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Unlock the sustained therapeutic efficacy of mRNA

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A B S T R A C T

mRNA therapies have emerged as a transformative class of medicines, offering immense potential across a diverse array of applications. This progress has been particularly evident in the wake of the success of lipid nanoparticle (LNP)-based mRNA vaccines during the COVID-19 pandemic. As these applications expand, the demand for sustained protein production has become increasingly critical. However, conventional mRNA therapies face significant challenges, including inherent RNA instability and suboptimal expression efficiency, often requiring repeated dosing to maintain therapeutic efficacy over time. This review highlights recent advances in strategies to prolong the therapeutic efficacy of LNP-mRNA systems. We focus on preclinical and emerging approaches aimed at extending the period of protein translation by engineering both the mRNA molecule and the LNP delivery system. Sustained protein expression is a cornerstone of mRNA-based therapeutics, and addressing this challenge is vital for unlocking their therapeutic potential. We hope this review provides valuable insights to guide the development of optimized delivery platforms for LNP-mRNA therapeutics.

1. Introduction

Endogenous mRNA, transcribed from genomic DNA, serves as the template for protein biosynthesis [1]. As early as the 1990s, scientists discovered that administering mRNA to animals could induce the production of target proteins, highlighting the potential of mRNA as a therapeutic agent [2]. However, naked mRNA is highly unstable and exhibits innate immunogenicity, which initially led to its classification as undruggable. As a transient molecule, mRNA is inherently designed for rapid degradation, ensuring tight regulation of protein expression. Mechanisms such as decapping [3], deadenylation [4], and ribonuclease-mediated cleavage [5] efficiently break down mRNA within minutes to hours. While this characteristic is crucial for maintaining cellular homeostasis, it poses a significant challenge in its use as a therapeutic agent, where sustained protein expression is often required.

To address these challenges, lipid nanoparticle (LNP)-based delivery systems have emerged as a transformative solution, encapsulating mRNA to protect it from degradation and ensuring its integrity in vivo [6]. The development of LNP-mRNA technologies has seen remarkable progress, particularly during the coronavirus disease 2019 (COVID-19) pandemic, which accelerated innovations in vaccine formulations. Many recent reviews have highlighted progress in areas such as LNPs [7–9], and mRNA vaccines [10–12].

The clinical success of mRNA vaccines highlights the potential of this technology, but therapeutic mRNA applications face additional hurdles, including the need for prolonged expression of target proteins [13]. mRNA-based therapies depend on the efficient translation of target proteins, which often require a sustained and sufficient presence of these proteins to exert their desired effects. Insufficient protein production or limited translation duration frequently necessitates repeated dosing, which can increase the risk of side effects and reduce patient compliance [14]. While different routes of administration for LNP-mRNA may lead to different expression kinetics, the duration of protein expression is generally limited to a few days [15]. In a Phase I clinical trial investigating VEGF mRNA, protein expression peaked within 5 h of mRNA translation and declined within 24 h after intradermal administration [16]. These findings highlight the importance of developing strategies to achieve sustained mRNA delivery and expression, which is vital for unlocking the full therapeutic potential of mRNA-based treatments.

This review provides an overview of recent advances in achieving sustained mRNA therapeutic efficacy, with a focus on biomaterial-based approaches for controlled release. We discuss strategies for enhancing mRNA stability and translation efficiency, as well as biomaterials integrated with LNPs. Finally, we outline future directions and critical challenges, providing valuable insights to advance the field of therapeutic mRNA.

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2. RNA engineering to enhance mRNA stability and translation

Achieving sustained therapeutic efficacy of mRNA involves addressing both extracellular and intracellular challenges [17]. Extracellular challenges refer to ensuring the precise delivery of mRNA into cells after administration, since it is vulnerable to enzymatic degradation in vivo. Intracellular challenges pertain to optimizing mRNA stability and translation after cellular uptake. The extracellular stability of mRNA could be maintained through biomaterial-based delivery systems [18,19], while intracellular delivery necessitates optimizing both translational efficiency and mRNA stability [20]. This section delves into in vitro transcribed (IVT) RNA engineering strategies to enhance intracellular stability and translation efficiency, pivotal for effective therapeutic applications. Significant progress in RNA engineering has been made by modifying critical mRNA regions, including the 5′ cap, coding region, untranslated regions (UTRs), and the poly(A) tail, all of which are pivotal for mRNA functionality [21]. Additionally, circular RNA (circRNA), distinguished by its enhanced stability and extended persistence relative to linear mRNA, holds significant promise for achieving extending mRNA therapeutic efficacy [22]. Similarly, self-

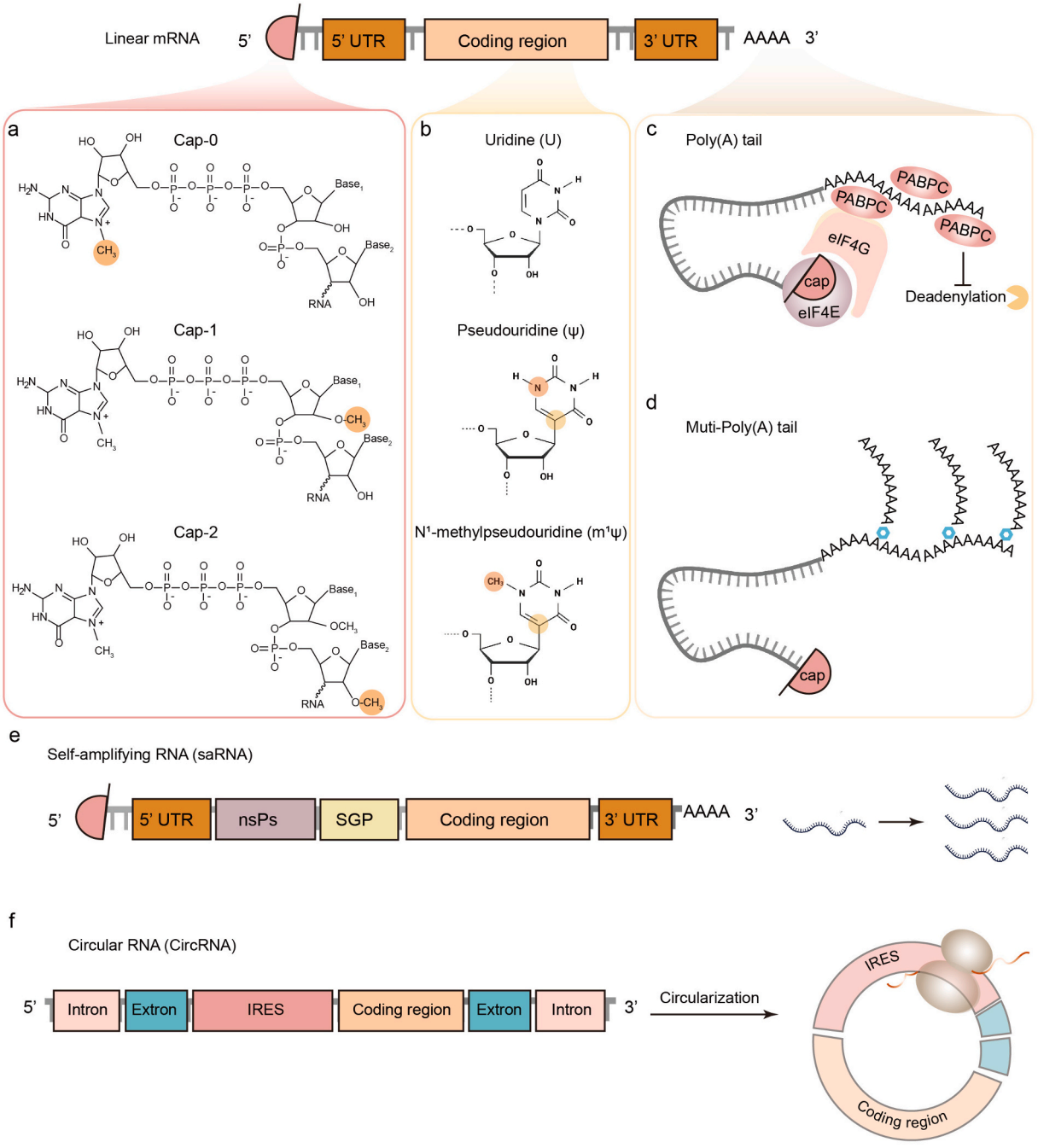

Fig. 1. Illustration of mRNA engineering to enhance mRNA stability and protein production. (a) The structures of Cap-0, Cap-1 and Cap-2, and the cap-dependent translation initiation. (b) The chemical structures of uridine, pseudouridine (Ψ) and N^1-methylpseudouridine ($m^1\Psi$). (c) The poly(A) tail binds to cytoplasmic poly (A)-binding protein (PABPC), forming a closed-loop structure by interacting with eukaryotic translation initiation factor 4G (eIF4G) and eIF4E. (d) The structure of chemically modified mRNA with multiple poly(A) tails. (e) The self-amplifying RNA (saRNA) expands on the structure of conventional linear mRNA by including two crucial additional elements: viral non-structural proteins (nsPs) and a subgenomic promoter (SGP). (f) Circular RNA (circRNA) is generated through the ligation of a linear precursor RNA and includes an internal ribosome entry site (IRES) to facilitate translation initiation. This figure was created using icons from BioRender.com.

amplifying RNA (saRNA), which leverages endogenous mechanisms to extend RNA durability, holds considerable potential for long-term expression [23].

2.1. 5′-cap

The 5′-cap structure is a universal modification found in nearly all the eukaryotic mRNAs, as it enhances transcript stability, prevents degradation, and facilitates cap-dependent translation [24,25]. It consists of a guanine nucleotide linked to the first transcribed nucleotide at the 5′ end of the mRNA via a unique 5′-to-5′ triphosphate bond. This guanine is subsequently methylated to form the 7-methyl-GpppNp (m^7GpppNp), known as the Cap-0 structure (Fig. 1a). The Cap-1 structure is generated by the additional methylation of the first nucleotide through (nucleoside-2′-O)-methyltransferase (2′OMTase). Further methylation of the second nucleotide results in the Cap-2 structure, which forms gradually over time and is therefore enriched on long-lived mRNAs [26].

The 5′-cap serves dual roles in protecting mRNA from 5′-to-3′ exonuclease-mediated degradation and modulating immune recognition. For instance, the Cap-2 structure contributes to the distinction between host mRNA and viral mRNA [27], a process closely linked to the relatively slow kinetics of methylation. Because methylation occurs relatively slowly, rapidly replicating viral mRNA often lacks a Cap-2 structure, as the methylation machinery cannot keep pace with its rapid synthesis. This deficiency leaves viral mRNA more vulnerable to immune detection. In contrast, uncapped mRNA or mRNA with a Cap-0 structure can activate innate immunity by engaging retinoic acid-inducible gene I (RIG-I) receptors, which recognize the 5′-triphosphate as a pathogen-associated molecular pattern (PAMP) and induce the type I interferon (IFN-I) response [28]. Although the Cap-1 structure reduces RIG-I activation, significant levels of Cap-1 mRNA can still trigger immune detection. In contrast, the Cap-2 structure more effectively prevents RIG-I binding, enabling mRNA to evade immune surveillance [26].

Beyond its roles in protecting mRNA from degradation and reducing immune recognition, the m^7G cap is indispensable for translation initiation in eukaryotic cells. During cap-dependent translation, multiple eukaryotic initiation factors (eIFs) sequentially recruit the ribosomal small subunit and initiator tRNA [29]. Specifically, eIF4E recognizes and binds to the m^7G cap, while eIF4G interacts with the poly(A) tail and bridges the mRNA to the 40S ribosomal subunit via eIF3 [30]. The ribosome then scans the mRNA to locate the start codon (AUG), and GTP hydrolysis by eIF-5 facilitates the joining of the 60S large subunit, ultimately forming an active 80S ribosome complex [31]. This process underscores the critical role of the 5′ cap in efficient translation initiation. Therefore, proper capping is a prerequisite for IVT mRNA to achieve sustained therapeutic efficacy, as it supports both translational efficiency and stability.

2.2. Coding region

The coding region in mRNA encodes genetic information for protein synthesis and comprises codons (triplets of nucleotides), each of which corresponds to either an amino acid or a stop signal. Mathematical modeling studies have shown that codon usage could explain up to 55 % of the variation in mRNA half-life [32]. This influence is primarily mediated through codon optimality, which determines the rate of translation elongation. Codons associated with abundant tRNAs enable rapid decoding, reduce ribosome pausing, and minimize the activation of mRNA surveillance and degradation pathways [33]. In contrast, rare or suboptimal codons slow ribosomal progression, increasing the likelihood of ribosomal collisions and decay. Therefore, codon optimization, by preferentially selecting codons matched to abundant tRNAs, not only enhances protein output but also contributes directly to extended mRNA half-life, supporting sustained protein expression in vivo [34]. Maintaining an optimal ribosome load, an appropriate density of ribosomes on a single mRNA molecule, is also crucial for sustained translation [35]. mRNAs enriched with optimal codons tend to maintain this balance, ensuring efficient elongation while avoiding ribosome traffic jams that can lead to premature decay. As such, codon engineering serves as a foundational strategy to enhance the duration and consistency of therapeutic protein production from IVT mRNAs.

In addition to codon usage, chemical modification of nucleoside bases in the coding region further improves mRNA performance. A notable breakthrough is the substitution of uridine with pseudouridine (Ψ) (Fig. 1b), recognized by the 2023 Nobel Prize in Physiology or Medicine [36]. Uridine can also be modified into N^1-methylpseudouridine ($m^1\Psi$), which significantly reduces immunogenicity and enhances translation efficiency. This modification has been successfully applied in COVID-19 mRNA vaccines, including Spikevax® (Moderna) and Comirnaty® (BioNtech/Pfizer). Mechanistically, $m^1\Psi$ alters mRNA secondary structures by reducing the formation of stable secondary folds, which could otherwise impede ribosome progression along the mRNA [37]. This structural adjustment facilitates smoother ribosome movement during translation, minimizing pauses and enhancing the overall efficiency of protein synthesis. Additionally, these structural modifications reduce activation of innate immune sensors, further contributing to enhanced protein expression [38].

2.3. 5′ and 3′ untranslated regions (UTRs) and poly(A) tail

The untranslated regions (UTRs) at the 5′ and 3′ ends of mRNA do not encode proteins but contain diverse sequence elements and structural motifs. These features interact with RNA-binding proteins (RBPs) and microRNAs, critically influencing mRNA stability, translation, and overall fate [39]. For example, incorporating liver-specific microRNA target sites into the UTRs can substantially suppress mRNA translation in the liver, thereby improving delivery efficiency and therapeutic effects in extrahepatic tissues [40].

The poly(A) tail, consisting of approximately 50 to 250 adenosine nucleotides, is appended to the 3′ end of eukaryotic mRNA and is critical for stability and efficient translation [41]. It facilitates translation initiation by binding to cytoplasmic poly(A)-binding protein (PABPC). This interaction forms a closed-loop structure by bridging the Poly(A) tail with the 5′ cap structure through translation initiation factors such as eIF4G (Fig. 1c). This closed-loop enhances ribosome recruitment, boosting translation efficiency and protein yield. Deadenylation, the enzymatic removal of the poly(A) tail, disrupts this structure by releasing PABPC. This weakens the interaction between eukaryotic initiation factor 4E (eIF4E) and the 5′ cap, leading to mRNA decapping and subsequent degradation. Thus, the poly(A) tail is pivotal in maintaining mRNA stability and regulating translation efficiency. Innovative designs, such as multitailed mRNA, have further enhanced mRNA stability. Wang et al. reported a multitailed mRNA by incorporating branched oligonucleotides modified with triazole linkages, with nuclease-resistant modifications introduced into each poly(A) tail (Fig. 1d) [42]. This branched poly(A) topology can engage in multimeric interactions with PABPC, thereby enhancing translation efficiency and stabilizing mRNA.

2.4. Self-amplifying RNA (saRNA)

Self-amplifying RNA (saRNA) represents an advanced RNA technology designed to significantly enhance protein production through the use of an engineered replicon system [43–45]. Unlike conventional mRNA, saRNA encodes replicase proteins, enabling self-replication within target cells (Fig. 1e). Once delivered into the cytoplasm, the replicase proteins initiate RNA replication, producing numerous copies of the original strand. This amplification of RNA templates fuels sustained and amplified protein expression over extended periods.

Current research emphasizes saRNA's capacity to achieve comparable or superior protein expression levels with a lower initial RNA dose

than traditional mRNA, thereby minimizing potential adverse effects, particularly in vaccine applications. Notably, Japan's regulatory authorities have recently approved the world's first saRNA-based COVID-19 vaccine, marking a significant milestone for this technology [46]. Beyond vaccines, saRNA is also being explored for therapeutic applications requiring elevated protein levels. For instance, Weiss et al. demonstrated the potential of saRNA encapsulated in LNPs to achieve high-level expression of IL-12 [47]. This approach showcased saRNA as a potent single-agent immunotherapy, capable of eradicating large tumors and inducing durable immune memory.

Despite its promise, saRNA faces several challenges. First, saRNA has high immunogenicity, primarily due to the production of double-stranded RNA (dsRNA) intermediates during replication. These dsRNA intermediates arise because the replicase enzyme synthesizes complementary RNA strands as templates for further replication. This process, while central to the amplification mechanism of saRNA, inadvertently generates dsRNA, which is recognized by the innate immune system, triggering inflammatory responses.

To mitigate inflammation and their impact on protein expression, several strategies have been developed. One approach involves co-delivering therapeutic saRNA with mRNAs encoding immune-evasive proteins to extend protein expression [48,49]. These immune-evasive proteins, derived from viruses, such as E3, K3, and B18R (EKB) proteins, help evade innate immune recognition and suppress IFN responses. In addition, Grinstaff et al. recently reported that substitutions of saRNA's native nucleotides with chemically modified nucleotides can enhance protein expression [50]. Importantly, introducing modified nucleotides did not disrupt downstream replication processes, thus maintaining the amplification efficiency of saRNA. Specifically, after screening a library of saRNA molecules with different modified nucleoside triphosphate (NTP) substitutions, 5-methylcytidine (m^5C) saRNA demonstrated superior transfection efficiency compared to both wild-type saRNA and $m^1\Psi$ saRNA across multiple cell lines. In vivo studies also confirmed that m^5C saRNA not only reduced IFN levels but also significantly prolonged protein expression.

Additionally, saRNA relies on replicase, derived from specific regions of non-structural proteins (nsPs) within the saRNA sequence, for self-replication. However, its performance may vary across cell types and environmental conditions, potentially affecting amplification efficiency. Thus, optimizing nsPs to elicit more compatible immune responses in target cells remains a promising strategy for improving saRNA efficacy [51].

2.5. Circular RNA (circRNA)

Circular RNA (circRNA) has increasingly recognized as a promising alternative to linear mRNA due to its enhanced stability and translational efficiency, in which enables prolonged protein expression [52,53]. Unlike linear mRNA, circRNA forms a closed-loop structure without free 5′ and 3′ ends (Fig. 1f), making it resistant to exonuclease degradation and substantially extending its half-life. Additionally, circRNA is less detectable by the innate immune system, as it evades Toll-like receptor (TLR) sensors, reducing immune activation [54].

Several methods have been developed for in vitro circRNA production, with permuted intron-exon (PIE) splicing as the most widely adopted method [55,56]. The PIE method generates circular RNA by splitting an intron into two segments and ligating the flanking exons [57]. However, its application to longer RNA sequences remains challenging, as it typically results in lower circRNA yields [58]. Anderson et al. optimized the PIE method, enabling the circularization of RNA sequences up to 5 kb [59]. Additionally, the introduction of bacterial sequences during the PIE process raises concerns about potential immunogenicity. To address these limitations, Du et al. developed a trans-ribozyme-based circularization (TRIC) approach that circumvents the need for bacterial sequences [60]. This approach not only supports the synthesis of circRNAs exceeding 8000 nucleotides but also demonstrates remarkably efficient translation. By engineering the IRES to eliminate stop codons, they achieved rolling circle translation, leading to dramatically higher protein expression compared to traditional single-round translation methods.

The absence of a conventional cap structure poses challenges for translation initiation. CircRNA relies on internal ribosome entry sites (IRES) to recruit ribosomes and initiate translation with the help of translation initiation factors (IFs) and IRES-transacting factors (ITAFs) [61]. The efficiency of IRES-mediated translation varies among cell types, facilitating tissue-specific translation. For example, Chen et al. utilized the IRES of human rhinovirus type 2 (HRV2) in engineered circRNA to selectively enhance translation in cancer cells, improving targeting specificity [62]. In different cancer cells, compared to linear RNA, circRNA-translated eGFP exhibited a 2-to 3-fold increase in half-life. Despite these advantages, IRES-driven translation generally exhibits reduced efficiency than cap-dependent mechanisms, limiting its broader application [63]. To address this, researchers are investigating strategies to enable cap-dependent translation initiation in circRNA. Wang et al. developed a panel of capped circRNA by incorporating a cap structure via a branched topology [64]. The addition of an m^7G cap to circRNA led to a 115-fold increase in luminescence intensity compared to unmodified circRNA. They further verified that this modification enhanced translational efficiency by enabling translation initiation via an eIF4E-dependent mechanism.

Moreover, as the translation mechanisms of circRNA and linear mRNA differ, traditional mRNA optimization strategies are often unsuitable. Researchers have focused on circRNA-specific approaches. Chang et al. established a modular cloning platform for circRNA, enabling systematic testing of a diverse range of sequence variants and independent optimization of multiple parameters [65]. Using this platform, they identified several strategies to enhance translation efficiency. They disrupted RNA secondary structures using locked nucleic acids, which modulate translation initiation by fine-tuning it, either enhancing or suppressing it as needed. Additionally, they demonstrated that leveraging synthetic aptamers to recruit eIF4G can effectively improve translation efficiency.

3. Biomaterial-integrated LNPs for sustained therapeutic efficacy

Owing to its intrinsic negative charge, mRNA is naturally repelled by the cell membrane, posing significant challenges for cellular uptake and subsequent translation. Developing delivery systems that protect mRNA and ensure its functional entry into the cytoplasm not only overcomes these barriers but also provides a viable method to realize sustained therapeutic efficacy from mRNA-based treatments. Various mRNA delivery systems have been developed, such as polymers, liposomes, exosomes, LNPs and proteolipids [66–71]. Among these, LNPs have emerged as one of the leading non-viral delivery platforms for mRNA-based therapeutics.

LNPs typically consist of four key components: ionizable lipids, phospholipids, cholesterol, and polyethylene glycol lipids (PEGylated lipids) [72].Phospholipids help stabilize the LNPs, while cholesterol modulates the fluidity of the lipid membrane [73]. PEGylated lipids increase the circulation time of LNPs by reducing protein adsorption and immune clearance [74]. The composition of LNPs can be further optimized to expand the possibilities for mRNA-based therapies [75]. Moreover, optimizing LNPs can extend circulation time, minimize immune clearance, and enhance cellular uptake. Targeted LNP delivery to specific tissues can also reduce unnecessary degradation and improve translation efficiency [76,77].

Ionizable lipids are considered a critical component of LNPs, as they are positively charged under formulation conditions, enabling mRNA encapsulation via electrostatic interactions. The pH-responsive groups in their chemical structures enable endosomal escape by promoting charge activation under acidic conditions, while remaining uncharged at

neutral pH, which is important for enhancing delivery efficiency. Ionizable lipids typically consist of a hydrophilic head and hydrophobic tails. In the design of hydrophobic tails, biodegradable chemical bonds (e.g., ester or acetal bonds) are often incorporated to further improve delivery efficiency and biocompatibility [78]. Notably, Onpattro® (patisiran) employs D-Lin-MC3-DMA as the ionizable lipid [79], Spikevax® (Moderna's COVID-19 vaccine) utilizes SM-102 [80], and Comirnaty® (Pfizer-BioNTech's COVID-19 vaccine) incorporates ALC-0315 [81]. All three ionizable lipids share the common feature of incorporating biodegradable ester bonds. Additionally, prior studies suggest that branched hydrophobic tails may enhance cellular delivery [82,83]. In the design of the hydrophilic head, ligand-mediated delivery may endow LNPs with active targeting capabilities. This can be achieved by introducing ligand-like small molecules into the head structure of ionizable lipids during their design. For example, Dong et al. incorporated small molecule ligands capable of crossing the blood-brain barrier into ionizable lipid structures, developing a series of LNPs [84]. Through in vivo tests, the LNP derived from an MK-0752-based ligand demonstrated the most efficient brain delivery following systemic administration.

Despite their effectiveness in facilitating cellular transfection and targeting, LNP-mRNA systems face challenges in achieving long-term protein expression. Once internalized, LNPs release mRNA into the cytoplasm, where it is rapidly translated into proteins. Although this ensures efficient protein production, the transient nature of mRNA expression limits prolonged expression. To further enhance the functionality of LNP-mRNA, particularly for sustained or controlled delivery, various novel biomaterials such as hydrogels, microparticles, implants, and microneedles can be integrated with LNP-mRNA [85–88]. Some of these biomaterials are well-established, with several already clinically translated for small and large molecular drugs [89,90]. Therefore, incorporating these biomaterials into mRNA therapeutics builds upon existing advancements, leveraging available resources to enhance their applications. This section summarizes the approaches of integrating biomaterials with LNPs for enhanced translation efficiency and improved mRNA durability.

3.1. *Microneedle embedded with LNP-mRNA*

Microneedles have been applied in drug delivery systems, providing a minimally invasive and virtually painless alternative to traditional injection-based administration [91]. These microscale needle arrays are engineered to enhance transdermal delivery efficiency while providing a more patient-friendly administration route. Additionally, microneedles act as a sustained-release drug delivery system, allowing the encapsulated drugs or nanoparticles to be gradually released as the microneedles degrade, ensuring controlled and prolonged therapeutic efficacy [92].

The efficacy of mRNA vaccines is inherently linked to immune

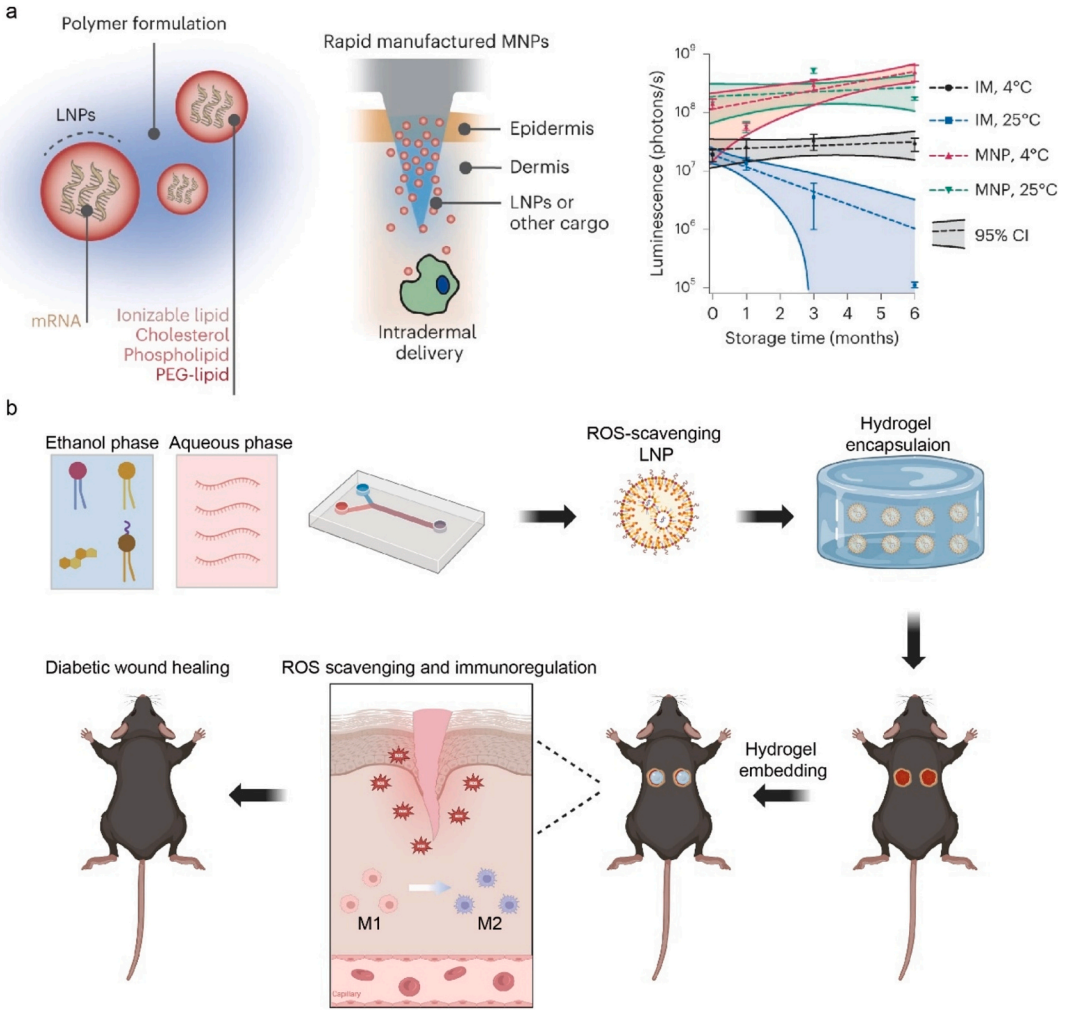

Fig. 2. (a) LNP-mRNA encapsulated in a dissolvable microneedle for intradermal delivery of vaccine. The microneedles maintain their capacity for high protein expression even after six months of storage at room temperature. MNPs refer to microneedle patches, while IM denotes intramuscular injection with conventional LNP-mRNA. Reproduced from Ref. [96] with permission from *Springer Nature*. © 2023. (b) LNP-mRNA incorporated into a hydrogel for diabetic wound healing therapy. Reproduced from Ref. [99] with permission from *Proceedings of the National Academy of Sciences*, under the CC BY-NC-ND.

memory, and intramuscular (i.m.) administration of LNPs has been shown to elicit a durable immune response [93,94]. Microneedles have additional advantages for LNP-mRNA vaccines, particularly in enhancing stability and simplifying storage requirements [95]. In 2023, Jaklenec et al. developed a microneedle system encapsulating COVID-19 mRNA within lipid nanoparticles (LNPs) [96]. They compared the immune responses induced by i.m. administration of LNP solution and transdermal administration using microneedle-LNP. The microneedle-LNP system generated titers comparable to those observed with i.m. administration of LNP-mRNA suspension, but with a delayed peak concentration. This indicated a delay in protein expression of LNP-mRNA after integration with the microneedle. More importantly, this formulation eliminates the need for cold-chain storage, allowing the vaccine to remain stable at room temperature for up to six months (Fig. 2a). Besides, a sustained-release transdermal patch has been developed by loading LNP-mRNA into silk fibroin matrices [97]. This patch has been shown to protect LNP-mRNA at 37 °C for up to two weeks. Beyond mRNA vaccines, microneedles can also enhance the duration of mRNA therapeutics. For instance, microneedles loaded with type I collagen-encoding mRNA particles can sustain therapeutic effects for up to 70 days, compared to 35 days with syringe injection [98].

3.2. Hydrogel loaded with LNP-mRNA

Hydrogels are biocompatible materials composed of three-dimensional polymer networks, including synthetic and natural polymers, capable of retaining significant amounts of water, often exceeding 90 % [100–102]. Their high-water content allows hydrogels to closely mimic the extracellular matrix of natural tissues, making them ideal for biomedical applications [103,104]. The three-dimensional network structure in hydrogels creates a mesh size that can serve as a drug reservoir, enabling controlled release of embedded drugs. However, direct encapsulation of mRNA within hydrogels often results in poor outcomes due to the uncertain functionality of mRNA in such water-rich environments.

To overcome this limitation, mRNA is typically encapsulated in nanoparticles before being incorporated into hydrogels. This combination provides a synergistic advantage: nanoparticles ensure efficient mRNA encapsulation and protect it from enzymatic degradation, while hydrogels enable controlled and sustained release. For instance, Appel et al. showcased this approach by embedding LNPs into an injectable hydrogel depot [105]. Following subcutaneous administration, the cells migrated into hydrogel and took up LNPs, enabling the hydrogel to function as a niche for cell regulation, particularly of immune cells. Eight weeks after a single immunization, measurable antibody titers were observed in 5 out of 6 animals in the hydrogel group, compared to only 1 out of 6 in the group receiving LNPs dissolved in PBS. Similarly, Mao et al. covalently crosslinked electrospun poly(ε-caprolactone) (PCL) nanofiber fragments with hyaluronic acid (HA) to develop a nanofiber-hydrogel [106]. They incorporated LNP-mRNA into the nanofiber-hydrogel to facilitate immune cell transfection, demonstrating its immune activation through a prophylactic vaccination model. This approach enabled single-dose immunization with efficacy comparable to the conventional three-dose of LNP regimen. The combination of hydrogel and LNP can enable a specific microenvironment that facilitates immune cell migration and LNP uptake, thereby enhancing the immune response. Additionally, the inclusion of immune adjuvants in hydrogels further amplifies local inflammation, augmenting the magnitude and quality of immune reactions.

Certain hydrogels exhibit dynamic properties, such as temperature sensitivity or shear-thinning behavior, making them suitable for injectable applications. These properties enable localized administration and precise spatial control over drug release [107,108]. For instance, Tzeng et al. encapsulated mRNA-loaded nanoparticles into a thermosensitive gel composed of the copolymer poly(lactic-*co*-glycolic acid)-poly (ethylene glyc*o*l)-poly(lactic-co-glycolic acid) (PLGA-PEG-PLGA) [109]. Following intratumoral injection, firefly luciferase expression was obviously localized at the injection site in the hydrogel-treated group, while off-target expression in organs such as the liver and spleen was significantly reduced compared to the non-hydrogel group. This localized application improves therapeutic effectiveness while minimizing systemic side effects. The sustained-release properties of hydrogels also protect mRNA-loaded nanoparticles from rapid clearance by immune cells, allowing for prolonged therapeutic effects.

The adaptability of hydrogels arises from their unique rheological properties, which allow hydrogels to integrate seamlessly with surrounding tissues, making them particularly suitable for specialized scenarios such as wound healing, bone reconstruction, and cranial defect repair [110,111]. This feature is also highly beneficial for mRNA therapeutics. For instance, Dong et al. developed a topically applied hydrogel system incorporating LNPs loaded with IL-4 mRNA for diabetic wound treatment [99]. They synthesized a library of reactive oxygen species (ROS)-responsive LNPs with the capability to scavenge excess ROS and integrated them into a hydrogel to modulate the pathological microenvironment of diabetic wounds (Fig. 2b). These LNP-hydrogel systems not only efficiently delivered mRNA to the wound site but also modulated the inflammatory microenvironment by reducing ROS levels. In another study, the same group integrated cell therapy with LNP-mRNA within a hydrogel for diabetic wound treatment via topical administration. In this approach, they utilized LNP-mRNA to engineer adipose stem cells, which were subsequently embedded in the hydrogel [49]. This design enabled adipose stem cells to sustain therapeutic protein expression, ensuring a continuous healing effect.

Other biomaterials can also be integrated with LNP-mRNAs formulation.

For example, Carvallo et al. developed a granular scaffold based on gelatin methacryloyl (GelMA) microporous structures for sustained mRNA release [112]. The GelMA scaffold's hydroxyl and carboxyl groups provide a negative charge, enabling electrostatic interactions with the positively charged LNP-mRNA complex. This interaction ensures uniform mRNA distribution within the GelMA scaffold. Besides, Murphy and colleagues proposed a novel “overexpress and sequester” mechanism [113]. This strategy employs mineral-coated microparticles (MCMs), specifically hydroxyapatite microspheres, to encapsulate LNP-mRNAs (Fig. 3a), allowing for extended therapeutic effects after a single dose. The “sequester” capability of MCMs was demonstrated using an engineered secretion-capable mCherry as a model protein. Following delivery via the MCM-LNP system, the mRNA encoding the secretion-capable mCherry protein was translated, resulting in red fluorescence in the cytoplasm within 12 h. As shown in Fig. 3b, the cytoplasmic fluorescence disappeared by 48 h, while the MCMs themselves began fluorescing red, signifying the sequestration of the secreted mCherry protein. To further investigate this process in vivo, mCherry-mRNA was delivered to one dermal wound in mice, either with or without MCMs, while saline was administered to the contralateral wound on the same animal. Delivery with MCMs resulted in stronger red fluorescence signal at the treated wound site compared to delivery without MCMs. This strategy shows that biomaterials can not only encapsulate LNP-mRNAs but also act as reservoirs, sustaining the release of expressed proteins over time.

4. Conclusion and perspective

mRNA therapeutics have made remarkable strides in recent years [114–117]. However, clinical translation remains challenges, with the need for sustained and efficient protein expression at lower dosing frequencies posing a major hurdle. Advances in mRNA engineering, including chemical modifications, self-amplifying RNA (saRNA), and circular RNA (circRNA), hold potential for overcoming these challenges. Nevertheless, these approaches still require further optimization. For instance, certain modifications may enhance translational efficiency but inadvertently trigger innate immune responses due to the incorporation

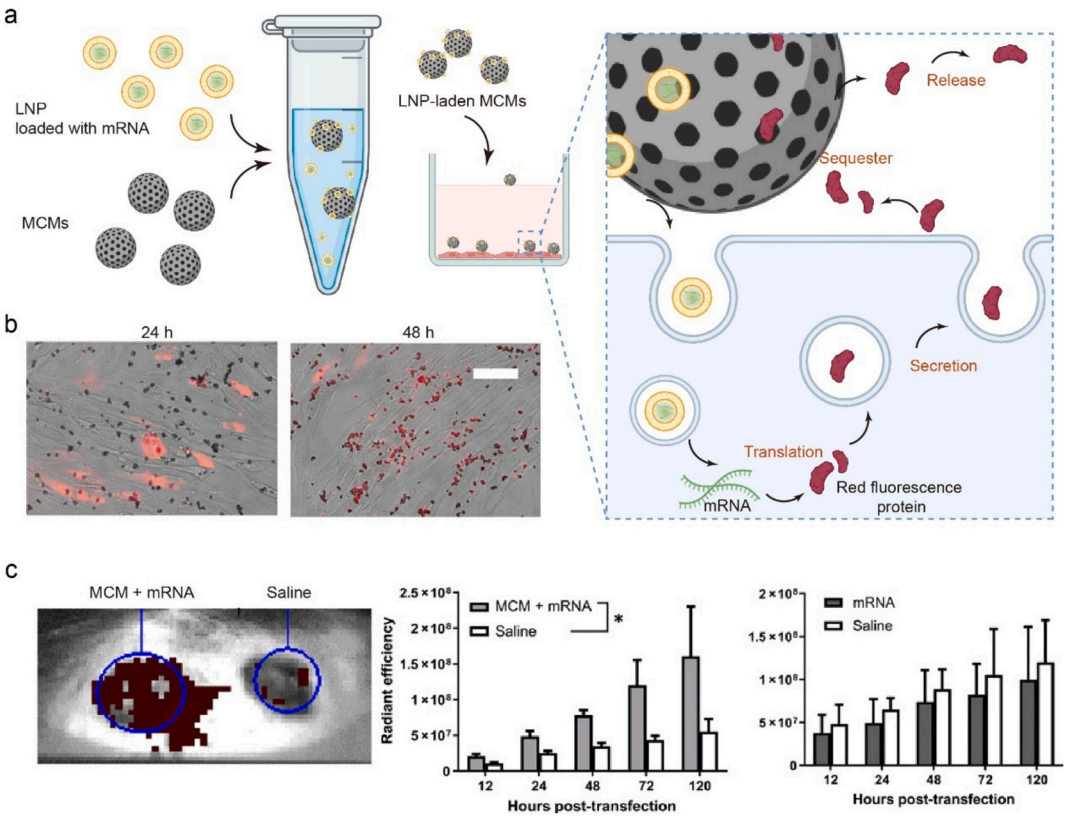

Fig. 3. (a) Schematic illustration of the preparation and delivery of LNP-laden mineral-coated microparticles (MCMs). (b) Following delivery of LNP-laden MCMs, red fluorescence was primarily localized in the cytoplasm at 24 h and predominantly accumulated within the MCMs by 48 h. (c) Representative IVIS images of skin wounds 48 h after treatment, comparing wounds treated with secreted MCM + mRNA and saline control. Quantitative comparison of mCherry fluorescence intensity over time at mouse skin wound sites treated with MCM + mRNA, mRNA alone, and saline as control. Reproduced from Ref. [113] with permission from The American Association for the Advancement of Science, © 2020. (a) was created using icons from BioRender.com. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of additional regulatory elements or structural motifs. Moreover, unlike traditional drugs, mRNA-based therapies rely on a translation step to produce functional proteins, a process that can vary across species and individuals. These variations affect the duration of protein expression and complicate the translation of preclinical findings from animal models to human applications. Addressing these complexities necessitates personalized strategies and well-designed clinical trials to ensure consistent therapeutic outcomes. Notably, in some cases, initial high mRNA translation efficiency does not guarantee prolonged protein expression [118]. Therefore, balancing translation intensity and expression duration is essential for achieving sustained efficacy of mRNA therapeutics.

Nanoparticles, such as LNPs, provide protection during transit and effective cellular uptake. To maximize the therapeutic potential of LNP-mRNA systems, LNPs can be engineered to enhance targeting specificity, cellular uptake, lysosomal escape, and prolonged circulation time [119–121]. However, LNPs still face several hurdles. Immunogenicity associated with PEG components and the complexity of LNP formulations present challenges for safety evaluation. Additionally, the ADME profiles of individual LNP components require comprehensive investigation. Another limitation is the sequestration of LNPs by the reticuloendothelial system (RES) or mononuclear phagocyte system (MPS), primarily in the liver [122]. This sequestration significantly reduces delivery efficiency to non-liver tissues, underscoring the need for novel strategies to achieve effective extrahepatic targeting.

Integrating LNPs with other biomaterials opens new possibilities (Fig. 4). For instance, microneedles combined with LNPs enable transdermal delivery, while hydrogels locally retain LNPs and regulate protein expression within surrounding tissues. Microspheres and implants serve as reservoirs for sustained protein release. However, combining LNPs with biomaterials poses multiple challenges. First, the compatibility between LNPs and the chosen biomaterial should be carefully evaluated. For example, it is crucial to ensure that microneedles retain sufficient mechanical strength to penetrate the stratum corneum after LNP incorporation. Likewise, preserving the structural stability of LNP-mRNA formulations within the microneedle matrix is vital to prevent aggregation. Additionally, incorporating LNPs may alter the physicochemical properties of the biomaterial itself. In the case of hydrogels, for instance, the presence of LNPs may interfere with hydrogel crosslinking, potentially compromising structural integrity and performance of the gel system.

Extending the duration of target protein expression is one of the key objectives in the development of mRNA-based therapies. For future studies, several important aspects warrant great attention. In particular, direct and unbiased evaluation of delivery efficiency and therapeutic performance is essential for meaningful interpretation of data. For instance, circRNAs often rely on IRES to initiate translation, rather than the conventional Cap1 structure. Since IRES-mediated translation generally exhibits lower efficiency than Cap1-dependent mechanisms, it is critical to clearly define the structural elements and functional properties of control mRNAs to ensure rigorous and accurate comparisons. Similarly, when evaluating expression levels and durability of mRNA in the context of novel delivery systems, it is equally important to select a well-established and reproducible delivery platform as a benchmark. In most cases, side-by-side comparisons with clinically validated LNPs serve as a valuable reference point for assessing the relative performance of experimental formulations.

In summary, sustained therapeutic efficacy of mRNA drugs depend

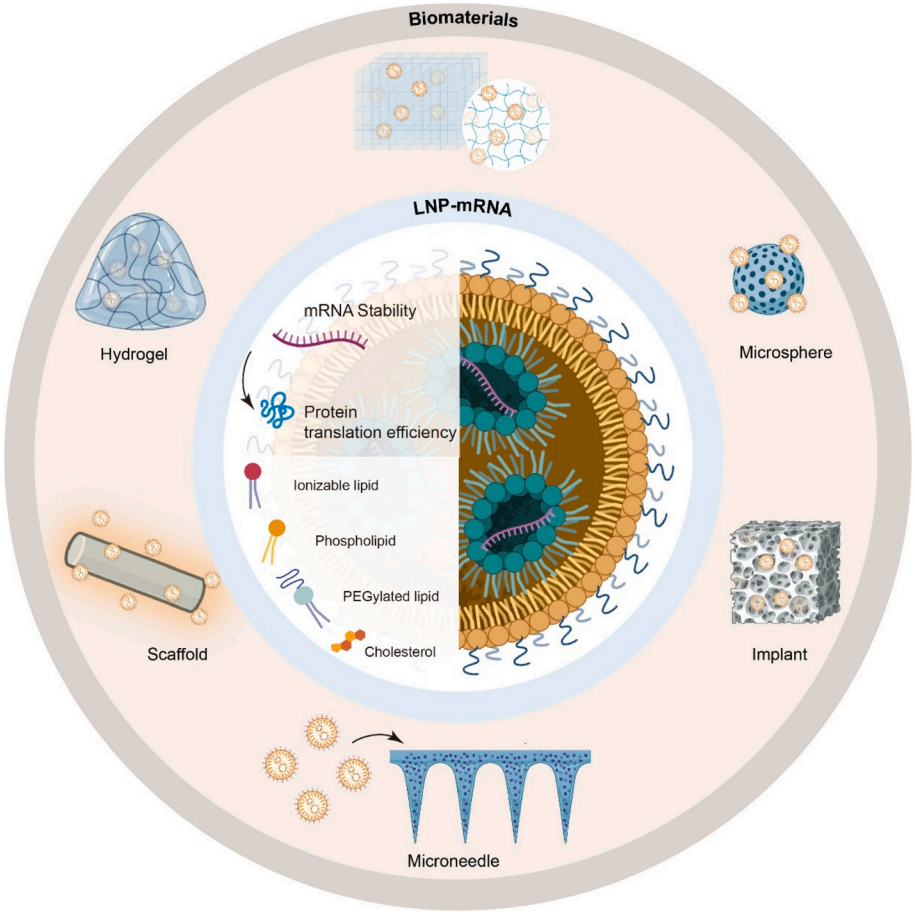

Fig. 4. Strategies for combining LNPs with macroscopic biomaterials to achieve sustained mRNA delivery. This figure was created using icons from BioRender.com.

on advancements in both mRNA molecular engineering and delivery systems. In comparison with other gene therapies, mRNA therapeutics encounter significant challenges in maintaining prolonged efficacy. However, leveraging innovative strategies that integrate multiple approaches offers promising avenues for overcoming these limitations. Future advancements in mRNA therapeutics could facilitate sustained protein expression following a single administration, revolutionizing treatment paradigms. Advances in emerging biomaterials and a deeper understanding of mRNA stability in vivo will be pivotal to overcoming these challenges. Successfully resolving these obstacles could pave the way for mRNA-based therapies targeting chronic diseases, ultimately reducing dosing frequency and enhancing patient compliance.

CRediT authorship contribution statement

Dinglingge Cao: Writing – review & editing, Writing – original draft, Conceptualization. **Meng Tian:** Writing – review & editing. **Zhengwei Liu:** Writing – review & editing. **Kaiyuan Guo:** Writing – review & editing. **Jonathan Peng:** Writing – original draft. **Anjali Ravichandra:** Writing – original draft. **Caroline Ferrell:** Writing – original draft. **Yizhou Dong:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition.

Declaration of competing interest

Y.D. is a scientific advisor for Arbor Biotechnologies. Y.D. is a cofounder and holds equity in Immunanoengineering Therapeutics. The other authors declare no competing interests.

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Data availability

Data will be made available on request.

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