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CELL & GENE THERAPY INSIGHTS

NON-VIRAL DELIVERY:
MANUFACTURING AND ANALYTICS

SPOTLIGHT

EXPERT INSIGHT

Overcoming mRNA medicine supply hurdles: distributed, continuous and multi-product mRNA manufacturing in a box at high quality and low cost

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There is a growing need for solutions to develop and manufacture high-quality, safe, and effective mRNA medicines in a disease-agnostic manner. Key barriers including scalability, high production costs, and limited access to GMP-compliant facilities lead to inequitable global access to mRNA medicines. To address these challenges, our team has been innovating and digitalizing mRNA medicines production processes, by: developing continuous flow IVT, continuous purification, and continuous LNP encapsulation processes; developing novel cost reduction strategies; developing advanced analytical methods; employing computational modeling to characterize a robust quality-by-design design space, guide process development, monitor the process (via soft sensors), and enable advanced automation (via digital twins). These innovations are being integrated into a GMP-compliant RNA-production platform process in a box: RNAbox™. This will provide rapid access to this transformative technology and enable the distributed, rapid production of high-quality, low-cost medicines to combat a wide range of diseases.

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THE NEED FOR SCALABLE AND EQUITABLE mRNA MANUFACTURING

The rapid development of mRNA vaccines during the COVID-19 pandemic demonstrated the transformative potential of this technology. However, the pillars for this success were laid decades earlier through academic research on mRNA medicines [1], which had languished primarily due to inadequate funding [2]. Despite its early promise, mRNA technology struggled to transition from academic discovery to clinical application due to limited government support and a lack of early-stage investment. Nevertheless, the worldwide COVID crisis accelerated mRNA recognition as a

cornerstone of modern medicine, leading to an unprecedented surge in research on RNA medicines, including RNA-based vaccines and therapeutics [3,4]. Therefore, a balanced, long-term strategy bridging academia, government, and industry is essential to sustain this momentum and enhance the flexibility of existing facilities to adapt to evolving health challenges.

Although current RNA vaccine supply meets demand and no regulatory-approved mRNA therapeutics exist, this landscape may shift as clinical trials and approvals expand. This revolutionary expansion into mRNA therapeutics research has encouraged manufacturers to reassess and refine their processes, guidelines, and production strategies to improve adaptability and efficiency [5,6]. Beyond establishing mRNA as a transformative medical technology, the pandemic also highlighted the potential of lipid nanoparticles (LNPs) as versatile non-viral delivery systems, exemplified by the success of mRNA-LNP vaccines in enabling rapid RNA-based therapeutic development.

A key advantage of mRNA medicines is their cell-free production process, which offers significant benefits over conventional vaccine platforms. In contrast, mRNA manufacturing enables a more streamlined, inherently scalable, and potentially more cost-effective process [7], making it a compelling alternative for rapid and economically viable response to emerging health threats [8]. Moreover, cell-free mRNA medicines production, encompassing *in vitro* transcription (IVT), purification, LNP formulation, and LNP purification, could function as a platform technology. This approach enables the production of diverse mRNA-based medicines targeting a broad range of diseases using the same standardized production processes, raw materials (excluding the specific DNA template sequence), standard operating procedures, and analytical methods [5].

The versatility of IVT-based manufacturing and the success of mRNA-LNP formulations have solidified their role in the future of vaccine development and therapeutic applications, paving the way for broader adoption beyond emerging health crises, such as infectious diseases [9]. Currently, mRNA medicines are being explored for a wide range of applications, including pathogens such as bacteria, parasites, and viral infections, as well as other conditions such as cancer and even hearing loss [10]. Examples include cytomegalovirus, Ebola, Epstein–Barr virus (preclinical stage), human immunodeficiency virus (HIV), human papillomavirus (HPV), influenza, Lyme disease, malaria, monkeypox, rabies, respiratory syncytial virus (RSV), rotavirus (preclinical stage), seasonal influenza, tuberculosis, varicella-zoster virus. Trials also cover a wide spectrum of advanced malignancies, including colorectal carcinoma, melanoma, lung cancer, pancreatic tumor, prostate cancer, and head and neck cancers [10,11]. Yet, to fully unlock mRNA's potential for future epidemics, chronic diseases, and oncology, it is essential to implement the use of a versatile platform for the scalable production of RNA medicines. This would enable efficient adaptation to varying demands: scalability upwards to accommodate high-volume production, downwards for process optimization and clinical trials tailored to smaller populations or regions, and outwards for parallelized manufacturing to support individualized or personalized medicine. Moreover, cost-effective manufacturing strategies that ensure global accessibility and rapid deployment as and when needed should be considered. The critical vulnerabilities in the supply chain, manufacturing infrastructure, and global distribution of these life-saving medicines have been exposed.

Despite the witnessed speed and efficacy of the developed mRNA vaccines, their high production costs, reliance on

centralized GMP-compliant facilities, and supply chain bottlenecks have created significant disparities in access to these medicines, particularly in low- and middle-income countries. In addition, new regulatory restrictions on genetic product export/import further complicate raw material procurement and distribution. Establishing localized, flexible GMP-compliant manufacturing hubs could mitigate these supply chain risks, improving the availability of mRNA-based medicines worldwide. Traditional GMP manufacturing facilities require significant capital investment, often exceeding $500 million, and are primarily concentrated in North America and Europe. In contrast, since the pandemic, the Serum Institute of India was the largest vaccine producer by doses, showcasing India's substantial capacity [12,13]. Additionally, China, along with other Asian countries and a few in Africa, are expanding their manufacturing capabilities, with plans to build more GMP facilities, diversifying global production capacity [14,15]. In the rest of the world, the lack of local production capacity means dependency on external supply chains, longer waiting times for procurement, and higher costs per dose. As of August 2024, low-income countries had received just 333 million doses, while high-income countries, despite having a smaller population share, administered 2.8 billion doses. Globally, 64.9% of the population has been fully vaccinated, with significant disparities between income groups: only 27.8% of individuals in low-income countries are fully vaccinated, compared to 59.8% in lower-middle-income countries and 74.3% in high-income countries (Figure 1) [16–18]. Nevertheless, even within high-income countries, scalability remains a challenge—namely out-scalability, for the parallelized production of individualized cancer therapeutics, as there is limited demand for up-scalability and GMP capacity is often underutilized.

Most mRNA drug product (DP) production is still batch-based, requiring multiple subsequent phases such as IVT, purification, LNP encapsulation, and formulation. This approach introduces inefficiencies, limiting production speed and increasing costs, while supply chain vulnerabilities, such as shortages of nucleotides, capping reagents, and specialized lipids, further constrain production capacity and raise costs. While several companies—including Quantoom Biosciences, Centillion, RiboPro, Nutcracker Therapeutics, TelesisBio, Dillico, CureVac, and BioNTech—are actively developing technologies to streamline or automate mRNA manufacturing, fully integrated and truly continuous solutions remain rare. Moreover, many of these systems are designed for specific scales, applications, or infrastructure constraints that may not be compatible with decentralised or multi-scale production settings.

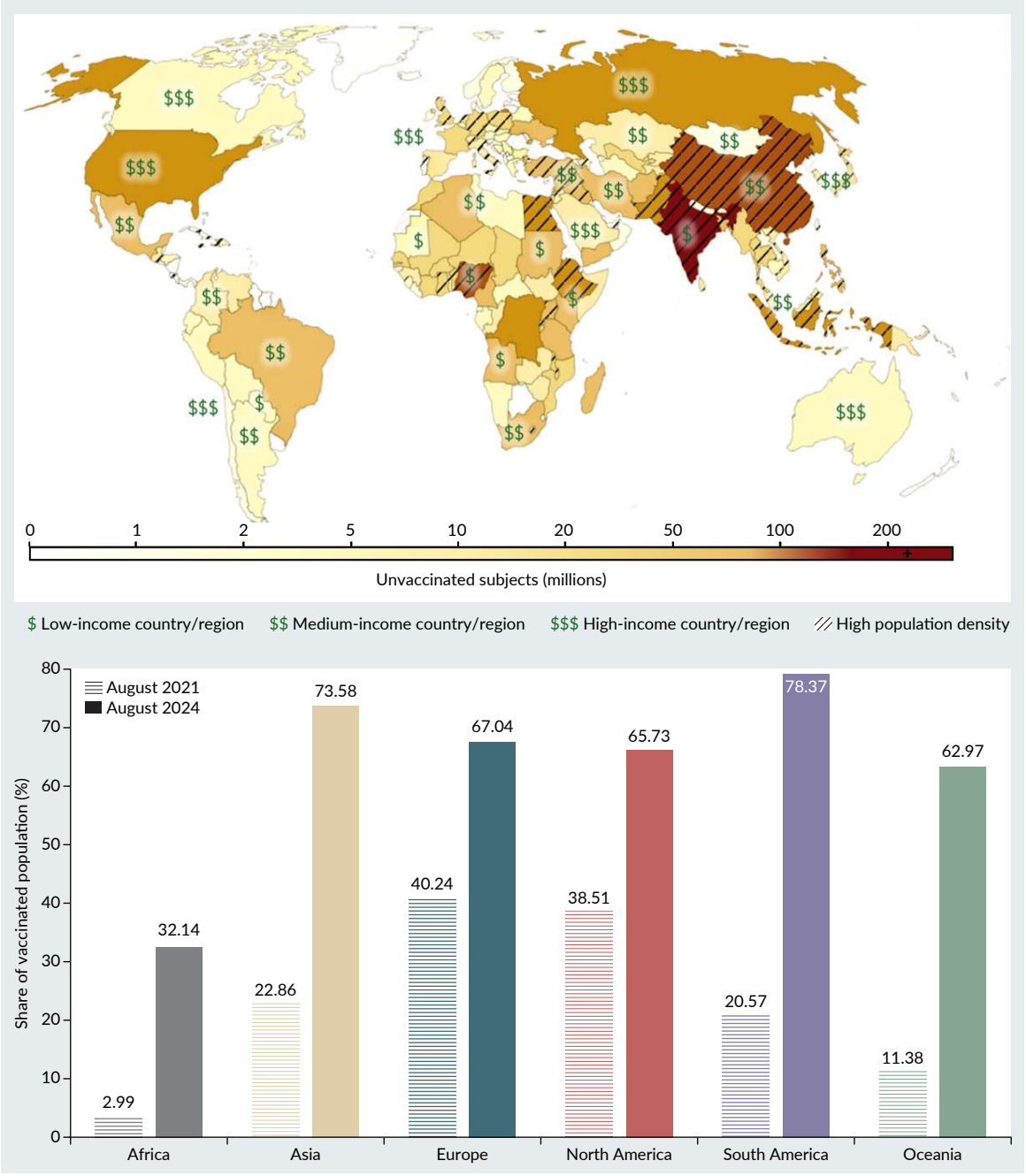
CHALLENGES IN TRANSITIONING TO CONTINUOUS MANUFACTURING

While batch-based production has enabled the initial success of mRNA vaccines, in the long term, it can pose manufacturing productivity limitations. Continuous manufacturing has revolutionized small-molecule and monoclonal antibody production, but the mRNA field still falls behind in fully integrating this approach.

The primary obstacle is that true continuous manufacturing is not yet widely implemented. Many so-called 'continuous' processes in mRNA production are semi-continuous or sequential batch processes, where unit operations run back-to-back rather than as a single uninterrupted workflow. In response, the pharmaceutical industry is currently exploring true continuous manufacturing, which integrates all production steps into a seamless, automated workflow, reducing downtime and improving efficiency. This transformation

FIGURE 1

Global distribution of unvaccinated populations by August 2024 (top), showing population density and income levels; and COVID-19 vaccine coverage by region (bottom).



Adapted from [17,18].

has been driven by bioprocess intensification, which aims to achieve higher productivity with fewer resources by leveraging smaller, modular facilities that maintain high efficiency while reducing operational complexity and cost [19]. Key technological advancements, such as single-use systems and plug-and-play equipment, have powered significant productivity gains over the past few decades. Additionally, the emergence of end-to-end integrated continuous biomanufacturing platforms, incorporating

N-1 perfusion-based bioreactors, multicolumn chromatography, simulated moving beds, true moving beds, and single-pass tangential flow filtration, has further propelled this trend towards continuous manufacturing [20–22]. The first breakthrough in continuous bioprocessing occurred in 2019, when BiosanaPharma successfully produced the first monoclonal antibody using a fully integrated continuous biomanufacturing process, demonstrating enhanced efficiency, increased yield, and significant cost reductions compared to traditional batch-based production [23,24].

Efficient mRNA production and purification strategies are critical for maintaining process performance, product integrity, and suitability for downstream operations, such as chromatography-based purification [25]. As mRNA-based therapeutics transition toward continuous manufacturing, it is imperative to ensure that Critical Quality Attributes (CQAs) remain within acceptable limits, as they directly impact patient safety and product efficacy. Figure 2 shows a list of CQAs for the mRNA drug substance (DS) after IVT, commonly referred to as 'naked mRNA'. It also includes the CQAs for mRNA formulated into lipid nanoparticles, known as the DP. These acceptance criteria, established through preclinical and clinical data, are guided by literature reviews, surveys of commercial manufacturers, United States Pharmacopeia guidelines, EMA, and WHO technical reports [26–29].

Given the rigorous purity requirements and high costs of mRNA production and purification, early impurity removal is essential to reduce downstream processing challenges and enhance scalability [30]. As continuous manufacturing advances, refining purification strategies will be crucial for achieving cost-effective and high-quality mRNA medicines. To maximize product quality and yield, it is essential to consider inputs such as plasmid DNA template quality (with enzymatic production being explored for greater purity), T7 RNA polymerase activity, and rate-limiting factors that influence IVT efficiency. These inputs directly impact CQAs like mRNA sequence identity, concentration, integrity, capping efficiency, and the length of the 3′ Poly(A) tail, as well as process-related impurities (e.g., residual DNA template, T7 RNA polymerase, free nucleosides) and product-related impurities (e.g., double-stranded RNA (dsRNA), aggregates, fragments). By carefully controlling these critical process parameters (CPPs) and critical material attributes (CMAs), one can optimize CQAs and key performance indicators (KPIs) such as cost, productivity and optimize CQAs. Furthermore, establishing a multi-product design space where various products can be manufactured with high quality and optimal KPIs.

The transition from batch chromatography towards continuous chromatography has demonstrated significant improvements in yield, purity, and efficiency. Mirroring successful adaptations of continuous chromatography in protein and other biopharmaceutical purification processes

FIGURE 2

Overview of CQAs for the mRNA DS and mRNA-LNP DP.

DS CQAs	DP CQAs
mRNA sequence identity	Lipid identity
5′ capping efficiency	Lipid content
3′ poly(A) tail length/ heterogeneity	Lipid nanoparticle size
RNA concentration	Lipid nanoparticle polydispersity
Double-stranded RNA	Zeta potential
mRNA integrity	Encapsulation efficacy
Fragment RNA percentage	Residual ethanol
Residual DNA template content	pH
Residual T7 RNA polymerase content	Endotoxin content
Residual NTP content	Potency
mRNA size	

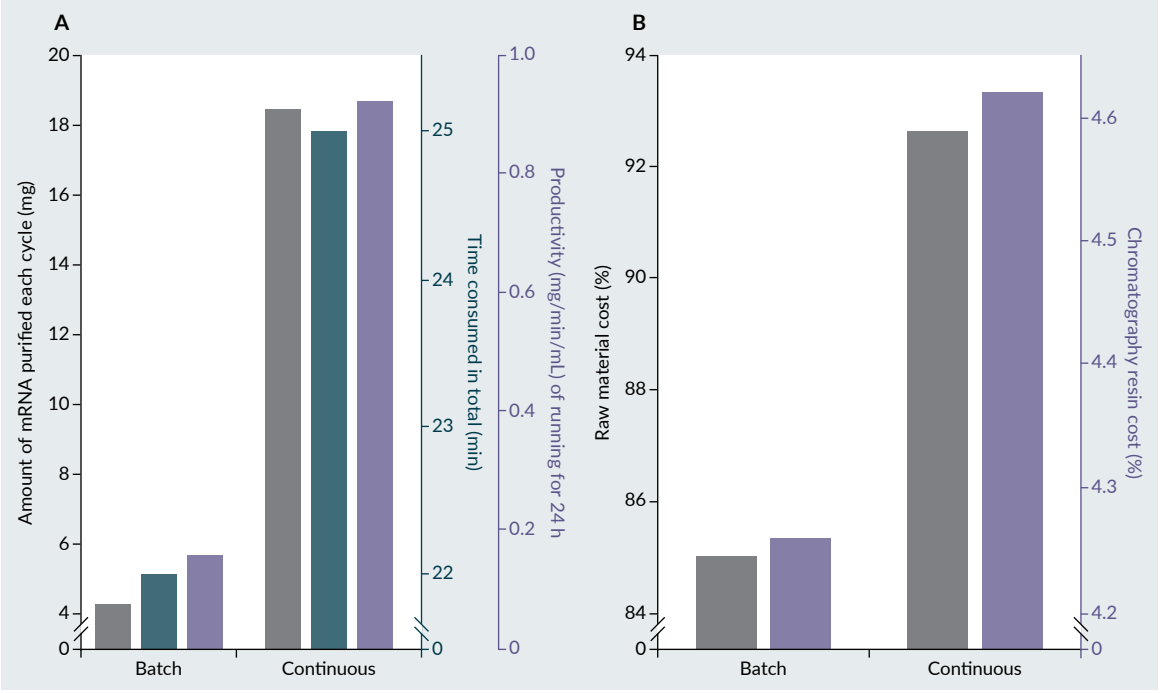
CQAs: Critical Quality Attributes. DP: drug product. DS: drug substance.

[30–32], multi-column oligo-dT chromatography has proven to be a versatile platform for purifying different mRNA sequences, including eGFP mRNA and SARS-CoV-2 Spike protein mRNA [33,34]. The ability to translate oligo-dT purification from batch to continuous mode demonstrates the feasibility of establishing a universal purification platform for multi-sequence, multi-product mRNA vaccine manufacturing.

In a comparative study of batch and continuous manufacturing carried out by our group [35], a high mRNA recovery yield of 93.62% was achieved, quantified by anion-exchange high-performance liquid chromatography (AEX-HPLC), with 41.44 mg of purified mRNA obtained from a 44.27 mg load. Additionally, mRNA integrity exceeded 95% (measured by capillary gel electrophoresis [CGE]), and overall purity surpassed 99% across all elution fractions, with no detectable nucleotide triphosphate (NTP) impurities in the elution fractions. These findings confirm that continuous chromatography enhances mRNA purification efficiency while maintaining high product quality. Moreover, the flow-through fractions collected during purification showed no presence of mRNA, reinforcing the high selectivity and efficiency of the continuous chromatography process. The productivity calculations indicated a rate of 0.92 mg/min/mL, which is significantly (5.75-fold) higher than the 0.16 mg/min/mL observed in batch chromatography (Figure 3) [36]. Furthermore, the entire continuous purification process lasted only 70 minutes, excluding equilibration and shutdown phases, further emphasizing the efficiency and scalability of the approach. However, this could be run for substantially longer time periods, ultimately limited by the life span of the chromatography columns and ligands therein. Notably, one of the key benefits of continuous chromatography is its potential to reduce operational

FIGURE 3

Comparative benchmarking details of mRNA manufacturing for batch vs continuous processes.



Adapted from [36].

costs. Initial cost assessments estimate a 15% reduction in operating expenses when transitioning from batch to continuous chromatography, mostly due to reduced losses and improved utilization of column capacity, but also reduced required facility footprint and infrastructure investments and lower labor costs, as continuous systems require less manual intervention due to automation.

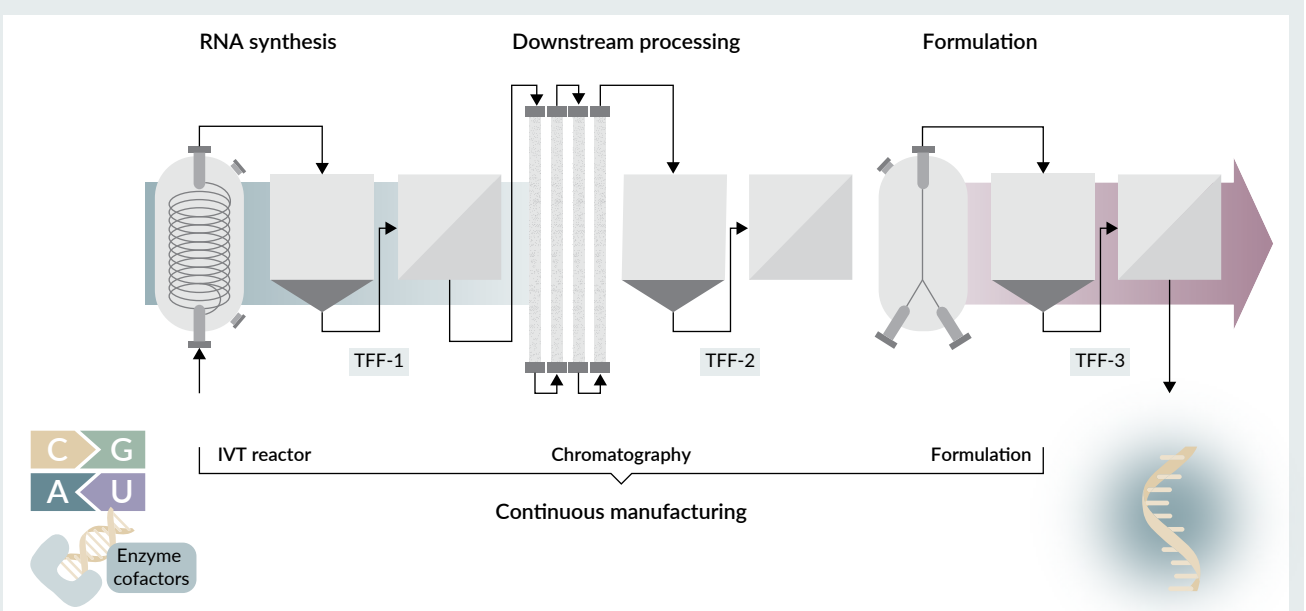
This study successfully confirmed the translation from batch to continuous purification of both mRNA transcripts utilized, eGFP mRNA (995 nucleotides) and SARS-CoV-2 Spike protein mRNA (4284 nucleotides). In addition, similar CPPs, CQAs, and KPIs were observed for both constructs. While DNA template encoding mRNA of various lengths is identified as a CMA with varying impacts on CQAs and KPIs, the overall conclusion is that this platform is suitable for purifying a wide range of mRNA molecules that contain a polyA tail directly from unpurified crude IVT.

Beyond chromatographic purification, tangential flow filtration (TFF) also impacts mRNA stability, an essential consideration for maintaining product efficacy, safety, and shelf-life. Funkner *et al.* investigated mRNA stability in both the IVT reaction mix and after purification via TFF [37]. Their findings indicated that TFF-purified mRNA exhibited significantly greater stability, maintaining high integrity for at least 7 days at room temperature, followed by a gradual decrease to 80% integrity by day 33. In contrast, non-purified mRNA stored in the IVT reaction mix showed rapid degradation, with integrity dropping to 51% within 14 days. These results highlight the importance of robust purification strategies in preserving mRNA quality for downstream formulation and delivery.

Ultimately, as mRNA therapeutics evolve, integrating continuous purification techniques with LNP formulation and purification will be key to enhancing scalability, maintaining quality, and ensuring

FIGURE 4

The integrated continuous production of RNA, encompassing an IVT continuous flow reactor, downstream purification via chromatography, and final formulation steps.



cost-effective production. By leveraging TFF for both purification and buffer exchange, manufacturers can streamline the stability of mRNA DP during storage by efficiently removing impurities and evaluating bleb formation, thereby optimizing the overall continuous manufacturing workflow (Figure 4) for next-generation RNA medicines. TFF is responsible for both bulk concentration and buffer exchange to ensure scalability, stability, and consistent product quality. Optimization of this step is essential due to the sensitivity of mRNA-LNPs to shear stress and process conditions. Recent comparative studies have shown that Hydrosart® ECO membranes outperform Ultracel® membranes, demonstrating up to 1.5x higher permeate flux and reduced process times, while maintaining the physicochemical integrity of the DP. Final mRNA-LNPs exhibited a Z-average particle size of 79.9 nm, a low polydispersity index (PDI) of 0.072, and an encapsulation efficiency above 95%, indicating a homogeneous and efficient formulation [38]. In terms of sterile filtration, no significant differences in size, PDI, or encapsulation efficiency were observed pre- and post-filtration using Sartoguard® and Sartopore® 2 filters, confirming process robustness [38]. Notably, cryo-TEM images revealed occasional crescent-shaped bleb structures. The biological relevance of these blebs remains uncertain: while Cheng *et al.* [39] associated them with enhanced *in vitro* transfection, studies by Henderson *et al.* [40] and Meulewaeter *et al.* [41] linked freeze-induced blebs with reduced potency. Their impact on *in vivo* efficacy and long-term stability is still under investigation [40–43]. Furthermore, in the context of continuous manufacturing, single-pass TFF has shown significant potential as an enabling technology for uninterrupted downstream processing, enabling a 10-fold concentration of mRNA over 12 hours using 100 kDa regenerated cellulose membranes while maintaining mRNA integrity [44]. This process intensification strategy, along with membrane optimization, could significantly enhance downstream efficiency in the production of next-generation RNA medicines.

Despite these advancements, significant knowledge gaps remain, as key aspects of LNP formulation, production, and properties (e.g., surface modifications, tailored lipids use, adjuvant incorporation, and bleb formation control) are often proprietary and not widely disclosed. These factors play a critical role in enhancing stability, optimizing cellular uptake, modulating immune response, and improving therapeutic efficacy. Addressing these through greater transparency and collaborative research is essential to further optimizing LNP design, ensuring process consistency, and advancing the scalability of mRNA therapeutics.

Overall, continuous manufacturing has demonstrated significant potential, yet further quantification and control of process- and product-related impurities remain critical. Future studies should focus on enhancing impurity detection and minimization, particularly for residual enzymes (T7 RNA polymerase, pyrophosphatase), RNase inhibitors, endotoxins from bacterial-derived materials [45], immunogenic dsRNA, abortive transcripts, truncated RNA, and RNA-DNA hybrids, which are especially relevant for therapeutic mRNA applications [44–47]. Addressing these challenges will require the development of more sensitive analytical methods with lower limits of detection (LOD) and quantification (LOQ) [45], as well as the potential adoption of enzymatically synthesized template DNA to further refine impurity profiles. Additionally, the purification and formulation of LNPs must be optimized to ensure consistent particle size, encapsulation efficiency, and stability, as these factors directly influence mRNA delivery, bioavailability, and therapeutic efficacy, with the goal of reducing the need for

readministration using RNA constructs like self-amplifying or circular RNA. By integrating these advancements with continuous purification strategies, RNA manufacturing can achieve higher productivity, enhanced efficiency, and reduced operational costs, ultimately accelerating the development of next-generation RNA medicines. As the field progresses, collaborative efforts among academia, industry, and regulatory bodies will be essential to refine these technologies, standardize best practices, and ensure the broad accessibility of high-quality mRNA-LNP medicines worldwide.

ENABLING INNOVATION: DIGITALIZATION AND ADVANCED PROCESS CONTROL

Enhancing continuous mRNA DP manufacturing requires real-time process monitoring and digitalization to track CQAs and key performance indicators (KPIs), enabling adaptive production and purification for optimal purity, yield, and translation. However, regulatory agencies (US FDA, MHRA, and EMA) recently introduced new RNA therapeutic guidelines focused on CQA standardization and large-scale consistency. Meeting these demands will require advanced process control strategies to ensure automation, precision, and reproducibility [35].

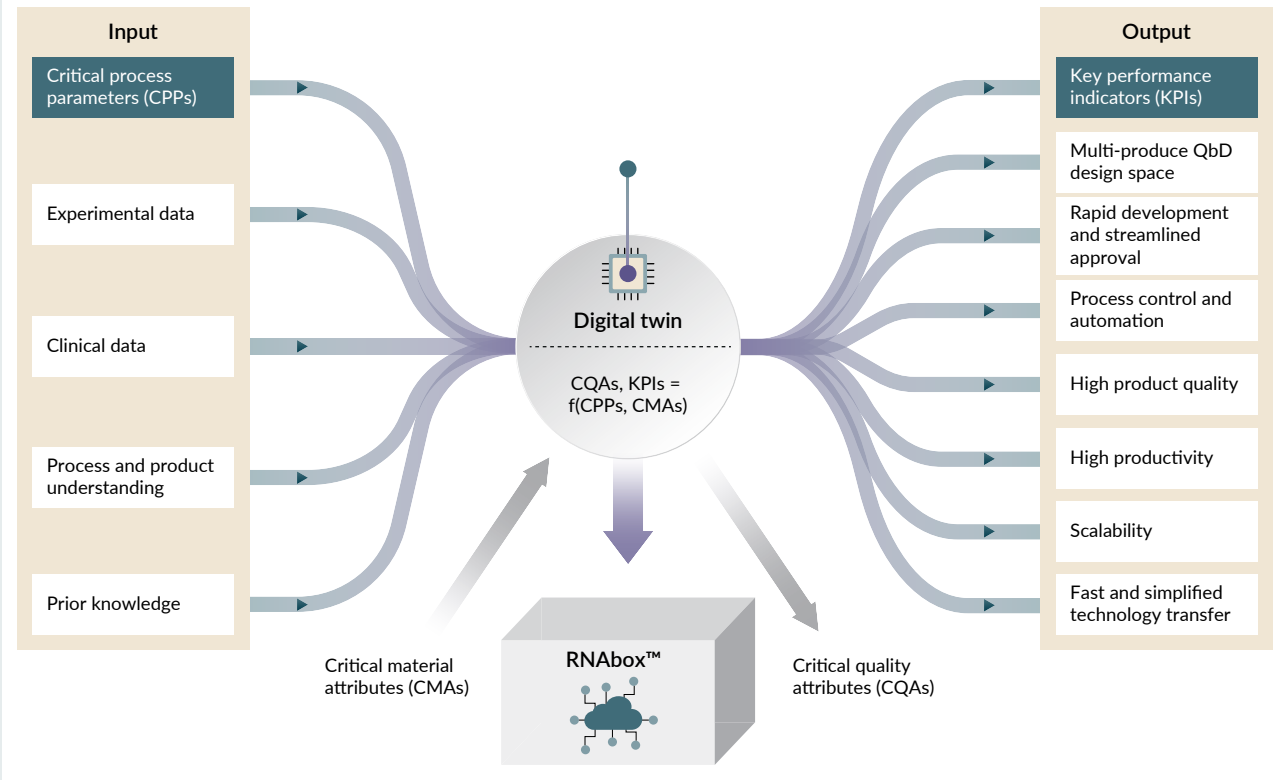
Evolving from traditional batch-based processes to fully continuous operations presents significant challenges, particularly in process control, quality assurance, and real-time monitoring. The QbD framework, introduced by the FDA in 2004, laid the groundwork for this shift by emphasizing that quality should be built into the process rather than tested into the final product [48]. QbD methodologies focus on defining CQAs, mapping CPPs, and using statistical modelling techniques such as Design of Experiments (DoE) to develop robust, reproducible processes [49]. However, implementing continuous manufacturing requires a more advanced approach—one that integrates real-time data acquisition, dynamic process control, and predictive modelling.

This need for real-time, adaptive control has driven the digitalization of biomanufacturing, enabling process optimization through Quality by Digital Design (QbDD; Figure 5). QbDD represents an evolution of QbD, leveraging digital tools, data-driven modelling, and process analytical technology (PAT) to improve process monitoring and decision-making [50]. The ultimate goal of digitalization is the creation of high-fidelity digital twins (DTs)—virtual replicas of manufacturing systems that integrate data, simulate process behavior, and provide predictive insights [51]. DTs enable manufacturers to optimize material usage, predict maintenance needs, and enhance automation, offering a significant return on investment. However, the biomanufacturing industry has yet to establish standardized data frameworks, predictive models and IT infrastructure necessary to fully harness DT capabilities. Also, this digital transformation extends beyond automation, aiming to create intelligent, self-optimizing production systems that enhance efficiency and scalability.

Building on this momentum, digital biomanufacturing has become a driving force in advancing next-generation productivity. Almost 5 years ago, Sanofi invested $4 billion to launch a digitally integrated biomanufacturing facility in Framingham, Massachusetts, pioneering data-driven, paperless production with continuous biologics manufacturing, integrated processes, digital twins, and augmented reality [52]. As the field of bioprocessing evolves, companies and research institutions worldwide are increasingly adopting DTs, artificial intelligence (AI), computer vision [53], and augmented reality (AR) [54] to optimize biomanufacturing workflows. However, despite these advancements, the field lacks

FIGURE 5

Digitalization of RNA biomanufacturing via QbDD, PAT, and digital twins for real-time monitoring, predictive modelling, and process optimization.



a unified vision and an operational framework that clearly defines how these technologies should converge to an integrated approach that strategically aligns automation, digitalization, and advanced analytics within a cohesive, quality-driven biomanufacturing framework.

Essentially, advancing continuous mRNA manufacturing will require a multifaceted approach encompassing process optimization, analytical innovation, and digital integration. Specifically, the scope of improvement should extend to:

- Enhancing the quality and efficiency of raw material, its recycling and reuse within the continuous RNA manufacturing process;
- Developing and implementing advanced analytical methodologies for in-line, on-line, or at-line automated CQA analysis (namely RNA integrity, 5′ capping efficiency, 3′ poly-A tail length, dsRNA impurities, residual nucleotide triphosphates (NTPs) and LNP physical properties);
- Refining the performance of digital tools through the application of the QbDD and (process analytical technology) PAT framework, ensuring robust process consistency, scalability, automation and regulatory compliance;
- Seamlessly integrating these technological and methodological

advancements to achieve a holistic and optimized manufacturing platform.

The convergence of the mentioned CQA, principles, real-time analytics, and automation is vital for the realization of high-yield, cost-effective, and globally scalable production of high-quality mRNA medicines.

TRANSLATION INSIGHTS: THE RNAbox™

To overcome the challenges of centralized, batch-based mRNA manufacturing, our team is developing the RNAbox: a compact, GMP-compliant manufacturing system designed for on-site, continuous mRNA production. Unlike conventional large-scale GMP facilities, RNAbox is a self-contained, modular platform that enables the rapid, distributed production of mRNA therapeutics anywhere in the world.

In addition to addressing cost, scalability, and automation, the RNAbox represents a breakthrough in preparedness and response. Conventional mRNA vaccine production demands complex coordination among numerous stakeholders, including product and assay developers, disease experts, immunologists, clinical trial specialists and volunteers, raw material and equipment suppliers, GMP manufacturers, and regulatory authorities. This extensive interdependence across manufacturing hubs, regulatory bodies, and distribution networks often causes critical delays, especially during public health emergencies. By contrast, RNAbox enables rapid, localized vaccine production, significantly accelerating response times in outbreak regions and strengthening global health security. Its integrated, automated design reduces reliance on specialized labor and multiple manual unit operations, making it a more sustainable and cost-effective solution for maintaining manufacturing readiness. Moreover, its ability to produce multiple RNA sequences within the same platform further enhances its versatility, making it suitable for developing vaccines against emerging infectious diseases, seasonal influenza strains, and novel pathogens.

Still, one of the most pressing challenges in mRNA manufacturing is cost. Traditional GMP facilities require substantial infrastructure investments and extensive labor, inflating production costs. RNAbox addresses this issue by eliminating the need for centralized mega-facilities, significantly reducing capital and operational costs. The system is designed to achieve manufacturing costs below $10,000 per gram of mRNA, with a production rate exceeding 50 µg/mL/min—far surpassing the efficiency of current batch-based methods. Beyond cost reduction, RNAbox offers an alternative to global supply chain dependency. Centralized mRNA production has exposed vulnerabilities, including raw material sourcing to multiple locations, export restrictions on genetic products, and logistical delays. By enabling on-site, distributed manufacturing, RNAbox helps mitigate select associated risks with centralized production. While it will not eliminate the need for raw materials and consumables, which must still be supplied by the commercializing company, its integrated and efficient design could reduce overall material usage and production losses, thereby lowering costs and easing supply constraints.

RNAbox will integrate continuous flow IVT to overcome the inefficiencies of batch-based RNA synthesis and significantly enhance production throughput. Automated multi-column chromatographic and TFF-based purification steps are expected to streamline downstream processing, followed by controlled LNP formation to ensure consistent particle size, high encapsulation efficiency, and robust product stability. Furthermore, real-time monitoring, driven by PAT and DTs, ensures precise control over CQAs, maintaining RNA

integrity, capping efficiency, and dsRNA impurity levels within target specifications. By integrating these advancements, RNAbox will provide a scalable and versatile manufacturing platform capable of adapting to varying production demands, whether for pandemic-scale vaccine deployment or small-batch personalized therapeutics. The integration of QbDD principles further enhances process efficiency by standardizing production workflows, simplifying regulatory compliance, and enabling seamless technology transfer between different sites. A key innovation within RNAbox is its advanced digital infrastructure, which includes soft sensors for real-time process, KPI and CQA monitoring and DTs for advanced automation. Soft sensors are computational models that infer process parameters, such as RNA yield and purity, based on real-time measurements, reducing the need for invasive sampling. DTs, on the other hand, serve as virtual replicas of the IVT reactor, purification system, and LNP formulation unit, enabling model-predictive control by forecasting future CQAs and KPIs. If predicted trends indicate potential deviations from specifications within a defined time frame (e.g., 5–10 minutes), the system can proactively adjust current process parameters to maintain product quality and process stability. These digital tools enable real-time monitoring and respond in less than five seconds, ensuring that CQAs remain within target ranges throughout the manufacturing process.

While the RNAbox concept offers significant advantages in terms of automation, decentralization, and cost reduction, several implementation challenges remain. The system will be engineered to minimize operator training and number of operators through automation. However, it will rely on established quality control infrastructure and regulatory frameworks to ensure compliance across different geographies. Power consumption, cleanroom compatibility, and environmental constraints will be addressed through isolated modular, energy-efficient design elements. Furthermore, real-time monitoring via PAT and digital twins will be embedded within an integrated software framework, with tailored sensor packages.

While the concept of continuous RNA production is not new, RNAbox distinguishes itself by integrating upstream and downstream processes (including IVT, chromatography, TFF, LNP formulation, and real-time PAT) into a modular, automated system designed for portability and decentralized deployment. The projected cost and production rates are based on internal process models calibrated with existing continuous process data, which are further being validated in the prototype development. The RNAbox is therefore a novel implementation of continuous, end-to-end RNA manufacturing in a self-contained format.

We aim to assemble a fully functional prototype capable of meeting stringent manufacturing KPIs and CQAs. These include a significantly reduced production cost, a productivity rate exceeding 50 µg/mL/min, and enhanced analytical resolution compared to current industry standards. Our goal is to achieve RNA integrity above 90%, 5′ capping efficiency above 90%, and dsRNA impurity levels below 0.1%. Additionally, RNAbox will support LNP formulation with a PDI below 0.15 and encapsulation efficiency exceeding 80%, ensuring high-quality RNA DP manufacturing.

Looking ahead, the success of mRNA therapeutics will rely on reimagining production systems that are scalable, cost-effective, and globally accessible. A paradigm shift in RNA medicines production can only be achieved through continued collaboration between academia, industry, and regulatory bodies. Whether for pandemic preparedness, decentralized vaccine manufacturing, or personalized medicine,

we strongly believe that a disease-agnostic RNAbox has the potential to democratize access to mRNA-based therapies and redefine the future of biomanufacturing.

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