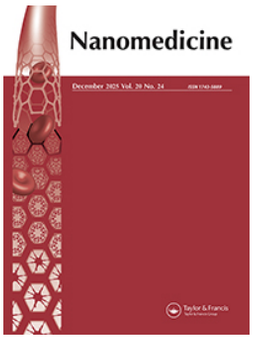

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REVIEW



# Advancing RNA delivery with Ionizable lipid nanoparticles: the roles of microfluidics and machine learning

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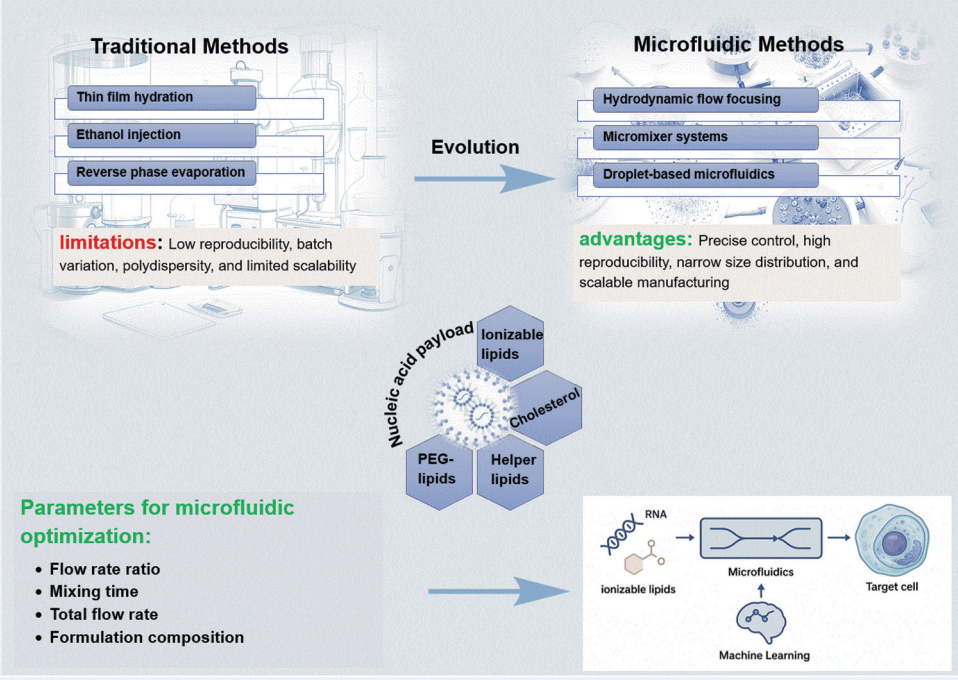
**ABSTRACT**

Ionizable lipid nanoparticles (iLNPs) have revolutionized Ribonucleic acid (RNA) therapeutics by enabling precise and efficient delivery of nucleic acids. However, their clinical translation remains challenged by batch-to-batch variability, complex lipid – RNA interactions, and stringent regulatory requirements. This review highlights how advanced microfluidic technologies address these issues by providing precise control over iLNP fabrication through engineered mixer geometries, optimized flow dynamics, and pH-dependent self-assembly. Comparative analyses of hydrodynamic flow focusing (HFF), and staggered herringbone mixers (SHM) demonstrate their distinct influence on particle size, polydispersity index (PDI), and encapsulation efficiency. Furthermore, the integration of design-of-experiments (DoE) methodologies, computational fluid dynamics (CFD) modeling, and machine learning (ML)-assisted optimization enables predictive formulation design and adaptive process control, enhancing reproducibility and scalability. Collectively, this review underscores microfluidics and ML as synergistic technologies that bridge laboratory innovation with Good Manufacturing Practice (GMP)-compliant, large-scale production paving the way for the next generation of intelligent, personalized RNA nanomedicines.

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Ionizable lipid nanoparticles; microfluidics; RNA delivery; mixing strategies; machine learning; scalable manufacturing; lab-on-a-chip

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**Article highlights**

- iLNPs revolutionize RNA therapeutics with pH-sensitive ionizable lipids, enabling high encapsulation efficiency ( > 90%) and low PDI ( < 0.1), but face challenges in scalability and reproducibility.
- Traditional methods like thin-film hydration yield heterogeneous particles, while microfluidics offers precise control via FRR and TFR for uniform iLNP production.
- Microfluidic mixers (HFF, SHM, toroidal) optimize particle size (20–-140 nm) and stability, with HFF excelling for siRNA and SHM for mRNA delivery.
- DoE and CFD modeling align iLNP production with QbD and ICH Q8–Q10 guidelines, ensuring cGMP-compliant manufacturing.
- ML models (XGBoost, random forest) reduce experimental iterations by 70% and predict organ-specific delivery with 90% accuracy.
- Microfluidics and ML bridge lab innovation to clinical translation, positioning iLNPs for next-generation vaccines and gene therapies.
- Challenges include lipid-RNA interactions and pH neutralization fusion, addressed by real-time inline sensors and parallelized platforms like glass-iLiNPs (160 mL/min).
- Future prospects integrate AI for adaptive control, enabling personalized RNA formulations and continuous manufacturing.

## 1. Introduction

LNPs have emerged as leading non-viral carriers for nucleic acid therapeutics due to their ability to encapsulate and protect fragile RNA molecules while facilitating intracellular delivery [1]. Over the past decades, extensive efforts have focused on optimizing lipid structures to improve delivery efficacy and safety [2]. Among these, ionizable lipids have demonstrated superior performance owing to their pH-sensitive behavior: they remain neutral at physiological pH minimizing systemic toxicity but become protonated within acidic endosomes, enabling endosomal membrane disruption and efficient cytosolic release [3,4]. This dual behavior makes iLNPs a cornerstone in the clinical delivery of therapeutics, particularly RNA-based ones. RNA therapeutics including small interfering RNA (siRNA), messenger RNA (mRNA), and clustered regularly interspaced short palindromic repeats (CRISPR) guide RNAs, offer powerful strategies for gene modulation [5]. Yet their large size, negative charge, and susceptibility to enzymatic degradation pose significant delivery challenges [6]. iLNPs address these hurdles by offering a clinically validated platform for effective RNA transport and cellular uptake. Their typical formulation includes four components: phospholipids and cholesterol, which provide membrane structure and fluidity; polyethylene glycol (PEG)-ylated lipids, which extend circulation; and ionizable lipids, which enable complexation and intracellular delivery [1,7]. Ionizable lipids differ from permanently cationic ones in safety and functionality. While cationic lipids bind nucleic acids efficiently, their persistent positive charge leads to high cytotoxicity and rapid clearance [8]. Ionizable lipids, in contrast, achieve charge-switching behavior, promoting endosomal escape via pH-triggered protonation without inducing systemic toxicity [9]. This makes them indispensable for mRNA vaccines and siRNA-based therapies.

Various methods have been employed for LNP fabrication, including ethanol injection, thin-film hydration, and reverse-phase evaporation. However, microfluidic mixing has gained prominence as the most advanced and scalable strategy, allowing precise control over particle size, composition, and encapsulation efficiency [10]. This technique involves rapid mixing of lipid-containing ethanol with an aqueous RNA solution under laminar flow, enabling uniform NP assembly typically in the 60–120 nm range, with low polydispersity and high reproducibility [11]. Beyond lab-scale benefits, microfluidics also aligns with continuous manufacturing standards and regulatory expectations, supporting clinical translation [12]. These microfluidic advancements have not only streamlined the production of iLNPs but also created opportunities for integrating cutting-edge computational tools to further enhance formulation efficiency and scalability. recent advances in combining microfluidic scale-out strategies with machine-learning – guided formulation have significantly accelerated the development and translation of iLNPs, enabling both rapid discovery of novel formulations and scalable industrial production. These innovations have paved the way for enhanced therapeutic applications, setting the stage for a deeper exploration of their impact on precision medicine and targeted drug delivery in the following sections [13].

In this review, we highlight the unique advantages of microfluidic platforms, particularly in the context of iLNP production, for the precise engineering of NPs physicochemical properties. We examine how mixer architecture, flow parameters such as the flow rate ratio (FRR) and total flow rate (TFR), and lipid composition collectively determine formulation outcomes and govern RNA delivery efficiency. Unlike previous reviews that have discussed these factors in isolation, this work provides an integrated perspective linking microfluidic design principles with ionizable lipid behavior and ML driven optimization strategies. Furthermore, we address key challenges related to scalability, reproducibility, and regulatory compliance, emphasizing how artificial intelligence (AI)-assisted, programmable microfluidic systems hold promise for enabling the next generation of intelligent, and adaptive.

## 2. iLNPs production: from traditional methods to microfluidics

The production of iLNPs has undergone a remarkable evolution from foundational traditional methods to the emergence of advanced microfluidic technologies that effectively overcome their inherent limitations. Early approaches, such as thin film hydration (TFH), ethanol injection, and reverse-phase evaporation, have shaped the field but come with notable limitations [14]. TFH, pioneered by Bangham et al. in 1965, involves dissolving lipids in an organic solvent, evaporating it to form a thin film, and hydrating it with an aqueous solution containing nucleic acid payloads. While foundational, this method often produces heterogeneous particles with low encapsulation efficiency [15]. The polarity shift in this method drives spontaneous NP formation, but it often suffers from inconsistencies between batches. As a result, the produced particles typically show wide size distributions and limited tunability in their physicochemical properties compared to modern microfluidic techniques [16]. Reverse-phase evaporation, developed by Szoka in 1978, forms water/oil emulsions with organic solvent removal under reduced pressure. This method provides high encapsulation efficiency for hydrophilic

molecules like mRNA or siRNA and allows for fine control of particle attributes through solvent manipulation, but still yields polydisperse populations [17]. These traditional methods struggle with inconsistent drug loading, inefficient mixing, and poor scalability, driven by uncontrolled factors such as local concentration gradients, mixing speeds, and temperature fluctuations. Moreover, achieving regulatory-compliant production under current GMP (cGMP) remains challenging due to variability in critical quality attributes (CQAs) like particle size, PDI, and encapsulation efficiency. The intricate interplay between ionizable lipid chemistry and RNA payloads further complicates formulation, as subtle changes in lipid structure or buffer pH can significantly alter endosomal escape and therapeutic performance.

The transition to microfluidics addresses the critical challenges of traditional methods, which include inconsistent drug loading, inefficient mixing, and limited scalability due to uncontrolled nanoprecipitation influenced by local concentration gradients, mixing speeds, and temperature fluctuations [18]. Microfluidic systems revolutionize iLNP production by offering precise spatial and temporal control through engineered channel geometries and laminar flow profiles, creating homogeneous mixing environments with controlled diffusion distances [19,20]. This precision leads to narrower PDI (PDI < 0.1), encapsulation efficiencies exceeding 90%, and exceptional batch-to-batch reproducibility, while also enabling scalability through parallelization or continuous flow [21]. Platforms like HFF, SHM, and toroidal mixers enable fine-tuned manipulation of FRR and TFR, directly influencing particle size and stability. For instance, HFF systems achieve uniform mixing by focusing aqueous and organic streams, producing iLNPs with sizes between 20–100 nm, ideal for RNA delivery. Microfluidics also supports scalability through channel parallelization and continuous flow processing, addressing industrial demands while maintaining batch-to-batch reproducibility. Real-time monitoring via inline sensors (e.g., dynamic light scattering (DLS)) and CFD modeling further enhance process control, minimizing variability and aligning with ICH Q8–Q10 guidelines for robust, cGMP-compliant production. Beyond technical superiority, microfluidics supports real-time monitoring, automated process control, and rapid formulation iteration with minimal material use, making it an ideal platform for personalized nanomedicine development and accelerating clinical translation [22–24].

## 3. Basic principles of microfluidics for iLNPs

Microfluidic devices are specialized platforms with engineered flow characteristics that precisely control fluid behavior in microscale channels, where laminar flow predominates due to low Reynolds numbers. These miniaturized tools manipulate fluids through applied pressure gradients or electric fields, creating predictable behaviors governed by the Navier – Stokes equations [25]. By leveraging the unique fluid dynamics at the microscale, these cost-effective and versatile systems enable rapid analysis within seconds, making them valuable for automated biomedical applications [26].

### 3.1. Fluid behavior at microscale

Microfluidics manages fluids in sub-millimeter channels where laminar flow dominates due to low Reynolds numbers ($Re = \rho V_{avg} L/\mu$: $\rho$ is the density of the fluid, V is the velocity of the flow, L is the characterizing length in the flow system, $\mu$ is the viscosity of the fluid), typically below 1.0, creating predictable fluid behavior [27]. Two primary flow mechanisms exist: pressure-driven flow, which uses pumps to propel fluids and produces parabolic velocity profiles due to the no-slip boundary condition at walls; and electrokinetic flow, which leverages charged channel walls to form an electric double layer resulting in nearly uniform velocity profiles. The critical property in microfluidic systems is not the total fluid volume, but rather the channel dimensions, which facilitate laminar flow where fluids move in smooth, non-turbulent layers. This predictable behavior, governed by the Navier – Stokes equations, enables precise molecular transport using minimal sample volumes and reagents, directly enhancing efficiency and precision in applications such as drug delivery. While pressure-driven systems offer cost-efficiency and reproducibility, electrokinetic systems avoid diffusion nonuniformities but require high voltages and are sensitive to surface property variations [28–30].

### 3.2. Broader applications of microfluidics

This subsection explores the diverse applications of microfluidics beyond iLNP production, highlighting its versatility in fields such as diagnostics, drug discovery, and synthetic biology, setting the stage for its specialized use in LNP and iLNP fabrication in later sections. Microfluidic platforms further distinguish themselves through elegant modular designs that seamlessly integrate multiple reaction steps, analysis protocols, and purification processes onto single, compact chips. This sophisticated integration enables complex, multi-step syntheses to proceed in continuous flow with real-time monitoring capabilities, dramatically reducing processing times while enhancing automation potential [31]. In addition to the application of microfluidics in medical diagnostics and the production of NPs, such as LNPs, there are several other examples of microfluidics applications (Table 1).

### 3.3. The materials for microfluidic devices

Various materials are employed in creating microfluidic devices, each offering distinct advantages and limitations. Glass and silicon were among the earliest materials used in microfluidics. Glass offers optical transparency, electrical insulation, high thermal conductivity, and stable electro-osmotic mobility. Glass capillary devices can provide three-dimensional, axisymmetric fluidic channels, enhancing production efficiency by minimizing fluid contact with inner walls. Silicon provides excellent chemical resistance, thermal stability, mechanical robustness, and semiconductor properties, enabling devices to tolerate extreme temperatures and pressures. However, its opacity and brittleness remain significant drawbacks [63].

Polydimethylsiloxane (PDMS) has recently gained popularity due to its low cost, optical clarity, biocompatibility, and

Table 1. Wide range of applications of microfluidic devices.

| Application area | Specific application | Highlights | References |
|---|---|---|---|
| Clinical diagnostics | Liquid biopsies | CTC-iChip for isolation of circulating tumor cells | [32] |
| | Blood analysis | Microfluidic blood cell separation techniques | [33] |
| | Infectious disease detection | Microfluidic diagnostic tools for global health | [34] |
| | Cancer diagnostics | Isolation of rare circulating tumor cells | [35] |
| Organ-on-a-chip | Lung-on-a-chip | Biomimetic microsystem that reconstitutes the lung alveolar-capillary interface | [36] |
| | Heart-on-a-chip | Human iPSC-derived cardiac microphysiological system | [37] |
| Drug discovery & development | High-throughput screening | Enabling rapid formulation optimization for mRNA delivery using droplet-based platforms. | [38] |
| | Drug delivery systems | Leveraging crossflow micromixing for scalable GMP-compliant manufacturing. | [39] |
| | Personalized medicine | Integrating machine-learning-guided design for targeted drug delivery and biomarker analysis. | [40] |
| Cell biology | Single-cell analysis | Microfluidic devices for single-cell manipulation | [41] |
| | Cell sorting | Label-free cell separation and sorting techniques | [42] |
| | Cell culture | Microfluidic environments for cell studies | [43] |
| | Study the movement of living cells | Microfluidics for stem cell differentiation | [44] |
| | Cellular microenvironment | Soft lithography for cell biology applications | [45] |
| Molecular biology & genetics | PCR & DNA amplification | Continuous-flow PCR on a chip | [46] |
| | Genetic screening | High-throughput genetic interaction mapping | [47] |
| Synthetic biology | Artificial cells | Construction of artificial cells using droplet microfluidics | [48] |
| | Protein synthesis | Cell-free protein synthesis in microfluidic devices | [49] |
| Environmental monitoring | Air pollution detection | Paper-based devices for air quality testing | [50] |
| | Toxin detection | Rapid detection of environmental toxins | [51] |
| Food safety & agriculture | Plant phenotyping | Microfluidics for plant biology | [52] |
| Chemical synthesis | Catalysis research | Microreactors for catalytic processes | [53] |
| | Process intensification | Smart scale-up of microchemical processes | [54] |
| Materials science | Material characterization | Analysis of material properties | [55] |
| | Polymer science | Synthesis of polymer particles | [56] |
| | Surface patterning | Patterning surfaces for material applications | [57] |
| Energy applications | Fuel cells | Microfluidic fuel cells | [58] |
| Wearable & implantable devices | Sweat analysis | Wearable sweat sensors | [59] |
| | Continuous monitoring | Skin-interfaced microfluidic devices | [60] |
| | Drug delivery | Implantable microchip for drug delivery | [61] |
| | Tissue engineering | Microfluidic scaffolds for tissue engineering | [62] |

mechanical flexibility. Despite these advantages, PDMS has significant limitations: it is prone to deformation under pressure and can be degraded by organic solvents, which may adversely affect NPs properties. As a result, researchers have begun exploring alternative materials. Several promising candidates have emerged: polymethyl methacrylate is a rigid, transparent thermoplastic that offers improved durability, though it is less flexible than PDMS. Cyclic olefin copolymers provide excellent optical clarity, low autofluorescence, and high chemical resistance, Polycarbonate is valued for its strength, transparency, and thermal resistance, making it suitable for high-durability applications, and Polystyrene, widely used in cell culture systems, offers excellent biocompatibility [62–64]. Accordingly, the selection of microfluidic device materials requires careful consideration of multiple factors. Researchers must balance chemical compatibility, durability, and transparency to ensure optimal system fabrication and performance.

### 3.4. Mechanistic basis of iLNPs formation in microfluidics

The assembly of iLNPs in microfluidic platforms hinges on the controlled interplay of solvent dynamics and molecular interactions at the organic-aqueous interface. Rapid mixing of lipid-containing organic phases with RNA-laden aqueous phases under laminar flow triggers solvent exchange, initiating lipid nucleation and encapsulation of nucleic acids [65]. Key process parameters, such as FRR and TFR, govern mixing kinetics and diffusion distances, directly shaping CQAs like particle size and encapsulation efficiency ( > 90%) [66]. Additionally, physicochemical factors, including buffer pH and ionic strength, modulate the ionization state of lipids (e.g., SM-102, pKa ~6.7), influencing electrostatic lipid-RNA interactions and particle morphology [21]. Notably, iLNP formation is a multi-step process: initial nucleation forms unstable intermediates, followed by particle fusion during pH neutralization (typically ~pH 6.5–7.4), which can increase size and reduce PDI [13]. These mechanistic insights underpin the rational design of microfluidic processes, enabling tailored, high-quality iLNP production for RNA therapeutics.

## 4. Microfluidics in iLNPs production

Microfluidic techniques offer highly precise control over NPs formation by manipulating fluids at the micro-scale under laminar flow conditions. These methods rely on diffusion-driven mixing and eliminate the need for post-processing steps [67]. At the laboratory scale, PDMS-based devices are widely used due to their affordability and suitability for rapid prototyping. To achieve production at an industrial scale, ongoing efforts focus on approaches like parallelizing microfluidic channels and designing high-throughput devices capable of handling elevated flow rates to satisfy commercial requirements. Also, the formation of LNPs occurs through self-assembly triggered by mixing lipid-containing solvent streams with aqueous solutions [68]. This mixing causes polarity shifts that promote aggregation of the lipid molecules. Several key methods enable this process,

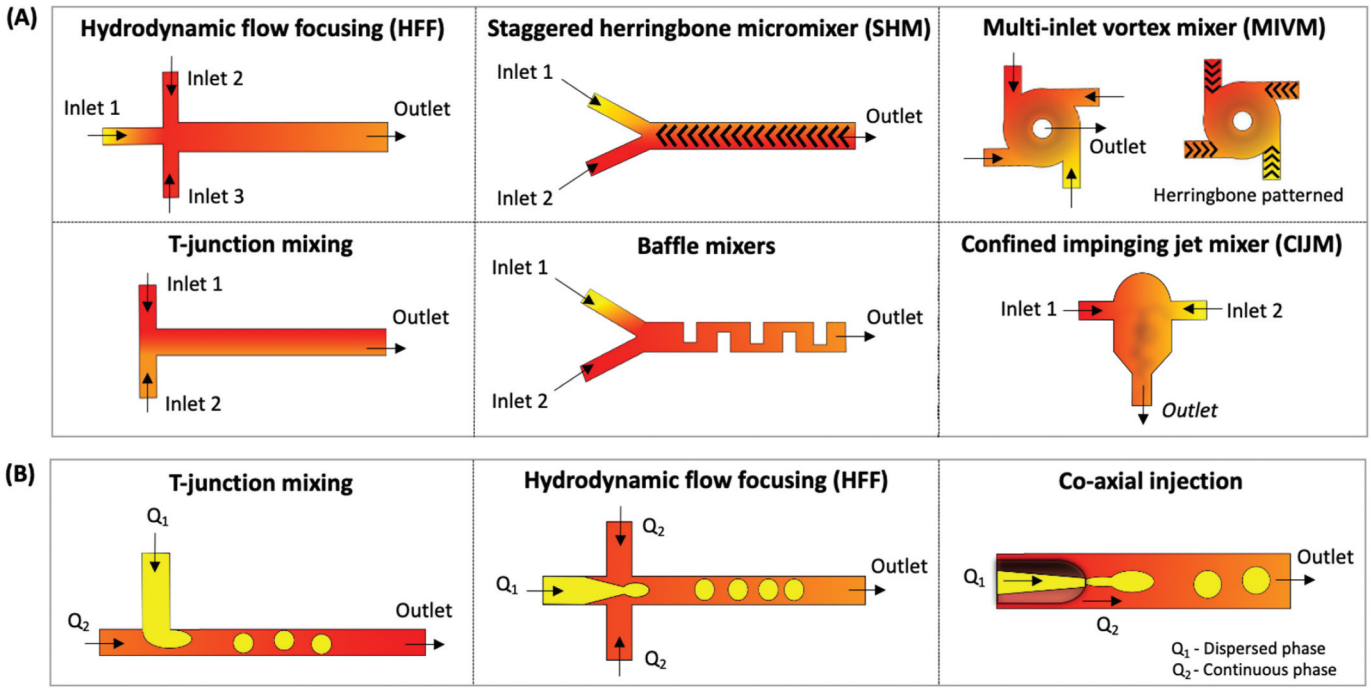


Figure 1. (A) Microfluidic strategies employed in the fabrication of drug and gene delivery NPs, including liposomes, LNPs, and core–shell nanostructures. (B) Droplet microfluidics utilized for encapsulating lipid-based nanostructures and constructing synthetic lipid vesicles resembling artificial cells. Reproduced from [69], under the terms of a creative commons attribution license (https://creativecommons.Org/licenses/by/4.0/).

including HFF, chaotic advection-based micromixers (such as SHM), and droplet-based microfluidics. These approaches enable reproducible synthesis of NPs tailored for biomedical applications (Figure 1) [69].

In other words, various microfluidic devices enable precise tuning of LNPs sizes ranging from 20 to 450 nm with resolution as fine as 10 nm can be achieved, which is especially critical for targeted drug delivery applications such as tumor accumulation and cellular uptake [70]. For instance, chaotic advection micromixers have successfully produced propofol-loaded liposomes (50–450 nm) with 41% drug loading efficiency combining production and encapsulation in a single step [19]. Similarly, baffle micromixers have yielded LNPs sized 20–100 nm, significantly enhancing siRNA delivery and hepatocyte targeting in vivo [71]. These microfluidic systems achieve encapsulation efficiencies of up to 80% for doxorubicin and 20–35% for proteins, while operating at room temperature to preserve sensitive agents such as mRNA and peptides [72]. Moreover, they support scalable and rapid production with real-time quality control, ensuring low polydispersity and high reproducibility [14].

Using HFF-based platforms, researchers have constructed libraries of single- and dual-ligand liposomes by finely tuning the FRR to control size, surface charge, and ligand density. One example involved dual-ligand liposomes conjugated with folic acid and TAT peptide, which enhanced cellular uptake, tumor spheroid penetration, and *in vivo* retention for up to 72 hours. In another study, a five-inlet microfluidic chip was used to develop PEGylated liposomes co-loaded with doxorubicin and umbelliprenin, yielding particles sized 100–250 nm with enhanced cytotoxicity against breast cancer cells compared to bulk mixing. Such examples demonstrate how microfluidics streamlines the creation of multifunctional, tumor-targeted delivery systems, accelerating their clinical translation [21,73–75]. Additionally, microfluidic technologies have optimized liposomal formulations for carboplatin delivery using the FLUIGENT MFCS-EZ system (Figure 2) [76].

In the context of nucleic acid therapeutics, microfluidics has proven indispensable for designing cationic LNPs with high encapsulation efficiency and strong transfection performance. Y-shaped SHM devices have been used to formulate monodisperse LNPs ( < 100 nm) for self-amplifying RNA vaccines against rabies, eliciting immune responses comparable to leading commercial formulations [67]. Similar devices have been adapted to formulate DNA-loaded LNPs for gene therapy, where tuning PEGylation and FRR significantly improved transfection efficiency while minimizing cytotoxicity in HEK-293 and CaSki cells. These findings highlight the power of microfluidic platforms to control LNPs physical properties precisely, while simultaneously optimizing biologically relevant performance outcomes [77].

## 5. Microfluidics for iLNPs fabrication

Microfluidics has revolutionized iLNP production by enabling precise control, high throughput, and scalable manufacturing. Various mixing strategies such as T-junction, SHM, HFF, and toroidal mixers – optimize their nanoprecipitation-driven self-assembly [14,67,78]. Each mixing method offers distinct flow profiles and mixing efficiencies, which directly impact NP properties and downstream therapeutic performance. A comparative study revealed the pivotal role of mixer design in determining iLNP functionality. For instance, in

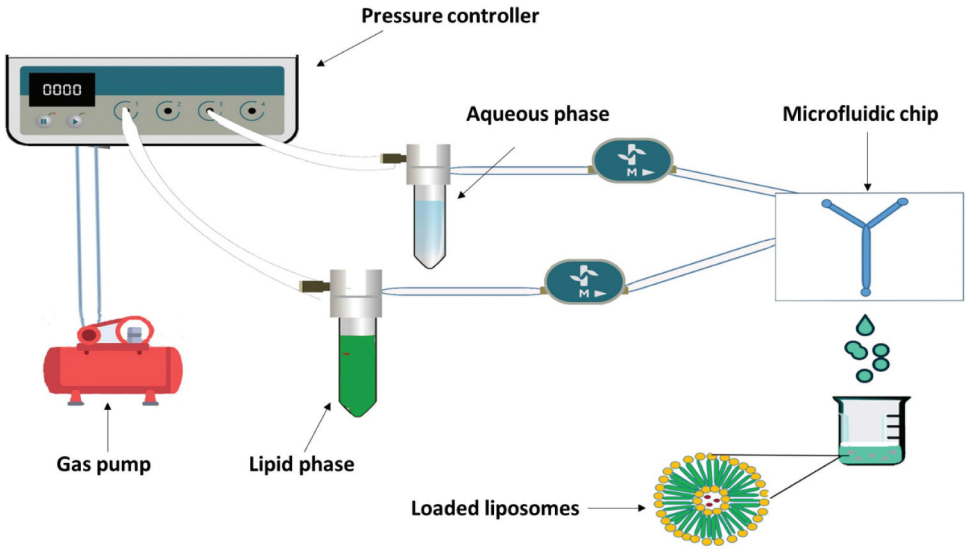


Figure 2. Schematic illustration of the microfluidic setup and the LNPs production process. The system includes a gas pump to generate pressure, a pressure regulator, and two reservoirs-one designated for the lipid phase and the other for the aqueous phase. These components are connected to a microfluidic chip, which functions as the mixing unit. Reproduced from [76], under the terms of a creative commons AttributionLicense (https://creativecommons.Org/licenses/by/4.0/).

siRNA delivery applications, HFF was identified as the most effective approach, producing particles with high encapsulation efficiency, narrow size distribution, and delivery efficacy in lung tissue comparable to clinically validated formulations. However, using the same lipid composition for mRNA delivery yielded successful outcomes only when fabricated via SHM, not HFF. This stark contrast underscores a critical insight: the success of RNA-loaded LNPs therapeutics is not solely dictated by lipid formulation, but is highly dependent on the microfluidic mixing architecture and flow regime employed during fabrication [78,79]. Recent advancements in microfluidic mixer designs, such as advanced crossflow micromixing, have further enhanced the scalability and reproducibility of iLNPs production, achieving consistent particle sizes and high encapsulation efficiencies for mRNA vaccines [13].

The intricate interplay between fluid dynamics and the physicochemical properties of nucleic acid cargo serves as a key determinant of both the structural integrity and functional efficacy of the resulting iLNPs. This relationship highlights the critical need for developing formulation strategies that are tailored to the specific molecular characteristics of different therapeutic payloads, such as siRNA, mRNA, or CRISPR components. Within microfluidic systems, two tunable parameters play an especially influential role: the FRR between the aqueous and organic phases and the TFR. By precisely modulating these parameters, researchers can fine-tune iLNP characteristics with a high degree of control. Extensive studies have demonstrated that systematic variation in FRR and TFR leads to significant, predictable changes in critical NP metrics particularly PDI and encapsulation efficiency. These physical properties, in turn, cascade into downstream biological effects, directly impacting the therapeutic potency, biodistribution, and clinical efficacy of the iLNPs formulations [80]. Moreover, integrating machine-learning-guided formulation with microfluidic platforms has enabled rapid optimization of iLNPs properties, enhancing targeted delivery for personalized therapeutics [81].

A groundbreaking *in vivo* screening platform was developed to systematically optimize iLNP formulations for mRNA delivery. In this approach, researchers used a microfluidic mixer to generate a diverse library of LNPs, each encapsulating barcoded mRNA (b-mRNA). The organic phase contained ionizable lipid (C12-200), helper lipid (DOPE), cholesterol, and PEG-lipid (C14-PEG2000), which was rapidly mixed with an aqueous solution of b-mRNA. The resulting iLNP approximately 83 nm in diameter were characterized using cryo-transmission electron microscopy and DLS Following intravenous administration in mice, the pooled library allowed for organ-level quantification of mRNA delivery, using barcode sequencing to track biodistribution across tissues such as the liver and spleen. This platform successfully identified top-performing formulations, which were further validated through luciferase reporter gene expression. Importantly, the results demonstrated that delivery efficiency is highly dependent on the type of nucleic acid cargo, reinforcing the need for payload-specific formulation strategies. Overall, this high-throughput, combinatorial approach marks a major advancement in the rational design of iLNPs, enabling rapid identification of optimized candidates for targeted organ-specific RNA delivery [82]. This approach has been further refined by incorporating de novo mRNA synthesis within microfluidic chips, streamlining vaccine development pipelines for rapid clinical translation [83].

In a separate study, researchers synthesized four structurally distinct ionizable lipids 306Oi10, 503Oi10, 306O10, and 514O6 employed them to formulate mRNA-loaded LNPs. Using a specialized microfluidic mixer, they evaluated the impact of various flow rates and mixing ratios on formulation performance. In vivo testing in mice revealed that 306Oi10-based iLNPs exhibited strong liver-targeting properties, whereas 503Oi10-based formulations showed broader biodistribution across multiple organs but with reduced activity. Interestingly, a single optimized microfluidic setting yielded

functional iLNPs across all lipid types; however, delivery efficiency still varied significantly when compared to manually mixed formulations, despite identical starting compositions. These findings underscore a critical insight: even minor structural differences in lipid architecture necessitate distinct microfluidic parameters, as the resulting particle composition and performance can diverge substantially between microfluidic and conventional mixing methods. This emphasizes the need for formulation-specific process optimization, balancing efficient mRNA delivery with precise organ targeting tailored to each therapeutic application [84]. These optimizations have accelerated next-generation mRNA vaccine development, with microfluidic platforms enabling rapid, reproducible reformulation to address emerging variants [85].

As previously noted, both ionizable lipid composition and microfluidic parameters play pivotal roles in defining iLNP physicochemical and functional attributes. In a comparative study, four ionizable cationic lipids DODAP, Dlin-MC3-DMA, ALC-0315, and SM-102 widely used in mRNA delivery, were formulated into iLNPs using a T-junction microfluidic mixer, where ethanol-dissolved lipids were rapidly combined with an acidic aqueous mRNA solution. The resulting particles exhibited similar physical characteristics, including particle size, PDI, surface charge (zeta potential), and encapsulation efficiency, and remained stable for at least one month under storage conditions. However, in vitro assays revealed functional differences, with SM-102-based iLNPs showing the highest levels of fluorescent protein expression and T-cell proliferation. These findings suggest that while microfluidic techniques ensure reproducible NP synthesis, the intrinsic chemical structure of the ionizable lipid remains a key determinant of biological performance [86]. Similarly, Sato et al. demonstrated the impact of microfluidic fine-tuning: under an FRR of 3 and TFR of 1.5 mL/min, changes in ionizable lipid type (e.g., CL15A6 vs. CL15H6) and helper lipid composition significantly altered particle size, cellular uptake, and gene silencing potency. Notably, substituting cholesterol reduced particle size from ~35 nm to 24 nm and improved silencing efficacy (Half maximal inhibitory concentration) $IC_{50}$) reduced from 20 nM to 7 nM) a result particularly relevant for applications like lymph node targeting, where precise size control is critical for immune engagement [80].

A deeper understanding of iLNP formation mechanisms is essential for optimizing microfluidic production strategies. Contrary to prior assumptions, iLNPs do not fully form during initial mixing, but rather during subsequent pH neutralization, where small, pH-sensitive liposomal intermediates fuse into larger, denser particles as pH shifts from acidic to physiological levels. A comprehensive study using DoE with Box-Behnken methodology investigated this transition using DODAP (pKa = 6.58) as the ionizable lipid. The formulation composed of DODAP, hydrogenated soy phosphatidylcholine, cholesterol, and PEG-lipid was produced via microfluidic mixing, systematically varying lipid concentration, FRR, and TFR. Although siRNA encapsulation efficiency remained consistently high, particle size and PDI were highly sensitive to lipid concentration and FRR. Notably, during dialysis-driven pH neutralization (from pH 4.0 to 7.4), DODAP neutralization around pH 6.5 induced LNP fusion, leading to increased size and reduced PDI, especially at higher FRRs (e.g., 3:1 vs. 1:1). Furthermore, siRNA-lipid electrostatic interactions buffered against ethanol-induced rearrangements, stabilizing particle size compared to empty iLNPs. Interestingly, different pH-neutralization approaches yielded divergent outcomes: for instance, iLNPs at FRR = 1 showed notable size increases when HEPES-buffered neutralization preceded dialysis. These findings highlight how post-mixing conditions, particularly pH dynamics, play a decisive role in dictating final iLNP structure and uniformity a consideration often overlooked in traditional formulation pipelines [87].

Scaling up iLNP production without compromising quality remains a major hurdle in translating bench-top discoveries into clinical applications. To address this, several advanced microfluidic platforms have been engineered to combine high-throughput capacity with nanoscale precision. One notable example is the glass-iLiNPs device, which supports the production of mRNA-loaded LNPs using clinically relevant ionizable lipids such as MC3 (pKa 6.35), SM-102 (pKa 6.68), and ALC-0315 (pKa 6.09). This platform achieves uniform particle sizes (~90–100 nm) and high encapsulation efficiencies ( > 90%) across all lipid types. Among them, SM-102-based iLNPs demonstrated superior biological performance, likely due to its higher pKa facilitating enhanced endosomal escape and mRNA release. What sets the glass-iLiNPs system apart is its scalable design based on 'piling-up' and 'numbering-up' strategies, enabling production rates up to 160 mL/min using eight parallelized units, while maintaining precise control of particle sizes (20–60 nm). This integration of throughput, precision, and lipid versatility positions glass-iLiNPs as a transformative platform for clinical-scale RNA therapeutics manufacturing [88]. This scalability is further evidenced by recent developments in parallelized microfluidic systems, which achieve production rates suitable for large-scale vaccine manufacturing while maintaining CQAs [89,90].

In parallel to the glass-iLiNPs system, the parallel microfluidic device (PMD) represents a next-generation platform for high-throughput iLNP production, particularly suited for RNA-based therapeutics and vaccines. Unlike conventional techniques, PMD consistently generates particles within the 70–140 nm range, maintaining tight size distributions and high encapsulation efficiency across a variety of formulations. In preclinical assessments, PMD-fabricated iLNPs achieved > 80% gene silencing in human cell cultures, mirroring the efficacy of small-scale platforms. Moreover, in murine models, these iLNPs induced a > 90% knockdown of a target blood protein and enhanced reporter gene expression by over fivefold compared to traditional bulk methods. A key advantage of the PMD lies in its modular and cost-efficient design, which supports production rates over 100-fold greater than single-channel systems, all while maintaining CQAs. These findings underscore the PMD's clinical relevance as a scalable, robust, and economically viable solution for large-scale RNA delivery applications [91]. Such platforms have been instrumental in rapidly adapting iLNPs formulations for emerging COVID-19 variants, demonstrating the flexibility of microfluidic systems in vaccine development [92,93].

Within mRNA vaccine development, microfluidic platforms have facilitated the rapid fabrication of iLNPs designed to address emerging COVID-19 variants. Formulations containing HEAH, DSPC, cholesterol, and DMG-PEG2000 achieved an encapsulation efficiency of 91.76%, with particle sizes averaging 112.67 nm and a PDI of 0.16 comparable to formulations using ALC-0315. These results demonstrate the scalability, flexibility, and reproducibility of microfluidic platforms in vaccine development pipelines. Additionally, microfluidic systems have enhanced the fabrication of ionizable nanoemulsions for RNA delivery, particularly using the NanoAssemblr™ platform. In this setup, researchers combined an organic phase (C12-200, DOPE, Vitamin E, and DMG-PEG2000) with an aqueous RNA phase, operating under controlled flow conditions (1:5 ratio, 12 mL/min). This precise control resulted in smaller, more uniform NPs than those produced by bulk mixing. When evaluated in HeLa cells, these iNEs exhibited reduced cytotoxicity while preserving transfection efficiency, highlighting how microfluidics enables fine-tuned engineering of NP properties to balance safety and functionality in RNA therapeutics (Figure 3) [94,95].

The NanoAssemblr™ platform has also been pivotal in producing bivalent mRNA vaccines, with recent studies demonstrating its ability to encapsulate mRNAs for multiple variants with high efficiency [96]. Importantly, microfluidic devices such as the NanoAssemblr™ platform were directly applied in the large-scale production of Pfizer – BioNTech and Moderna vaccines, enabling rapid, consistent, and regulatory-compliant manufacturing of billions of doses during the pandemic. This demonstrated the unique value of microfluidics in bridging laboratory-scale iLNP formulations to real-world clinical translation, ensuring reproducibility and rapid adaptation to novel SARS-CoV-2 variants [97].

A particularly sophisticated application of microfluidic technology was demonstrated in cancer immunotherapy, where TRAIL mRNA-loaded iLNPs were designed to selectively target the tumor microenvironment. The LNPs were formulated using the ionizable lipid C12-200, combined with helper components including DOPE, cholesterol, and C14-PEG2000. Employing a 3:1 ethanol-to-aqueous phase ratio, the microfluidic mixing process enabled the precise, scalable, and reproducible generation of homogeneous iLNPs with controlled physicochemical properties. The resulting NPs exhibited a hydrodynamic diameter of 121.1 ± 0.56 nm, low PDI (0.146 ± 0.04), a slightly negative surface charge (−2.82 ± 0.60 mV), and encapsulation efficiency of ~84%. Significantly, the iLNPs demonstrated an apparent pKa of 7.96, suggesting a high ionization potential at endosomal pH, which is essential for membrane fusion and endosomal escape. This facilitated the efficient cytosolic release of TRAIL mRNA upon cellular uptake. The ability of microfluidics to finely tune formulation variables was crucial in achieving structural uniformity and functional performance in vivo. Ultimately, these iLNPs induced potent tumor cell apoptosis via TRAIL-mediated pathways, delivering precise antitumor activity while minimizing the systemic side effects typically associated with protein-based TRAIL delivery.

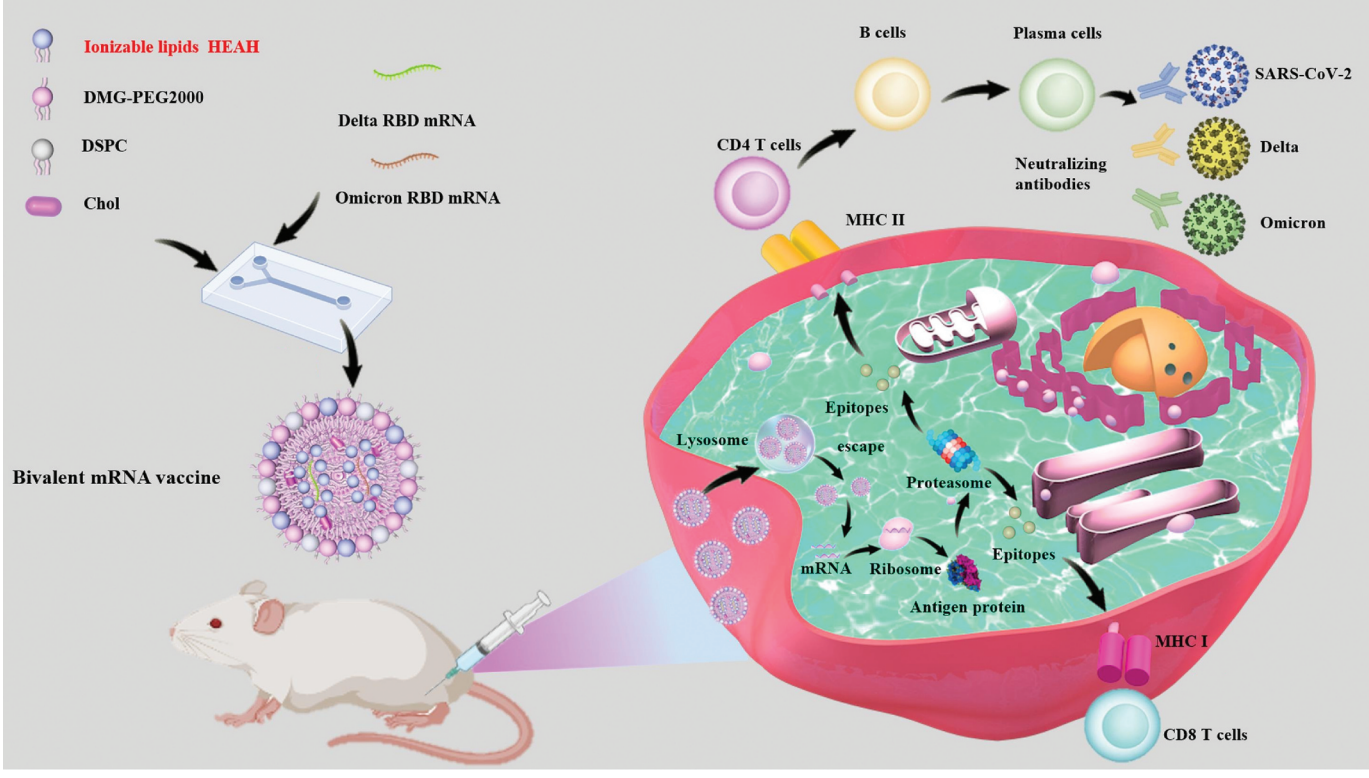


Figure 3. Schematic depiction of a bivalent mRNA vaccine facilitated by ionizable lipid heah. Receptor-Binding Domain (RBD) mRNAs from Delta and Omicron variants are encapsulated within HEAH-based LNPs using a microfluidic platform, resulting in a bivalent mRNA formulation. Upon administration, the vaccine is internalized by antigen-presenting cells, where the released mRNAs are translated into their respective RBD proteins via ribosomal machinery. These proteins are subsequently processed into antigenic peptides, which are presented on MHC class I and II molecules, thereby eliciting robust cellular and humoral immune responses. Reproduced with permission [95]. Copyright 2023, Elsevier.

This example highlights how microfluidic platforms not only enhance manufacturing precision but also unlock therapeutic potential through the rational design of targeted, high-performance iLNP formulations (Figure 4) [98]. Recent advancements have further leveraged microfluidic platforms to optimize TRAIL mRNA delivery, achieving enhanced tumor-specific targeting through fine-tuned lipid compositions [99]. Importantly, these microfluidic advances in cancer nanomedicine not only improve experimental precision but also demonstrate a clear translational trajectory, where scalable and reproducible iLNPs manufacturing could support the clinical development of RNA-based immunotherapies alongside vaccines.

In summary, the translational potential of microfluidic-based iLNP manufacturing has become increasingly evident, bridging laboratory-scale discoveries to industrial and clinical applications. Unlike bulk mixing approaches, microfluidic systems enable continuous, reproducible, and contamination-controlled production under conditions compatible with cGMP standards, ensuring consistent CQAs such as particle size, PDI, and encapsulation efficiency [67,100]. This has been exemplified by their integration into large-scale vaccine manufacturing pipelines, most notably in the Pfizer – BioNTech (BNT162b2) and Moderna (mRNA-1273) COVID-19 vaccines, where microfluidic platforms such as NanoAssemblr™ enabled precise formulation and scalable throughput [23,101,102]. These advances highlight how microfluidic production facilitates regulatory compliance, accelerates process validation, and minimizes batch-to-batch variability – factors essential for clinical translation. Furthermore, microfluidic systems are being adapted for personalized medicine, including ex vivo RNA delivery for gene editing and in vivo cancer immunotherapy, where controlled formulation and reproducibility are critical for clinical success [102,103]. Collectively, these translational developments demonstrate that microfluidics is not only a tool for nanoscale engineering but a cornerstone for next-generation, patient-tailored RNA therapeutics.

### 5.1. Comparative analysis of RNA delivery platforms

Microfluidic technology has fundamentally reshaped the landscape iLNP-based RNA therapeutics. To appreciate its advantages, it is essential to compare iLNPs with other RNA delivery platforms. iLNPs incorporating advanced ionizable lipids such as SM-102 and ALC-0315 excel in microfluidic systems by achieving encapsulation efficiencies exceeding 90%, PDI below 0.1, and transfection efficiencies of 70–95%, attributed to their finely tuned pH-dependent self-assembly and precise control over flow dynamics (e.g., HFF, SHM) [1,104,105]. For instance, SM-102–based iLNPs demonstrated superior T-cell proliferation and protein expression in mRNA vaccine formulations, underpinning the clinical success of the BNT162b2 (Pfizer – BioNTech) and mRNA-1273 (Moderna) vaccines [97].

In contrast, traditional LNPs that lack ionizable components typically yield 80–90% encapsulation efficiency but exhibit broader size distributions (PDI ≈0.2), which limits reproducibility for complex RNA cargos such as CRISPR – Cas systems

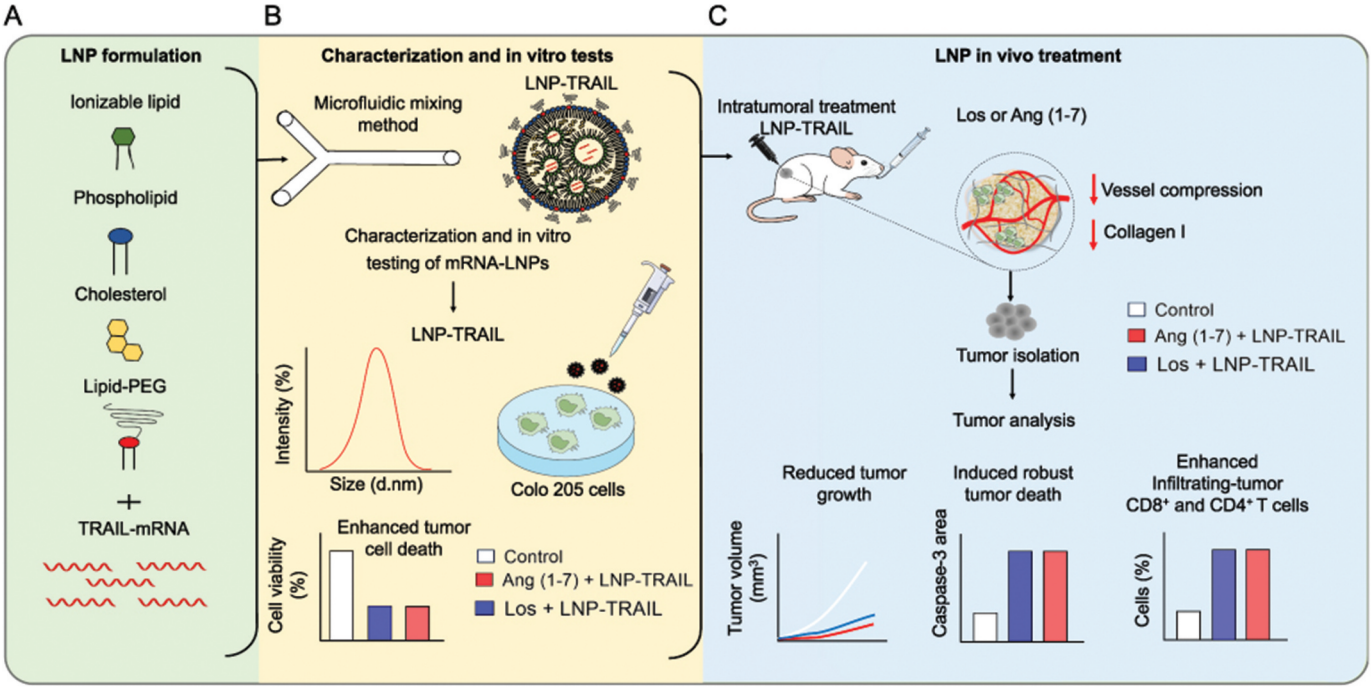


Figure 4. Illustrative workflow outlining the development of an iLNP system for targeted delivery of trail mRNA to the tumor microenvironment, aiming to induce cancer cell apoptosis. (A) Diagram showing the key components involved in the formulation of LNPs. (B) Fabrication of TRAIL-mRNA-loaded LNPs using microfluidic mixing technology. The resulting NPs were evaluated using dls for characterization, and their therapeutic potential was tested *in vitro* using Colo 205 colorectal cancer cells. (C) Illustration of an *in vivo* experimental setup using humanized NSG mice implanted with human colorectal tumor cells. Mice received oral administration of either Los or Ang (1–7), alongside direct intratumoral injection of LNP-TRAIL. Tumor progression was monitored throughout the study, and subsequent analyses focused on tumor cell apoptosis and immune cell infiltration. Reproduced with permission from [98], under the terms of a Creative Commons Attribution - Non Commercial License (https://creativecommons.org/licenses/by-nc/3.0/).

[105,106]. Viral vectors provide near-complete transduction efficiency yet suffer from significant immunogenicity, preexisting immunity, and high manufacturing costs, restricting their scalability for RNA delivery [107,108]. Polymeric NPs achieve moderate encapsulation (60–80%) and transfection efficiencies (50–70%) but are hindered by cytotoxicity and limited clinical progression beyond early-phase trials [109]. Similarly, exosomes offer excellent biocompatibility and low immunogenicity but face challenges related to loading efficiency (50–70%), isolation, and standardization, which impede their large-scale therapeutic translation [110]. Lipid – polymer hybrid NPs balance stability and delivery efficiency (80–90% encapsulation); however, their complex and multistep synthesis compromises scalability and cost-effectiveness compared to iLNPs [111]. Inorganic NPs, such as gold or silica-based systems, exhibit relatively low RNA loading (40–60%) and transfection rates (30–50%) and are mainly confined to preclinical investigations due to high production costs and poor biodegradability [112].

In comparison, microfluidic-produced iLNPs stand out for their scalability, reproducibility, and alignment with regulatory standards, particularly under Quality-by-Design (QbD) and ICH Q8–Q10 frameworks [113]. These platforms enable precise control of CQAs such as particle size, PDI, and encapsulation efficiency through real-time modulation of flow parameters (FRR, TFR, mixing intensity). The continuous-flow nature of microfluidic systems inherently supports cGMP-compliant manufacturing, minimizing batch-to-batch variability and facilitating rapid process validation [21,114]. Consequently, microfluidic iLNPs production not only enhances therapeutic consistency but also accelerates clinical translation demonstrated by its pivotal role in mRNA vaccines and emerging applications in cancer immunotherapy [97].

## 6. Challenges and optimization of microfluidics in iLNPs production

Scaling the production of iLNPs from lab-scale experiments to clinical-grade manufacturing presents significant challenges. The most critical issue lies in maintaining tight control over NP characteristics – such as particle size, PDI, and encapsulation efficiency – while increasing throughput. Traditional single-channel microfluidic systems are incapable of meeting industrial demands. When scaled, they often yield iLNPs with inconsistent quality. To address this limitation, advanced systems like the glass-iLiNPs platform and the PMD employ channel parallelization, achieving impressive flow rates up to 160 mL/min while maintaining particle sizes between 20–140 nm and encapsulation efficiencies exceeding 90%. However, ensuring uniform mixing across multiple channels remains technically complex. Real-time flow monitoring, CFD modeling, and in-line quality control systems are increasingly essential for reducing batch variability and ensuring clinical-grade consistency [88].

A key axis of optimization lies in precise control of flow parameters, especially the FRR and TFR. These variables directly impact nucleic acid encapsulation efficiency, particle size, and PDI. For example, an FRR of 3:1 and TFR of 1.5 mL/min reduced particle size from 35 to 24 nm in certain formulations, enhancing cellular uptake and gene silencing efficiency. However, these optimal conditions vary with cargo type (e.g., siRNA vs. mRNA) and ionizable lipid structure, necessitating formulation-specific tuning. Recent high-throughput screening systems using barcoded mRNA libraries have proven effective in rapidly identifying optimal flow and lipid parameters for various therapeutic goals [80].

Beyond process parameters, the chemical structure of ionizable lipids introduces another optimization challenge. Lipids such as SM-102 (pKa 6.68) outperform others like ALC-0315 (pKa 6.09) due to better endosomal escape properties, but their performance is highly sensitive to microfluidic mixing conditions. Notably, iLNP formation primarily occurs not during initial mixing, but during pH neutralization, where particle fusion at ~pH 6.5 significantly alters size and PDI. This highlights the importance of understanding post-formation dynamics. DoE strategies, such as Box – Behnken designs, are increasingly used to optimize multiple factors simultaneously, but they require extensive experimentation to account for complex lipid – nucleic acid interactions [87]. The optimization of microfluidic-based iLNP production closely aligns with the QbD principles defined in ICH Q8–Q10, promoting a systematic and risk-based framework for pharmaceutical development. DoE approaches such as the Box – Behnken design, critical process parameters including FRR, TFR, and buffer pH are optimized to control CQAs such as particle size, PDI, and encapsulation efficiency. Integrating real-time flow monitoring, CFD modeling, and machine-learning-assisted feedback enables continuous process verification and minimizes variability during scale-up, consistent with ICH Q9 and Q10 guidelines. Collectively, these QbD-driven and data-informed strategies ensure cGMP-compliant manufacturing of iLNPs with high reproducibility, robust quality, and regulatory readiness for clinical translation [115–118].

Furthermore, achieving reproducibility across diverse RNA payloads (e.g., self-amplifying RNA, guide RNA) and therapeutic contexts (e.g., vaccines vs. cancer) remains a persistent bottleneck. Addressing these challenges increasingly calls for advanced, adaptive control strategies. In this context, emerging approaches that integrate AI and ML into microfluidic platforms capable of predictive modeling, real-time parameter adjustment, and device-level optimization CFD offer a promising pathway toward intelligent and scalable iLNPs manufacturing, as discussed below.

## 7. ML-Assisted optimization strategies

ML-assisted optimization is emerging as a transformative tool to address these multifactorial challenges. Predictive ML models such as gradient boosting and random forests can be trained on experimental datasets linking input parameters (e.g., lipid composition, FRR, TFR, buffer pH, ionic strength, organic solvent polarity) to output metrics (e.g.,

particle size, PDI, encapsulation efficiency). Studies have shown that such models can predict liposome or iLNPs size with correlation coefficients above 0.90, enabling the pre-selection of optimal conditions before conducting physical experiments [119,120]. This approach drastically reduces trial-and-error cycles and experimental costs. Beyond formulation, ML is also applied to device engineering: generative design algorithms and reinforcement learning have been used to create novel microfluidic mixer geometries that outperform conventional designs in mixing efficiency and flow uniformity [121,122]. Some groups have demonstrated closed-loop systems where inline sensors (e.g., DLS) feed real-time particle size data into ML models, which then adjust FRR and TFR on-the-fly to maintain target specifications critical for scaling up while preserving quality [81]. Recent case studies vividly illustrate the transformative power of ML in optimizing iLNPs, bridging the gap between theoretical models and practical, scalable applications in RNA therapeutics. These examples not only demonstrate ML's precision but also highlight its ability to reduce experimental burdens, enhance predictability, and accelerate clinical translation. For instance, Maharjan et al. demonstrated XGBoost and Bayesian optimization algorithms to meticulously fine-tune microfluidic parameters such as FRR, total TFR, and buffer pH in mRNA- LNPs vaccine formulations. By analyzing vast datasets of lipid compositions and process variables, their model achieved encapsulation efficiencies exceeding 95% and PDI below 0.1, resulting in significantly boosted vaccine immunogenicity and stability, paving the way for faster development of next-generation vaccines against infectious diseases [123]. Similarly, Palanki et al. employed regression-based ML models to screen comprehensive LNPs libraries for fetal gene delivery, where the algorithms predicted NP size, surface charge, and cellular uptake with an impressive 80% accuracy. This approach identified optimal ionizable lipids for targeted RNA transport across placental barriers, minimizing off-target effects and enhancing therapeutic precision in prenatal gene editing applications [124]. In another compelling study, Ding et al. integrated deep learning into LNPs design for mRNA delivery, using convolutional neural networks to analyze structural data of over 1,000 lipid variants; this led to a 70% reduction in experimental iterations while forecasting transfection efficiency with greater than 85% accuracy, ultimately yielding formulations with superior endosomal escape properties for cancer immunotherapy [125]. Bae et al. further advanced this by applying random forest regression to ionizable lipid selection, optimizing pKa values and helper lipid ratios to double mRNA expression levels in vivo, demonstrating ML's role in tailoring vaccines for enhanced immune responses against viral pathogens [126]. Also, Sato et al. focused on manufacturing processes, utilizing supervised ML to dissect microfluidic mixing dynamics, which improved encapsulation efficiency to 92% and PDI to under 0.08, enabling reproducible, high-throughput RNA-LNPs production suitable for large-scale clinical trials [127]. Lee et al. employed generative ML models for formulating ionizable lipids, generating novel structures that accelerated nucleic acid transfection by 50% in cellular models [128]. Rafiei et al. leveraged ML classifiers to design immunomodulatory LNPs for mRNA delivery to microglia, achieving 65% repolarization of hyperactivated cells in neurodegenerative disease models, highlighting ML's potential in neurotherapeutics [129]. Further, Li et al. combined ML with combinatorial chemistry to discover high-potency ionizable lipids, boosting mRNA delivery by 3.5-fold while cutting design time by 40%, a breakthrough for personalized medicine [130]. Finally, Xu et al. introduced the AGILE deep learning platform, which streamlined LNPs engineering for organ-specific targeting with 90% predictive success, facilitating rapid adaptation for liver, lung, or tumor-selective RNA delivery [131]. Collectively, these studies exemplify how ML not only demystifies complex interactions in iLNP fabrication but also propels the field toward intelligent, efficient, and patient-centric nanomedicine solutions.

Building on these ML-driven advancements, the integration of process optimization and innovative microfluidic platforms further enhances the precision and adaptability of iLNPs production for diverse therapeutic applications. So, by combining process parameter optimization with device-level innovation, ML offers a pathway toward intelligent, adaptive, and application-specific iLNPs manufacturing systems. Tailoring iLNP formulations for organ-specific delivery adds further complexity. For example, lipids like 306Oi10 yield liver-targeted delivery, while 503Oi10 distributes more broadly [132]. These findings reveal that even small changes in lipid structure demand distinct microfluidic conditions. Versatile microfluidic platforms like NanoAssemblr™ have enabled rapid adaptation across applications, producing iLNPs with excellent encapsulation efficiency and low PDI [96]. However, achieving reproducibility across diverse RNA payloads (e.g., self-amplifying RNA, guide RNA) and therapeutic contexts remains a persistent bottleneck. Looking forward, several innovations hold promise for overcoming current limitations and advancing the next generation of microfluidic platforms for iLNPs production. Integrating AI and ML with microfluidic systems could enable real-time adjustment of critical parameters such as FRR, TFR, and temperature based on feedback from inline sensors [40].

Moreover, the development of modular or programmable microfluidic chips with interchangeable mixing units may facilitate rapid customization across various lipid types and nucleic acid cargos, enhancing flexibility in formulation design [133]. To ensure successful clinical translation, it is also essential to integrate these platforms into regulatory-compliant, cGMP-compatible production lines that incorporate robust validation protocols and scalable hardware. Ultimately, optimizing microfluidic-based iLNPs manufacturing will require a synergistic approach that combines high-throughput experimentation, predictive modeling, and AI-driven feedback systems to create intelligent, scalable, and personalized NPs production workflows (Figure 5).

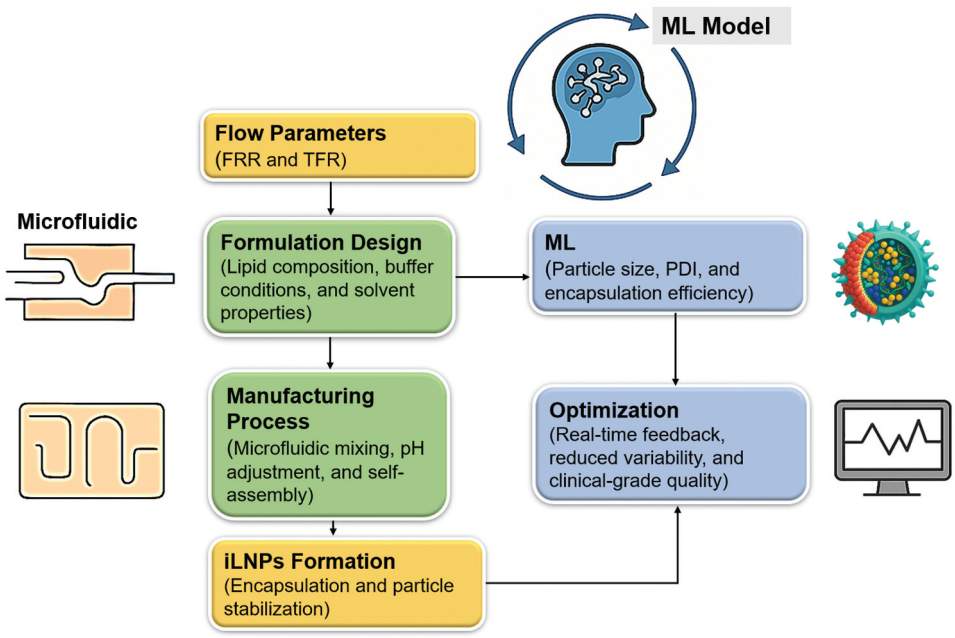


Figure 5. Integration of ML with microfluidic iLNPs production. Predictive models forecast particle size, PDI, and encapsulation efficiency from flow and formulation inputs, enabling real-time optimization and reproducible large-scale manufacturing.

## 8. Conclusion

Microfluidic technology has fundamentally redefined the fabrication of iLNPs, enabling precise regulation of particle size, PDI, and encapsulation efficiency, while offering a scalable and reproducible pathway toward clinical-grade manufacturing. Continuous progress in microfluidic mixer design, flow regime optimization, and lipid formulation chemistry has expanded the therapeutic applicability of iLNPs across mRNA vaccines, gene editing, and cancer immunotherapy. Despite these advances, several critical challenges persist. The translation of microfluidic processes from laboratory-scale to industrial production demands solutions for device parallelization, batch uniformity, and real-time quality assurance. Moreover, the chemical diversity and pH-sensitivity of ionizable lipids complicate universal optimization, necessitating formulation-specific tuning under cGMP frameworks. From a regulatory perspective, the absence of standardized analytical and validation guidelines for microfluidic-based RNA delivery systems continues to hinder widespread clinical adoption.

## 9. Future perspectives

Looking forward, the integration of AI with adaptive process control systems is expected to transform the landscape of iLNPs manufacturing. These intelligent frameworks can enable real-time process optimization, predictive modeling of NPs stability, and self-correcting, fully automated production pipelines, ensuring consistent quality from bench to clinical scale. Moreover, the convergence of ML, organ-on-a-chip technologies, and high-throughput microfluidic screening promises to accelerate the design of patient-specific RNA formulations while minimizing preclinical experimentation. As regulatory standards evolve toward continuous manufacturing and data-driven validation, such digitalized, modular systems will underpin the next generation of precision nanomedicine one characterized by personalized, scalable, and QbD compliant iLNPs platforms that bridge innovation and clinical translation.

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## Author contributions statement

N.R: conceptualization and writing – original draft. M.A, H.B, F.N, H.N, S.H, V.U: writing – review and editing. T.G: supervision, validation, and writing – review and editing.

## Disclosure statement

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