Advancing mRNA Vaccine Manufacturing: Innovations, Challenges, and Solutions

Claire Chong | Technical Application Specialist

EXPERTISE IN BIOPHARMACEUTICAL

Contents

1 Introduction to mRNA Vaccines

What is mRNA

❖ **What is mRNA?**

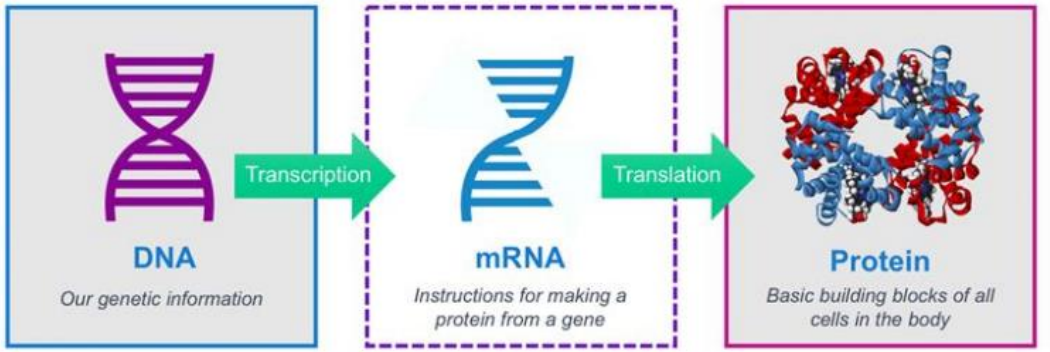
- mRNA (***messenger RNA***) is a molecule that carries instructions from DNA in the cell nucleus to the ribosomes, where proteins are made.
- It acts as a messenger, allowing genetic information from DNA to be used to build proteins.

❖ **How is mRNA Made?**

- **Transcription:**
  - The process of mRNA synthesis begins in the cell's nucleus.
  - An enzyme called ***RNA polymerase*** reads a section of DNA (a gene) and creates a complementary strand of mRNA.
- The mRNA strand carries the gene's instructions for making a specific protein and then leaves the nucleus.

❖ **Where Does mRNA Function?**

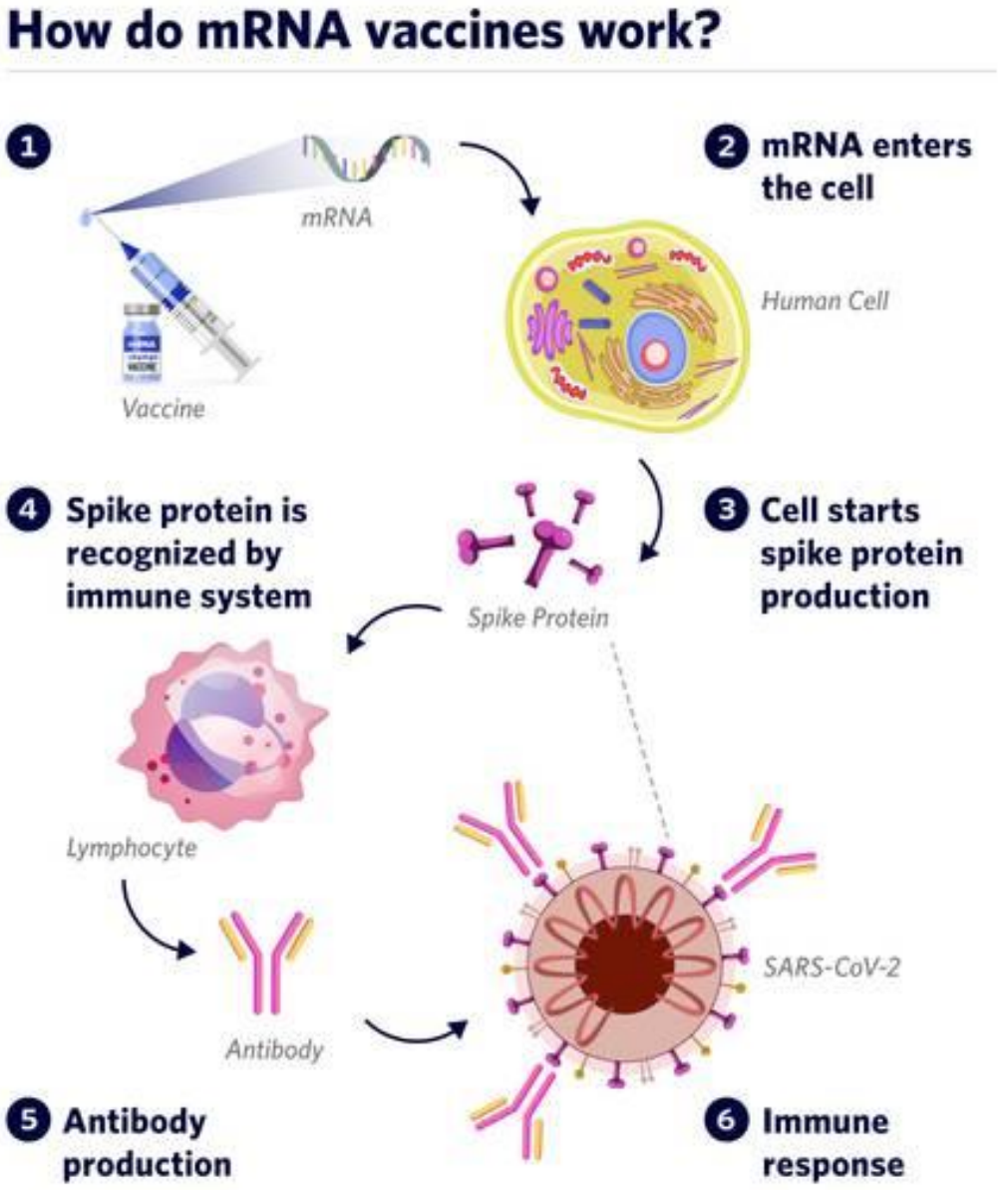
- **Translation** in the Cytoplasm:
  - After exiting the nucleus, mRNA travels to ribosomes in the cell's cytoplasm.
  - Ribosomes read the mRNA sequence and use it to assemble amino acids into a protein in a process called translation.



Introduction to mRNA Technology in Vaccines

❖ Introduction to mRNA Vaccines:

- mRNA vaccines use **synthetic mRNA** to instruct cells to produce a protein that **triggers an immune response**.
- Unlike traditional vaccines, mRNA vaccines do not use live virus components, making them faster to develop.
- Rapid development during the COVID-19 pandemic showcased mRNA vaccines' flexibility, speed, and efficacy.
- mRNA vaccines offer an opportunity to address both endemic and pandemic diseases.



Source: https://beyond.ubc.ca/nutshell-how-vaccines-work

mRNA Vaccine Showing Satisfying Performance

Higher delivery rate than DNA vaccine

DNA is supposed to penetrate nucleus to allow transcription to happen, while translation happens in cytoplasm, where is easier to penetrate.

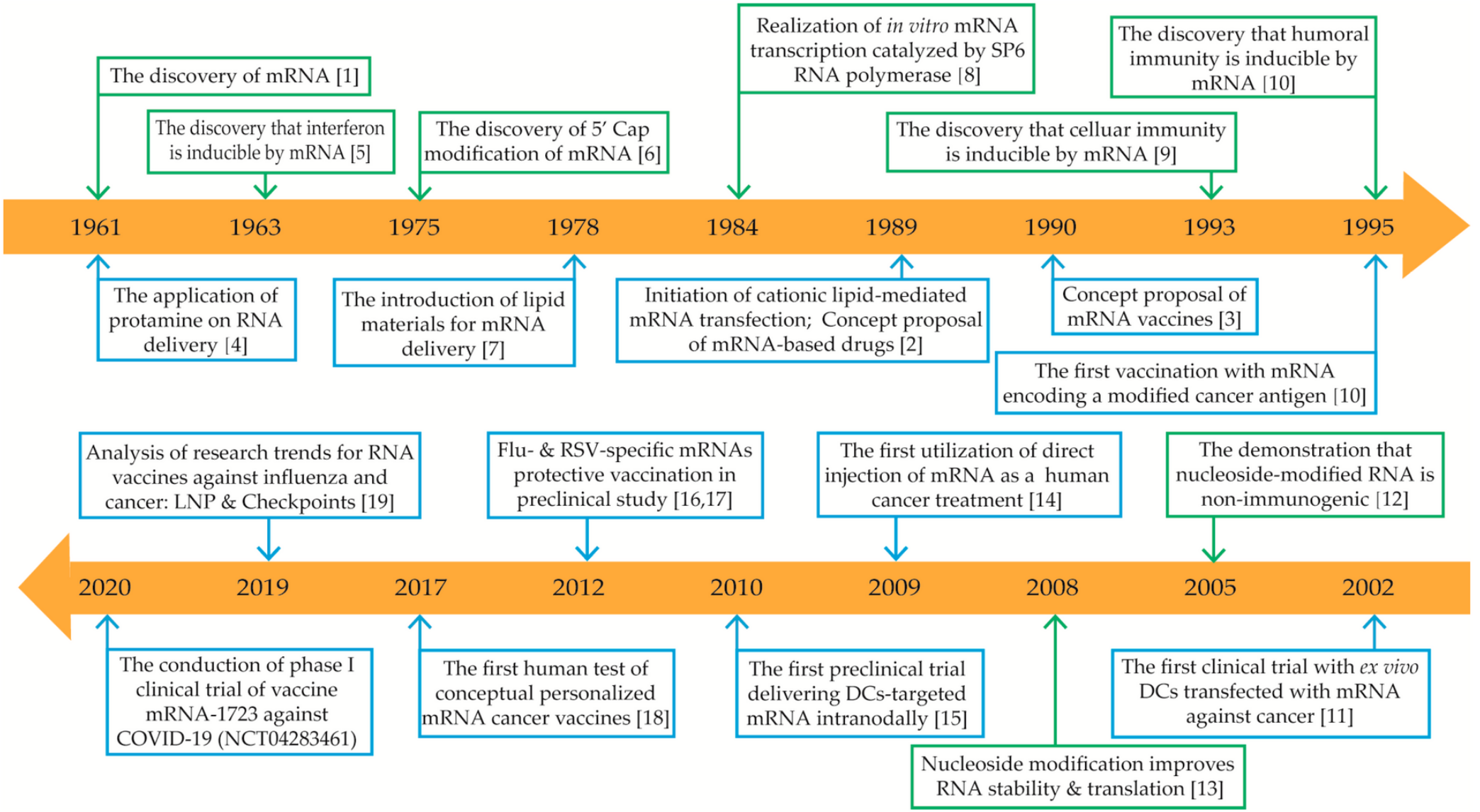
Faster to manufacture, easier to manufacture in large quantities

Produced by high yields of *in vitro* transcription reactions, potential for rapid, inexpensive and scalable manufacturing.

Higher Safety and efficacy

1. Manufacturing process does not involve toxic chemicals or cell culture, avoid adventitious viruses;
2. Short manufacturing time presents few opportunities to introduce contaminating microorganisms.

Timeline of some key discoveries and advances in the development of mRNA-based drug technology



Source: mRNA Vaccine Era—Mechanisms, Drug Platform and Clinical Prospection. Int. J. Mol. Sci. 2020, 21, 6582. https://doi.org/10.3390/ijms21186582

2 End-to-End Manufacturing Process

mRNA Manufacturing Process

Plasmid preparation

1 E.coli seed

2 Flask

3 Expansion

4 Fermentation

5 Harvest

6 Lysis

7 Clarification

8 UF/DF

9 Chromatography (2-3 steps)

10 UF/DF

11 VF

12 FFT

mRNA preparation

13 Plasmid linearization

14 Purification (1-2 steps)

15 UF/DF

16 IVT

17 Purification

18 UF/DF

Formulation

19 LNP

20 UF/DF

21 Sterile filtration

Fill & Finish

22 Fill/finish

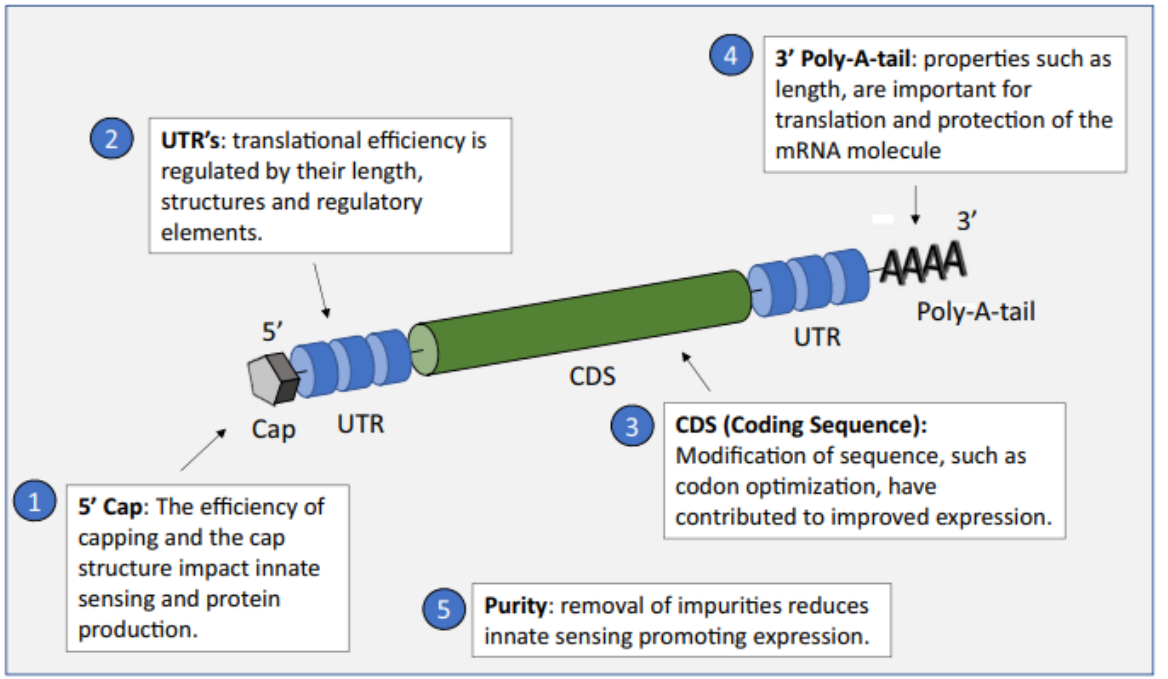
23 Packing

24 Storage

3 Challenges and Technological Innovations

5 Critical Quality Attributes

Source: The promise of mRNA vaccines: a biotech and industrial perspective. npj Vaccines 5, 11 (2020). https://doi.org/10.1038/s41541-020-0159-8

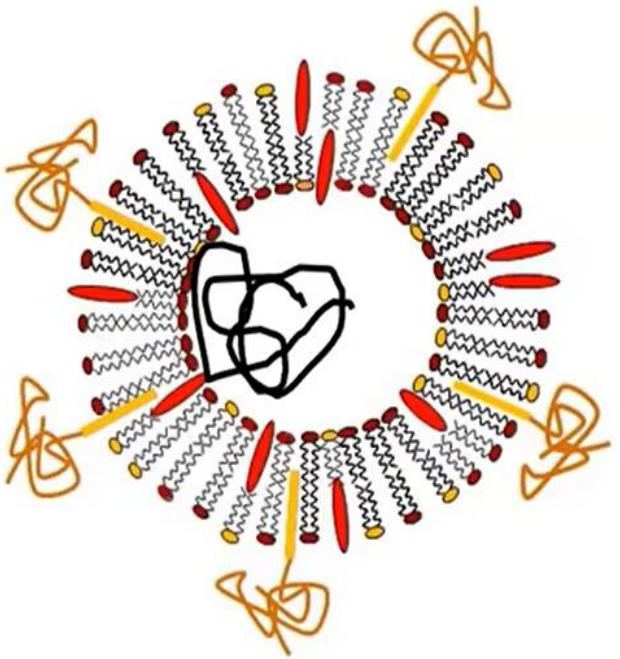


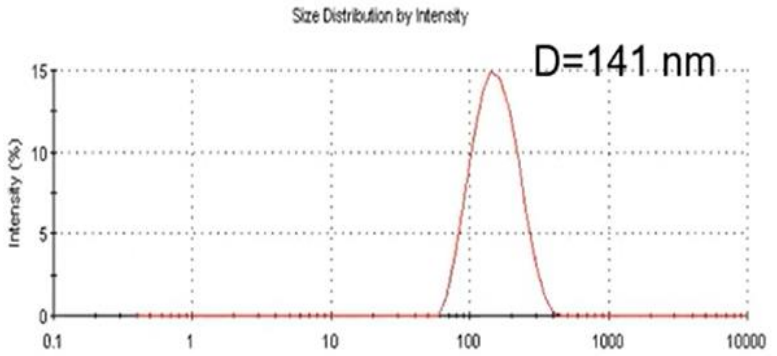
1. **Capping**
   - The 5' cap (m7G cap) is essential for stability and translation. It **prevents degradation** and **enhances protein production**, impacting both effectiveness and immune response.
2. **5' and 3' UTR Regions**
   - The UTR regions at both ends of the mRNA help **maximize gene expression** and stability. Their length and specific elements affect how efficiently the mRNA is translated into protein.
3. **Coding Sequence (CDS)**
   - Optimization of the mRNA sequence itself, like choosing specific codons, improves how well the mRNA is translated into protein.
4. **Poly-A Tail**
   - The 3' Poly-A tail protects mRNA and ensures efficient translation. Its length also impacts the stability and **longevity of the mRNA.**
5. **Purity of mRNA**
   - Ensuring high purity reduces impurities that can trigger unwanted immune responses. Impurities can come from incomplete RNA production.

Technical Challenges in mRNA Vaccine Manufacturing

- **mRNA Stability and Degradation**
  - ☹ Challenge: Sensitive to degradation by enzymes, requires strict contamination control
  - ✓ Solution: Specialized equipment and controlled conditions; use of stabilizing elements like 5' cap and Poly-A tail
- **Efficient Transcription Process**
  - ☹ Challenge: In vitro transcription produces by-products (e.g., double-stranded RNA) that can trigger immune reactions
  - ✓ Solution: Optimized IVT conditions and advanced purification to reduce impurities
- **Purity of Final mRNA Product**
  - ☹ Challenge: High purity needed to avoid immune reactions and ensure efficacy
  - ✓ Solution: Use of advanced purification methods like chromatography and filtration
- **Lipid Nanoparticle (LNP) Formulation**
  - ☹ Challenge: Requires precise control over lipid composition and assembly for stability and cell uptake
  - ✓ Solution: Rigorous control over formulation conditions to achieve consistent particle size and efficacy

LNP-Encapsulation





Component		Function
Neutral Lipid		Particle base
Cationic Lipid		RNA Loading
Cholesterol		Particle stabilization
PEG - Lipid		Particle stabilization Charge shielding
Self-replicating RNA		Antigen expression

Technical Challenges in LNP Formulation for mRNA Vaccines

- **Precise Composition & Size Control**
  - Need for uniform particle size and lipid ratios for stability and effectiveness.
- **LNP Stability**
  - Sensitive to temperature and mechanical stress, requiring ultra-cold storage to prevent degradation.
- **Encapsulation Efficiency**
  - High efficiency needed to reduce wastage and ensure effective dosing.
- **Consistency & Scalability**
  - Difficult to scale up while maintaining batch-to-batch consistency.
- **Shear Sensitivity**
  - LNPs are vulnerable to mechanical stress, risking mRNA payload integrity.

Single-Use Systems as Solutions in mRNA Vaccine Manufacturing

❖ **Enhanced Sterility**

- Pre-sterilized, disposable systems minimize contamination risk and protect mRNA integrity.

❖ **Flexible Scalability**

- Modular, adaptable setups enable quick scale-up from R&D to full-scale production.

❖ **Low-Shear Environments**

- Designed for low-shear mixing to protect LNP integrity and mRNA delivery.

❖ **Streamlined Quality Control**

- Eliminates cross-contamination, reducing time and cost in quality assurance.

❖ **Cold Chain Compatibility**

- Integrates easily with cold storage requirements to maintain mRNA stability.

Practical Single-Use Setup for mRNA Vaccine Production

❖ Plasmid Production Stage

- **50L Single-Use Fermentor (SUS)** for E. coli plasmid production (~20g plasmid DNA).
- **SUS Mixing Bag (50–100L)** for alkali lysis and neutralization.
- **Small SUS Depth Filtration** (~0.5–1m²) for debris removal.
- **Mini SUS Chromatography Skid** with pre-packed columns for plasmid purification (~15–18g purified plasmid).
- **Small SUS TFF System** for plasmid concentration and buffer exchange.

❖ Linearization and IVT

- **SUS Mixer (20–50L)** for enzymatic plasmid linearization.
- **SUS IVT Reactor (10–20L)** for mRNA synthesis (~15–18g mRNA).
- **Benchtop SUS TFF + Chromatography** for mRNA purification (~12–15g pure mRNA).

❖ LNP Encapsulation and Formulation

- **Small Microfluidic Mixer** (20–100 mL/min) for LNP encapsulation (~11–14g final encapsulated mRNA).
- **Post-LNP SUS TFF System** for polishing.
- **SUS Formulation Mixer** and small **SUS Fill-Finish Skid** (500–1000 vials/hr).
- **Cryogenic Storage (-80°C)** for final vaccine product.

Yield and Dose Estimation from 50L mRNA Production Line

Parameter	Estimate
Final mRNA Amount (after losses)	~11–14 g*
Pfizer-BioNTech doses (30 µg/dose)	~370,000 to 470,000 doses
Moderna doses (100 µg/dose)	~110,000 to 140,000 doses

**Includes practical process losses (~20–30%) during IVT, purification, and encapsulation*

Key Takeaways

✅ 50L fermentation enables **commercial-scale mRNA vaccine production**.

✅ **Single-Use Systems (SUS)** support the entire process from fermentation to fill-finish.

✅ **Small footprint, rapid deployment, lower capital cost**, and **high agility**.

✅ **Ideal for pandemic response, personalized vaccines, and regional manufacturing hubs**.

Other Challenges in mRNA Vaccine Manufacturing

❖ Cold Chain Requirements

- Challenge: Ultra-cold storage (-70°C) is needed for stability, making distribution difficult, especially in regions without robust cold chain infrastructure.
- Solution: Research into formulations for higher temperature stability is ongoing.

❖ Scaling Up Production

- Challenge: Scaling up requires specialized equipment and consistent quality control, needing extensive optimization.
- Solution: Single-use bioreactors, modular facilities, and automation improve efficiency but are capital-intensive.

❖ Quality Control and Consistency

- Challenge: Consistent batch quality is essential, requiring tests for purity, potency, and stability at each stage.
- Solution: Advanced analytics ensure quality but increase production costs and require sophisticated lab setups.

❖ Regulatory Compliance

- Challenge: Evolving guidelines create uncertainty, with varying standards across countries.
- Solution: Collaboration with regulators and adapting to new guidelines is critical but complex.

4 Tech Transfer Considerations

Challenges in Tech Transfer for mRNA Vaccine Production

❖ Process Complexity and Standardization

- mRNA vaccine production involves intricate steps (e.g., IVT, capping, purification, LNP encapsulation) with many sensitive parameters.
- Lack of standardized processes across the industry adds challenges in scaling and replicating methods in new facilities.

❖ Specialized Equipment and Infrastructure

- Lipid Nanoparticle (LNP) Formulation: Requires specialized microfluidic mixing equipment; precise control of mixing ratios, rates, and temperature is critical.
- Single-use vs. stainless steel systems: Single-use technology is preferred for sterility and flexibility, yet not all facilities have compatible setups..

❖ Workforce Training and Knowledge Transfer

- Specialized Skill Requirements: mRNA production demands proficiency in RNA handling, sterility practices, and complex purification processes.
- Documentation & SOPs: Comprehensive transfer of SOPs, batch records, and troubleshooting guides are crucial to minimize process variability.

Overcoming mRNA Vaccine Tech Transfer Challenges

❖ Scalable and Standardized Equipment Solutions

- **Modular, Single-Use Systems:** Support sterility and flexibility for various production scales.
- **Specialized LNP Encapsulation:** Provide microfluidic mixers for precise LNP formulation and consistent quality.
- **Integrated Process Platforms:** Standardized setups reduce customization needs and align production steps..

❖ Process Standardization and Knowledge Transfer

- **Validation and Optimization Services:** Pilot testing and parameter optimization for process consistency.
- **Real-Time Monitoring and Data Sharing:** Digital platforms for seamless process data transfer between sites.
- **Comprehensive Documentation and Training:** User manuals, SOPs, video tutorials, and troubleshooting guides.

❖ Training and On-Site Technical Support

- **Cross-Site Training Programs:** Hands-on support and training with technical experts for smooth setup and operation.
- **Dedicated Support Teams:** Real-time troubleshooting to overcome operational challenges quickly.

❖ Reliable Supply Chain and Compliance Support

- **Supply Chain for Consumables:** Ensure a reliable supply of single-use components to avoid delays.
- **Regulatory Compliance Assistance:** Documentation support for GMP and regional compliance requirements.

Tofflon Bio-engineering

Bio Tech


SU. Cell filling system


Programmed thermostat


Manual liquid Nitrogen canister


Auto liquid nitrogen storage system


Parallel bioreactor


Glass bioreactor


SU. glass mixer


Pump


Lab chromatography system


Lab chromatography column


GMP cell drug preparation station


Beehive incubation system


SU. Cell processing system

Bio Process


Cell expansion system


Single use bioreactor


Single use fermenter


SU. filtration system


SU. chromatography system

Pre-packed column


SU. tangential flow filtration system


Compounding isolator for ADC


Disc centrifuge

Bio Pharma


SS. Bioreactor


Bio-processing Piping & Vessel System (BPVS)


Tangential flow filtration system


Depth filtration system


Chromatography system


Manual chromatography column


Auto chromatography column

Tofflon Bio-engineering

Medium | **SU. bags** | **Resin** | **Filters**





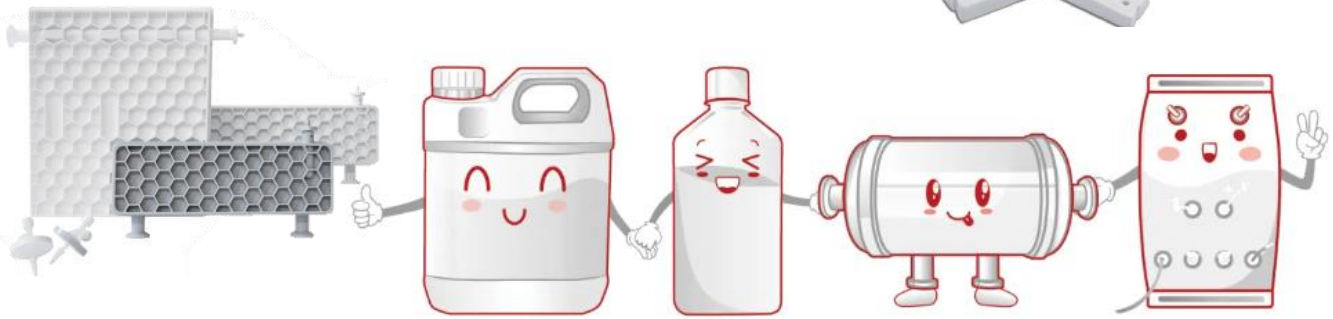










5 Case Studies and Future Outlook

Case Study 1: CSPC Pharmaceutical Group Co, Ltd.

Project Introduction

Location: Shijiazhuang, Hebei, China

Product: mRNA

Production Scale: 200L

Equipment scope of this project:

Continuous alkali lysis neutralization system, IVT transcription system, 2.5㎡ ultrafiltration system, 5㎡ ultrafiltration system, 10㎡ ultrafiltration system;

ID100, ID200 manual chromatography column, PC homogenate tank, LNP system

Case Study 2: InnoRNA (Shenzhen) Co., Ltd

Project Introduction

Location: Shenzhen, China

Product: mRNA

Equipment scope of this project:

1L Gradient chromatography, 3L Gradient chromatography

0.5-2.5㎡ Microfiltration system, 0.5-2.5㎡ Ultrafiltration system

Future Outlook of mRNA Vaccines

❖ **Expanding Applications**

- Infectious Diseases: Vaccines for influenza, HIV, Zika, CMV, and more in development.
- Cancer Vaccines: Personalized mRNA vaccines targeting tumor antigens.

❖ **Innovations on the Horizon**

- Combination Vaccines: Single-shot protection for multiple diseases (e.g., COVID-19 + influenza).
- Self-Amplifying mRNA (saRNA): Enhanced immune response with lower doses.

❖ **Overcoming Challenges**

- Improved Stability: Innovations for easier storage and transport.
- Advanced Delivery Systems: Better lipid nanoparticles for stability and efficacy.

❖ **Impact**

- mRNA technology is transforming vaccine development, offering rapid, adaptable solutions for global health.

Summary of Key Points

- **Revolutionary Impact:** mRNA vaccines offer high adaptability and faster development.
- **Process Essentials:** Key steps—synthesis, encapsulation, and formulation—ensure quality and efficacy.
- **Production Challenges**: Scalability, stability, and cold chain logistics are critical obstacles.
- **Innovative Solutions**: Advanced LNP formulations, single-use systems, and effective downstream filtration enhance production.
- **Tech Transfer Needs**: Success requires consistent quality, regulatory alignment, and adaptability to diverse facilities and expertise.

Tofflon

Tofflon Vision:

Smart Pharma Factory Builder