Nanoscale

REVIEW

View Article Online
View Journal | View Issue

Cite this: *Nanoscale*, 2025, **17**, 23822

Microfluidic-based nanocarriers for overcoming biological barriers in therapeutic delivery systems

Alireza Gharatape, [a,b] Zahra Niasari-Naslaji,[c] Jeanne Leblond Chain, [d] Nasrollah Tabatabaei *[a] and Reza Faridi-Majidi *[a,b]

Microfluidic technologies have revolutionized the synthesis of nanocarriers for drug and gene delivery, providing unparalleled precision and efficiency in the production of therapeutic nanoparticles. This review highlights recent advancements in microfluidic systems, emphasizing their role in addressing critical challenges such as poor targeting, low bioavailability, and systemic toxicity associated with conventional delivery systems. By enabling the controlled production of nanocarriers with customizable size, composition, and release profiles, microfluidic platforms have represented a powerful tool in improving therapeutic efficacy and targeting capabilities. Key innovations discussed include the use of droplet microfluidics, flow-focusing techniques, and the incorporation of stimuli-responsive materials. Additionally, the integration of AI and machine learning has further enhanced the optimization and scalability of microfluidic synthesis processes. Nanocarriers represent a transformative approach to overcoming biological barriers in gene/drug delivery, enabling enhanced targeting, intracellular transport, and therapeutic efficacy, particularly for challenging conditions like central nervous system disorders and cancer. Despite ongoing challenges, such as scalability and cost-effectiveness, the future of microfluidic nanocarrier synthesis appears promising, with potential applications extending beyond drug and gene delivery to imaging, diagnostics, and personalized medicine. This comprehensive review underscores the transformative role of microfluidic-based nanocarriers in advancing nanomedicine and highlights the need for continued research and development in this rapidly evolving field.

Received 31st May 2025,
Accepted 14th September 2025

DOI: 10.1039/d5nr02322j

rsc.li/nanoscale

1. Nanotechnology and microfluidic engineering

Therapeutic delivery systems (TDS) like genetic materials and drugs have revolutionized modern medicine by offering transformative solutions for treating complex diseases such as cancer, genetic disorders, and infectious diseases.[1–3] By enabling targeted delivery of therapeutic agents, these systems not only reduce systemic side effects but also improve overall treatment efficacy, making a significant advancement over traditional methods.[4,5] Despite these benefits, conventional TDS continue to face critical challenges, including limited targeting specificity, low bioavailability of therapeutic agents, and the risk of systemic toxicity, which constrain their clinical success.[6,7] These challenges significantly hinder the full therapeutic potential of TDS, especially in complex disease settings where precise drug delivery is crucial. Such limitations underscore the need for more advanced, precise, and efficient delivery approaches. To this end, next-generation multifunctional delivery systems—particularly lipid nanoparticles (LNPs) – lipid based nanocarrier that designed to encapsulate and transport therapeutic agents like nucleic acids such as mRNA, siRNA, and DNA into target cells. Engineered with surface ligands and responsive elements—are gaining attention for their ability to address these limitations with improved precision and functionality.[8,9]

The development of effective therapeutic agents, especially nucleic acids and proteins, is hindered by complex biological barriers that limit their bioavailability and therapeutic performance. These barriers span extracellular and intracellular environments, including enzymatic degradation, rapid systemic clearance, endosomal entrapment, and restricted nuclear access. To address these challenges, nanocarriers have emerged as versatile platforms capable of navigating the biological milieu with precision. By tailoring their physico-

[a]*Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran. E-mail: ntabatabaei@tums.ac.ir, refaridi@sina.tums.ac.ir*
[b]*Advanced Nanocarrier Engineering Laboratory (ANEL), Department of Medical Nanotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran*
[c]*School of Life Science Engineering, College of Interdisciplinary Science and Technology, University of Tehran, Tehran, Iran*
[d]*Université de Bordeaux, CNRS, Inserm, ARNA, UMR 5320, U1212, F-33000, Bordeaux, France*

chemical properties and leveraging advanced engineering strategies—such as pH-responsive designs, receptor-mediated targeting, and biomimetic approaches—nanocarriers can enhance the solubility, stability, and selective delivery of therapeutic payloads. In particular, LNPs with post-formulation modifications—such as ligand post-insertion and surface engineering—are increasingly used to fine-tune targeting efficiency while preserving particle integrity.[8,10] Although LNPs are clinically leading nanocarrier systems for RNA and drug delivery, many other types of nanocarriers (*e.g.*, inorganic nanoparticles such as silica or iron oxide, gold nanoparticles, and polymeric nanoparticles) have been extensively studied preclinically.[11–13] Inorganic nanoparticles can be tuned to have desirable physicochemical properties while maintaining stability; however, concerns over long-term accumulation, cytotoxicity, and clearance have limited their clinical translation. In particular, gold nanoparticles possess unique optical properties that enable imaging and photothermal therapy, but potential immunogenicity, lack of biodegradability, and manufacturing constraints limit the widespread therapeutic application.[14,15] Polymeric nanoparticles can be designed with structural diversity for versatility while potentially offering controlled release; however, challenges include batch-to-batch reproducibility, suboptimal transfection efficiency for nucleic acids, and incomplete safety profiling.[16,17] This review highlights recent innovations in nanocarrier design, focusing on their ability to overcome critical barriers like the blood–brain barrier (BBB) and endosomal sequestration, while emphasizing the need for personalized and disease-specific strategies to advance clinical translation and improve patient outcomes.[18–20] In response, innovative strategies integrating nanotechnology and microfluidic platforms have emerged, offering enhanced precision, efficiency, and scalability in both drug and gene delivery.[21–23]

Microfluidic technologies offer promising approaches to overcome the limitations of conventional TDS by enabling precise control over fluid dynamics and nanoparticle synthesis at the nanometer scale.[24–27] These systems allow for the controlled production of therapeutic nanoparticles, such as liposomes and LNPs, with customizable size, composition, and release profiles.[28–30] In addition, microfluidic systems facilitate the generation of nanoparticles with controlled surface properties, which are crucial for enhancing targeting capabilities and minimizing off-target effects.[31] Furthermore, microfluidic platforms support scalable production while maintaining uniformity and reproducibility, both critical factors for clinical translation.[32–34] Despite the precise control over particle size, polydispersity, and encapsulation efficiency that microfluidics offers, several regulatory and manufacturing hurdles must be addressed before these systems can achieve widespread clinical adoption. A primary challenge involves compliance with Good Manufacturing Practice (GMP) standards, which require robust, reproducible, and scalable production processes. Regulatory barriers can further complicate clinical translation, as guidelines are continuously evolving. The U.S. Food and Drug Administration (FDA) and other global regulatory agencies require detailed characterization of nanocarrier composition, stability, and safety, alongside validated manufacturing protocols. This dynamic regulatory landscape may pose additional challenges for clinical development planning, although ongoing collaboration between researchers and regulators aims to establish robust and reliable pharmacopeia. Nonetheless, current workflows often overlook downstream processing steps—such as post-insertion of targeting ligands, dialysis, and surface modification—that significantly impact nanoparticle performance but are poorly integrated into microfluidic frameworks.[10] Comparative analysis and key differences of microfluidic synthesis and conventional methods represented in Table 1.[35–37]

By harnessing these advantages, microfluidic platforms serve as a gold standard for the reproducible and scalable fabrication of TDS designed to overcome biological barriers such as poor tissue penetration and lack of specificity. However, challenges regarding cost-effectiveness, regulatory compliance, and adapting to the complex *in vivo* environment.[38,39]

Microfluidics has long demonstrated versatility and efficiency in Therapeutics delivery. For instance, as early as 2016, Bottaro *et al.* explored cost-effective microfluidic systems

Alireza Gharatape

Alireza Gharatape, Ph.D. is a researcher in Nanomedicine from Tehran University of Medical Sciences (TUMS), with a focus on gene delivery using polymeric and lipid-based nanocarriers for cancer immunotherapy and non-viral gene delivery systems. His research integrates nanotechnology, molecular biology, and immunoengineering to develop targeted therapeutic platforms, particularly for T cell engineering.

Zahra Niasari-Naslaji

Zahra Niasari-Naslaji received her bachelor's degree in Biotechnology from the University of Tehran and is currently pursuing a master's degree in Industrial Biotechnology at the same institution. Her research focuses on the design of Aptasensors. She has also contributed to research on nanoparticle synthesis and gene delivery.

for liposome production, employing T-junction and cross-flow geometries to optimize liposome size and polydispersity index (PDI). Their findings demonstrated the ability to fine-tune liposome properties by adjusting flow rate ratios (FRR) and total flow rates (TFR), ultimately achieving liposomes with superior uniformity compared to traditional methods.[40] Similarly, Riewe *et al.* examined the synthesis of lipid nanoparticles using various micromixers, achieving precise size control and demonstrating the scalability of microfluidics in producing nanoparticles essential for poorly soluble drugs.[41] However, it is important to note that the inclusion of targeting moieties is often achieved *via* post-insertion techniques rather than co-formulation, a crucial consideration when evaluating microfluidic compatibility and design.[42] Extending this work, Leung *et al.* used microfluidics to encapsulate bacteriophages for treating antibiotic-resistant bacteria, highlighting improved encapsulation efficiency and reduced phage inactivation compared to conventional methods.[43] Collectively, these examples reflect the growing potential and adaptability of microfluidic technology to address some of the longstanding TDS challenges, including size control and scalability, and highlight its promise for diverse therapeutic needs.

Innovative microfluidic platforms for gene delivery have also been developed. For instance, Balbino and colleagues designed a microfluidic device for producing cationic liposomes and lipoplexes, reducing reagent waste while enabling scalable production. Their device generated lipoplexes with sizes ranging from 140 to 250 nm and low PDIs, achieving transfection efficiencies comparable to bulk synthesis methods.[44] Expanding on this progress, López and colleagues utilized a Periodic Disturbance Mixer (PDM) combined with a Design of Experiments (DoE) approach to control liposome characteristics. They succeeded in producing stable liposomes with diameters between 52 and 200 nm, attaining monodisperse populations under specific FRR and TFR conditions. Notably, an FRR of 8.56 and TFR of 18 mL h^{-1} resulted in the smallest liposomes (41 nm), maintaining stability over six months. The study also demonstrated that zeta potential remained unaffected by operational parameters, confirming the robustness and scalability of microfluidic liposome production for diverse applications.[45] Obeid *et al.* further emphasized the influence of solvent properties on niosome synthesis using microfluidic devices, revealing that organic solvent polarity significantly affects both particle size and encapsulation efficiency, with hydrophilic and hydrophobic drugs responding differently to these variations.[46] It is also important to differentiate between commercially available microfluidic systems designed for different user profiles. Some commercial microfluidic systems are designed as plug-and-play platforms, targeting users with limited experience in nanoparticle formulation. These systems prioritize ease of use and rapid setup, often at the cost of operational flexibility and customization. Others are modulable systems tailored for expert users in LNP formulation or pharmaceutical development, offering fine control over a wide range of parameters to optimize both process and product characteristics. This divergence should be considered when evaluating cost-effectiveness and scalability. Given this distinction, a universal assessment of cost-effectiveness becomes challenging. While beginner-friendly systems currently remain somewhat costly, their simplified architecture—typically comprising a microchip and basic pump mechanisms—suggests that they are likely to become significantly more affordable as market adoption increases. Therefore, it is crucial to differentiate between these user cases when evaluating the economic and practical feasibility of microfluidic technologies in nanocarrier development.[17,47]

Jeanne Leblond Chain

Jeanne Leblond Chain, Ph.D. is a senior researcher at French Institute of Health and Medical Research working in ARNA laboratory in Bordeaux, France. Chemist by training, she is pharmaceutical scientist expert specializing in stimuli responsive drug and gene delivery systems. Dr. Leblond Chain's lab explores smart delivery systems that combine lipids and nucleic acid aptamers as multifunctional components. These systems serve as drug carriers, targeting ligands, and dynamic release triggers, responsive to disease markers. Her technical expertise spans organic synthesis, lipid nanoparticle formulation, physico chemical characterization, aptamer chemistry, intracellular delivery systems, and translational evaluation—from design through in vitro and in vivo testing.

Nasrollah Tabatabaei

Nasrollah Tabatabaei, Ph.D. is a biomedical engineer, his doctoral research investigated the use of magnetic nanoparticle hyperthermia to transiently open the blood–brain barrier for targeted drug delivery, followed by post-doctoral work on liposomal drug delivery to the retina. Dr. Tabatabaei's research lies at the interface of nanomedicine, microfluidics, and translational therapeutics. He leads TabaLab, an interdisciplinary research group focused on developing microfluidic systems for diagnostics, drug delivery, and organ-on-chip platforms. His ongoing projects include the design of nanostructured lipid carriers for neurological disorders, microfluidic chips for sperm selection, extracellular vesicle and cell-free DNA isolation, and wearable artificial kidney technologies.

Although prior review articles have thoroughly examined microfluidic TDS, recent rapid advancements and emerging challenges necessitate a fresh perspective. Specifically, this review will focus on the development and challenges of multifunctional LNPs produced *via* microfluidic platforms, with attention to post-insertion techniques, downstream processing, and ligand-functionalized systems. Moreover, it addresses outstanding challenges, such as optimizing manufacturing costs, ensuring regulatory compliance, and adapting to complex *in vivo* environments, that remain inadequately explored. By examining unresolved issues, such as cost, scalability, and clinical translation, this review aims to illuminate the transformative role of microfluidic-based nanocarriers, which enables a highly controllable and scalable strategy for the rational design of nanomedicine, in advancing drug and gene delivery systems, helping bridge the gap between laboratory innovation and clinical application.

2. Nanocarriers and biological barriers

The delivery of therapeutic agents, particularly nucleic acids and proteins, is challenged by biological barriers. These include enzyme degradation, immune system removal, limited endothelial passage, and issues with endosomal entrapment and nuclear entry, all reducing their bioavailability and effectiveness (Fig. 1).[48,49]

As Qiu *et al.* explain, one of the most formidable intracellular barriers is the endosomal/lysosomal system and strategies to enhance endosomal escape should be considered.[50] Wang *et al.* complement this by introducing a pH-responsive peptide system specifically engineered to enhance endosomal escape, achieving over 90% transfection efficiency *in vitro* and increased *in vivo* antitumor activity through chirality inversion

Reza Faridi-Majidi

Reza Faridi-Majidi, Ph.D. is a Professor of Organic Chemistry and the current Dean of the School of Advanced Technologies in Medicine (SATiM) at Tehran University of Medical Sciences (TUMS). His research focuses on nanofibers, electrospinning, polymers, and biomedical nanotechnology, with more than 100 scientific publications and significant citation impact. Combining expertise in organic chemistry and medical nanotechnology, he has contributed to the development of advanced biomaterials and scaffolds. As Dean, he promotes interdisciplinary research, innovation, and the integration of advanced technologies into medical science.

Table 1 Comparative performance of conventional and microfluidic methods in nanoparticle formulation. This table highlights key advantages of the microfluidic swirl mixer over conventional synthesis techniques in terms of particle size control, polydispersity, reproducibility, scalability, encapsulation efficiency, and mixing mechanism. Data are derived from experimental results and literature cited in the study

Parameter								
Methods	Particle size control	Size distribution & PDI	Reproducibility	Scalability	Production throughput	Encapsulation efficiency	Morphology uniformity	Mixing mechanism
Conventional Methods	Limited; inconsistent particles	Broad distribution	Low; high batch-to-batch variation	Poor; difficult to scale up	Low; time-consuming, multi-step processes	Variable	Heterogeneous; irregular shapes	Passive or mechanical mixing
Microfluidic Methods	High; tunable size	Narrow distribution	High; continuous flow enables consistent, production	Excellent; supports high flow rates	High; continuous, one-step production	High; due to rapid self-assembly	Homogeneous; spherical, uniform morphology	Active swirling flow; rapid and homogeneous

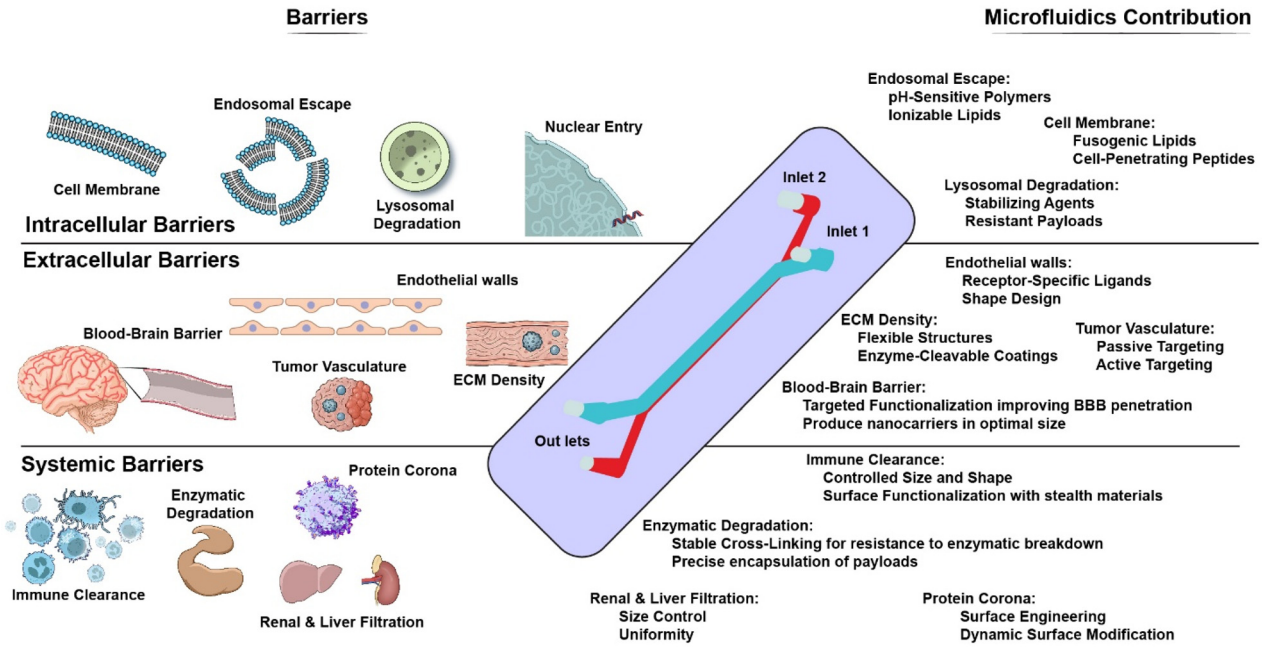

Fig. 1 Microfluidic contributions to overcome biological barriers for nanocarrier delivery. The figure illustrates the key biological barriers encountered by nanocarriers at systemic, extracellular, and intracellular levels, along with the specific microfluidic technologies and strategies employed to address each barrier. The left side depicts the barriers (*e.g.*, immune clearance, blood-brain barrier, lysosomal degradation), while the right side highlights the corresponding microfluidic contributions, such as controlled size and shape, targeted functionalization, and precise encapsulation of payloads. This integrated approach demonstrates how microfluidics enables tailored solutions to enhance nanocarrier efficacy across various biological challenges.

and modular peptide design.[51] Additionally, Passos Gibson *et al.* emphasize the importance and application of reliable high-throughput assays to study endosomal escape in LNP systems, noting that mechanistic gaps hinder optimization and clinical translation Targeting endosomal escape represent an interesting idea that focused on development of a novel class of cationic switchable lipids designed for efficient siRNA delivery, leveraging a pH-triggered conformational switch that activates in the acidic environment of endosomes. Cationic switchable lipids disrupt the LNP structure in response to acidic pH, enhancing endosomal escape and cytoplasmic release of siRNA. Among the tested candidates, lipid 3 showed the most effective gene silencing. This study underscores the potential of pH-responsive lipid systems for improving nucleic acid delivery.[52] Tabatabaei and *et al.*, found that co-encapsulating miR-181a and melphalan in lipid nanoparticles significantly boosts treatment efficacy for retinoblastoma. This study presents a LNP-based co-delivery system for miR-181a and melphalan to treat vitreous-seeded retinoblastoma. The cationic switchable LNPs (171 nm, 93–98% encapsulation efficiency, from DSPC and DSPE-PEG2000 lipids) enable efficient cellular uptake and endosomal escape, delivering both agents directly into retinoblastoma cells. miR-181a acts as a tumor suppressor by downregulating anti-apoptotic Bcl-2 and anti-proliferative MAPK1, while upregulating pro-apoptotic BAX. Melphalan provides direct cytotoxic DNA damage. Co-encapsulation creates a synergistic effect: miR-181a sensitizes cancer cells to melphalan, allowing lower drug doses and reducing toxicity. *In vitro* and *in vivo* results show significantly enhanced tumor cell death, with 72% reduction in viable tumor cells in a rat model compared to controls. This co-delivery strategy improves therapeutic efficacy and offers a promising approach for treating advanced retinoblastoma.[53]

By precisely controlling nanocarriers physicochemical characteristics—such as particle size, surface charge, and material composition—nanocarriers can improve the solubility of therapeutic agents, extend their presence in systemic circulation, and promote selective accumulation in target tissues. Additionally, these carriers enhance intracellular transport mechanisms.[54]

Various engineered nanocarrier systems, including lipid-based nanoparticles, polymeric assemblies, and inorganic structures, have shown strong potential to navigate the biological milieu with high specificity. Beyond merely shielding therapeutic cargo from enzymatic degradation, these carriers actively interact with cellular processes, enabling more efficient transport to intracellular compartments and even to the nucleus when necessary.[55,56] Nuclear-targeting strategies using nuclear localization signals (NLSs) modified nanoparticles have been comprehensively reviewed before.[57,58] Moreover, Li *et al.* highlight how nanoparticles, once in biological fluids, form a protein corona (PC) that significantly affects their fate. While traditionally considered a hindrance, the PC can be engineered to enhance circulation time and cellular uptake using 'de-opsonins' such as albumin, transferrin, and apolipoproteins.[59] Nakamura *et al.* explored the impact of

size and surface charge on the lymphatic transport of LNPs. Their findings showed that 30 nm, negatively charged nanoparticles efficiently reached lymph nodes and penetrated deeper regions, emphasizing the importance of both physical dimensions and surface chemistry in targeted delivery.[60] These findings reinforce the importance of precisely engineering LNPs for targeted delivery, especially when post-formulation surface modifications are required to enhance biodistribution profiles.[61]

The incorporation of nanotechnology into precision medicine has initiated a fundamental transformation in the development of personalized therapeutic approaches. Through advances in nanoparticle bio interface engineering, researchers have been able to design intelligent nanocarriers that can sense and adapt to the distinct physiological conditions associated with specific diseases and patient populations. These smart systems offer the potential for more selective and effective treatment by aligning drug delivery with individual biological profiles.[54] For instance, Liu, J.-p., *et al.* examine smart nanoparticles that respond to tumor-specific cues like acidic pH or matrix metalloproteinases (MMPs), thereby achieving precise drug release in resistant tumors.[62] Fukuta and Kogure further present a biomimetic approach, developing leukocyte-mimicking liposomes capable of crossing inflamed endothelium by intermembrane protein transfer, improving drug accumulation in tumor tissue.[63] Xiong *et al.*, engineered ATP-responsive tumor targeted LNPs for targeted siRNA delivery against melanoma. Using a microfluidic chip-based approach, siRNA targeting the undruggable MITF oncogene was efficiently encapsulated within LNPs. The PBA ligands enabled dual tumor targeting *via* sialic acid recognition and ATP-triggered intracellular siRNA release. Additionally, LNPs accumulated passively in tumor tissue through the enhanced permeability and retention (EPR) effect. The combined targeting and controlled release significantly improved *in vivo* gene silencing and anti-tumor efficacy in melanoma models. This platform exemplifies a promising strategy to overcome extrahepatic delivery challenges and enhance the therapeutic potential of RNAi-based cancer treatments.[64]

Even so, ongoing innovations in nanoparticle engineering, coupled with a deeper understanding of biological delivery barriers, are expected to significantly improve therapeutic outcomes and expand the clinical utility of nanomedicine. The treatment of central nervous system (CNS) disorders continues to pose significant challenges, largely because of the stringent and highly selective properties of the blood–brain barrier (BBB). Although the BBB plays a critical role in preserving the brain's internal environment and ensuring neuronal stability, it also acts as a major obstacle to the delivery of therapeutic agents. This barrier restricts the passage of most drugs, including those targeting neurodegenerative conditions and brain malignancies, thereby limiting their therapeutic efficacy within the CNS.

Sadat Razavi *et al.* provide a focused review on organic nanoparticles such as chitosan-based carriers, liposomes, and lipid nanoparticles, designed to cross the BBB *via* receptor-mediated transcytosis and paracellular modulation. They emphasize the importance of surface functionalization—through ligand attachment or charge tuning—and the strategic use of disease-induced BBB disruptions to enhance delivery in conditions like Alzheimer's, Parkinson's, and glioblastoma. These systems also offer high biocompatibility and controlled drug release, supporting clinical translation.[65] Expanding the landscape, Kulkarni *et al.* examine both invasive and non-invasive methods. They categorize nanocarriers, and highlight the critical role of surface modifications in determining both BBB permeability and therapeutic specificity, emphasizing the potential of multifunctional nanoparticles to carry multiple drug payloads while maintaining structural integrity.[66] Incorporating targeting ligands such as peptides or antibodies can further improve specificity to target cells or tissues, demonstrating the potential of microfluidic systems to produce nanocarriers with enhanced functionality and safety. The related study developed peptide-functionalized lipid nanoparticles (LNPs) for targeted systemic mRNA delivery to the brain, addressing the challenge of blood-brain barrier (BBB) penetration. Using a microfluidic mixing system, LNPs were synthesized and functionalized with brain-targeting peptides (RVG29, T7, AP2, mApoE), which significantly enhanced neuronal transfection and minimized hepatic uptake. Among these, RVG29-LNPs showed the highest efficiency, demonstrating the potential of peptide-mediated functionalization for brain-specific mRNA delivery.[67]

Ahlawat *et al.* mentioned that nanoparticles like carbon dots and peptide-conjugated carriers can exploit multiple pathways, reducing off-target toxicity and counteract metabolic degradation.[68] Xie *et al.* emphasize using disease-specific BBB changes in stroke, glioblastoma, and Alzheimer's to adjust nanocarrier properties for optimized biodistribution.[69] However, despite promising results in preclinical studies, the translation of these technologies into clinical practice remains limited, mostly due to differences in nanoparticle behavior across species, as well as the variability among human patients.[55]

3. Microfluidic for controlled synthesis

Microfluidic technology, a multidisciplinary field bridging chemistry, physics, biology, and engineering, has emerged as a powerful tool for synthesizing nanocarriers with unparalleled precision and efficiency.[70] By facilitating fluid manipulation in micrometer-scale channels, microfluidics establishes laminar flow conditions that enable highly controlled mixing and reactions-conditions crucial for the size, uniformity, and functionality of nanoparticles.[71–73] These attributes directly influence their therapeutic efficacy, cellular uptake, and drug release kinetics. Consequently, microfluidic systems can produce highly monodisperse nanoparticle populations, critical for maintaining consistent performance in clinical settings.[74] This level of control helps overcome the shortcomings of tra-

ditional bulk methods, which often yield heterogeneous nanoparticles with inconsistent properties, positioning microfluidics as a powerful tool—among several emerging methods—for producing uniform, reproducible, and scalable nanocarriers tailored to specific applications.[75,76] Moreover, these systems offer compatibility with post-formulation processes, such as surface ligand insertion and downstream modification, which are essential for engineering multifunctional LNPs for advanced targeting and stimuli responsiveness.[10,77]

Among various platforms, droplet microfluidics and flow-focusing techniques are most extensively utilized for nanocarrier fabrication.[78,79] Droplet microfluidics generates emulsions by forming droplets of immiscible fluids at microfluidic junctions, thereby allowing precise control over particle size and composition (Fig. 2).[80,81] This method is particularly well suited for encapsulating therapeutic agents in lipid-based nanoparticles, such as liposomes and LNPs.[82] By tuning droplet size and interfacial tension, droplet microfluidics enables high-throughput synthesis of nanoparticles with uniform sizes, an essential feature for consistent drug delivery.[83] Flow focusing, on the other hand, employs a central liquid stream that is hydrodynamically focused by outer streams, resulting in the formation of monodisperse nanoparticles. While droplet microfluidics excels in achieving particle uniformity, its scalability and throughput are often limited. Conversely, flow-focusing systems are more suitable for large-scale production but may compromise particle uniformity.[83–85] Recent advancements in flow-focusing designs, such as the use of multiple microchannels and enhanced fluid dynamics, have helped address these limitations, enabling higher throughput without sacrificing uniformity.[86]

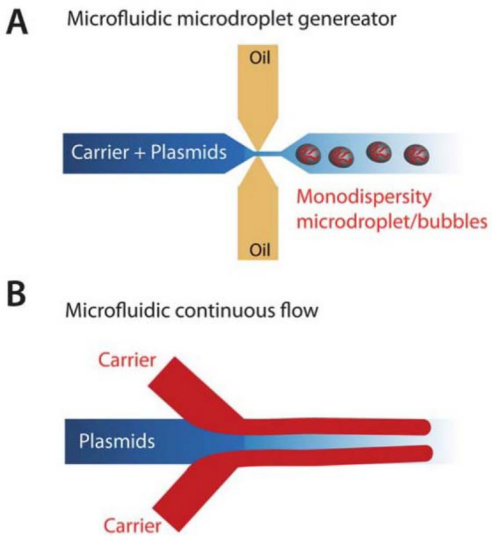

Fig. 2 Microfluidic approaches for nanocarrier synthesis. (A) Schematic representation of a microfluidic microdroplet generator. This method involves the formation of monodisperse microdroplets/bubbles by encapsulating a solution containing carriers and genetic materials within an immiscible oil phase. The uniform size and composition of these droplets enable precise control over nanocarrier synthesis. (B) Illustration of a microfluidic continuous flow system. In this approach, carriers and plasmids are introduced into separate channels and mixed in a controlled manner to facilitate the synthesis of nanocarriers. The continuous flow design allows for scalable and reproducible production of nanocarriers with high efficiency. Reprinted with permission from ref. 81, Copyright (2022) Royal Society of Chemistry.

Other advanced platforms, including centrifugal microfluidics, herringbone mixers, and vortex-based systems, offer specialized capabilities that cater to different nanocarrier requirements, expanding the possibilities for synthesis optimization.[87–89] These systems are particularly relevant for manufacturing LNPs intended for RNA-based therapies, where batch-to-batch consistency and control over biophysical properties are essential for regulatory approval and clinical translation.[90,91]

Recent advancements underscore the potential of microfluidic systems to enhance nanocarrier production. Ahn *et al.*, for instance, utilized a polydimethylsiloxane (PDMS) microfluidic device to generate LNPs with precise size control by adjusting the flow rate of the continuous phase and the diameter of the micro-orifice. Their findings revealed that smaller nanoparticles exhibited higher cellular uptake, although this also increased toxicity.[92] Similarly, Han and colleagues developed a Microfluid Vortex Focusing (MVF) system that combined hydrodynamic focusing with vortex mixing, enabling high-throughput liposome production at over 20 grams per hour (Fig. 3). The system yielded liposomes with adjustable sizes (27–100 nm) while maintaining precise control over their characteristics.[93] Such scalability is particularly advantageous for clinical and industrial applications, as it balances large-scale production demands with strict requirements for nanoparticles quality. However, integration of post-synthesis steps such as targeting ligand grafting or PEGylation remains underexplored in these scalable systems, presenting a key area for innovation.[94]

Another noteworthy innovation is the solvent-free method introduced by Kulkarni *et al.*, which enables siRNA encapsulation in LNPs without the use of ethanol. By employing single-phase and two-phase mixing techniques, the authors successfully generated nucleic acid-loaded LNPs with potential applications in personalized medicine.[95]

The integration of auxiliary techniques into microfluidic systems has further expanded their capabilities. Kotouček *et al.* examined the role of lipid membrane fluidity, influenced by cholesterol content, affects liposome size in herringbone mixers. Their work showed that increased bilayer fluidity led to smaller liposomes, whereas higher cholesterol concentrations, particularly in unsaturated lipids, resulted in larger particles.[96] Owing to their unique microstructural patterns, herringbone mixers facilitate effective mixing and enhance the synthesis of liposomes with controlled membrane characteristics, making them ideal for liposome-based TDS. Bolze and colleagues introduced ultrasound-assisted antisolvent precipitation, achieving particle sizes as small as 26 nm, though persistent challenges such as fouling and leakage remain.[97]

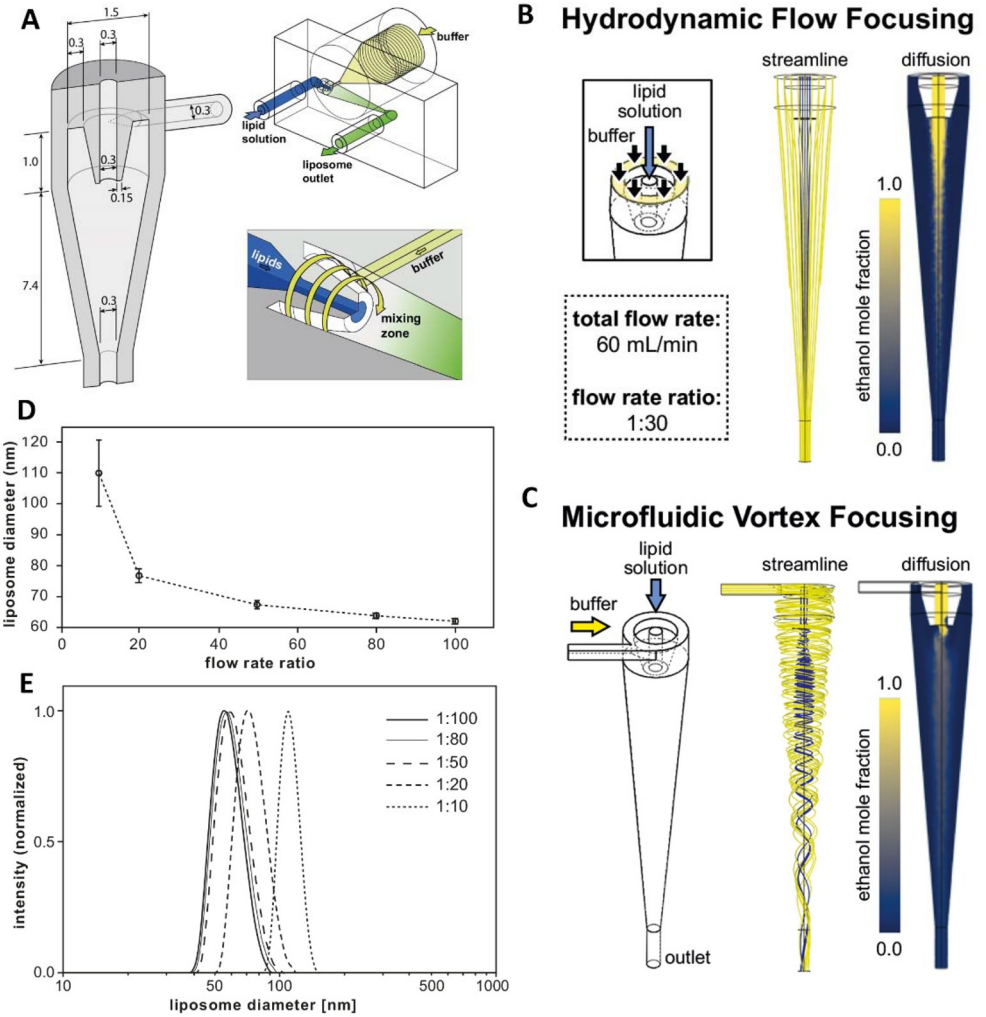

Fig. 3 Design and manufacturer of a microfluidic vortex focusing (MVF) device. (A) The schematic illustrates the fluidic architecture responsible for vortex generation and flow focusing, with all dimensions indicated in millimeters. The MVF device consists of two inlets merging at an annular junction, followed by a conical mixing zone and an outlet. A close-up of the annular junction is also shown. (B) Computational simulations of co-flowing water and ethanol. The flow streamlines and ethanol concentration profiles are visualized in numerical simulations of hydrodynamic focusing. (C) The radial solvent concentration profiles are shown at various distances from the lipid injection point for both simulation cases. The total flow rate used in these simulations was 60 mL min^{-1}, with a flow rate ratio of 1 : 30 (ethanol to water). (D) Average liposome diameter. (E) Normalized size distribution plots. Both the total flow rate (60 mL min^{-1}) and initial lipid concentration (10 mM) were kept constant. The data represent at least six measurements ($N \geq 6$), with error bars indicating ± 1 standard deviation. Reprinted with permission from ref. 93, Copyright (2022) Springer Nature.

Complementing these efforts, Petersen highlighted that optimizing microfluidic mixing parameters significantly enhances *in vivo* delivery efficiency compared to manual mixing.[98] Ultrasound-assisted microfluidics presents an especially promising approach for refining particle size and enhancing the encapsulation efficiency of hydrophobic drugs, which are often difficult to formulate using conventional methods.

Additionally, Kastner *et al.* employed multivariate data analysis to evaluate the relationship between flow parameters and nanoparticle characteristics. They demonstrated that higher FRRs produced smaller liposomes but also increased PDI, while TFRs had minimal influence on particle size or transfection efficiency.[99] These findings emphasize the importance of optimizing flow parameters not only to control nanoparticle size but also to ensure homogeneity, both of which are critical for ensuring consistent therapeutic performance. Notably, such optimization becomes even more critical in the context of multifunctional nanoparticles, where changes in particle size or distribution can affect the efficiency of ligand presentation and subsequent targeting accuracy.[100]

Despite these advances, unresolved challenges such as fouling, scalability, and the complexity of clinical translation persist.[101] Combining experiments with modeling offers valuable insight, predicting synthesis outcomes and reducing trial-and-error to speed up nanoparticle optimization.[73,102] Further development of *in silico* tools capable of simulating downstream processes—such as dialysis, surface ligand post-insertion, and purification—could bridge existing knowledge gaps

and aid in designing closed-loop microfluidic systems.[103] As microfluidic technology continues to evolve, its potential to revolutionize nanocarrier synthesis and enable personalized, targeted therapeutics becomes increasingly evident, signaling a significant leap forward in gene and drug delivery.

4. Recent advances in microfluidic methods

Recent advancements in microfluidic synthesis of nanocarriers for gene and drug delivery have revolutionized nanomedicine by enabling the creation of highly precise, functional, and scalable delivery systems.[76,104] Microfluidic systems precisely control nanocarrier traits and, combined with emerging tools, drive innovations in therapeutic delivery.[105] While prior reviews have addressed early-stage microfluidic synthesis, this section focuses on recent developments enabling multifunctional LNPs, particularly those incorporating targeting ligands and stimuli-responsive features relevant to personalized therapeutics.

A key innovation is the use of stimuli-responsive polymers in microfluidic nanocarriers, enabling payload release triggered by factors like pH, temperature, or enzymatic activity.[106,107] Recent advances have also focused on the development of hybrid materials combining inorganic nanoparticles with organic polymers, enhancing the mechanical stability and drug-loading capacity of the carriers. For instance, Hirai *et al.* developed a charge-reversible lipid derivative, DOP-DEDA, capable of modulating its surface charge under acidic conditions, thereby improving siRNA delivery and minimizing systemic toxicity.[108] Similarly, Da Costa *et al.* utilized a one-step hydrodynamic focusing process to produce anionic and stealth anionic liposomes (SALs) with remarkable size control and reproducibility.[109]

Recent studies by López *et al.* have demonstrated the optimization of liposome synthesis using microfluidic technologies. By manipulating FRR and TFR, they achieved smaller and more uniform liposomes, with elevated temperatures further reducing liposome size through enhanced control of lipid bending energy. Notably, the zeta potential remained stable under different processing conditions, underscoring the robustness of the method. This approach also supports scalable production, making it highly promising for applications requiring precise and stable liposome synthesis.[110] Another breakthrough in microfluidic nanocarrier synthesis was reported by Jo *et al.*, who developed a technique to produce monodispersed liposomes approximately 100 nm in diameter by optimizing the FRR between non-aqueous and aqueous phases and incorporating ionic surfactants and biodegradable polymers. This strategy significantly enhanced colloidal stability, allowing the liposomes to remain stable for over 30 days. This highlights the critical role of fine-tuning flow parameters and using stabilizing agents for long-term stability.[96] These methods allow sequential addition of functional elements, such as ligands or PEG layers, after stabilizing size and charge.[111] Another study used a passive targeting method to functionalize lipid nanoparticles. Patel *et al.* created amino acid-modified LNPs for siRNA delivery, achieving 70% gene silencing efficacy with minimal cytotoxicity. By modifying lipid headgroups with histidine and lysine, they enhanced cellular uptake and endosomal escape, resulting in significant tumor growth reduction.[112]

Advancements in microfluidic design have also enhanced both the precision and functionality of nanocarrier synthesis. Tomeh *et al.* introduced a novel microfluidic swirl mixer to enhance large-scale production of nanoparticles for drug delivery (Fig. 4). This mixer demonstrated multiple advantages over existing designs, such as higher flow rates, customizable components, and better control over particle size that make it suitable for the production of biopolymer and lipid-based formulations, including silk and lipid nanoparticles.[113] The study emphasized the importance of optimizing processing parameters and understanding formulation characteristics to achieve desired nanoparticle properties for clinical translation.

Notably, devices like the swirl mixer also allow plug-and-play integration with downstream units for post-formulation steps such as ligand grafting or purification, which are underrepresented in conventional microfluidic designs.[114] Scalability and reproducibility represent major challenges in nanocarrier production, yet significant progress has been made to address these concerns. Firmino *et al.* developed a high-flow-rate microfluidic device (HFR-MD) that yielded nanoliposomes with high uniformity and productivity, surpassing 2 grams of lipid per hour. This accomplishment underscores the scalability of microfluidic systems for industrial applications.[115] Maeki *et al.* further demonstrated the scalability of microfluidic approaches by introducing a five-layered glass-based system for producing RNA-loaded LNPs. By employing a "piling-up and numbering-up" strategy, their method allowed precise control over particle size while increasing production capacity, enabling the efficient manufacturing of RNA therapeutics for clinical applications.[116,117] These scalable designs are especially important for producing therapeutic LNPs in expert-oriented systems, whereas "plug-and-play" versions serve the needs of academic or preclinical users looking to adopt microfluidics without technical expertise.[118,119]

Eş *et al.* combined chaotic advection-based mixing with a centrifugal vacuum concentrator to produce stealth cationic liposomes (SCLs) with minimal micelle formation and high structural integrity, qualities vital for scalable production.[120] Terada *et al.* employed a systematic DoE approach to optimize LNP-siRNA systems, determining that FRRs and lipid concentrations played crucial roles in determining particle size and PDI. Additionally, the use of anionic polymers suppressed size increases in siRNA-loaded LNPs, highlighting the importance of fine-tuning synthesis variables.[121] Further design innovations, such as microfluidic chips equipped with modular flow paths or programmable mixing zones, hold promise for more sophisticated, multi-functional nanocarriers.[122,123]

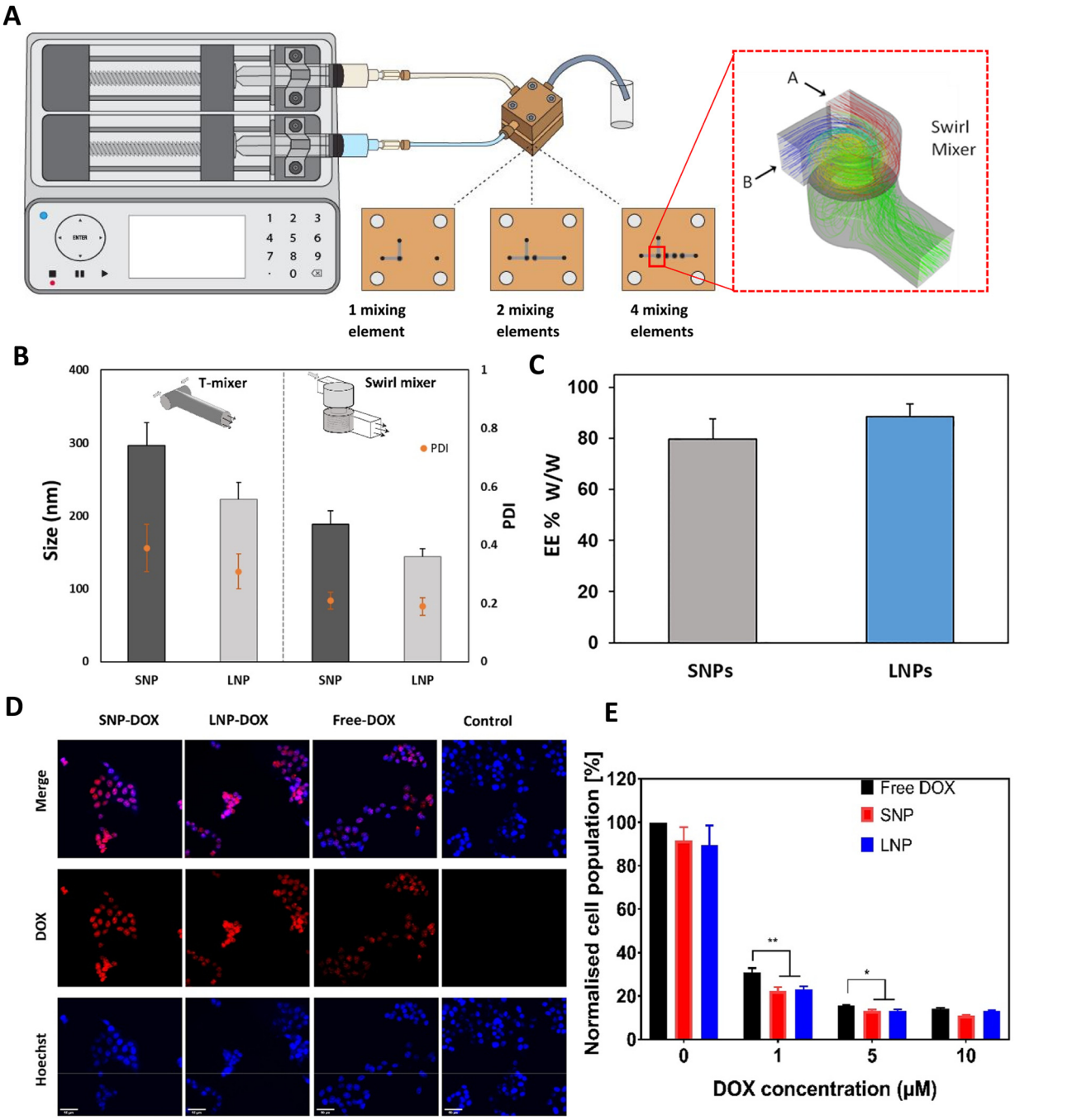

Fig. 4 A: Schematic representation of the microfluidic swirl mixer design, showing the adjustable number of mixing elements (1, 2, or 4) integrated into the device. (A) The swirl mixer is designed to generate rapid and homogeneous mixing by creating a swirling flow pattern within the microchannels. This design allows for precise control over the mixing process, enabling reproducible nanoparticle production with tunable particle size and polydispersity index (PDI). The mixer is connected to a dual syringe pump system, which ensures consistent flow rates and pressures during operation. (B) Mean particle size and PDI of SNPs and LNPs produced by both mixers. Error bars represent standard deviation (n = 3). The swirl mixer consistently produces particles with smaller mean sizes and narrower size distributions compared to the T-mixer, indicating improved control over nanoparticle characteristics. (C) Encapsulation efficiency (% w/w) of doxorubicin (DOX) in SNPs and LNPs prepared using the swirl mixer. The encapsulation efficiency is shown to be high for both formulations, demonstrating the effectiveness of the swirl mixer in drug loading applications. (D) Fluorescence microscopy images (×20 magnification) showing the cellular uptake of free DOX, SNPs loaded with DOX (SNP-DOX), and LNPs loaded with DOX (LNP-DOX) in HCT 116 cells after 24 hours at a DOX concentration of 1 μM (scale bar = 40 μm). (E) Normalized cell population in HCT 116 cells following treatment with free DOX, SNP-DOX, and LNP-DOX. Reprinted with permission from ref. 113, Copyright (2022) from Elsevier.

In addition to facilitating robust synthesis, microfluidic systems have also facilitated the exploration of new methods for screening and optimizing nanocarriers. Cui *et al.* developed a high-throughput automated platform capable of screening 384 LNP formulations per plate, significantly accelerating the identification of promising candidates for mRNA delivery. Their system demonstrated strong correlations with conventional microfluidic mixing methods, revealing novel ionizable lipids that outperformed existing formulations, a testament to the efficiency gains that high-throughput screening platforms provide.[124] Furthermore, the integration of computational models to predict nanocarrier behavior at the cellular level could further streamline candidate selection processes.

Finally, the convergence of microfluidic systems with other emerging technologies offers additional avenues for creating advanced nanocarriers. Guimaraes *et al.* utilized a barcoded mRNA (b-mRNA) system to evaluate organ-specific delivery efficiency of LNPs, uncovering distinctive structural requirements for effective b-mRNA and DNA *versus* DNA-based carriers.[125] Perli *et al.* investigated the impact of ionic strength on liposome production, demonstrating that increased ionic strength improved both size uniformity and stability by enhancing liposome self-assembly and preventing micelle formation (Fig. 5).[126]

Despite these advancements, challenges remain, regarding scaling up production and maintaining reproducibility, particularly for clinical applications. The continued integration of experimental techniques with computational modeling and the design of novel devices offers promising strategies to these hurdles. As microfluidic technology continues to evolve, it is poised to play an ever-increasing role in refining nanocarrier synthesis for gene and drug delivery, ultimately paving the way for personalized medicine through safer, more enabling precise, efficient, and patient-tailored therapies.

5. Design strategies for next-generation microfluidic nanocarriers

Recent advancements in TDSs have been transformative, driven by the integration of microfluidic technologies, stimuli-responsive materials, and advanced nanocarrier design.[127,128] These innovations have enhanced the precision and efficacy of therapeutic systems, by mitigating off-target effects and boosting drug bioavailability.[129] Notably, recent work has focused on multifunctional lipid nanoparticles (LNPs), particularly those incorporating both stimuli-responsive features and surface-bound targeting ligands—components often introduced through post-formulation modification rather than during initial synthesis.[130]

5.1. Drug delivery

Co-encapsulation of therapeutic agents with targeting ligands and stimuli-responsive materials has become a pivotal strategy for achieving site-specific release. Incorporating ligands such as antibodies, peptides, or aptamers into nanocarriers enables specific binding to target cells or tissues, enhancing the therapeutic precision.[131–134] However, in many cases, targeting ligands are introduced after initial particle formulation through post-insertion or surface modification techniques to avoid encapsulation within the nanoparticle core, a challenge particularly relevant for LNPs synthesized *via* microfluidic platforms.[135,136] For example, Alizadeh *et al.* developed pH-sensitive nanocarriers using chitosan and alginate to deliver gefitinib, an anticancer drug, demonstrating significantly lower IC_{50} values compared to free drugs in the acidic microenvironment of tumor tissues.[131] Further control over drug release kinetics can be attained through dual-functional stimuli-responsive systems triggered by multiple parameters

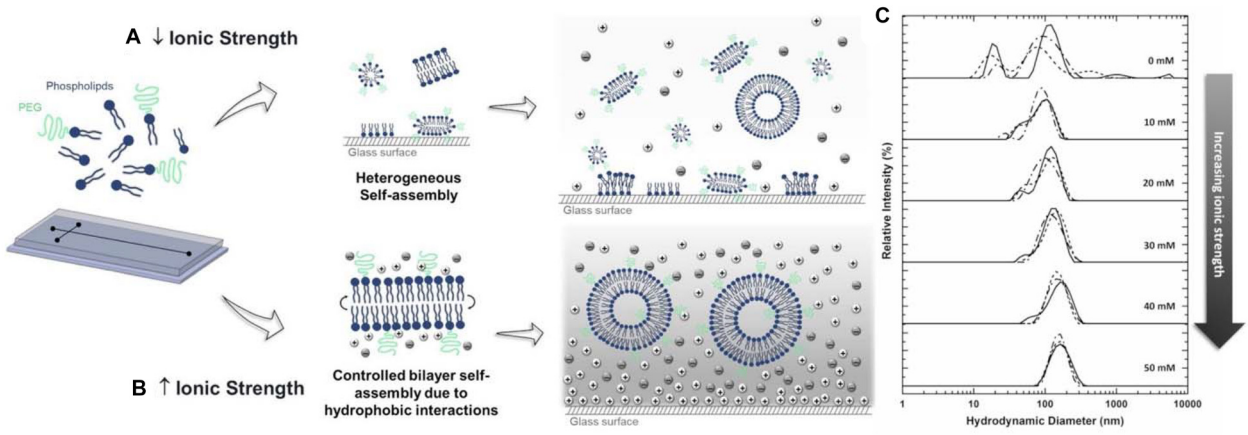

Fig. 5 Depiction of the effects of low (A) and high (B) ionic strength environments on the formation of stealth cationic liposomes (SCLs), incorporating poly(ethylene glycol) (PEG)-lipids, using microfluidic hydrodynamic flow-focusing platforms. (C) Intensity-weighted size distribution of SCLs (containing 1% DSPE-PEG2000) synthesized under varying ionic strength in the side streams, achieved by introducing PBS concentrations ranging from 0 mM (using only ultrapure water) to 50 mM. The curves represent data from independent replicates (n = 3). Reprinted with permission from ref. 126, Copyright (2022) from Elsevier.

(*e.g.*, pH and temperature). Khademzadeh *et al.* illustrated this concept with a microfluidic approach for creating pH-sensitive nanocarriers encapsulating gefitinib for non-small cell lung cancer treatment. They used a microfluidic chip to generate aerosolized droplets that mixed gefitinib with chitosan, enabling on-chip nucleation and rapid self-assembly of drug–polymer nanoparticles. Their system showed a high encapsulation efficiency of 77.8% and demonstrated significantly enhanced therapeutic efficacy, as evidenced by a lower IC_{50} value compared to free gefitinib. This pH-sensitive release mechanism underscores the potential of microfluidic technologies in developing targeted therapies for cancer treatment.[137]

Microfluidic platforms have been particularly effective in producing homogeneous nanocarriers by enabling rapid and reproducible mixing at the microscale. Chiesa *et al.* utilized advanced micromixer geometries within a microfluidic platform to achieve lipid nanocarriers with encapsulation efficiencies exceeding 70%.[132] This study used a passive micromixing-based microfluidic strategy combined with systematic DoE optimization to develop lipid nanocarriers with tailored characteristics. In this study, microfluidics allowed for structure-function studies of LNPs, where subtle changes in size were linked directly to *in vivo* behavior and ability to cross tissue and cellular barriers. Similarly, Jaradat *et al.* optimized the synthesis of paclitaxel-loaded PEGylated liposomes, achieving sustained release profiles and sub-200 nm particle sizes, making them suitable for cancer therapy.[133] Such platforms are particularly well-suited for producing scalable batches of uniform LNPs; however, the incorporation of ligands post-synthesis still requires further development in microfluidic-compatible downstream processes such as dialysis, filtration, or controlled post-grafting.[138] Introduced a microfluidic device with integrated dialysis so that liposome formation and solvent/impurity removal happen on the chip. This one-step form-and-purify design continuously strips ethanol/unencapsulated drug while particles form, giving biocompatible, tightly sized liposomes—crucial for safe systemic delivery and barrier crossing.

In addition to fabrication techniques, the role of lipid composition and stereochemistry in optimizing nanocarrier performance has been highlighted by Da Silva Sanchez *et al.*, who studied ionizable lipids for mRNA delivery. Their findings revealed that stereochemical variations in lipid molecules, such as the C12-200-S variant, could enhance delivery efficiency by 2.8- to 6.1-fold without altering physical attributes like size or PDI.[139] This underscores the importance of molecular design in improving both the efficacy and tolerability of LNPs. Such molecular-level refinements, combined with microfluidic control over particle assembly, offer a promising pathway for tailoring LNPs to match tissue-specific delivery requirements.[140] The integration of lipid-based nanocarriers with hydrophilic polymers further enhances the stability and bioavailability of hydrophobic drugs, offering a versatile platform for diverse drug delivery applications. To fully realize their potential, future systems must also incorporate modular workflows that allow surface functionalization in post-synthesis stages—facilitating the production of multifunctional particles within continuous microfluidic frameworks.[141,142]

5.2. Gene delivery

Microfluidic-based fabrication methods have proven instrumental in creating tailored nanocarriers optimized to protect and efficiently transport various nucleic acids like DNA and RNA.[139,143–146] These examples highlight microfluidics' capacity to create multifunctional nanocarriers that not only protect genetic material but also selectively target specific cell types.

On the same line, the study of Okuda *et al.* underscores the critical role of nanoparticle size in gene delivery outcomes. The chip is the experimental engine that yields predictable, scalable size control—the central lever for crossing endothelial barriers. RNA-loaded LNPs were synthesized using a microfluidic-based strategy, where precise control over formulation parameters—such as TFR, FRR, buffer pH, lipid and PEG-lipid concentrations, and particularly salt concentration—enabled tunable size regulation based on Derjaguin–Landau–Verwey–Overbeek (DLVO) theory and Hofmeister effects. By modulating electrostatic interactions and colloidal stability, the researchers successfully generated homogenous LNPs with sizes exceeding 200 nm. Their findings demonstrated that larger RNA-loaded LNPs exhibited higher transgene expression and greater activation of splenic immune cells in mouse models, suggesting that microfluidic-enabled, size-controlled nanoparticles may be a key strategy for enhancing the efficacy of RNA-based cancer vaccines.[147]

Several studies have explored innovative methods to optimize nanoparticle size, composition, and release profiles. Matsuura-Sawada *et al.* showed that higher lipid concentrations in paclitaxel-loaded liposomes can lead to multilamellar structures with extended drug release.[143] Naidu *et al.* designed and screened a combinatorial library of ionizable LNPs to achieve cell type-specific mRNA delivery *in vivo*. By systematically varying hydrophobic tail chains and linkers (hydroxylamine/ethanolamine) in novel amino ionizable lipids and using microfluidic mixing to formulate stable LNPs, they identified key structure–function relationships governing biodistribution and cellular targeting. The study revealed that subtle structural modifications significantly influence delivery efficiency and cell specificity. Lipid 23 emerged as a highly efficient, liver-tropic LNP, outperforming the reference lipid (Lipid 6) in hepatic mRNA expression, while Lipid 16 demonstrated remarkable specificity for macrophages across the spleen, lung, and liver—without the need for additional targeting ligands. This macrophage tropism enabled enhanced mRNA delivery to tumor myeloid cells in a B16F10 melanoma model, underscoring its potential for cancer immunotherapy. This work highlights how rational lipid engineering can drive precise, extrahepatic mRNA delivery, offering a powerful strategy for cell-selective therapeutic applications. More details represented in Fig. 6.[144] In a related approach, Eş *et al.* utilized diffusion-driven microfluidics to synthesize lipid-based nanocarriers (LNCs) and lipoplexes (LPXs) for siRNA delivery. They

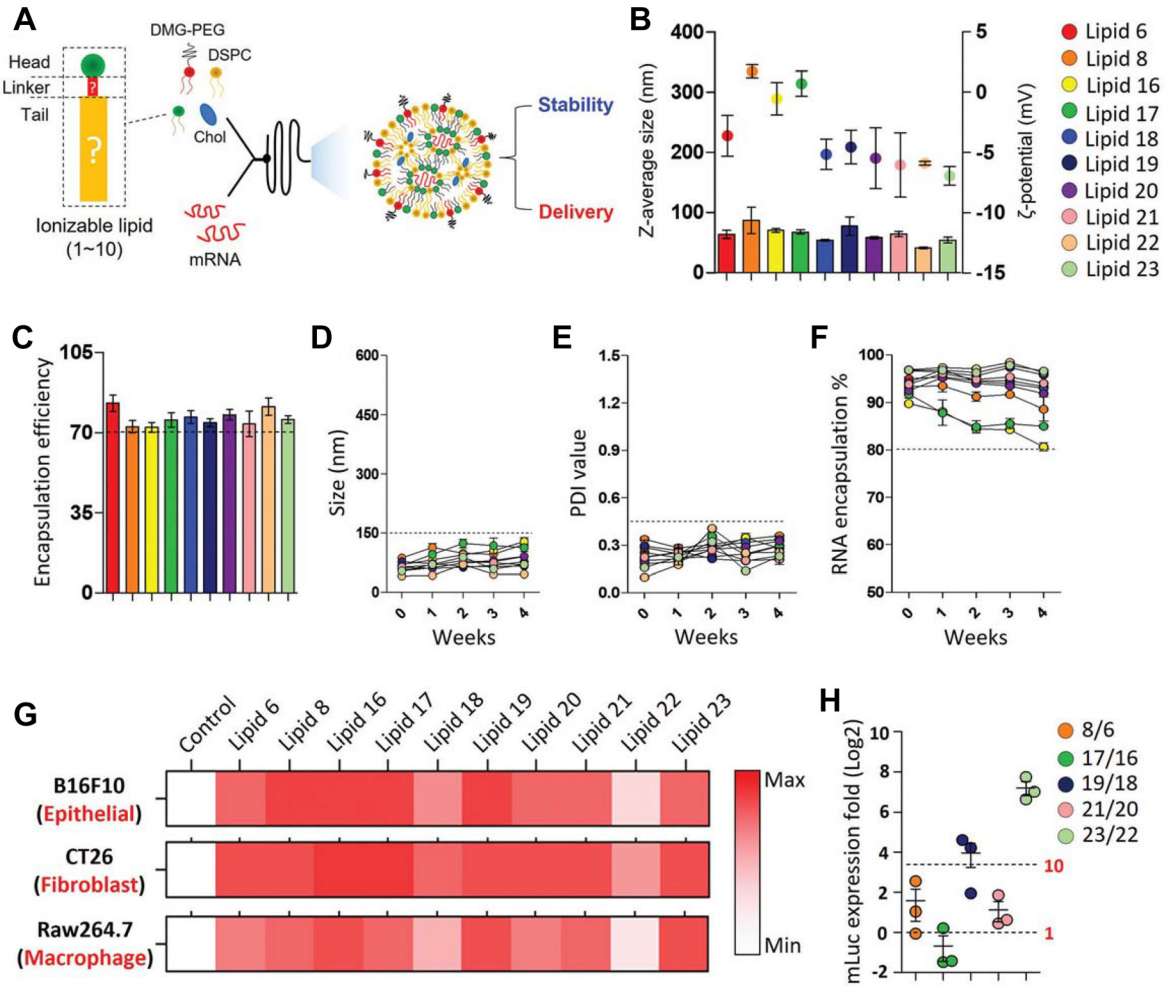

Fig. 6 Designing cell-type-specific lipid nanoparticles for mRNA delivery. (A) Schematic representation of the ionizable lipid nanoparticle (LNP) structure, highlighting key components such as the ionizable lipid, cholesterol (Chol), distearoylphosphatidylcholine (DSPC), and polyethylene glycol (PEG) for stability. The figure emphasizes the importance of the lipid tail structure in determining both LNP stability and mRNA delivery efficiency. (B) Physicochemical characterization of LNPs formulated with different lipids. The graph shows the *z*-average size (left *y*-axis) and ζ-potential (right *y*-axis) of LNPs over time. Each lipid is represented by a distinct color, illustrating variations in particle size and surface charge among the formulations. (C) Encapsulation efficiency of mRNA in LNPs. The bar chart demonstrates that all lipid formulations achieved high encapsulation efficiencies (>70%), indicating effective mRNA loading across the library. (D) Size distribution of LNPs over time. The plot shows the mean diameter of LNPs at various time points (weeks), revealing stable particle sizes for most formulations, with minimal changes over four weeks. (E) Polydispersity index (PDI) values of LNPs over time. The graph indicates consistent PDI values close to 0.3, suggesting uniform particle size distributions and good colloidal stability. (F) RNA encapsulation percentage of LNPs over time. The line graph illustrates the retention of mRNA within LNPs, showing slight decreases over four weeks but maintaining high encapsulation levels above 80%. (G) *In vitro* luciferase assay results in three cell lines: B16F10 (epithelial), CT26 (fibroblast), and Raw264.7 (macrophage). The heatmap depicts relative mRNA delivery efficiency, with darker red indicating higher expression. Lipid pairs (*e.g.*, 17/16, 23/22) are compared, highlighting differences in delivery efficacy across cell types. (H) Quantitative comparison of mRNA delivery efficiency between lipid pairs in the three cell lines. The scatter plot shows the log2 fold change in firefly luciferase (mLuc) expression, with error bars representing standard deviation. This analysis further validates the cell-specific delivery profiles observed in the heatmap.

demonstrated that variations in lipid composition and microfluidic processing can significantly affect particle size and surface charge, ultimately affecting the "stealth" properties and delivery efficiency.[120]

Biodistribution and gene expression are strongly linked to nanoparticle size. Di *et al.* showed that larger LNPs localize preferentially in the liver and spleen, achieving higher transgene expression.[145] Similarly, Evers *et al.* demonstrated the potential of LNPs to deliver modified RNA (modRNA) for heart repair post-ischemia-reperfusion injury, resulting in significant gene expression and therapeutic benefit in damaged cardiac tissues.[146]

Other studies have highlighted the importance of process parameters and material properties in nanocarrier performance. Jaradat *et al.* demonstrated that modifying PEG concentration in PEGylated liposomes influences particle size, drug release rates, and overall stability.[148] The use of microfluidics was not just for synthesis, but for engineering LNPs with

tunable, optimal physicochemical properties specifically designed to enhance tumor penetration and cellular uptake. Sarode *et al.* focused on PEG-lipid composition, demonstrating that specifically tailored formulations can enhance the gene silencing efficacy of antisense oligonucleotide-loaded LNPs.[149] Additionally, Aliakbarinodehi *et al.* explored how pH and protein corona formation affect LNP–membrane interactions, revealing that lower pH levels promote LNP disintegration and facilitate mRNA release.[150] Meanwhile, emerging studies focus on the impact of surface charge and hydrophobicity in influencing cellular uptake, which is key for improving both drug and gene delivery efficiency.

The integration of stimuli-responsive materials offers an additional innovative dimension. For instance, Jin *et al.* developed temperature-sensitive liposomes loaded with Ansamitocin P-3 and Indocyanine Green (ICG) where upon near-infrared (NIR) laser stimulation, these liposomes released their payload, achieving tumor inhibition rates as high as 97% *in vitro*.[151] The microfluidic control produced small, uniform temperature-sensitive liposomes (TSLs) that penetrate tumor tissue, then release AP-3 on near-infrared heating, enhancing intratumoral delivery beyond vascular and interstitial barriers while sparing healthy tissue. Such advances highlight the potential of combining external triggers with nanocarrier design to achieve on-demand drug release. Incorporating nano-sensors or fluorescent markers within nanocarriers could enable real-time monitoring of drug release and distribution, facilitating more personalized treatment regimens.

Despite these remarkable advancements, challenges remain in scaling up microfluidic platforms and integrating complex functionalities into nanocarriers. Published research, such as the study of El-Mayta *et al.* on mRNA-LNPs for *in vivo* gene delivery and Rana *et al.* on galactose (GAL)-conjugated liposomes for liver-targeted siRNA delivery, steadily progressing toward clinical translation.[152] Addressing regulatory barriers and establishing robust quality control protocols will be paramount for bringing microfluidic-based nanocarrier technologies into widespread clinical use. Additional studies relevant to these themes are summarized in Table 2.

By merging microfluidics with innovative materials and precise targeting strategies, drug and gene delivery systems are poised to deliver highly effective, patient-specific therapies, marking a significant leap forward in medical science.

6. Gaps and challenges

Although microfluidic techniques offer substantial advantages for nanocarrier synthesis, challenges related to scalability, cost-effectiveness, and system complexity continue to hinder their widespread adoption in clinical and pharmaceutical applications.[21,153,154] Fig. 7 illustrates several key elements, challenges, and opportunities related to the synthesis of microfluidic nanocarriers, which are discussed in detail below.

Scalability is a key challenge in microfluidic nanocarrier production using microfluidic technologies. While microfluidic systems excel in producing small batches of highly uniform nanocarriers, precise size control, and reproducible physicochemical properties, transitioning these processes from laboratory-scale synthesis to industrial-scale manufacturing poses considerable difficulties. The inherently small channel dimensions and low throughput of microfluidic devices limit the production volume, making it challenging to meet the large-scale demands required for preclinical and clinical applications. Moreover, scaling up is not simply a matter of operating multiple devices in parallel; it often necessitates redesigning system architecture, optimizing fluid dynamics, and ensuring consistent mixing conditions across all production units.[32,128,153,155] A fundamental limitation is the low production throughput inherent to microfluidic devices, which is largely dictated by microscale channel dimensions and the laminar flow regime under which they operate. In microfluidic systems, the Reynolds number (Re) typically remains well below unity, indicating a laminar flow profile where viscous forces dominate over inertial forces. While this regime facilitates highly controlled and predictable fluid behavior, it also constrains the mixing process to diffusive mechanisms unless chaotic advection strategies are employed, thereby limiting production rates.[156,157] Channel geometry plays a critical role in determining both mixing efficiency and device performance. Variations in channel cross-section, length, and curvature can significantly influence fluid velocity distribution, residence time, and shear stress—all of which impact nanoparticle size, polydispersity, and encapsulation efficiency.[128,158,159] For instance, serpentine or herringbone channel designs can enhance transverse mixing through induced secondary flows, improving particle homogeneity without compromising the gentle processing conditions needed for sensitive biomolecules such as RNA or proteins. However, the fabrication of such complex geometries often requires advanced microfabrication techniques, increasing production costs and complicating scale-up.[70,160]

The intricate design features and fine control parameters that are beneficial at the microscale become cumbersome and less efficient when applied to large-volume production. Buttitta and colleagues addressed this scalability challenge by developing a systematic DoE approach combined with microfluidics to produce highly monodisperse liposomes on a scale that could potentially meet clinical demands. Their findings demonstrated that microfluidic systems, when optimized with appropriate design strategies, could be scaled up without sacrificing liposome uniformity or product quality.[161] However, scaling up without compromising product quality often requires extensive process validation, which may not always be feasible for commercial-scale production. Future work should focus on developing standardized scale-up protocols and modular microfluidic architectures that can be seamlessly integrated into GMP-compliant manufacturing pipelines, ensuring reproducibility across global production sites.

The cost of integrating such complex systems can raise concerns about the overall feasibility in large-scale adoption. Similarly, Turkmen Koc and colleagues optimized the flow rate

Table 2 Summary of microfluidic-based polymeric and lipid nanoparticle (LNP) and nanocarrier formulations: device parameters, carrier and cargo characteristics, and delivery performance

			The feature of delivery systems				
Nanocarrier structure	Microfluidic device	Control conditions	Loaded cargo	Size (nm)	PDI	Descriptions	Ref.
LNP DSPC	Herringbone micromixer	FRR: 5 : 1 TFR: 15 mL min^{-1}	Metformin glipizide	Approximately 80–90 nm	0.11 to 0.22	Co-encapsulation of metformin and glipizide in liposomes resulted in a faster release of both drugs compared to their single-drug formulations	213
LNP		TFR set at 1.0 mL min^{-1}	Docetaxel (DTX)	From 112–162 nm	PDI was lowest in formulations with lower lipid-to-drug ratios (5 : 1)	**Entrapment efficiency:** DOPC : Chol LNPs showed high entrapmentDPPC and DSPC LNPs had 55% entrapment non-purified LNPs released DTX rapidly, while purified liposomes showed a slower, sustained release	214
DOPC : Chol : DSPE-PEG2000		FRR: 40 : 1, 20 : 1, 10 : 1 and 5 : 1		PEGylated were smaller (112–126 nm)		Non-purified LNPs were more cytotoxic at 24 hours due to rapid drug release. DTX-loaded LNP exhibited dose- and time-dependent cytotoxicity in 3D tumor models	
DPPC : Chol : DSPE-PEG2000		LNPs were elevated temperatures at 60 °C for DOPC and DPPC; 65 °C for DSPC					
DSPC : Chol : DSPE-PEG2000 Lipid : DTX							
LNP DSPC or DMPC	Staggered herringbone micromixer	TFR: 1, 6, and 20 mL min^{-1}	Atenolol (AT)	DMPC/cholesterol (1 : 1): ~363.67 ± 39.78 nm	PDI decreased with higher TFR and FRR; FRR 5 : 1 formulation showed lowest PDI	Atenolol demonstrated slower release in DSPC-based liposomes, likely due to the longer lipid chain length in contrast, quinine exhibited a faster release in DSPC formulations	215
		FRR: 1 : 1, 3 : 1, 5 : 1. Optimal size achieved at FRR 5 : 1 and TFR 20 mL min^{-1}	Quinine (Q)	DMPC/cholesterol (2 : 1): ~217.83 ± 18.33 nm DSPC/cholesterol (1 : 1): ~251.83 ± 46.55 nm DSPC/cholesterol (2 : 1): ~266.83 ± 48.43 nm.			

Table 2 *(Contd.)*

			The feature of delivery systems				
Nanocarrier structure	Microfluidic device	Control conditions	Loaded cargo	Size (nm)	PDI	Descriptions	Ref.
LNP pH-sensitive cationic lipids (YSK05), cholesterol, PEGylated lipids, and other phospholipids depending on the specific LNP system used	iLiNP device, a two-dimensional microchannel.	TFR: 50–500 μL min^{-1} FRR: 3 to 9	siRNA	20–100 nm	Less than 0.1 for most sizes	siRNA-loaded LNPs effectively mediated gene silencing in hepatocytes, showing no significant adverse effects	216
Nanolipomer (NLP), consisting of a polymeric core made of PLGA and a lipid coating of DSPE-PEG	Staggered herringbone micromixer, NanoAssemblr Benchtop instrument for high-flow nanoprecipitation	TFR: 12 mL min^{-1} FRR: 1 : 1. Polymer concentration: 10 mg mL^{-1} PLGA. Lipid concentration: 10% DSPE-PEG in 4% ethanol	Curcumin	100 nm	0.126	The encapsulation of curcumin ensured controlled release kinetics, contributing to sustained drug release over time	217
Budesonide-loaded solid lipid	Acoustically enhanced microfluidic mixer	TFR: 8 μL min^{-1} Frequency: 177.6 kHz to induce mixing Solvent/antisolvent ratio: 1 : 4 Concentration: budesonide 0.2–1 mg mL^{-1} in ethanol, purified water with flow rate of 20 μL per minute	Budesonide	Mean: 135.7 nm	0.044	Specific values for entrapment and release efficiency. **Cell viability and biological testing:** were not reported	218
LNP: DSPC, DMPC and cholesterol, forming bilayers with an aqueous core	Staggered herringbone micromixer chip	Lipid concentrations ranged from 0.3 to 10 mg mL^{-1} FRR: 1 : 1, 3 : 1, and 5 : 1 TFR: 5–20 mL min^{-1}	Proteins including ovalbumin (OVA), insulin, and bovine serum albumin (BSA)	50 and 100 nm	<0.2	Slower release rates were observed in LNPs with longer hydrocarbon tails or higher cholesterol content. No structural changes occurred in the proteins following encapsulation	219
LNP, PEGylated with DSPE-PEG2000, containing DOPC, DPPC, or DSPC as phospholipids, combined with cholesterol	Staggered herringbone micromixer	FRR: varied between 1 : 1 and 5 : 1 TFR: 2 mL min^{-1} Temperature: 65 °C	Doxorubicin	~100 nm	Below 0.2	The entrapment efficiency was higher than 80%	220

Table 2 *(Contd.)*

			The feature of delivery systems				
Nanocarrier structure	Microfluidic device	Control conditions	Loaded cargo	Size (nm)	PDI	Descriptions	Ref.
PLGA microspheres embedded with mesoporous silica nanoparticles (MSNs)	A capillary-based three-phase microfluidic device, using a water-in-oil-in-water (W/O/W) double emulsion template	Flow rates: innermost water phase at 1 mL h^{-1}, middle oil phase at 2 mL h^{-1}, and outermost water phase at 4 mL h^{-1} Solidification: evaporation of dichloromethane (DCM) at ambient temperature with constant stirring	Rhodamine B (RB)	56 μm	Coefficient of variation (CV) of 4.91%	PLGA-MSNs provided sustained drug release for approximately four months without burst release No significant cytotoxicity was observed at various drug concentrations	221
POPC, cholesterol and pH-sensitive cationic lipid (YSK05), DSPE-PEG2000 and DMG-PEG2000	A baffle device with staggered mixing structures	Initial flow rate: 50–500 μL min^{-1} FRR: 3 : 1 Ethanol concentration reduced from 25% to 1%	siRNA	POPC LNPs: 25–40 nm siRNA-loaded LNPs: 33–35 nm	<0.2	The formulation led to a greater than 80% reduction in gene expression in hepatocytes, demonstrating effective gene delivery and silencing capability	222
LNP: HSPC/DSPC, cholesterol, and DSPE-PEG2000	Staggered herringbone micromixer	Lipid concentration: 20–40 mg mL^{-1} FRR: 1.5 : 1 TFR: 12 mL min^{-1} Temperature: doxorubicin active loading performed at 60 °C for 10 minutes	Doxorubicin, with vincristine and acridine orange	80–100 nm	<0.2	Drug release in PBS at 37 °C was minimal, with only approximately 3% released over 8 hours. In PBS with 1% human serum, the release was slightly lower at around 1.5%	223
LNP	Staggered herringbone micromixers	FRR: 3 : 1	Proteins such as ovalbumin (OVA)	50 nm to 100 nm	<0.2	The entrapment efficiency for proteins (OVA) ranged from 26% to 36%, depending on the LNPs formulation. Doxorubicin had a higher entrapment efficiency of 95%	224

Table 2 *(Contd.)*

			The feature of delivery systems				
Nanocarrier structure	Microfluidic device	Control conditions	Loaded cargo	Size (nm)	PDI	Descriptions	Ref.
DSPC : Chol		TFR: ranged from 10 to 200 mL min^{-1}. Temperature: ambient temperature	Nucleic acids (PolyA as surrogate)			The release of the encapsulated drugs ranged from 20% to 30% within the first 24 hours, increasing to 70% to 85% by 120 hours	
DSPC : Chol : DOPS HSPC : Chol : DSPE-PEG2000			Doxorubicin				
LNP : Chol : DSPE-PEG2000	Dolomite microfluidic system	FRR: 1 : 10 or 1 : 16	Doxorubicin	80 nm to 250 nm	<0.25	The thin-film method resulted in approximately 30% drug release over 48 hours, while the microfluidic method achieved around 90% release within 48 hours	225
		TFR: 500 μL min^{-1}, temperature: 60 °C	Umbelliprenin			Free doxorubicin exhibited greater cytotoxicity compared to the LNPs formulations. Co-loaded LNPs prepared using the microfluidic method showed higher cytotoxicity, as indicated by a lower IC_{50}, compared to those prepared by the thin-film method	
LNP composed of PEG-lipids, ionizable or cationic lipids, cholesterol, and neutral or cationic helper lipids	Microfluidic channel fabricated with polydimethylsiloxane using soft lithography	The LNP stability was assessed under nebulization conditions. The best formulations were stable for at least four days at 4 °C in PBS	mRNA	Less than 200 nm	Indicated mono-dispersity	The nanoparticle demonstrated good *in vivo* tolerability, with minimal inflammatory response. There was no significant weight loss observed in treated mice	226
1LNP ionizable cationic lipids, helper lipids, cholesterol, and PEG-lipids	NanoAssemblr™ microfluidic system	Lipid-to-siRNA weight ratio was 10 : 1	siRNA targeting the BCR-ABL fusion oncogene	55 nm	Low polydispersity index (PDI) values	The LNPs exhibited minimal toxicity in normal CD34+ cells from healthy donors. In contrast, they significantly induced apoptosis in leukemia cells, with Annexin V positivity reaching 80–82%	227
		siRNA concentrations ranged from 0.25 to 2 μg ml^{-1} for *in vitro* and up to 5 mg kg^{-1} for *in vivo* studies	Various concentrations of siRNA were tested (0.25, 0.5, 1, and 2 μg ml^{-1})				

Table 2 *(Contd.)*

			The feature of delivery systems				
Nanocarrier structure	Microfluidic device	Control conditions	Loaded cargo	Size (nm)	PDI	Descriptions	Ref.
LNP: ionizable lipid (C12-200 or C14-4) Helper lipids: DOPE or DSPC, cholesterol Lipid-PEG, molecular weights from 1000 to 5000 Da	Herringbone	Lipid-to-DNA weight ratios (5 : 1 and 10 : 1), cholesterol-to-lipid-PEG molar ratios, and PEG molecular weights	siRNA and mRNA	~80 to 200 nm	Below 0.2	The LNPs exhibited tissue-specific accumulation in the liver and spleen, where they facilitated efficient mRNA transfection. No adverse effects were reported	228
LNP DMPC, DSPC Cholesterol, DSPE-PEG2000	Automated dolomite microfluidic system	FRR 1 : 10 TFR: 500 μL min^{-1} Temperature varied for lipid formulations: DMPC liposomes at 40 °C and DSPC liposomes at 60 °C	Doxorubicin hydrochloride	LNP size ranged from ~80 to 266 nm: DMPC: ~82 nm DSPC: ~266 nm	Between 0.13 and 0.22	DSPC100 nanoparticles exhibited a burst release, with over 85% of the drug released within 48 hours. In contrast, DMPC100 showed a slower, sustained release profile, with around 45% release within 48 hours. Cytotoxicity was evaluated in three human breast cancer cell lines (MCF-7, MDA-MB-231, and BT-474)	229

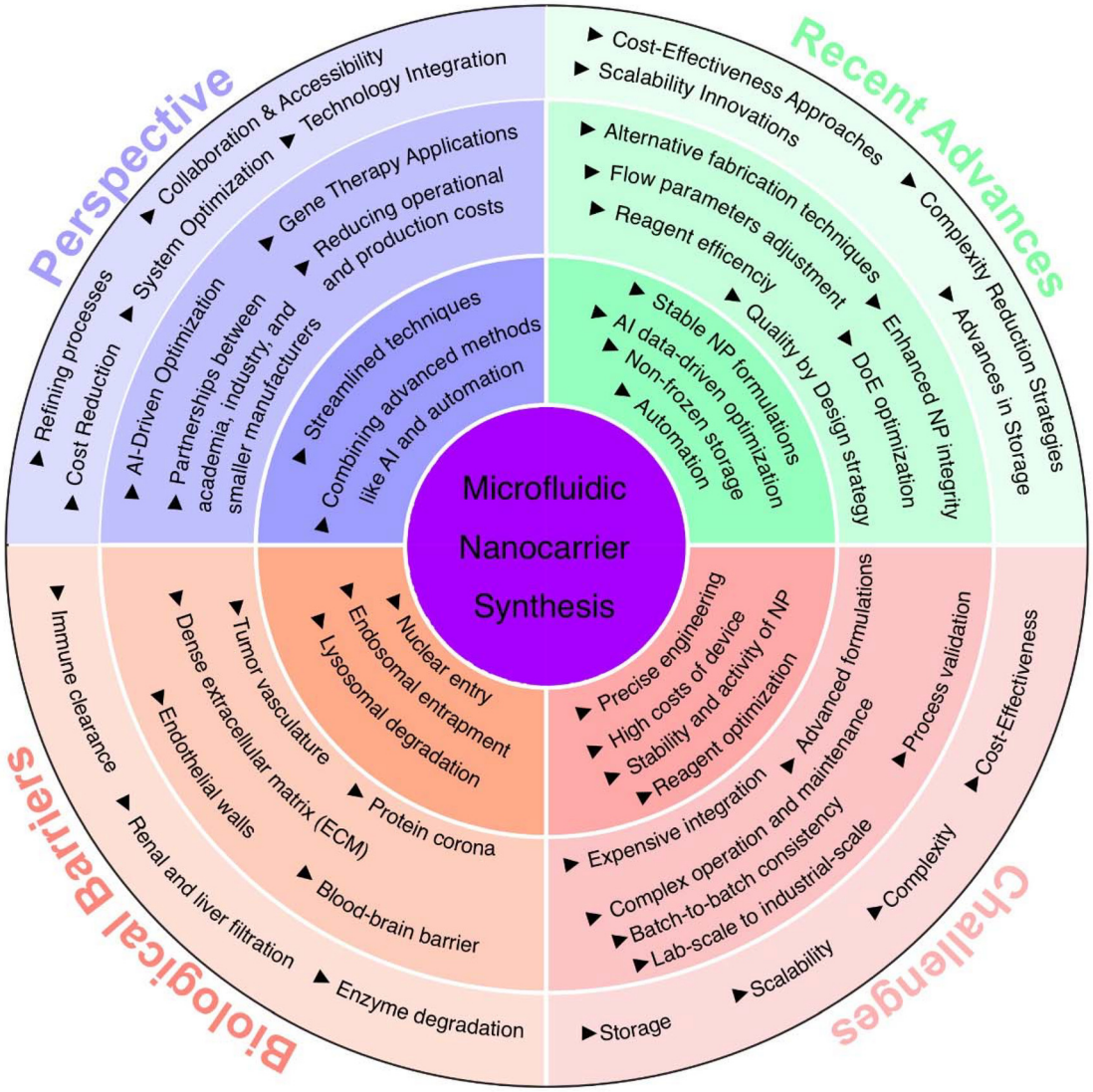

Fig. 7 Presents a clear and comprehensive model of microfluidic nanocarrier synthesis, highlighting its main process along with the key challenges, recent advancements, biological barriers, and future directions. The central focus is the synthesis process itself, surrounded by four interconnected areas. The model first outlines major challenges such as difficulties in scaling up production, high costs of devices and materials, complex automation, and issues related to stability and storage. It then highlights recent advancements, including the use of quality by design (QbD), improved flow rate control, development of stable nanoparticle formulations, and storage methods that do not require freezing. The model also considers biological barriers that nanoparticles face during both laboratory testing and interactions within the body. Finally, it offers a forward-looking perspective, emphasizing the need for system improvements, cost reduction, integration of new technologies, and stronger collaboration between academic and industrial sectors. This model provides a practical and forward-thinking guide to overcoming current limitations and advancing the use of microfluidic platforms in pharmaceutical and therapeutic applications.

and flow rate ratio to produce uniform, stable poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles loaded with 5-fluorouracil (5-FU) using microfluidics, highlighting their potential for industrial-scale cancer therapeutics.[162] However, maintaining batch-to-batch consistency at such scales, remains a hurdle.

Cost-effectiveness also poses a significant challenge in the adoption of microfluidic methods for nanocarrier production. The fabrication of microfluidic devices often involves the use of high-grade materials such as glass, silicon, or specialized polymers, which can be costly, especially when precision engineering and high-resolution fabrication techniques are required. Additionally, the production process frequently relies on advanced manufacturing methods, such as photolithography, soft lithography, or micromachining, which demand sophisticated cleanroom facilities and skilled technical expertise. Maintenance of these systems can also be resource-intensive, as microchannels are prone to clogging, fouling, or damage, necessitating frequent replacement or meticulous cleaning protocols. Achieving cost-effective solutions will require innovations in device design, material selection, and manufacturing strategies to make microfluidic nanocarrier production economically viable for large-scale clinical and

industrial applications.[154,163] These factors make microfluidic systems less economically viable compared to traditional bulk synthesis methods, which benefit from economies of scale. However, several studies have demonstrated ways to make microfluidic systems more cost-effective. For instance, Terada *et al.*, designed a microfluidic system to achieve high encapsulation efficiency and long-term stability, crucial for drug-loaded nanoparticles. Their approach utilized poly(ethylene glycol) (PEG) within a microfluidic platform to precisely control nanoparticle formation, resulting in uniform particle sizes and enhanced solubility of poorly soluble drug compounds. This method not only improved the stability and bioavailability of the nanoparticles but also demonstrated the potential for scalable, efficient, and cost-effective production. By optimizing the formulation process, their study offers a promising pathway toward the affordable large-scale manufacturing of therapeutic nanoparticles, addressing key challenges in drug delivery.[164] Similarly, Yenduri and colleagues utilized a continuous processing platform and Quality by Design (QbD) principle to identify critical parameter such as cholesterol and phosphatidylglycerol content, that influence liposome size and uniformity, thereby facilitating more flexible and economical liposomal manufacturing.[165] Despite these advances, there is a need for comprehensive techno-economic analyses that account for long-term operational costs, raw material supply chains, and device lifetime performance to guide industry-scale decision-making. Additionally, Lee and colleagues employed a microfluidic approach guided by QbD frameworks to produce lipid-based nanocarriers with controlled sizes, high drug encapsulation efficiency, and low cytotoxicity, making it a promising method for cost-effective drug delivery.[166] However, targeting ligands such as peptides and antibodies are rarely co-encapsulated during synthesis; instead, they are often added through post-insertion techniques to preserve orientation and bioactivity—a key consideration in microfluidic process design post-insertion steps within continuous microfluidic lines to enable truly multi-functional LNP production.[136] Nevertheless, achieving cost-effectiveness remains challenging when considering the costs of reagents, maintenance, and operational overhead associated with microfluidic platforms. Future research should explore integrated synthesis–functionalization workflows that can incorporate targeting moieties, imaging agents, and stimuli-responsive components in a single continuous process without compromising yield or bioactivity.[167,168]

Novel fabrication techniques have further expanded the scope of microfluidic systems. For instance, De *et al.* introduced thermocycling as an alternative to conventional microfluidic methods, achieving LNPs characterized by high encapsulation efficiency and extended shelf life, a cost-effective and scalable approach that challenges the need for specialized equipment.[169] Pratsinis *et al.* examined the effects of lipid composition and manufacturing methods on LNP characteristics, noting that microfluidic mixing generally yields smaller, more uniform particles than solvent-injection methods, although the latter offered advantages in structural order. Their work underscores the importance of selecting appropriate lipid components and synthesis techniques for specific therapeutic objectives.[170] However, a major unresolved question is how microfluidic and non-microfluidic methods compare in long-term stability, *in vivo* biodistribution, and clinical performance across diverse patient populations—a gap that warrants systematic head-to-head studies.[171,172]

The inherent complexity of microfluidic systems presents a significant barrier to their broader adoption. These devices require precise engineering and expert knowledge to design, operate, and maintain, limiting their accessibility to a wider audience.[128,173,174] Addressing this challenge may involve two complementary strategies: simplifying the hardware design to create more user-friendly, "plug-and-play" microfluidic systems with fewer adjustable parameters, and developing automation tools that standardize processes where feasible. Jin and colleagues highlighted the high precision of microfluidic systems for producing glycoprotein-imprinted nanospheres—applicable in biomarker detection and protein enrichment—but also noted that maintaining this precision during automation remains a significant hurdle. While automation can improve reproducibility, it often requires skilled personnel for programming and system maintenance, underscoring the need for balanced innovation between usability and technical performance.[175] In the future, combining AI-driven process control with self-calibrating microfluidic modules could reduce operator dependency and improve reproducibility across multi-site production.

Further illustration of this complexity emerges from Maeki *et al.*'s work on polymer–lipid hybrid nanoparticles designed to improve the transfection efficiency of large plasmid DNA. By optimizing cationic polymer and lipid components in a microfluidic device, they achieved a 4-fold increase in transfection efficiency relative to traditional lipid nanoparticles systems.[176] They developed a polymer–lipid hybrid LNP system for large-sized pDNA transfection, incorporating preformed polycation–DNA complexes prior to microfluidic encapsulation. This two-step strategy—distinct from conventional single-phase mixing—adds design complexity, especially in optimizing charge balance and nanoparticle structure, yet enables enhanced transfection performance for difficult-to-deliver plasmids. Passos Gibson *et al.* also developed a two-step microfluidic-inspired method to fabricate lipid-coated chitosan nanogels (NLG) with enhanced stability and biocompatibility. Chitosan nanogels were first prepared *via* ionic gelation, then coated with DMPG : cholesterol lipids using a controlled mixing platform. The lipid layer reversed surface charge, increased particle size, and preserved monodispersity. Compared to uncoated nanogels, NLGs showed improved colloidal stability in physiological media and significantly reduced cytotoxicity in HeLa, U87, and b.End3 cells. This approach offers a scalable and biocompatible platform for hybrid nanoparticle-based drug and gene delivery systems.[177] However, multi-step processes increase manufacturing time, regulatory validation requirements, and potential for batch variability, highlighting the need for streamlined multi-functionality within single-step continuous systems.

In another illustration of microfluidic innovation, Mo and colleagues introduced Light-Activated siRNA Endosomal

Release (LASER) technology using porphyrin lipid nanoparticles. Their system used near-infrared (NIR) light to enhance endosomal escape of siRNA, demonstrating a fourfold increase in gene silencing efficiency.[178] Although the porphyrin-LNPs in this study were synthesized *via* microfluidic rapid mixing, the subsequent LASER relies on external NIR irradiation. This external activation step—while enhancing delivery precision—introduces additional engineering and operational complexity beyond the microfluidic platform itself. The integration of microfluidic synthesis with downstream activation technologies will require harmonized regulatory pathways and compatibility testing to ensure clinical readiness.

The exploration of novel carrier materials can likewise introduce additional complexities. Carvalho *et al.* investigated the potential of combining cationic carriers with anionic biopolymers, specifically Chondroitin sulfate (CS), to improve the targeting efficiency and reduce the cytotoxicity of gene delivery systems.[179] The study showed that CS could mitigate the cytotoxicity associated with cationic carriers while enhancing their stability and transfection efficiency. However, the incorporation of such biopolymers adds complexity to the overall formulation, as it typically requires additional purification and quality control steps to ensure reproducibility and product uniformity. Digiacomo *et al.*'s comparative study on lipoplexes prepared by microfluidic mixing and bulk self-assembly revealed key insights into gene delivery system optimization. Although bulk self-assembly showed higher transfection efficiency, the microfluidic mixing method resulted in lower cytotoxicity. In contrast, microfluidic-prepared lipoplexes showed lower TE but markedly better cytocompatibility. These differences are not attributable to variations in size or zeta potential, which were comparable between both systems, but rather to deeper physicochemical distinctions. Specifically, bulk-prepared lipoplexes were multilamellar and carried more DNA per complex, resulting in higher TE per cell but greater toxicity due to excess cationic lipid accumulation. This highlights the necessity of balancing efficacy and safety in the development of gene delivery systems, emphasizing the advantages of microfluidic techniques in achieving this balance.[180] This emphasizes the need for design frameworks that optimize the efficacy–toxicity balance early in development, potentially leveraging predictive *in silico* modeling to reduce trial-and-error experimentation.

Storage and transportation introduce another layer of complexity, particularly for mRNA-based therapeutics. Reinhart *et al.* investigated the stability of mRNA LNPs produced *via* microfluidic mixing and their ability to maintain functionality during storage at various temperatures. The synthesized LNPs *via* microfluidic mixing exhibited remarkably stable physicochemical properties—such as size, polydispersity index (PDI), and zeta potential—over nine weeks of storage at 2–8 °C and 25 °C. A key contribution of microfluidics to stability in this context is the generation of homogenous LNP populations with tightly packed lipid arrangements, which may reduce mRNA leakage and degradation. This research is particularly relevant given the rise of mRNA-based vaccines and therapeutics, underscoring the potential of microfluidic systems for producing stable, transportable nanocarriers.[181] However, controlling and monitoring temperature conditions can raise supply-chain expenses. Developing nanocarriers with inherent thermostability, or integrating lyophilization-compatible formulations into microfluidic synthesis, could substantially reduce cold-chain dependence.

Finally, while microfluidic methods for nanocarrier synthesis offer significant advantages in terms of control over particle properties and uniformity, addressing the challenges of scalability, cost-effectiveness, and complexity is essential for their successful integration into clinical and pharmaceutical applications. By overcoming these challenges through system optimization, automation, and integration with other technologies, microfluidic platforms could play a pivotal role in advancing the production of nanocarriers for therapeutic use.

7. Future perspectives and applications

The future of microfluidic nanocarrier synthesis will likely be defined by significant advancements in techniques, materials, and computational integration.[70,182–185] These developments promise to expand applications beyond conventional drug and gene delivery, enabling innovations in imaging, diagnostics, and personalized medicine.

Recent innovations in microfluidic devices have revolutionized nanocarrier production, allowing for precise control over size, shape, and composition. Specifically, the hybridization of microfluidics with 3D bioprinting has enabled the fabrication of complex nanocarrier systems with integrated cellular models, offering a more physiologically relevant environment for drug testing. Tiboni *et al.* developed 3D-printed microfluidic chips featuring zigzag structures and circular sub-channels to facilitate passive mixing, producing polymeric nanoparticles and liposomes with tunable properties and efficient drug loading.[186] Ballacchino *et al.* utilized 3D-printed microfluidic devices to produce curcumin-loaded liposomes with enhanced encapsulation efficiency and stability. Their work underscores the importance of advanced fabrication methods in improving the scalability and reproducibility of liposome production.[230]

Similarly, Weaver *et al.* leveraged UV LCD printing to fabricate microfluidic chips with sub-micrometer resolutions, enabling the synthesis of liposomes with fine-tuned properties.[187] Their work highlighted the potential of advanced printing techniques to create custom microfluidic systems for diverse biomedical applications. Moreover, the integration of flexible and stretchable materials into microfluidic systems could enable real-time adjustments to nanocarrier production, further advancing the precision and functionality of the synthesis process.

Beyond structural innovations, microfluidic nanocarriers are being engineered for applications in imaging and diagnostics. Lou *et al.* further demonstrated how microfluidic techniques could optimize liposome size for improved cellular uptake and tissue retention, suggesting broader applications

in disease imaging and detection.[188] Emerging applications for microfluidic nanocarriers also include their use in personalized medicine, where patient-specific characteristics could guide the design of nanocarriers for tailored therapeutic and diagnostic interventions. In this context, the need for small, customized batches aligns well with the limited-scale production capabilities of current microfluidic systems, making scalability less of a barrier compared to mass production scenarios. The integration of artificial intelligence (AI) into microfluidic systems is an exciting development that could help overcome some of the challenges discussed. Liu *et al.* explored how AI can optimize microfluidic processes for nanomedicine and material synthesis. AI can be used to predict nanoparticle properties and improve synthesis parameters, which could enhance the scalability and uniformity of microfluidic production.[195] However, AI still faces challenges, such as the need for large labeled datasets and its application being primarily focused on cancer diagnosis and treatment.

The integration of AI into microfluidic systems represents a promising frontier with the potential to address several of the scalability, cost-effectiveness, and process optimization challenges previously discussed.[189] By enabling real-time monitoring, predictive modeling, and adaptive process control, AI can facilitate more consistent nanocarrier synthesis, reduce batch-to-batch variability, and accelerate the design–build–test–learn cycle. In particular, machine learning (ML) algorithms can be trained to optimize key process parameters—such as total flow rate, flow rate ratio, and channel geometry—to achieve desired particle sizes, polydispersity indices, and encapsulation efficiencies with minimal experimental iterations.[190,191] Despite these benefits, the integration of AI into microfluidic-based nanocarrier production still faces notable hurdles. One of the most significant is the need for large, high-quality labeled datasets to train robust and generalizable models. In the microfluidics field, experimental datasets are often small, heterogeneous, and lack standardized reporting formats, making it difficult to leverage ML methods effectively.[192,193] Moreover, much of the existing AI research in microfluidics has focused on biomedical diagnostics—particularly cancer detection and treatment—rather than on drug and gene delivery applications. As a result, the adoption of AI for therapeutic nanocarrier synthesis remains relatively limited, with most demonstrations still at the proof-of-concept stage.[189,194]

To fully realize the potential of AI in this context, future efforts should focus on developing open-access microfluidics datasets, establishing standardized data acquisition and annotation protocols, and exploring transfer learning approaches to adapt models trained in diagnostic applications for use in therapeutic nanocarrier production. Furthermore, integrating AI-driven design optimization with closed-loop microfluidic systems—capable of autonomous feedback control—could pave the way for scalable, cost-effective, and high-precision manufacturing of clinically relevant nanocarriers.[195–197,198,199] Rebollo *et al.* employed a DoE approach combined with artificial neural networks (ANNs) to fabricate LNPs with optimal properties, including sizes below 100 nm and low polydispersity.[199] By varying parameters such as cholesterol concentration, NaCl levels, TFR, FRR, and temperature, their ANN model accurately predicted LNP characteristics, significantly improving production efficiency. Similarly, Maharjan *et al.* used an ANN-DoE model to optimize the synthesis of mRNA-loaded LNPs, identifying key factors such as flow rate ratios and lipid composition that influenced particle size, zeta potential, and encapsulation efficiency. This computational approach streamlined the production process while achieving high reproducibility and precision.[200] Looking forward, AI could be further applied in predicting the optimal combinations of materials for nanocarriers, as well as simulating the interactions between nanocarriers and biological systems, to enhance efficacy and safety. Future efforts in device automation, AI-driven optimization, and innovative manufacturing strategies could help pave the way for more accessible and clinically relevant microfluidic nanocarrier systems.

The predictive capabilities of AI-driven approaches were further validated by Damiati *et al.*, who used ANNs to predict the size of poly(lactic-*co*-glycolic acid) (PLGA) microparticles. Their model achieved a prediction accuracy of 0.99, demonstrating its robustness in optimizing microparticle production (Fig. 8).[201] In addition to previous study, Damiati *et al.* exemplified these possibilities by using 3D flow focusing microfluidic chips and artificial neural networks (ANNs) to engineer PLGA microparticles encapsulating indomethacin. Their system achieved high drug loading efficiency and sustained release over nine days, demonstrating the effectiveness of coupling microfluidic techniques with computational tools to optimize drug delivery systems.[202]

In another study, Ocampo *et al.* compared ANN models to regression-based DOE models for liposome size prediction, finding that ANNs provided superior accuracy, with correlation coefficients of 0.98147 for training data compared to 0.882 for the DOE model.[203] These findings highlight the ability of AI to reduce experimental effort while maintaining high-quality outcomes. The integration of AI with real-time monitoring systems in microfluidic devices could facilitate continuous process optimization, ensuring consistent product quality during large-scale production.

In addition to conventional materials, researchers are exploring novel materials and methods to expand the versatility of microfluidic-synthesized nanocarriers. Kastner *et al.* demonstrated a scalable microfluidic method for producing propofol-loaded liposomes, achieving high drug loading capacities and stability over extended periods.[204] These studies exemplify how microfluidic technologies can address the limitations of traditional production methods while maintaining cost-effectiveness. Additionally, Tanaka *et al.* introduced an innovative freeze-dried LNP platform for encapsulating IVT-mRNA (RtoU), which exhibited durable structural stability and functionality for at least three months, underscoring the future possibilities of mRNA therapeutics in gene therapy.[205]

Sansare *et al.* used ANNs to optimize the continuous manufacturing process of liposomes, focusing on critical quality attributes such as particle size and polydispersity index. Their

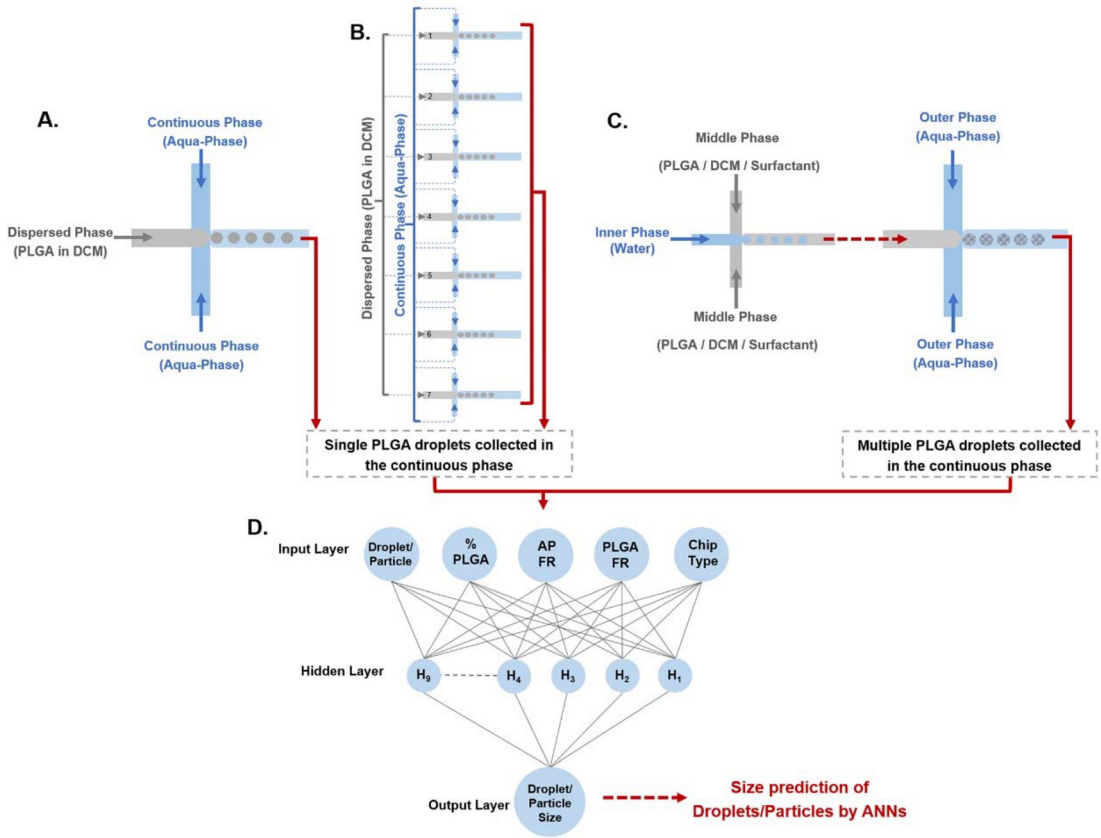

Fig. 8 Schematic illustration of the production of monodisperse PLGA droplets. Droplets are generated either in a single-emulsion format using single-junction devices. (A) or microfluidic devices with seven parallel junctions, (B) or in a multiple-emulsion format, (C) using two sequentially connected devices. Droplets were visualized at the orifice of the flow-focusing region within the microfluidic chips. The resulting data were subsequently utilized to train artificial neural network (ANN) models. A schematic representation of one of the developed ANN models (ANN-ABC) is shown, (D) abbreviations: AP, aqueous phase; FR, flow rate. Reprinted with permission from ref. 201, Copyright (2020) from Springer Nature.

computational models demonstrated superior accuracy in predicting liposome properties, further reducing the reliance on traditional experimental approaches.[206] The combination of microfluidics with microarray-based platforms could enable high-throughput screening of nanocarrier formulations for a wide range of drug and gene delivery applications.[207]

Emerging microfluidic techniques also offer improved mixing and ligand modification strategies. Sugimoto *et al.* demonstrated that their novel PEGylated liposomes maintained high cellular association with cancer cells even with a short mixing time of one minute, emphasizing the scalability and precision of their approach.[208] Further developments in microfluidic devices that integrate multiple functionalities, such as particle size control, drug loading, and surface modification in a single platform, will greatly simplify the synthesis of multifunctional nanocarriers for complex therapeutic applications.

Looking forward, interdisciplinary approaches combining AI, 3D printing, and novel materials will further enhance microfluidic nanocarrier synthesis.[199,209,210] As demonstrated by studies like those by Maharjan *et al.*, these technologies have the potential to expand applications beyond drug and gene delivery into areas such as disease diagnostics, imaging, and personalized medicine.[200] Additionally, the integration of real-time data analytics and AI-driven feedback systems could allow for the dynamic adjustment of microfluidic parameters, making nanocarrier production more adaptive and responsive to changing conditions. By integrating computational tools and scalable fabrication methods, researchers can accelerate the development of nanocarriers with precise properties, addressing the growing demand for next-generation biomedical solutions.[197,199,211]

Recent innovations in microfluidic nanocarrier synthesis—particularly those leveraging AI-driven optimization—have enabled the precise engineering of nanocarriers with application-specific properties, enhancing their efficacy across a range of biomedical contexts.[212]

8. Conclusion

Microfluidic technologies have revolutionized drug and gene delivery by providing precise control over the synthesis of therapeutic nanoparticles. These advancements have helped

address limitations of conventional drug delivery methods—such as poor targeting, low bioavailability, and high toxicity—not solely due to the use of microfluidics, but because the technology facilitates the controlled production of increasingly functionalized and optimized nanoparticles.

A chief advantage of microfluidic systems lies in their ability to produce uniform and reproducible nanocarriers, crucial for clinical applications. These technologies support scalable production of complex nanocarriers like liposomes, LNPs and polymeric nanoparticles with targeted delivery and controlled release. This has paved the way for personalized medicine, where the precise control offered by microfluidic platforms enables the production of tailored nanocarriers that match individual patient profiles and therapeutic needs. Microfluidics allows for the fine-tuning of parameters such as particle size, surface charge, and payload composition, which are critical for optimizing biodistribution and therapeutic efficacy. This level of customization is particularly valuable in applications like mRNA-based vaccines, cancer immunotherapies, and rare genetic disorders, where patient-specific formulations can significantly enhance treatment outcomes. Beyond drug and gene delivery, microfluidic nanocarriers could also be adapted for theranostics, biosensing, and patient-specific treatment platforms, expanding their role in precision medicine and integrated healthcare solutions.

Despite these benefits, widespread adoption faces notable barriers, including the high cost and complexity of microfluidic system design and operation. Ongoing research focuses on optimizing processes, automating production, and reducing costs through several approaches, including the use of low-cost and reusable materials, simplified chip designs, and the development of scalable manufacturing strategies for microfluidic devices. These innovations aim to make microfluidic production of LNPs more economically viable for both clinical and industrial applications. Additionally, translating these technologies into clinical and industrial settings requires rigorous validation and standardization to meet regulatory standards.

Future developments in microfluidic nanocarrier synthesis include the integration of AI and machine learning to optimize production and enhance efficiency. The exploration of new materials and fabrication techniques like 3D printing and photolithography will further improve the scalability and versatility of microfluidic systems. Finally, microfluidic technologies represent a transformative advancement in drug and gene delivery, offering precise, efficient, and scalable solutions. As research continues, these technologies have the potential to revolutionize personalized medicine, improving patient care and therapeutic outcomes.

Author contributions

Alireza Gharatape: Conceptualization, Investigation, Methodology, Literature Search, Data Curation, Visualization, Writing – Original Draft, Writing – Review & Editing. Zahra Niasari-Naslaji: Literature Search, Methodology, Data Curation, Writing – Original Draft, Writing – Review & Editing. Jeanne Leblond Chain: Conceptualization, Methodology, Writing – Review & Editing. Nasrollah Tabatabaei: Conceptualization, Methodology, Visualization, Funding acquisition, Project administration, Resources, Supervision, Writing – Review & Editing. Reza Faridi-Majidi: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – Review & Editing.

Conflicts of interest

The corresponding author on behalf of all the authors declares no potential conflict of interest.

Data availability

No primary data, software, or code were generated or analyzed in this review.

Acknowledgements

This work received funding from the Tehran University of Medical Sciences under grant code 1403-1-148-71508.

References

1 A. Vikal, *et al.*, From Conventional to Cutting-edge: A Comprehensive Review on Drug Delivery Systems, *Drug Delivery Lett.*, 2024, **14**(3), 226–243.
2 S. J. Soni, Review on advanced drug delivery systems: innovations in formulation and delivery technologies, *J. Multidiscip. Res.*, 2024, 6–13.
3 M. Kim, *et al.*, Transformative Impact of Nanocarrier-Mediated Drug Delivery: Overcoming Biological Barriers and Expanding Therapeutic Horizons, *Small Sci.*, 2024, **4**(11), 2400280.
4 M. T. Manzari, *et al.*, Targeted drug delivery strategies for precision medicines, *Nat. Rev. Mater.*, 2021, **6**(4), 351–370.
5 A. A. Yetisgin, *et al.*, Therapeutic nanoparticles and their targeted delivery applications, *Molecules*, 2020, **25**(9), 2193.
6 A. Al Ragib, *et al.*, Current advanced drug delivery systems: Challenges and potentialities, *J. Drug Delivery Sci. Technol.*, 2022, **76**, 103727.
7 M. Lorscheider, *et al.*, Challenges and opportunities in the delivery of cancer therapeutics: update on recent progress, *Ther. Delivery*, 2021, **12**(1), 55–76.
8 L. Xu, *et al.*, Lipid Nanoparticles for Drug Delivery, *Adv. NanoBiomed Res.*, 2022, **2**(2), 2100109.
9 H. Sabit, *et al.*, Nanocarriers: A Reliable Tool for the Delivery of Anticancer Drugs, *Pharmaceutics*, 2022, **14**, 1566.

10 C. Wang, Y. Zhang and Y. Dong, Lipid Nanoparticle–mRNA Formulations for Therapeutic Applications, *Acc. Chem. Res.*, 2021, **54**(23), 4283–4293.
11 H. Huang, *et al.*, Inorganic nanoparticles in clinical trials and translations, *Nano Today*, 2020, **35**, 100972.
12 Y. Liu, *et al.*, Nanoparticles advanced from preclinical studies to clinical trials for lung cancer therapy, *Cancer Nanotechnol.*, 2023, **14**(1), 28.
13 B. Li, *et al.*, Drug-Loaded Polymeric Nanoparticles for Cancer Stem Cell Targeting, *Front. Pharmacol.*, 2017, **8**, 51.
14 M. Chehelgerdi, *et al.*, Progressing nanotechnology to improve targeted cancer treatment: overcoming hurdles in its clinical implementation, *Mol. Cancer*, 2023, **22**(1), 169.
15 A. Gharatape and R. Salehi, Recent progress in theranostic applications of hybrid gold nanoparticles, *Eur. J. Med. Chem.*, 2017, **138**, 221–233.
16 M. A. Beach, *et al.*, Polymeric Nanoparticles for Drug Delivery, *Chem. Rev.*, 2024, **124**(9), 5505–5616.
17 S. J. Shepherd, D. Issadore and M. J. Mitchell, Microfluidic formulation of nanoparticles for biomedical applications, *Biomaterials*, 2021, **274**, 120826.
18 A. G. Hamilton, K. L. Swingle and M. J. Mitchell, Biotechnology: Overcoming biological barriers to nucleic acid delivery using lipid nanoparticles, *PLoS Biol.*, 2023, **21**(4), e3002105.
19 J. C. Gonzalez, *et al.*, Nano Approaches to Nucleic Acid Delivery: Barriers, Solutions, and Current Landscape, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2025, **17**(2), e70010.
20 A. Yousfan, *et al.*, A Comprehensive Study on Nanoparticle Drug Delivery to the Brain: Application of Machine Learning Techniques, *Mol. Pharm.*, 2024, **21**(1), 333–345.
21 S. E. Alavi, *et al.*, Microfluidics for personalized drug delivery, *Drug Discovery Today*, 2024, **29**(4), 103936.
22 Q. Zhang, *et al.*, Tailoring drug delivery systems by microfluidics for tumor therapy, *Mater. Today*, 2024, 151–178.
23 B. Ferreira, *et al.*, Microfluidic Technologies for Precise Drug Delivery. Drug Discovery Today, *Lab Chip*, 2024, 313–333.
24 F. Tian, *et al.*, Microfluidic technologies for nanoparticle formation, *Lab Chip*, 2022, **22**(3), 512–529.
25 L. Chen, *et al.*, Millifluidics, microfluidics, and nanofluidics: manipulating fluids at varying length scales, *Mater. Today Nano*, 2021, **16**, 100136.
26 I. Shaw, *et al.*, Integrating Artificial Intelligence and Microfluidics Technology for Psoriasis Therapy: A Comprehensive Review for Research and Clinical Applications, *Adv. Intell. Syst.*, 2025, **7**(4), 2400558.
27 L. Mathur, *et al.*, Microfluidics as an Enabling Technology for Personalized Cancer Therapy, *Small*, 2020, **16**(9), 1904321.
28 G. Prakash, *et al.*, Microfluidic fabrication of lipid nanoparticles for the delivery of nucleic acids, *Adv. Drug Delivery Rev.*, 2022, **184**, 114197.
29 A. Bezelya, B. Küçüktürkmen and A. Bozkır, Microfluidic Devices for Precision Nanoparticle Production, *Micro*, 2023, **3**(4), 822–866.
30 J. Hong, *et al.*, Size-Controllable and Monodispersed Lipid Nanoparticle Production with High mRNA Delivery Efficiency Using 3D-Printed Ring Micromixers, *ACS Appl. Mater. Interfaces*, 2024, **16**(35), 46044–46052.
31 A. Forigua, *et al.*, Recent advances in the design of microfluidic technologies for the manufacture of drug releasing particles, *J. Controlled Release*, 2021, **333**, 258–268.
32 M. Yu, *et al.*, Microfluidics for Formulation and Scale-Up Production of Nanoparticles for Biopharma Industry, in Microfluidics in Pharmaceutical Sciences: Formulation, Drug Delivery, Screening, and Diagnostics, *Microfluidics Pharm. Sci.*, 2024, 395–420.
33 E. Jaradat, *et al.*, Microfluidics technology for the design and formulation of nanomedicines, *Nanomaterials*, 2021, **11**(12), 3440.
34 D. Khorsandi, *et al.*, *Lab-on-a-chip: Unit Operations to Scale-up Strategies*, 2024. DOI: **10.1039/9781837673476-00560**.
35 H. Zhang, *et al.*, Microfluidics for nano-drug delivery systems: From fundamentals to industrialization, *Acta Pharm. Sin. B*, 2023, **13**(8), 3277–3299.
36 S. Gimondi, *et al.*, Microfluidic Devices: A Tool for Nanoparticle Synthesis and Performance Evaluation, *ACS Nano*, 2023, **17**(15), 14205–14228.
37 S. Siavashy, *et al.*, A Comprehensive Review of One Decade of Microfluidic Platforms Applications in Synthesis of Enhanced Carriers Utilized in Controlled Drug Delivery, *Adv. Mater. Technol.*, 2022, **7**(10), 2101615.
38 U. A. Gurkan, *et al.*, Next generation microfluidics: fulfilling the promise of lab-on-a-chip technologies, *Lab Chip*, 2024, **24**(7), 1867–1874.
39 P. Ranjan, *et al.*, *Commercialization of microfluidic point-of-care diagnostic devices*, in *Advanced Microfluidics Based Point-of-Care Diagnostics*, CRC Press, 2022, pp. 383–398.
40 E. Bottaro and C. Nastruzzi, "Off-the-shelf" microfluidic devices for the production of liposomes for drug delivery, *Mater. Sci. Eng., C*, 2016, **64**, 29–33.
41 J. Riewe, *et al.*, Antisolvent precipitation of lipid nanoparticles in microfluidic systems - A comparative study, *Int. J. Pharm.*, 2020, **579**, 119167.
42 R.-M. Lu, *et al.*, Current landscape of mRNA technologies and delivery systems for new modality therapeutics, *J. Biomed. Sci.*, 2024, **31**(1), 89.
43 S. S. Y. Leung, *et al.*, Microfluidic-assisted bacteriophage encapsulation into liposomes, *Int. J. Pharm.*, 2018, **545**(1–2), 176–182.
44 T. A. Balbino, *et al.*, Integrated microfluidic devices for the synthesis of nanoscale liposomes and lipoplexes, *Colloids Surf., B*, 2017, **152**, 406–413.
45 R. R. López, *et al.*, Surface Response Based Modeling of Liposome Characteristics in a Periodic Disturbance Mixer, *Micromachines*, 2020, **11**(3), 235.
46 M. A. Obeid, S. Haifawi and I. Khadra, The impact of solvent selection on the characteristics of niosome nanoparticles prepared by microfluidic mixing, *Int. J. Pharm.: X*, 2023, **5**, 100168.

47 X. Han, *et al.*, Plug-and-play assembly of biodegradable ionizable lipids for potent mRNA delivery and gene editing *in vivo*, *bioRxiv*, 2025, preprint, DOI:DOI: 10.1101/2025.02.25.640222.
48 E. Blanco, H. Shen and M. Ferrari, Principles of nanoparticle design for overcoming biological barriers to drug delivery, *Nat. Biotechnol.*, 2015, **33**(9), 941–951.
49 J. Liu, H. Cabral and P. Mi, Nanocarriers address intracellular barriers for efficient drug delivery, overcoming drug resistance, subcellular targeting and controlled release, *Adv. Drug Delivery Rev.*, 2024, **207**, 115239.
50 C. Qiu, *et al.*, Advanced Strategies for Overcoming Endosomal/Lysosomal Barrier in Nanodrug Delivery, *Research*, 2023, **6**, 0148.
51 Z. Wang, *et al.*, Overcoming Endosomal Escape Barriers in Gene Drug Delivery Using De Novo Designed pH-Responsive Peptides, *ACS Nano*, 2024, **18**(14), 10324–10340.
52 V. Passos Gibson, *et al.*, Switchable Lipid Provides pH-Sensitive Properties to Lipid and Hybrid Polymer/Lipid Membranes, *Polymers*, 2020, **12**(3), 637.
53 S. N. Tabatabaei, *et al.*, Co-delivery of miR-181a and melphalan by lipid nanoparticles for treatment of seeded retinoblastoma, *J. Controlled Release*, 2019, **298**, 177–185.
54 S. Waheed, *et al.*, Engineering nano-drug biointerface to overcome biological barriers toward precision drug delivery, *J. Nanobiotechnol.*, 2022, **20**(1), 395.
55 M. J. Mitchell, *et al.*, Engineering precision nanoparticles for drug delivery, *Nat. Rev. Drug Discovery*, 2021, **20**(2), 101–124.
56 X. Hou, *et al.*, Lipid nanoparticles for mRNA delivery, *Nat. Rev. Mater.*, 2021, **6**(12), 1078–1094.
57 J. Yao, *et al.*, Strategies on the nuclear-targeted delivery of genes, *J. Drug Targeting*, 2013, **21**(10), 926–939.
58 R. Goswami, *et al.*, Nuclear localization signal-tagged systems: relevant nuclear import principles in the context of current therapeutic design, *Chem. Soc. Rev.*, 2024, **53**(1), 204–226.
59 H. Li, *et al.*, The protein corona and its effects on nanoparticle-based drug delivery systems, *Acta Biomater.*, 2021, **129**, 57–72.
60 T. Nakamura, *et al.*, The Effect of Size and Charge of Lipid Nanoparticles Prepared by Microfluidic Mixing on Their Lymph Node Transitivity and Distribution, *Mol. Pharm.*, 2020, **17**(3), 944–953.
61 S. Priya, V. M. Desai and G. Singhvi, Surface Modification of Lipid-Based Nanocarriers: A Potential Approach to Enhance Targeted Drug Delivery, *ACS Omega*, 2023, **8**(1), 74–86.
62 J.-p. Liu, *et al.*, Smart nanoparticles improve therapy for drug-resistant tumors by overcoming pathophysiological barriers, *Acta Pharmacol. Sin.*, 2017, **38**(1), 1–8.
63 T. Fukuta and K. Kogure, Biomimetic Nanoparticle Drug Delivery Systems to Overcome Biological Barriers for Therapeutic Applications, *Chem. Pharm. Bull.*, 2022, **70**(5), 334–340.
64 L. Xiong, *et al.*, ATP-responsive tumor targeted lipid nanoparticle for enhanced siRNA delivery and improved treatment efficacy in melanoma, *J. Controlled Release*, 2025, **382**, 113622.
65 Z. Sadat Razavi, *et al.*, Advancing neurological disorders therapies: Organic nanoparticles as a key to blood-brain barrier penetration, *Int. J. Pharm.*, 2025, **670**, 125186.
66 M. Kulkarni, *et al.*, Nanomaterials as drug delivery agents for overcoming the blood-brain barrier: A comprehensive review, *ADMET DMPK*, 2024, **12**(1), 63–105.
67 E. L. Han, *et al.*, Peptide-Functionalized Lipid Nanoparticles for Targeted Systemic mRNA Delivery to the Brain, *Nano Lett.*, 2025, **25**(2), 800–810.
68 J. Ahlawat, *et al.*, Nanocarriers as Potential Drug Delivery Candidates for Overcoming the Blood-Brain Barrier: Challenges and Possibilities, *ACS Omega*, 2020, **5**(22), 12583–12595.
69 J. Xie, *et al.*, Nanomaterial-based blood-brain-barrier (BBB) crossing strategies, *Biomaterials*, 2019, **224**, 119491.
70 S. Siavashy, *et al.*, A comprehensive review of one decade of microfluidic platforms applications in synthesis of enhanced carriers utilized in controlled drug delivery, *Adv. Mater. Technol.*, 2022, **7**(10), 2101615.
71 S. Cheng, W. Chen and P. J. G. Zhang, Developing advanced polymer films based on microfluidic laminar flow, *Giant*, 2022, **9**, 100091.
72 Y. Song, *et al.*, Microfluidic programmable strategies for channels and flow, *Lab Chip*, 2024, **24**, 4483–4513.
73 M. Ferreira, *et al.*, Advances in Microfluidic Systems and Numerical Modeling in Biomedical Applications: A Review, *Micromachines*, 2024, **15**(7), 873.
74 S. Gimondi, *et al.*, Microfluidic devices: a tool for nanoparticle synthesis and performance evaluation, *ACS Nano*, 2023, **17**(15), 14205–14228.
75 P. Shrimal, *et al.*, A review on novel methodologies for drug nanoparticle preparation: Microfluidic approach, *Chem. Eng. Res. Des.*, 2020, **153**, 728–756.
76 Y. Liu, *et al.*, Microfluidic nanoparticles for drug delivery, *Small*, 2022, **18**(36), 2106580.
77 A. Pratsinis, *et al.*, Impact of non-ionizable lipids and phase mixing methods on structural properties of lipid nanoparticle formulations, *Int. J. Pharm.*, 2023, **637**, 122874.
78 S. M. Giannitelli, *et al.*, Droplet-based microfluidic synthesis of nanogels for controlled drug delivery: tailoring nanomaterial properties via pneumatically actuated flow-focusing junction, *Nanoscale*, 2022, **14**(31), 11415–11428.
79 C. Shao, *et al.*, Droplet microfluidics-based biomedical microcarriers, *Acta Biomater.*, 2022, **138**, 21–33.
80 Z. Liu, *et al.*, Dynamic mechanism of double emulsion droplets flowing through a microfluidic T-junction, *Phys. Fluids*, 2023, **35**(7), DOI: 10.1063/5.0155427.
81 J. Kim, *et al.*, Microfluidic approaches for gene delivery and gene therapy, *Lab Chip*, 2011, **11**(23), 3941–3948.
82 Y. Cheng, *et al.*, Microfluidic technologies for lipid vesicle generation, *Lab Chip*, 2024, **24**(20), 4679–4716.

83 G. T. Vladisavljević, Droplet Microfluidics for High-Throughput Screening and Directed Evolution of Biomolecules, *Micromachines*, 2024, **15**(8), 971.
84 M. K. Mulligan and J. P. Rothstein, Scale-up and control of droplet production in coupled microfluidic flow-focusing geometries, *Microfluid. Nanofluid.*, 2012, **13**(1), 65–73.
85 S. Cleve, *et al.*, Microbubble formation by flow focusing: role of gas and liquid properties, and channel geometry, *J. Fluid Mech.*, 2023, **972**, A27.
86 W. D. Wong, *et al.*, Enhancement of mixing and reaction efficiency of various fluids applications at different microfluidic configuration and design, *Chem. Eng. Process.*, 2024, 109729.
87 C. M. Abreu, *et al.*, Microfluidic platforms for extracellular vesicle isolation, analysis and therapy in cancer, *Lab Chip*, 2022, **22**(6), 1093–1125.
88 Q. Zhao, *et al.*, A Review of Secondary Flow in Inertial Microfluidics, *Micromachines*, 2020, **11**(5), 461.
89 A. Naseri Karimvand, *et al.*, Machine Learning and Genetic Algorithms in the Microfluidic Design of Highly Efficient Micromixers, *Ind. Eng. Chem. Res.*, 2024, **63**(43), 18626–18638.
90 Y. H. Hwang, *et al.*, Robust, Scalable Microfluidic Manufacturing of RNA-Lipid Nanoparticles Using Immobilized Antifouling Lubricant Coating, *ACS Nano*, 2025, **19**(1), 1090–1102.
91 D. Jung, *et al.*, Automated Microfluidic Systems Facilitating the Scalable and Reliable Production of Lipid Nanoparticles for Gene Delivery, *BioChip J.*, 2025, **19**(1), 79–90.
92 G.-Y. Ahn, *et al.*, Continuous production of lipid nanoparticles by multiple-splitting in microfluidic devices with chaotic microfibrous channels, *Colloids Surf., B*, 2023, **224**, 113212.
93 J. Y. Han, J. N. La Fiandra and D. L. DeVoe, Microfluidic vortex focusing for high throughput synthesis of size-tunable liposomes, *Nat. Commun.*, 2022, **13**(1), 6997.
94 Y. Sugimoto, *et al.*, Microfluidic Post-Insertion Method for the Efficient Preparation of PEGylated Liposomes Using High Functionality and Quality Lipids, *Int. J. Nanomed.*, 2022, **17**, 6675–6686.
95 J. A. Kulkarni, *et al.*, Spontaneous, solvent-free entrapment of siRNA within lipid nanoparticles, *Nanoscale*, 2020, **12**(47), 23959–23966.
96 J. Kotouček, *et al.*, Preparation of nanoliposomes by microfluidic mixing in herring-bone channel and the role of membrane fluidity in liposomes formation, *Sci. Rep.*, 2020, **10**(1), 5595.
97 H. Bolze, *et al.*, Continuous Production of Lipid Nanoparticles by Ultrasound-Assisted Microfluidic Antisolvent Precipitation, *Chem. Eng. Technol.*, 2021, **44**(9), 1641–1650.
98 D. M. Strelkova Petersen, *et al.*, The mixing method used to formulate lipid nanoparticles affects mRNA delivery efficacy and organ tropism, *Eur. J. Pharm. Biopharm.*, 2023, **192**, 126–135.
99 E. Kastner, *et al.*, High-throughput manufacturing of size-tuned liposomes by a new microfluidics method using enhanced statistical tools for characterization, *Int. J. Pharm.*, 2014, **477**(1), 361–368.
100 R. Ran, A. P. J. Middelberg and C. X. Zhao, Microfluidic synthesis of multifunctional liposomes for tumour targeting, *Colloids Surf., B*, 2016, **148**, 402–410.
101 R. Natu, *et al.*, A Systematic Analysis of Recent Technology Trends of Microfluidic Medical Devices in the United States, *Micromachines*, 2023, **14**(7), 1293.
102 K. Nathanael, *et al.*, Computational modelling and microfluidics as emerging approaches to synthesis of silver nanoparticles–A review, *Chem. Eng. J.*, 2022, **436**, 135178.
103 H. Shan, *et al.*, Dialysis-functionalized microfluidic platform for *in situ* formation of purified liposomes, *Colloids Surf., B*, 2024, **236**, 113829.
104 M. A. Tomeh and X. J. M. P. Zhao, Recent advances in microfluidics for the preparation of drug and gene delivery systems, *Mol. Pharm.*, 2020, **17**(12), 4421–4434.
105 R. Su, F. Wang and M. C. McAlpine, 3D printed microfluidics: advances in strategies, integration, and applications, *Lab Chip*, 2023, **23**(5), 1279–1299.
106 A. Gharatape, *et al.*, Nanocarrier-based gene delivery for immune cell engineering, *J. Mater. Chem. B*, 2024, **12**(14), 3356–3375.
107 A. Gharatape, *et al.*, Recent advances in polymeric and lipid stimuli-responsive nanocarriers for cell-based cancer immunotherapy, *Nanomedicine*, 2024, **19**(30), 2655–2678.
108 Y. Hirai, *et al.*, Charge-reversible lipid derivative: A novel type of pH-responsive lipid for nanoparticle-mediated siRNA delivery, *Int. J. Pharm.*, 2020, **585**, 119479.
109 O. M. M. M. Da Costa, *et al.*, One-step Production of Sterically Stabilized Anionic Nanoliposome Using Microfluidic Device, *J. Oleo Sci.*, 2022, **71**(4), 515–522.
110 R. R. López, *et al.*, Parametric study of the factors influencing liposome physicochemical characteristics in a periodic disturbance mixer, *Langmuir*, 2021, **37**(28), 8544–8556.
111 O. Sartipzadeh, *et al.*, Microfluidic-assisted synthesis and modeling of stimuli-responsive monodispersed chitosan microgels for drug delivery applications, *Sci. Rep.*, 2022, **12**(1), 8382.
112 P. Patel, *et al.*, Development of amino acid-modified biodegradable lipid nanoparticles for siRNA delivery, *Acta Biomater.*, 2022, **154**, 374–384.
113 M. A. Tomeh, *et al.*, Optimization of large-scale manufacturing of biopolymeric and lipid nanoparticles using microfluidic swirl mixers, *Int. J. Pharm.*, 2022, **620**, 121762.
114 R. Xu, *et al.*, Novel microfluidic swirl mixers for scalable formulation of curcumin loaded liposomes for cancer therapy, *Int. J. Pharm.*, 2022, **622**, 121857.
115 P. C. O. S. Firmino, *et al.*, 3D micromixer for nanoliposome synthesis: a promising advance in high mass productivity, *Lab Chip*, 2021, **21**(15), 2971–2985.
116 M. Maeki, *et al.*, Mass production system for RNA-loaded lipid nanoparticles using piling up microfluidic devices, *Appl. Mater. Today*, 2023, **31**, 101754.

117 S. J. Shepherd, *et al.*, Scalable mRNA and siRNA Lipid Nanoparticle Production Using a Parallelized Microfluidic Device, *Nano Lett.*, 2021, **21**(13), 5671–5680.
118 K. Wei, *et al.*, A plug-and-play microfluidic device for hydrogel fiber spinning, *Lab Chip*, 2025, **25**(6), 1575–1585.
119 S. T. Sanjay, *et al.*, A reusable PMMA/paper hybrid plug-and-play microfluidic device for an ultrasensitive immunoassay with a wide dynamic range, *Microsyst. Nanoeng.*, 2020, **6**(1), 28.
120 I. Eş, *et al.*, High-throughput conventional and stealth cationic liposome synthesis using a chaotic advection-based microfluidic device combined with a centrifugal vacuum concentrator, *Chem. Eng. J.*, 2020, **382**, 122821.
121 T. Terada, *et al.*, Characterization of Lipid Nanoparticles Containing Ionizable Cationic Lipids Using Design-of-Experiments Approach, *Langmuir*, 2021, **37**(3), 1120–1128.
122 Z. Liu, *et al.*, Advancements in Programmable Lipid Nanoparticles: Exploring the Four-Domain Model for Targeted Drug Delivery, *arXiv*, 2024, preprint, DOI: 10.48550/arXiv.2408.05695.
123 K. Zoeller, *et al.*, Design of charge converting lipid nanoparticles via a microfluidic coating technique, *Drug Delivery Transl. Res.*, 2024, **14**, 3173–3185.
124 L. Cui, *et al.*, Development of a high-throughput platform for screening lipid nanoparticles for mRNA delivery, *Nanoscale*, 2022, **14**(4), 1480–1491.
125 P. P. G. Guimaraes, *et al.*, Ionizable lipid nanoparticles encapsulating barcoded mRNA for accelerated *in vivo* delivery screening, *J. Controlled Release*, 2019, **316**, 404–417.
126 G. Perli, *et al.*, Ionic strength for tailoring the synthesis of monomodal stealth cationic liposomes in microfluidic devices, *Colloids Surf., B*, 2019, **179**, 233–241.
127 Y. Huang, *et al.*, Microfluidic shear processing control of biological reduction stimuli-responsive polymer nanoparticles for drug delivery, *ACS Biomater. Sci. Eng.*, 2020, **6**(9), 5069–5083.
128 M. K. Alam, Nanocarrier–Based Drug Delivery Systems using Microfluidic–Assisted Techniques, *Adv. NanoBiomed Res.*, 2023, **3**(11), 2300041.
129 J. Ahn, *et al.*, Microfluidics in nanoparticle drug delivery; From synthesis to pre-clinical screening, *Adv. Drug Delivery Rev.*, 2018, **128**, 29–53.
130 J. Majumder and T. Minko, Multifunctional and stimuli-responsive nanocarriers for targeted therapeutic delivery, *Expert Opin. Drug Delivery*, 2021, **18**(2), 205–227.
131 H. Alizadeh, M. Ahmadi and O. Heydari Shayesteh, On chip synthesis of a pH sensitive gefitinib anticancer drug nanocarrier based on chitosan/alginate natural polymers, *Sci. Rep.*, 2024, **14**(1), 772.
132 E. Chiesa, *et al.*, Effect of Micromixer Design on Lipid Nanocarriers Manufacturing for the Delivery of Proteins and Nucleic Acids, *Pharmaceutics*, 2024, **16**(4), 507.
133 E. Jaradat, *et al.*, Synthesis and Characterization of Paclitaxel-Loaded PEGylated Liposomes by the Microfluidics Method, *Mol. Pharm.*, 2023, **20**(12), 6184–6196.
134 S. Khorshid, *et al.*, Microfluidic manufacturing of tioconazole loaded keratin nanocarriers: Development and optimization by design of experiments, *Int. J. Pharm.*, 2023, **647**, 123489.
135 J. Yoo, *et al.*, Active Targeting Strategies Using Biological Ligands for Nanoparticle Drug Delivery Systems, *Cancers*, 2019, **11**(5), 640.
136 L. E. Swart, *et al.*, A robust post-insertion method for the preparation of targeted siRNA LNPs, *Int. J. Pharm.*, 2022, **620**, 121741.
137 N. Khademzadeh, *et al.*, Microfluidic aerosol-assisted synthesis of gefitinib anticancer drug nanocarrier based on chitosan natural polymer, *J. Drug Delivery Sci. Technol.*, 2024, **99**, 105992.
138 H. Shan, *et al.*, Dialysis-functionalized microfluidic platform for *in situ* formation of purified liposomes, *Colloids Surf., B*, 2024, **236**, 113829.
139 A. J. Da Silva Sanchez, *et al.*, Substituting racemic ionizable lipids with stereopure ionizable lipids can increase mRNA delivery, *J. Controlled Release*, 2023, **353**, 270–277.
140 Y. Eygeris, *et al.*, Chemistry of Lipid Nanoparticles for RNA Delivery, *Acc. Chem. Res.*, 2022, **55**(1), 2–12.
141 A.-G. Niculescu, *et al.*, Nanomaterials Synthesis through Microfluidic Methods: An Updated Overview, *Nanomaterials*, 2021, **11**(4), 864.
142 F. Yao, *et al.*, Synthesis of nanoparticles via microfluidic devices and integrated applications, *Mikrochim. Acta*, 2023, **190**(7), 256.
143 Y. Matsuura-Sawada, *et al.*, Controlling lamellarity and physicochemical properties of liposomes prepared using a microfluidic device, *Biomater. Sci.*, 2023, **11**(7), 2419–2426.
144 G. S. Naidu, *et al.*, A Combinatorial Library of Lipid Nanoparticles for Cell Type-Specific mRNA Delivery, *Adv. Sci.*, 2023, **10**(19), e2301929.
145 J. Di, *et al.*, Biodistribution and Non-linear Gene Expression of mRNA LNPs Affected by Delivery Route and Particle Size, *Pharm. Res.*, 2022, **39**(1), 105–114.
146 M. J. W. Evers, *et al.*, Delivery of modified mRNA to damaged myocardium by systemic administration of lipid nanoparticles, *J. Controlled Release*, 2022, **343**, 207–216.
147 K. Okuda, *et al.*, On the size-regulation of RNA-loaded lipid nanoparticles synthesized by microfluidic device, *J. Controlled Release*, 2022, **348**, 648–659.
148 E. Jaradat, *et al.*, Microfluidic paclitaxel-loaded lipid nanoparticle formulations for chemotherapy, *Int. J. Pharm.*, 2022, **628**, 122320.
149 A. Sarode, *et al.*, Predictive high-throughput screening of PEGylated lipids in oligonucleotide-loaded lipid nanoparticles for neuronal gene silencing, *Nanoscale Adv.*, 2022, **4**(9), 2107–2123.
150 N. Aliakbarinodehi, *et al.*, Interaction Kinetics of Individual mRNA-Containing Lipid Nanoparticles with an Endosomal Membrane Mimic: Dependence on pH, Protein Corona Formation, and Lipoprotein Depletion, *ACS Nano*, 2022, **16**(12), 20163–20173.

151 Y. Jin, *et al.*, Microfluidic fabrication of photo-responsive Ansamitocin P-3 loaded liposomes for the treatment of breast cancer, *Nanoscale*, 2023, **15**(8), 3780–3795.
152 R. El-Mayta, *et al.*, Testing the *In Vitro* and *In Vivo* Efficiency of mRNA-Lipid Nanoparticles Formulated by Microfluidic Mixing, *J. Visualized Exp.*, 2023, (191), e64810, DOI: 10.3791/64810.
153 C.-S. Lee, Grand Challenges in Microfluidics: A Call for Biological and Engineering Action, *Front. Sens.*, 2020, **1**, 583035.
154 P. Pattanayak, *et al.*, Microfluidic chips: recent advances, critical strategies in design, applications and future perspectives, *Microfluid. Nanofluid.*, 2021, **25**(12), 99.
155 G. Liu, *et al.*, Design automation for continuous-flow microfluidic biochips: A comprehensive review, *Integration*, 2022, **82**, 48–66.
156 G. Wang, F. Yang and W. Zhao, There can be turbulence in microfluidics at low Reynolds number, *Lab Chip*, 2014, **14**(8), 1452–1458.
157 H. Bruus, *Theoretical microfluidics*, Oxford university press, 2007, vol. 18.
158 Y. Song, *et al.*, *Fundamental concepts and physics in microfluidics*, in *Microfluidics: Fundamental, Devices and Applications*, 2018. DOI: 10.1002/9783527800643.ch2.
159 S. Y. Kashani, *et al.*, Microfluidics for core–shell drug carrier particles–a review, *RSC Adv.*, 2021, **11**(1), 229–249.
160 E. Chiesa, *et al.*, Effect of micromixer design on lipid nanocarriers manufacturing for the delivery of proteins and nucleic acids, *Pharmaceutics*, 2024, **16**(4), 507.
161 G. Buttitta, *et al.*, Scalable microfluidic method for tunable liposomal production by a design of experiment approach, *Int. J. Pharm.*, 2024, **662**, 124460.
162 S. N. Turkmen Koc, *et al.*, Production of 5-fluorouracil-loaded PLGA nanoparticles with toroidal microfluidic system and optimization of process variables by design of experiments, *Int. J. Pharm.*, 2024, **662**, 124501.
163 H. Jiang, *et al.*, A Modular and Cost-Effective Droplet Microfluidic Device for Controlled Emulsion Production, *Polymers*, 2024, **16**(6), 765.
164 T. Terada, *et al.*, Microfluidic Preparation of Nanoparticles Using Poly(ethylene Glycol)-distearoylphosphatidylethanolamine for Solubilizing Poorly Soluble Drugs, *J. Pharm. Sci.*, 2022, **111**(6), 1709–1718.
165 G. Yenduri, *et al.*, Impact of critical process parameters and critical material attributes on the critical quality attributes of liposomal formulations prepared using continuous processing, *Int. J. Pharm.*, 2022, **619**, 121700.
166 C. Y. Lee, *et al.*, Formulation Development of Doxycycline-Loaded Lipid Nanocarriers using Microfluidics by QbD Approach, *J. Pharm. Sci.*, 2023, **112**(3), 740–750.
167 M. Yew, *et al.*, A Review of State-of-the-Art Microfluidic Technologies for Environmental Applications: Detection and Remediation, *Global Challenges*, 2019, **3**(1), 1800060.
168 M. Kumar Thimmaraju, *et al.*, Microfluidic revolution and its impact on pharmaceutical materials: A review, *Mater. Today: Proc.*, 2023, DOI: 10.1016/j.matpr.2023.03.096.
169 A. De and Y. T. Ko, Single pot organic solvent-free thermocycling technology for siRNA-ionizable LNPs: a proof-of-concept approach for alternative to microfluidics, *Drug Delivery*, 2022, **29**(1), 2644–2657.
170 A. Pratsinis, *et al.*, Impact of non-ionizable lipids and phase mixing methods on structural properties of lipid nanoparticle formulations, *Int. J. Pharm.*, 2023, **637**, 122874.
171 I. U. Khan, *et al.*, Microfluidics: a focus on improved cancer targeted drug delivery systems, *J. Controlled Release*, 2013, **172**(3), 1065–1074.
172 S. T. Sanjay, *et al.*, Recent advances of controlled drug delivery using microfluidic platforms, *Adv. Drug Delivery Rev.*, 2018, **128**, 3–28.
173 A. Enders, A. Grünberger and J. Bahnemann, Towards Small Scale: Overview and Applications of Microfluidics in Biotechnology, *Mol. Biotechnol.*, 2024, **66**(3), 365–377.
174 N. K. Khosla, *et al.*, Simplifying the complex: accessible microfluidic solutions for contemporary processes within *in vitro* diagnostics, *Lab Chip*, 2022, **22**(18), 3340–3360.
175 Y. Jin, *et al.*, A microfluidic approach for rapid and continuous synthesis of glycoprotein-imprinted nanospheres, *Talanta*, 2022, **239**, 123084.
176 M. Maeki, *et al.*, Development of Polymer–Lipid Hybrid Nanoparticles for Large-Sized Plasmid DNA Transfection, *ACS Appl. Mater. Interfaces*, 2024, **16**(2), 2110–2119.
177 V. Passos Gibson, *et al.*, Lipid Coating of Chitosan Nanogels for Improved Colloidal Stability and *In Vitro* Biocompatibility, *AAPS PharmSciTech*, 2021, **22**(5), 159.
178 Y. Mo, *et al.*, Light-Activated siRNA Endosomal Release (LASER) by Porphyrin Lipid Nanoparticles, *ACS Nano*, 2023, **17**(5), 4688–4703.
179 B. G. Carvalho, *et al.*, Hybrid polymer/lipid vesicle synthesis: Association between cationic liposomes and lipoplexes with chondroitin sulfate, *Colloids Surf., B*, 2022, **210**, 112233.
180 L. Digiacomo, *et al.*, Cationic lipid/DNA complexes manufactured by microfluidics and bulk self-assembly exhibit different transfection behavior, *Biochem. Biophys. Res. Commun.*, 2018, **503**(2), 508–512.
181 A.-G. Reinhart, *et al.*, Investigations into mRNA Lipid Nanoparticles Shelf-Life Stability under Nonfrozen Conditions, *Mol. Pharm.*, 2023, **20**(12), 6492–6503.
182 A. Agha, *et al.*, A review on microfluidic-assisted nanoparticle synthesis, and their applications using multiscale simulation methods, *Discover Nano*, 2023, **18**(1), 18.
183 M. B. Kulkarni and S. J. N. E. Goel, Microfluidic devices for synthesizing nanomaterials—a review, *Nano Express*, 2020, **1**(3), 032004.
184 S. Mehraji and D. L. DeVoe, Microfluidic synthesis of lipid-based nanoparticles for drug delivery: recent advances and opportunities, *Lab Chip*, 2024, **24**(5), 1154–1174.
185 A.-G. Niculescu, *et al.*, Nanomaterials synthesis through microfluidic methods: An updated overview, *Lab Chip*, 2021, **11**(4), 864.

186 M. Tiboni, *et al.*, Microfluidics for nanomedicines manufacturing: An affordable and low-cost 3D printing approach, *Int. J. Pharm.*, 2021, **599**, 120464.
187 E. Weaver, *et al.*, The manufacturing of 3D-printed microfluidic chips to analyse the effect upon particle size during the synthesis of lipid nanoparticles, *J. Pharm. Pharmacol.*, 2023, **75**(2), 245–252.
188 G. Lou, *et al.*, A novel microfluidic-based approach to formulate size-tuneable large unilamellar cationic liposomes: Formulation, cellular uptake and biodistribution investigations, *Eur. J. Pharm. Biopharm.*, 2019, **143**, 51–60.
189 J. Zhou, *et al.*, High-throughput microfluidic systems accelerated by artificial intelligence for biomedical applications, *Lab Chip*, 2024, **24**(5), 1307–1326.
190 Q. Jiang, *et al.*, Applications of artificial intelligence-driven microfluidics in medical laboratory science, *Interdiscip. Med.*, 2025, e20240135.
191 R. Rebollo, *et al.*, Microfluidic Manufacturing of Liposomes: Development and Optimization by Design of Experiment and Machine Learning, *ACS Appl. Mater. Interfaces*, 2022, **14**(35), 39736–39745.
192 D. McIntyre, *et al.*, Machine learning for microfluidic design and control, *Lab Chip*, 2022, **22**(16), 2925–2937.
193 R. Eugster, *et al.*, Leveraging machine learning to streamline the development of liposomal drug delivery systems, *J. Controlled Release*, 2024, **376**, 1025–1038.
194 Z. Liu, *et al.*, Microfluidic biosensors for biomarker detection in body fluids: a key approach for early cancer diagnosis, *Biomark. Res.*, 2024, **12**(1), 153.
195 L. Liu, *et al.*, Artificial intelligence-powered microfluidics for nanomedicine and materials synthesis, *Nanoscale*, 2021, **13**(46), 19352–19366.
196 J. Park, *et al.*, Closed-loop optimization of nanoparticle synthesis enabled by robotics and machine learning, *Matter*, 2023, **6**(3), 677–690.
197 D. McIntyre, *et al.*, Machine learning for microfluidic design and control, *Lab Chip*, 2022, **22**(16), 2925–2937.
198 A. Mottafegh, *et al.*, AI-assisted autonomous manufacturing of tailored drug-loaded nanoparticles by multi-step continuous-flow platform, *Chem. Eng. J.*, 2024, **500**, 157454.
199 R. Rebollo, *et al.*, Microfluidic manufacturing of liposomes: development and optimization by design of experiment and machine learning, *ACS Appl. Mater. Interfaces*, 2022, **14**(35), 39736–39745.
200 R. Maharjan, *et al.*, Comparative study of lipid nanoparticle-based mRNA vaccine bioprocess with machine learning and combinatorial artificial neural network-design of experiment approach, *Int. J. Pharm.*, 2023, **640**, 123012.
201 S. A. Damiati, *et al.*, Artificial intelligence application for rapid fabrication of size-tunable PLGA microparticles in microfluidics, *Sci. Rep.*, 2020, **10**(1), 19517.
202 S. A. Damiati and S. Damiati, Microfluidic synthesis of indomethacin-loaded plga microparticles optimized by machine learning, *Front. Mol. Biosci.*, 2021, **8**, 677547.
203 I. Ocampo, *et al.*, Comparative Evaluation of Artificial Neural Networks and Data Analysis in Predicting Liposome Size in a Periodic Disturbance Micromixer, *Micromachines*, 2021, **12**(10), 1164.
204 E. Kastner, *et al.*, Microfluidic-controlled manufacture of liposomes for the solubilisation of a poorly water soluble drug, *Int. J. Pharm.*, 2015, **485**(1), 122–130.
205 H. Tanaka, *et al.*, Ready-to-use-type lyophilized lipid nanoparticle formulation for the postencapsulation of messenger RNA, *ACS Nano*, 2023, **17**(3), 2588–2601.
206 S. Sansare, *et al.*, Artificial neural networks in tandem with molecular descriptors as predictive tools for continuous liposome manufacturing, *Int. J. Pharm.*, 2021, **603**, 120713.
207 R. T. Aggarwal, L. Lai and H. J. A. B. Li, Microarray fabrication techniques for multiplexed bioassay applications, *Anal. Biochem.*, 2023, **683**, 115369.
208 Y. Sugimoto, *et al.*, Microfluidic Post-Insertion Method for the Efficient Preparation of PEGylated Liposomes Using High Functionality and Quality Lipids, *Int. J. Nanomed.*, 2022, **17**, 6675–6686.
209 F. Yao, *et al.*, Synthesis of nanoparticles via microfluidic devices and integrated applications, *Microchim. Acta*, 2023, **190**(7), 256.
210 Y. Li, *et al.*, In silico design and 3D printing of microfluidic chips for the preparation of size-controllable siRNA nanocomplexes, *Int. J. Pharm.*, 2020, **583**, 119388.
211 V. Carvalho, *et al.*, Computational simulations in advanced microfluidic devices: A review, *Micromachines*, 2021, **12**(10), 1149.
212 J. Zhou, *et al.*, High-throughput microfluidic systems accelerated by artificial intelligence for biomedical applications, *Lab Chip*, 2024, **24**(5), 1307–1326.
213 S. Joshi, *et al.*, Microfluidics based manufacture of liposomes simultaneously entrapping hydrophilic and lipophilic drugs, *Int. J. Pharm.*, 2016, **514**(1), 160–168.
214 S. Pereira, *et al.*, Docetaxel-loaded liposomes: The effect of lipid composition and purification on drug encapsulation and *in vitro* toxicity, *Int. J. Pharm.*, 2016, **514**(1), 150–159.
215 M. Guimarães Sá Correia, *et al.*, Microfluidic manufacturing of phospholipid nanoparticles: Stability, encapsulation efficacy, and drug release, *Int. J. Pharm.*, 2017, **516**(1), 91–99.
216 N. Kimura, *et al.*, Development of the iLiNP Device: Fine Tuning the Lipid Nanoparticle Size within 10 nm for Drug Delivery, *ACS Omega*, 2018, **3**(5), 5044–5051.
217 A. Gdowski, *et al.*, Optimization and scale up of microfluidic nanolipomer production method for preclinical and potential clinical trials, *J. Nanobiotechnol.*, 2018, **16**(1), 12.
218 N. H. A. Le, *et al.*, Acoustically enhanced microfluidic mixer to synthesize highly uniform nanodrugs without the addition of stabilizers, *Int. J. Nanomed.*, 2018, **13**, 1353–1359.
219 N. Forbes, *et al.*, Rapid and scale-independent microfluidic manufacture of liposomes entrapping protein incorporating in-line purification and at-line size monitoring, *Int. J. Pharm.*, 2019, **556**, 68–81.

220 C. C. L. Cheung and W. T. Al-Jamal, Sterically stabilized liposomes production using staggered herringbone micromixer: Effect of lipid composition and PEG-lipid content, *Int. J. Pharm.*, 2019, **566**, 687–696.
221 J. Zhou, *et al.*, Microfluidic preparation of PLGA composite microspheres with mesoporous silica nanoparticles for finely manipulated drug release, *Int. J. Pharm.*, 2021, **593**, 120173.
222 N. Kimura, *et al.*, Development of a Microfluidic-Based Post-Treatment Process for Size-Controlled Lipid Nanoparticles and Application to siRNA Delivery, *ACS Appl. Mater. Interfaces*, 2020, **12**(30), 34011–34020.
223 C. B. Roces, *et al.*, Rapid scale-up and production of active-loaded PEGylated liposomes, *Int. J. Pharm.*, 2020, **586**, 119566.
224 C. Webb, *et al.*, Using microfluidics for scalable manufacturing of nanomedicines from bench to GMP: A case study using protein-loaded liposomes, *Int. J. Pharm.*, 2020, **582**, 119266.
225 L. Gkionis, *et al.*, Manufacturing drug co-loaded liposomal formulations targeting breast cancer: Influence of preparative method on liposomes characteristics and *in vitro* toxicity, *Int. J. Pharm.*, 2020, **590**, 119926.
226 M. P. Lokugamage, *et al.*, Optimization of lipid nanoparticles for the delivery of nebulized therapeutic mRNA to the lungs, *Nat. Biomed. Eng.*, 2021, **5**(9), 1059–1068.
227 N. Jyotsana, *et al.*, Lipid nanoparticle-mediated siRNA delivery for safe targeting of human CML *in vivo*, *Ann. Hematol.*, 2019, **98**(8), 1905–1918.
228 R. Zhang, *et al.*, Helper lipid structure influences protein adsorption and delivery of lipid nanoparticles to spleen and liver, *Biomater. Sci.*, 2021, **9**(4), 1449–1463.
229 L. Gkionis, *et al.*, Microfluidic-assisted fabrication of phosphatidylcholine-based liposomes for controlled drug delivery of chemotherapeutics, *Int. J. Pharm.*, 2021, **604**, 120711.
230 G. Ballacchino, Manufacturing of 3D-Printed Microfluidic Devices for the Synthesis of Drug-Loaded Liposomal Formulations, *Int. J. Mol. Sci*, 2021, **22**(15), 8064.