reconfiguration to accommodate different manufacturing scenarios for optimized facility utilization across different products and at different scales. While not required for process development, employing single-use equipment for cGMP production may be beneficial to expedite technology transfer and scale up to manufacturing for clinical use.

Filling operations

There can be a strategic benefit to having an in-house filling platform that can solely support advancement of drug candidates within your own pipeline instead of being reliant on outsourced organizations who are juggling the priorities of many clients. As mRNA therapeutics move towards the personalized scale there will be a greater need for process control and risk reduction. Regulatory agencies place greater emphasis on process control as the scale decreases.

DOI: 10.18609/cgti.2022.095

Filling machines can generate particles that can contaminate the fill containers used, which is especially challenging with a translucent product as with mRNA–LNP formulations where visual product checks aren't possible. As well, conventional filling solutions often have issues with tipping or broken vials requiring manual intervention, which presents opportunities for product contamination. Robotic filling systems capable of closed, aseptic operations mitigates the risk of particulate contamination and need for manual correction, while also enhancing process control, and provide flexibility for multi-products. Moreover, the current mRNA–LNP formulations require storage at -80 C to extend their shelf-life, therefore the final container and closure combinations must be capable of maintaining their integrity under these conditions. There have been also efforts to enhance mRNA–LNP stability when stored at 2–8 °C or even at ambient temperature, including lyophilization strategies [25], which is expected to reduce the cost on cold-chain logistics and drug storage.

As important as flexible and scalable platform technologies are to the rapid development of mRNA-based therapeutics, they would not be possible without rapid, robust, accurate, sensitive, and scalable analytical technologies. In the next section, we discuss key requirements and considerations for analytical technologies used for process development and in-process and product-release testing.

3. Analytics

However controlled and reproducible the manufacturing process, confirmation of the critical quality attributes (CQA) is an essential part of batch release and similar information informs process decisions at the critical control points (CCP). As such, fit-for-purpose analytics needs to be demonstrated to have the required performance characteristics for the intended use. This is essential to provide crucial information with respect to process performance and product quality during manufacturing to ensure the quality, purity, potency, safety, and stability of mRNA therapeutics. Where there is a choice of analytical method, the merits of in-line, at-line, and off-line testing can be balanced against the impact of the proposed assay on the process flow. For example, off-line assays may be slow but may provide greater accuracy or sensitivity and are more acceptable for final batch release than in-process testing. Whereas the analytical method chosen for in-process measurements may prioritize turnaround time given comparable sensitivities, especially for process operations that cannot proceed until the analytical results are available.

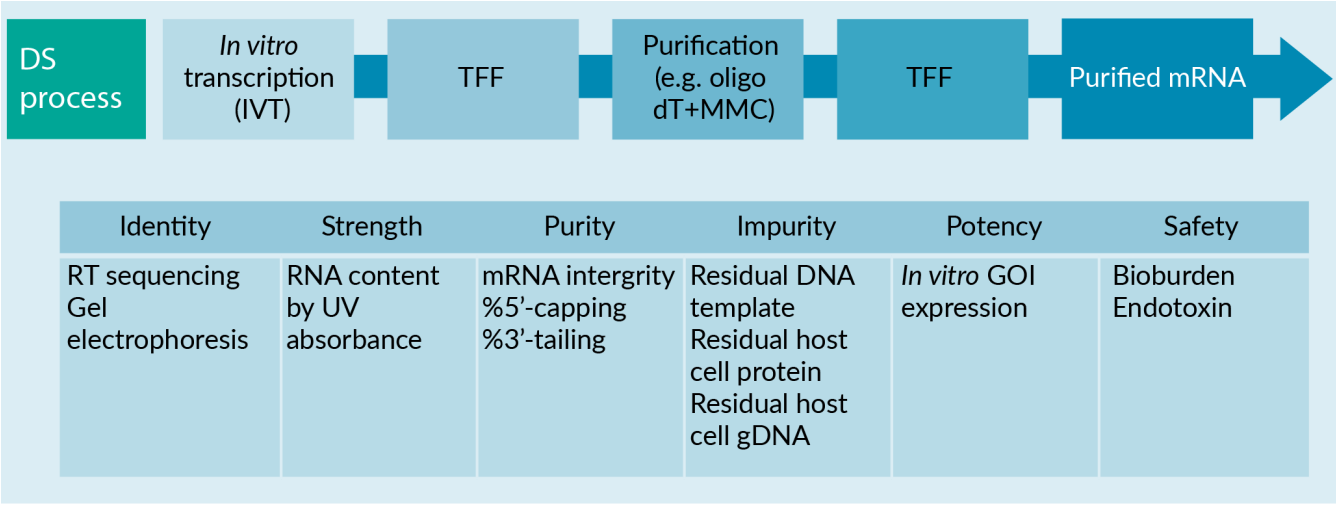
The manufacturing process of mRNA-based modalities involves linearized plasmid DNA as starting material, purified mRNA as drug substance, and formulated mRNA–LNP as the drug product, all of which require analytical testing for in-process controls, product release, and stability programs. Plasmid DNA and mRNA are large molecules and LNPs are complex nanostructures. As a result, a suite of complementary tools and technologies are required to cater to the wide range of product quality attributes testing with the resolution and speed needed in a manufacturing setting. Figure 3 and Figure 4 below depicts examples of analytical methods that are typically required for drug substance and formulated bulk processes.

Analytical methods for the drug substance process

During the drug substance process, mRNA molecules are synthesized via enzymatic reactions resulting in a mixture of product variants, including different 5′-cap structures (i.e., Cap0 vs Cap1), variable 3′-polyA tail length, and truncated mRNA transcripts. Other notable impurities include double-stranded RNA molecules and residual plasmid DNA templates. Additionally, the incorporation efficiency of modified nucleotides, if used in the IVT process, should be checked. Despite purification steps to remove these unwanted

FIGURE 3

Analytical methods for drug substance process (i.e. mRNA IVT and its purification).



Identity	Strength	Purity	Impurity	Potency	Safety
RT sequencing Gel electrophoresis	RNA content by UV absorbance	mRNA intergrity %5'-capping %3'-tailing	Residual DNA template Residual host cell protein Residual host cell gDNA	*In vitro* GOI expression	Bioburden Endotoxin

byproducts, a small portion may be carried through the process. Therefore, such product quality attributes should be characterized during in-process and lot release testing of the purified mRNA.

Analytical methods for the formulated bulk process

During the formulated bulk process, lipids are introduced into the process stream to be combined with the purified mRNA molecules. During formulation, the mRNA will be encapsulated into the LNPs forming a large and complex nanostructure, with different physical and chemical characteristics compared to the individual parent mRNA and lipid molecules. Advanced particle analytical assays are required to assess particle sizing and polydispersity index (PDI), which can impact final biological function. In addition, the surface charge on LNP has critical impact on the gene expression. Analytical assays that characterize the surface charge, such

FIGURE 4

Analytical methods for formulated bulk process (i.e., mRNA encapsulation and formulation).

Identity	Strength	Purity	Impurity	Potency	Safety
mRNA ID Lipid ID	RNA content by florescence assay	Encapsulation efficiency LNP size (mean and PDI) Lipid purity	Residual DNA template Residual DNA template Residual host cell gDNA Double-stranded RNA Lipid-related impurities	*In vitro* GOI expression	Bioburden Endotoxin

DOI: 10.18609/cgti.2022.095

as TNS assay and Zeta potential assay, should be included in the product characterization package [26].

In-process analytics

In mRNA processes, product concentration determination is required to quantify the performance of chromatography and TFF steps, to ascertain the load conditions, and to calculate the step yield. Spectroscopic methods to determine concentration determination, such as UV absorbance or fluorimetry are relatively rapid and easy analytical procedures. On the other hand, quantitative profiling of purity-related product quality attributes is more complex due to the large size and near-identical physical and chemical characteristics of the mRNA molecules and their variants. For example, mRNA transcripts with Cap0 and Cap1 on the 5′ end only differ by 14 Da in their molecular masses [27]. As a result, high resolution analytical technology is needed to resolve such subtle differences.

For nucleic acid analysis including pDNA and mRNA, separation sciences such as capillary electrophoresis (CE), high performance liquid chromatography (HPLC), and coupled technologies such as liquid chromatography-mass spectrometry (LC-MS), are powerful tools for high resolution analysis. Different analytical methods can be implemented on advanced CE systems [28], depending on the separation mechanism (i.e., capillary gel electrophoresis (CGE), capillary isoelectric focusing (cIEF)). CE with UV or laser-induced fluorescence (LIF) enables separation of molecules based on their hydrodynamic radius. Generally, pDNA for clinical applications should be > 80% supercoiled (vs. open or circular) [29]. CE-LIF has been demonstrated to be an automated and reproducible method for the rapid quantitative analysis of pDNA purity and to distinguish pDNA isoforms. Similarly, CE-LIF can be used to analyze the size variants of mRNA transcripts (i.e., intact, or truncated transcripts, poly-A tail length profiling). Advanced LC-MS technologies [28] combine the capability of high-resolution separation by LC and accurate mass detection by MS to offer a multi-purpose analytical platform well-suited for mRNA characterization. This includes mRNA 5′-cap analysis, poly-A tail analysis, and lipid identification based on accurate mass of both the intact molecular ions and their associated fragment ions. It should be noted that mRNA drug substance and formulated LNP samples have significant differences in the characteristics of their sample matrices. Therefore, assay optimization is required to ensure fit-for-purpose performance of the advanced analytical technologies.

It should be also noted that mRNA–LNP based therapeutics are a novel class of advanced therapies. Thanks to the COVID-19 pandemic, several mRNA vaccines have been brought to the market via Emergency Use Authorization (EUA) and later received fully marketing authorization. Given the relative novelty of the technologies and accelerated development timelines, we have limited experience with respect to their CMC, non-clinical, and clinical performances, product quality analysis and specification. While there are sporadic reports on the quality aspects of some mRNA assets [30], there is a lack of regulatory and industry-level guidance and standardization for the specific critical quality attributes to be targeted, release criteria and specifications setting strategies, which is still an actively evolving area. That said, establishing a comprehensive suite of analytical technologies and testing strategies to provide critical insights into product quality and to inform process development and manufacturing decisions, will continue to benefit the advancement of new development programs.

4. Data management

With the relatively short processing time for mRNA there is a potential to have high throughput workflows with many batches per year in the manufacturing line. As a result, data management and batch release can become a potential bottleneck, regardless of the

production scale. Investment into automated data management solutions can support efficient batch release and enable readiness for future production capacity. Manufacturing equipment with integrated software that can log data electronically can be implemented into existing workflows across a continuum, from discrete islands of automation, to fully integrated and connected platforms. Resource planning systems, electronic production records, process control systems, data historian, Manufacturing Execution System (MES) systems and centralized data repository (and off-site backup) are key technologies for GMP manufacturing. They also enable robust data management to support process development, characterization, and efficient tech transfer. These digital platforms can provide on-demand access to process data that can streamline product manufacturing, testing, and release.

5. Facility considerations

The sensitivity of mRNAs to RNase contamination dictates that the mRNA manufacturing should be separated from other cell-based processes. If possible, it might be beneficial to build a dedicated manufacturing environment for mRNA altogether to eliminate the risk. Multi-product, -process designs can be incorporated if there is a need to manufacture multiple mRNA products in the same facility/manufacturing line, that can support efficient changeover between products as well as reducing the risk of contamination can increase facility flexibility.

As an important side note, the mRNA–LNP is formulated using a solvent injection technique, and thus manufacturing facilities need to be designed to handle this specialized process as well as the volumes of reagent needed for the intended manufacturing scale. Be aware that different global regulations apply, making it necessary to work with the local authorities to get the correct approvals.

mRNA based vaccines have a great potential for pandemic preparedness and localized vaccine manufacturing. Modular prefabricated facility designs have become a consideration for these initiatives either as stand-alone or nested into existing facility space. They offer a pathway to decentralized vaccine production and to bring it to the point of need with speed, flexibility, and predictability. Several modular solutions have been announced lately in collaboration with vaccine manufacturers to improve global production of the COVID-19 vaccine. However, as the applications for RNA therapeutics continues to broaden, the most flexibility will be achieved with an end-to-end technology provider that can cover the entire process from IVT to filling and that is agnostic to what product will be manufactured in the facility.

WHAT'S NEXT?

As stated earlier, mRNA–LNPs are manufactured using technologies that were developed originally for traditional biologics such as mAbs and viruses. Manufacturing technologies designed specifically to meet the production scale and maximize productivity of mRNA–LNPs are needed as demand continues to increase for vaccine development and other modalities. Drug developers and solution providers will need to work together to understand the emerging needs to find the best fit solutions.

Notably, IVT is currently the most cost-driving step in the mRNA process where the raw materials (i.e., RNA polymerase and nucleotide triphosphates) represent 60–65% of the total cost of goods. Alternatives to batch production with different vessel designs, or eventually a continuous reaction flow, could be of interest to potentially increase the overall process productivity and result in the lowest utilization of costly reagents.

The pDNA template is an essential raw material for IVT, which is also used in viral vector applications, another growing area of biologics. The converging demand of pDNA has strained the supply of GMP quality pDNA, which is creating bottlenecks

DOI: 10.18609/cgti.2022.095

for manufacturing. One possible solution to overcome this limitation would be to use a cell-free technology, such as rolling circle amplification, to generate the DNA template. Such technologies offer a faster, simpler, and cleaner process that may be of interest for personalized therapies as well.

LNPs represent another area of focus for further development and optimization to define formulations to improve thermostability. Currently available mRNA vaccines must be stored frozen, resulting in a complex supply chain that limits their utilization in low-income countries. In addition, engineering tissue specificity can improve targeted mRNA drug delivery with the potential to reduce off-target effects, dosage levels to better manage drug safety/toxicity [12]. LNP surface functionalization, including antibody conjugation, may also present future opportunities for improving tissue targeting of mRNA–LNP for their broader applications in various therapeutic areas. Developing the LNPs systematically, by screening libraries and optimizing parameters, and then modeling the full unit operation at small scale can facilitate these goals and de-risk future manufacturing.

Some leading mRNA companies are also partnering with artificial intelligence (AI) companies to employ rational design approaches often used in biomolecular engineering to mRNA design. Sequence design, prediction modeling, manufacturability analysis, and other metrics are bringing insights and opportunities for example in the development of a personalized cancer vaccine/ immunotherapy, where tumor genome sequencing is used to create a patient-specific drug regimen. In this case predictive algorithms compares the genomic sequence of the tumor to healthy tissue and identifies the set of tumor-specific neoantigens that would elicit the strongest anti-tumor immune response with the lowest side effects for the patient. The drug manufacturer can then use this information to develop RNA vaccines and bring truly personalized medicines to realization. Another example of partnerships is between RNA companies and machine learning and cloud providers to efficiently and quickly be able to design research experiments, find insights, automate laboratory and manufacturing processes, simplify technology transfer and more easily comply with regulations during production and testing of vaccine and therapeutics candidates. This has been demonstrated in an ongoing collaboration between Moderna and Amazon Web Services (AWS) [31].

Finally, from a regulatory perspective, because of the novel composition of the RNA vaccines, regulatory criteria and standards have yet to be fully defined. Regulatory agencies are constantly modifying their guidance on both the manufacturing drug product requirements. To overcome this, more effort in product characterization and analytics are needed. A globally harmonized standard and implementation of manufacturing control strategies is crucial for the wide adoption of mRNA–LNP as a novel modality. Experience from biologics modalities reveals some noticeable divergence of interpretation of ICH guidelines related to control strategy [32]. Future mRNA–LNP development should leverage the experience learned from the accepted regulatory framework established by the COVID-19 vaccines as part of the EUA, to accelerate regulatory approvals across different countries and agencies.

CONCLUSION

There are numerous tools in the genomic medicine toolbox, and it will be crucial to identify the right tool for a given application (disease to be treated, tissue to be targeted, duration of expression needed, level of reactogenicity) while balancing the benefit and risk to the patient and cost burden to the health care system. mRNA–LNPs represent a promising addition to the expanding repertoire but, with the relative newness of the technology there is still uncertainty surrounding the best approaches to process development and manufacturing with the best process economics. We have highlighted

some key challenges and areas for improvement for mRNA–LNP manufacturing and emphasize the value of partnering with end-to-end solution providers who can provide evolving and tailored strategies to support changing industry needs. As manufacturing challenges are resolved, we will truly see influence of mRNA technology in the future trajectory of vaccine development, oncology, and personalized medicine.

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AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *This paper was a collaborative work of Pall Corporation, Cytiva, and Precision Nanosystems.*

Disclosure and potential conflicts of interest: *E Cameau and P Zhang are an employees of Pall Corporation, S Ip is an employee of Precision Nanosystems, L Mathiasson and K Stenklo are employees of Cytiva. The authors disclose that Pall Corporation, Cytiva and Precision Nanosystems owns patents relevant to the Biotech Industry. The authors have no other conflicts of interest.*

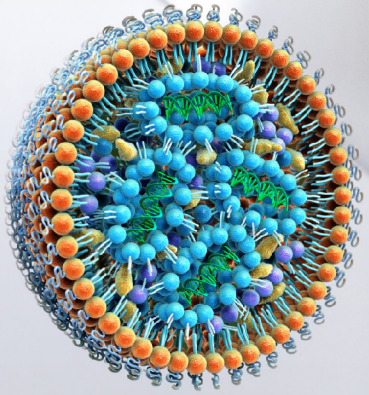
Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *May 11 2022;* ***Revised manuscript received:*** *Jun 9 2022;* ***Publication date:*** *Jul 13 2022.*

DOI: 10.18609/cgti.2022.095



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