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Processing challenges in realizing the future potential of mRNA vaccines and therapeutics

After the physician and biochemist Robert Malone first posited the medicinal potential of mRNA in the late 1980s, progress in mRNA vaccines and therapeutics was slow [1]. However, with the success of mRNA vaccines on the proving ground of the COVID-19 pandemic, mRNA products have cemented their future in biotherapeutics. mRNA biotherapeutics have significant clinical potential not only as vaccines, but also as a form of gene therapy and the treatment of cancers and are growing rapidly across the biopharmaceutical industry. As of 2020, mRNA therapeutics had a global market value of roughly $360 million, and the sector is expected to see a compound annual growth rate of 16.8% from 2020 to 2026 [2]. Developing or scaling up mRNA therapeutics, therefore, can help manufacturers stay competitive in a growing biopharmaceutical industry and revolutionize treatments for challenging diseases.

Messenger RNA (mRNA) is an endogenous cellular component composed of a single strand of nucleic acids. It is a critical component of cellular gene expression, as it instructs cells to create particular proteins. This function can be exploited for vaccines by using mRNA to teach cells to craft a protein characteristic of the virus or bacteria that is being vaccinated against, such as the spike protein of SARS-CoV-2. Likewise, mRNA can instruct cells to make an endogenous protein to compensate for a protein that is missing or defective, thereby treating the illness caused by the lack or defect of that protein. Additionally, mRNA could also be used to instruct patient cells to make protein therapeutics, such as monoclonal antibodies, effectively turning the cell into the API manufacturer.

Because mRNA is an endogenous cellular component—meaning that it originates within cells—mRNA vaccines and therapies do not induce an unwanted immune response. The proteins resulting from the mRNA biotherapeutic's introduction into cells produce a targeted effect by either replacing necessary proteins or inducing antibodies to fight a particular protein, like the spike protein of SARS-CoV-2. So far, mRNA vaccines against COVID-19 are the only FDA-approved mRNA therapies. In the future, however, mRNA may be used to prevent or treat other infectious diseases or cancers, alleviate allergies, and replace missing or defective proteins.

Much of the potential benefit of mRNA therapeutics comes thanks to a robust, simple and scalable manufacturing process, compared to other biotherapeutics like recombinant proteins or gene therapies. Despite this processual simplicity, manufacturers of mRNA therapeutics face significant challenges, many of which stem from the inherent instability of mRNA molecules and a limited supply of high-quality chemicals and materials. In this article, we will explore pain points in the major stages of the mRNA bioprocessing workflow.

GMP plasmid manufacturing

mRNA therapeutic production starts with the production of plasmid DNA (pDNA)—small, circular DNA sequences found in bacteria. The gene of interest is inserted into a plasmid, which is then inserted into a bacterial cell, usually E. Coli, by transformation that may include electroporation. The cells replicate during fermentation before they are harvested and the pDNA is extracted and purified.

pDNA production is often outsourced to contract manufacturing organizations (CMOs) and therefore can be expensive and have long lead times, but one main pain point is the purification stage.

Chromatography plays the key role in pDNA purification, and as such, advancements in chromatography techniques will provide solutions to many purification problems. For example, one major challenge impacting chromatography involving plasmid DNA is due to the large size of the molecule, which inhibits its ability to diffuse into the pores of chromatography resins [3]. Addressing this challenge through optimizing the pore size will increase the accessibility of the pDNA, resulting in higher dynamic binding capacities and increased production efficiency. Chromatography of pDNA is further complicated by the fact that the molecule's intrinsic highly negative charge is also characteristic of impurities such as RNA. This similarity in charge between pDNA and impurities makes it difficult to separate contaminants using traditional anion-exchange (AEX) chromatography resins. Typically, these resins would separate negatively charged impurities from a positively charged desired product, or vice versa. Because this difference in charge does not exist between pDNA and the impurities that result from its production, this method is ineffective. Multimode ion exchange (IEX) resins can increase selectivity in favor of pDNA over any impurities.

In vitro transcription

In vitro transcription (IVT) is a critical step in mRNA vaccine and therapy production. This process, the enzymatic conversion of linearized pDNA template to mRNA, requires polymerase, nucleotides and capping enzymes. IVT is relatively uniform between companies because a few particular enzymes are key to the process. As such, availability of raw materials is crucial to the maintenance of a production timeline. As mRNA production scaled up during the pandemic, the challenges of raw material availability became apparent, as supply of plasmids and enzymes currently struggle to match demand. Global plasmid supply is only a fraction of what would be needed to produce just one billion doses of an mRNA vaccine, and enzyme availability suffers not only from short supply in general but also from variable quality [4].

Furthermore, a reliable supply of consistently high-purity cGMP reagents, such as dithiothreitol, magnesium chloride, Tris HCl and sodium chloride that are well characterized and mitigate the risk of impurity contamination during mRNA production, is crucial. The issue of material availability is exacerbated by the fact that, while being relatively standard, IVT processes for mRNA biotherapeutics are not optimal. As processes evolve, fewer raw materials may be needed. In the meantime, however, it is crucial that the supply chain improves to meet manufacturers' needs since shortages of raw materials will likely be a major pain point as mRNA biotherapeutics come into greater use.

mRNA purification

Following the IVT reaction, mRNA must be purified and contaminants, such as IVT reaction components and solvents, must be removed. The main purification challenge for mRNA-based vaccines is lack of well-established and cost-effective large-scale process [3]. Several purification techniques are in use, such as precipitation, tangential flow filtration, or chromatographic separations. However, precipitation and size exclusion chromatography (SEC) are not cost-effective when scaled up. Affinity chromatography resins for oligonucleotides—short single strands of DNA or RNA used in biologic applications, including mRNA for therapeutics-can be used as a capture step, but this process is unable to separate single-stranded RNA (ssRNA) from double-stranded RNA (dsRNA) and has a low binding capacity [3, 4]. Hydrophobic interaction chromatography (HIC) and anion exchange (AEX) chromatography can be used and are often combined in capture or intermediate purification stages, but large-scale production requires careful optimization of conditions [3, 4].

Chromatography resins will continue to be an instrumental tool in the purification of biologic molecules, but improvements will need to be made to better serve the industry, especially for the purification of plasmid DNA and RNA. As upstream processing continues to increase titers of these biologic molecules, improving the current chromatography resins to increase the dynamic binding capacity while enhancing separation to remove process-related impurities will be critical for manufacturing of these biologic therapeutics.

Formulation, Fill, and Finish

Before mRNA products can be deployed as a therapeutic, the mRNA itself must be encapsulated to protect it from degradation and to facilitate its delivery into the body. The most common formulation for mRNA vaccines and therapies is lipid nanoparticle (LNP) encapsulation. A wide range of buffers, sugars, salts and surfactants prevent mRNA degradation during manufacturing and handling. These products also improve the stability of mRNA vaccines and therapies, prolonging shelf-life.

Beyond the availability of key reagents and excipients, the encapsulation stage faces challenges in mixing required components within closed fluid handling systems. For example, T-junction mixing—a common method for generating emulsions that involves the meeting of two perpendicular streams at a T-junction—provides greater control than other production methods, but appropriate scale of the T-junction is difficult to achieve due to the high flowrates required to achieve rapid mixing. [7] Furthermore, clogging of the T-junction is a common problem manufacturers face at this stage. Off-the-shelf single-use components may not meet the unique needs of a particular workflow, but not all suppliers have the ability to design and product single-use fluid handling systems.

Conclusion

The potential of mRNA pharmaceuticals is vast, but there many process challenges remain to be solved. Not the least among these is availability of cGMP chemicals and materials, especially because small vendors have monopolized individual reagents. The many starting materials required to mRNA vaccine or therapy manufacture may come from several different companies. This can provide supply chain flexibility, but it can also be a risk if the supplier of a necessary process component is unable to meet demand. As such, it is crucial that researchers and manufacturers work closely with suppliers to meet evolving needs. A globally integrated supply chain with built-in redundancies will be key for ensuring the adaptability and resilience of production processes. Just as important will be access to process experts who can identify challenges and work toward solutions across the workflow, ensuring quality and efficiency.

As scientists and manufacturers work to develop innovative mRNA pharmaceuticals, it is crucial that they have access to cGMP chemicals and materials. Avantor and our Innovation Centers around the world are monitoring emerging opportunities and challenges in mRNA research and production, including scale up, and crafting solutions to facilitate progress. Toward our mission of setting science in motion to create a better world, Avantor supplies a wide range of GMP chemicals and reagents, chromatography

resins and aseptic fluid handling solutions that, coupled with the expert support of our associates, helps researchers and manufacturers develop and scale up mRNA vaccines and therapies by addressing pain points in the process. Our associates work to create end-to-end solutions to simplify all aspects of the mRNA workflow, enabling researchers and manufacturers to stay competitive in the biopharmaceutical industry and focus on developing and producing solutions to health problems.

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ABOUT THE AUTHORS

Dr. Navjot Kaur
Business Segment Manager

Navjot specializes in mRNA and gene therapy workflows to provide solutions in the biomanufacturing of mRNA vaccines and therapeutics. She has a Ph.D. in Biology and twenty-plus years of experience in biopharma/biotechnology spanning from automation in early discovery to synergizing workflows for commercial production.

Dr. Jonathan Fura
Manager, Research & Development

In his current role, Jonathan focuses on developing technologies that are utilized to advance the development and manufacturing of antibody-based biopharmaceuticals and diagnostics. Jonathan holds a Ph.D. in chemistry with a focus on developing immunomodulation therapeutic strategies.