Article published online: 2024-05-23

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Review Article

Research Strategies for Precise Manipulation of Micro/Nanoparticle Drug Delivery Systems Using Microfluidic Technology: A Review

Jie Liu[1] Qinghui Fu[1] Qin Li[1] Yani Yang[1] Yue Zhang[1] Kaili Yang[1] Guohao Sun[1] Jiayu Luo[1] Weigen Lu[1]* Jun He[1]*

[1] National Advanced Medical Engineering Research Center, China State Institute of Pharmaceutical Industry, Shanghai, People's Republic of China

Pharmaceut Fronts

Address for correspondence Weigen Lu, PhD, National Advanced Medical Engineering Research Center, China State Institute of Pharmaceutical Industry, 285 Gebaini Road, Shanghai 201203, People's Republic of China (e-mail: sipiluwg@163.com).

Jun He, PhD, National Advanced Medical Engineering Research Center, China State Institute of Pharmaceutical Industry, 285 Gebaini Road, Shanghai 201203, People's Republic of China (e-mail: chinaynhe@163.com).

Abstract

Microfluidic technology facilitates precise control over fluid mixing and interactions between the components, including self-assembly and precipitation. It offers new options for accurately manufacturing particles and holds significant potential in advancing micro/nanoparticle drug delivery systems (DDSs). Various microchannel/microfluidic chips have been explored to construct micro/nanoparticle DDSs. The precise manipulation of particle size, morphology, structure, stiffness, surface characteristics, and elasticity through microfluidic technology relies on specific microchannel geometrical designs and the application of exogenous energy, adhering to the principles of fluid motion. Consequently, this enables reproducible control over critical quality attributes (CQAs), such as particle size and distribution, encapsulation efficiency, drug loading, *in vitro* and *in vivo* drug delivery profiles, Zeta potential, and targeting capabilities, for micro/nanoparticle DDSs. In this review, we categorize microfluidic techniques and explore recent research developments in novel microchannel structures spanning the past 5 years (2018–2023) and their applications in micro/nanoparticle DDSs. Additionally, we elucidate the latest manipulation strategies of microfluidic techniques that impact foundational structures related to the CQAs of micro/nanoparticle DDSs. Furthermore, we offer insights into the industrial applications and challenges microfluidic techniques face in the context of novel micro/nanoparticle DDSs.

Keywords

- microfluidic technology
- micro/nanoparticle drug delivery systems
- precision manufacturing
- industrialization

Introduction

Microfluidic technology involves precise fluid flow control in microchannels characterized by diverse geometries and sizes ranging from micrometers to millimeters. This technology holds significant potential across various domains, including drug screening,[1] drug discovery,[2] drug delivery,[3–9] biology sciences,[10] drug analysis,[11,12] and preclinical evaluation.[13,14] Notably, the application of microfluidics in drug delivery systems (DDSs), such as lipid nanoparticles (LNPs), has witnessed rapid advancement during the coronavirus disease 2019 (COVID-19) pandemic.

received
September 10, 2023
accepted
April 2, 2024

DOI https://doi.org/10.1055/s-0044-1786180.
ISSN 2628-5088.

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany

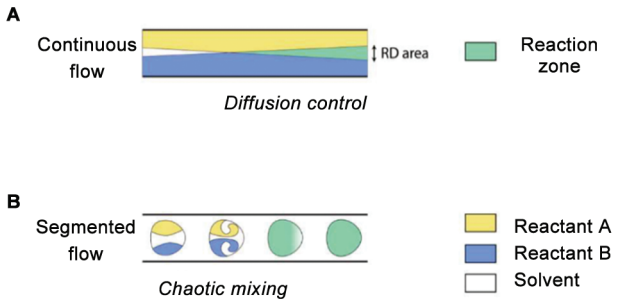

Fig. 1 Comparison of the mixing process of reactants in devices with (**A**) continuous and (**B**) segmented flow. The image is reproduced with permission from Ref. [29], copyright 2018 MDPI.

The physicochemical properties of micro/nanoparticles primarily depend on their foundational structures, encompassing morphology, size, structure, surface characteristics, stiffness, and elasticity. These attributes play a crucial role in determining the CQAs of micro/nanoparticles, which comprise particle size, polydispersity index (PDI), encapsulation performance, drug loading (DL), zeta potential, release behaviors, targeting capabilities, and stability. An ideal micro/nanoparticle DDS should demonstrate high loading capacity, a controllable preparation process, a narrow particle size distribution, controllable internal structure, surface characteristics, minimal batch-to-batch variation,[15] and, whenever possible, environmentally friendly and sustainable qualities.

Microfluidic technology, with its capacity for achieving uniform and rapid fluid mixing, establishes stable and optimal environments for the self-assembly or nucleation of micro/nanoparticles.[16–18] Furthermore, the conditions and mixing order of the reaction become conveniently and flexibly adjustable with the application of microfluidic technology.[19] Consequently, microfluidic technology demonstrates outstanding controllability and multifunctionality, enabling the stable and precise control of CQAs by manipulating particle size, internal structure, morphology, stiffness, surface characteristics, and elasticity.[19–24] Furthermore, the preparation of micro/nanoparticles through microfluidics presents numerous advantages, including a straightforward process, minimal reagent consumption, environmental friendliness, and a requirement for minimal workspace for the equipment.[25]

As scientists explore the intricacies of fluidic behavior within microchannels, their understanding expands, leading to increased utilization of the diverse modes of action of microchannels with distinct architectures for specific micro/nanoparticle DDSs (e.g., liposomes, LNPs, microspheres, nanocrystals, etc.).

Microfluidics can be categorized into two types based on the flow mechanisms of fluids: continuous-flow systems and droplet-based segmented-flow systems.

Diffusive mixing, the foundation of continuous-flow systems, primarily relies on the laminar diffusion of one or more solvents. In this process, molecules transition from areas of higher concentration to lower concentration via Brownian motion, resulting in progressive homogenization (►**Fig. 1A**).

The droplet-based segmented-flow system relies on mixing two immiscible phases, specifically the dispersed and continuous phases. In this system, the immiscible continuous phase separates the dispersed phase into multiple microdroplets, each serving as an individual mixing unit (►**Fig. 1B**).[26] Through the application of microfluidic technology, it becomes possible to engineer droplets of diverse sizes and morphologies, including single-emulsion droplets, double-emulsion droplets, multi-emulsion droplets, and multiple cores' droplets. This engineering capability facilitates the creation of particles with varied internal structures and encapsulation patterns (►**Fig. 2**). Because the droplet reactor remains unexposed to the microchannel's inner surface, the system prevents material from settling on the inner wall. This characteristic renders the droplet-based segmented-flow system resistant to contamination and compatible with extreme conditions, such as high temperatures or corrosive reagents. Consequently, this system allows for the preparation of micro/nanoparticles from a wide range of materials.[27]

►**Fig. 3** shows schematic diagrams illustrating liquid flow patterns in typical microchannels with continuous and segmented systems, respectively.[28,29]

Microfluidics can be further categorized into passive and active mixing systems, depending on the requirement for introducing external driving forces and the driving mode.[30]

Passive mixing systems rely solely on the energy of liquid flow to drive, guide, and facilitate mixing and separation.[31] In contrast, active mixing systems utilize external forces, such as acoustic,[31–39] electric,[40–42] magnetic,[43,44] thermal,[45] and optical fields,[46] to disrupt the laminar flow. This disruption induces rapid homogenization to enhance mixing performance or prompts specific substances to aggregate or precipitate within a designated time frame. The objective is to achieve faster homogenization, thereby improving mixing performance and generating particles with a smaller size and a

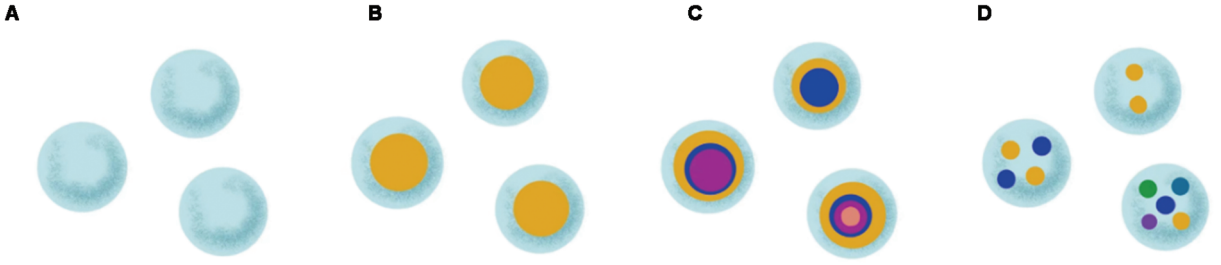

Fig. 2 The morphologies of droplets encompass (**A**) single emulsion droplets, (**B**) double emulsion droplets, (**C**) multiple emulsion droplets, and (**D**) droplets with multiple cores.

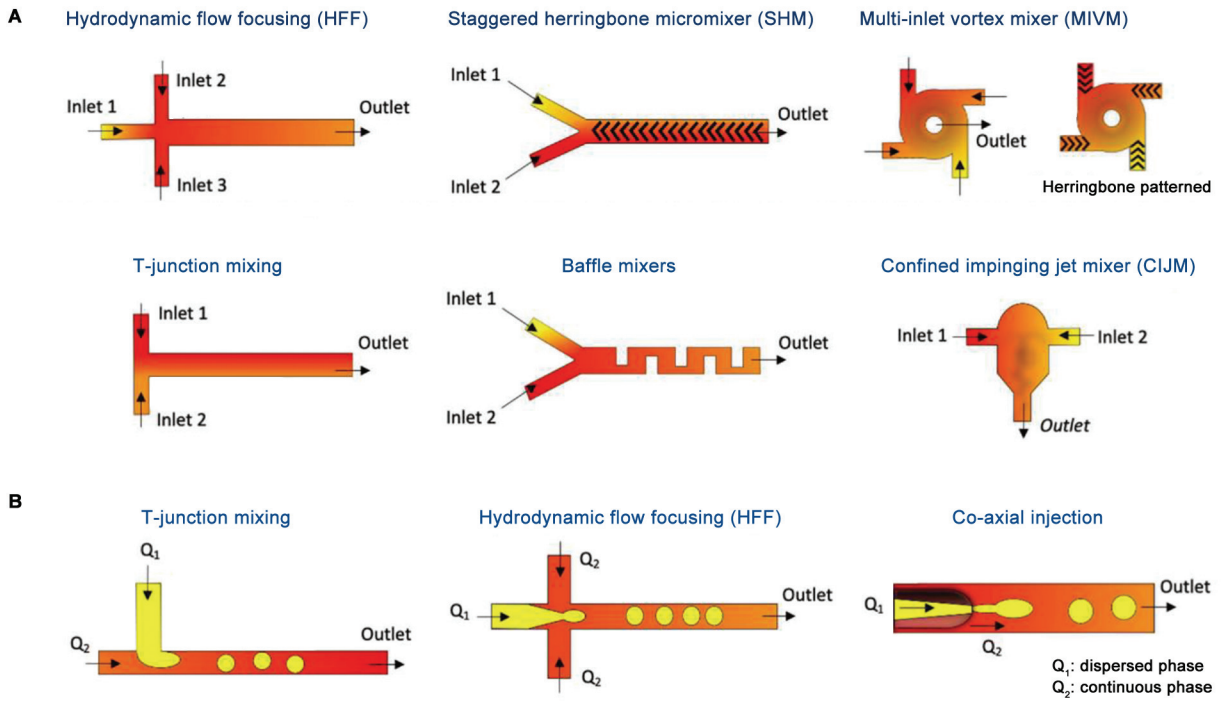

Fig. 3 Schematic diagram of liquid flow patterns in typical microchannels of (**A**) continuous- and (**B**) segmented-flow systems. The image is reproduced with permission from Ref. [28], copyright 2022 MDPI.

narrower distribution.[31] When applied in the construction of micro/nanoparticle DDSs, passive and active mixing systems exhibit distinct characteristics, as detailed in ►**Table 1**–[47–50] below. The combination and integration of microfluidics with external forces present the possibility of preparing high-quality, versatile micro/nanoparticles with complex structures. An example of the application of an active mixing system is illustrated in ►**Fig. 4**.[51,52]

Currently, abundant evidence from published research papers attests to the widespread use of microfluidics in constructing micro/nanoparticle DDSs. Leveraging specialized microchannel configurations, microfluidic methodologies are systematically displacing conventional approaches in the fabrication of micro/nanoparticles.

A comprehensive literature search was performed to analyze the potential applications of microfluidics in producing micro/nanoparticle DDSs. PubMed and Web of Science databases were searched, utilizing a combination of keywords to optimize search outcomes. The search criteria encompassed terms such as microfluidic or micromixer or mixing or microchannel or microfluidics or chip (title) and drug OR pharmaceutical OR medical OR drug delivery OR pharmaceutics OR medicine (topic) and particle or particles (topic), not Machine OR micromachine OR engineer OR engineering OR screen OR sensor OR analysis OR separation (topic) and Patent (exclude—document types). The literature search was restricted to articles published between 2018 and 2023.

This review outlines the various types of microchannel structures and their design philosophies utilized in constructing high-quality micro/nanoparticle DDSs from 2018 to 2023. Additionally, we summarize the most recent manipulation strategies employed by microfluidic techniques to influence the foundational constructive features (size, morphology and structure, stiffness, surface characteristics, and elasticity) of micro/nanoparticle DDSs. Finally, we present an outlook on potential industrial applications and the challenges associated with microfluidic techniques in developing innovative micro/nanoparticle DDSs.

Microchannel Structures Employed for Micro/Nanoparticle DDSs

The widespread utilization of microfluidic techniques in micro/nanoparticle DDSs currently encounters three challenges that require attention:

- Traditional microchannel configurations, such as T-shaped/Y-shaped structures, require prolonged mixing times or paths for complete homogenization, impeding efficient micro/nanoparticle fabrication. Therefore, microstructures must be designed to enhance mixing efficacy and reduce mixing durations.
- Some microchannel structures with intricate configurations, like the staggered herringbone mixer (SHM), display superior mixing owing to their internal microstructures. However, these configurations often introduce significant resistance, hindering the high-throughput production of micro/nanoparticles.
- Owing to the relatively enclosed nature of microchannels, integrating microfluidic chips with upstream and downstream equipment in the production process for continuous manufacturing, online monitoring, and intelligent feedback for sustainable product lifecycle management poses a challenge. This integration is also a hurdle in constructing high-quality, intelligent micro/nanoparticle DDSs.

Table 1 Characteristics of passive and active mixing systems in preparing micro/nanoparticle DDSs[31]

Mixing systems	Characteristics
Passive mixing systems	• Simple and low cost • The performance of the system depends on its geometry; its mixing efficiency can usually only be controlled by adjusting the TFR or FRR • Typical parabolic velocity profile causes the residence times of the fluids undergoing mixing to be unequal, which probably leads to a wider particle size distribution • Passive mixing is usually achieved by applying abrupt changes in the geometry of the channel (e.g., sharp turns), which may lead to fouling and clogging of the channels
Active mixing systems	• Facilitates the generation of particles with smaller sizes and narrower distributions and simplifies the preparation of multifunctional micro/nanoparticles with complex structures and special qualities • Acoustic fields increase the nucleation rate and significantly reduce the average size and polydispersity of the nanoparticles produced compared with the conventional preparation methods[34–36,38,47] • Electric fields facilitate the loading of substances[48] and promote the preparation of multilayered nanoparticles[40] • Magnetic fields trap and release magnetic precursors or nanoparticles on demand at designated locations, allowing for controlled measures[43,44] • Application of thermal fields enables precise manipulation of monodisperse nanoparticles[45,49,50] • Microfluidic systems integrated with light fields achieve photoinduced polymerization and efficiently mediate particle synthesis[46]

Abbreviations: DDSs, drug delivery systems; FRR, flow rate ratio; TFR, total flow rate.

In response to these limitations, pharmaceutical scientists, leveraging various fluidic principles, have constructed various micro/nanoparticle DDSs using microchannels based on different designs. Examples include the T-type/Y-type mixer, hydrodynamic flow focusing mixer (HFF mixer), SHM, invasive LNP production (iLiNP) device, capillary-based coaxial-flow mixer (CFM), confined impingement jet mixer (CIJM), multi-inlet vortex mixer (MIVM), and others. The following sections will introduce commonly used microchannel structures in the drug delivery domain.

Microchannel for Enhancing Mixing

T-Type/Y-Type Mixer

T-type/Y-type mixers, characterized by laminar flow patterns, represent the simplest laminar microchannel configurations. The utilization of T-type/Y-type mixers successfully addresses the heterogeneity inherent in traditional processes. Nevertheless, these designs require extended channels for achieving thorough mixing, leading to larger particle sizes attributable to slow diffusion rates. Consequently, these configurations are progressively being replaced by more sophisticated microchannel structures.

Hydrodynamic Flow Focusing Mixer

Expanding upon the single-layer laminar flow patterns of T-type/Y-type mixers, researchers have advanced the field by developing a multi-layer laminar flow HFF mixer. This innovation involves adjusting the number of channel inlets and introducing three-dimensional (3D) geometric features.

Studies indicate that, compared with traditional T-type/Y-type mixers, the 2D HFF mixer improves the diffusion interface between the two-phase fluids, leading to enhanced mixing efficacy.[53] Siavashy et al prepared cisplatin-loaded magnetic chitosan nanoparticles (NPs) using traditional batch methods and microfluidic techniques based on the HFF mixer. The results demonstrated that NPs fabricated via the HFF mixer exhibited smaller sizes with a narrower distribution than those produced by traditional methods. Furthermore, the microfluidic approach achieved higher encapsulation efficiency and controllable drug release.[53]

Furthermore, the 3D-HFF promotes interfacial contact between different phase fluids within a 3D space, thereby expanding the diffusion area of the fluids. This leads to a further enhancement in mixing efficiency compared with the two-dimensional (2D) HFF. ►**Fig. 5**–[54] provides visual examples of various 2D and 3D HFF microchannels utilized to fabricate NPs.

Split-and-Recombine Mixer

The split-and-recombine (SAR) mixer denotes a process where the fluid undergoes continuous splitting and recombination during flow, thereby increasing diffusion opportunities and enhancing mixing efficacy. SAR mixers are typically designed in three dimensions, encompassing symmetrical and asymmetrical configurations. Symmetrical SAR mixers employed in micro/nanoparticle DDSs include the Tesla structure,[55] fluidic trap mixer,[56] and toroidal mixer (TrM; developed by Precision Nano Systems Inc.).[57] Ma et al have developed a 3D-inertial microfluidic mixer, serving as a SAR mixer. This mixer facilitates the scalable synthesis of LNPs, and concurrently, the authors have proposed a theoretical prediction method to ensure equal mixing times across chips of different sizes, further guaranteeing consistency in the particle size and PDI of LNPs (►**Fig. 6**).[58] ►**Fig. 7** provides a schematic illustration of a typical SAR mixer.

The TrM structure developed by Precision Nano Systems Inc. (PNI) is a commercially available SAR mixer. Webb et al discovered that liposomes prepared using both the TrM and SHM mixers exhibited no significant differences in size and shape.[57]

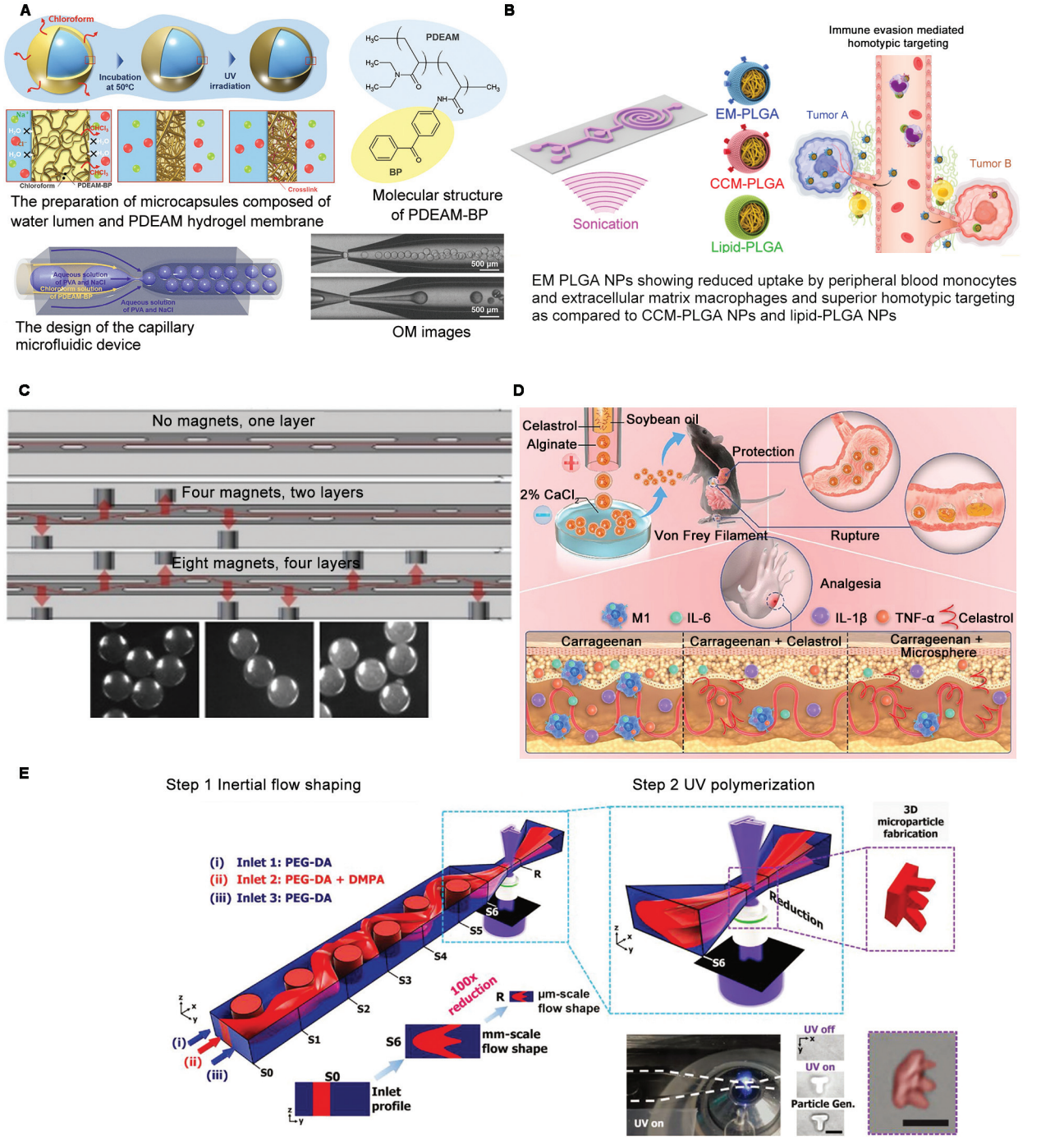

Fig. 4 Examples of applications of active mixing systems. (**A**) A thermal field: the microfluidic-thermal responsive technology is used to prepare temperature-sensitive microcapsules with adjustable permeability. The image is reproduced with permission from Ref. [45], copyright 2021 Wiley. (**B**) An acoustic field: natural membrane-encapsulated bio-nanoparticles are prepared in one step using the microfluidics-ultrasonic technology. The image is reproduced with permission from Ref. [32], copyright 2019 American Chemical Society. (**C**) A magnetic field: the microfluidic-magnetic responsive technology could reduce coating time and achieve successive layers of adsorption in a single step without experiencing disturbing diffusion and mixing of chemicals. The image is reproduced with permission from Ref. [51], copyright 2023 Elsevier. (**D**) An electric field: the microfluidic electrospray is used to prepare alginate microspheres encapsulated celastrol. The image is reproduced with permission from Ref. [52], copyright 2021 Elsevier. (**E**) An optical field: photo-fluidics based on microfluidic-ultraviolet irradiation-induced polymerization is used to achieve customized production of particles with complex 3D shapes. The image is reproduced with permission from Ref. [46], copyright 2018 Wiley.

Obstacle-Based Mixer

SHM Based on Embedded Obstacle Design

In 2002, Stroock first introduced the concept of SHM with embedded obstacles on the inner walls of the channel.[59] The repetitive herringbone-staggered structures induce fluid folding, resulting in chaotic laminar flow. This chaotic flow significantly enhances the mixing efficiency compared with traditional free diffusion. Subsequently, scientists have increasingly integrated SHM in constructing various NP

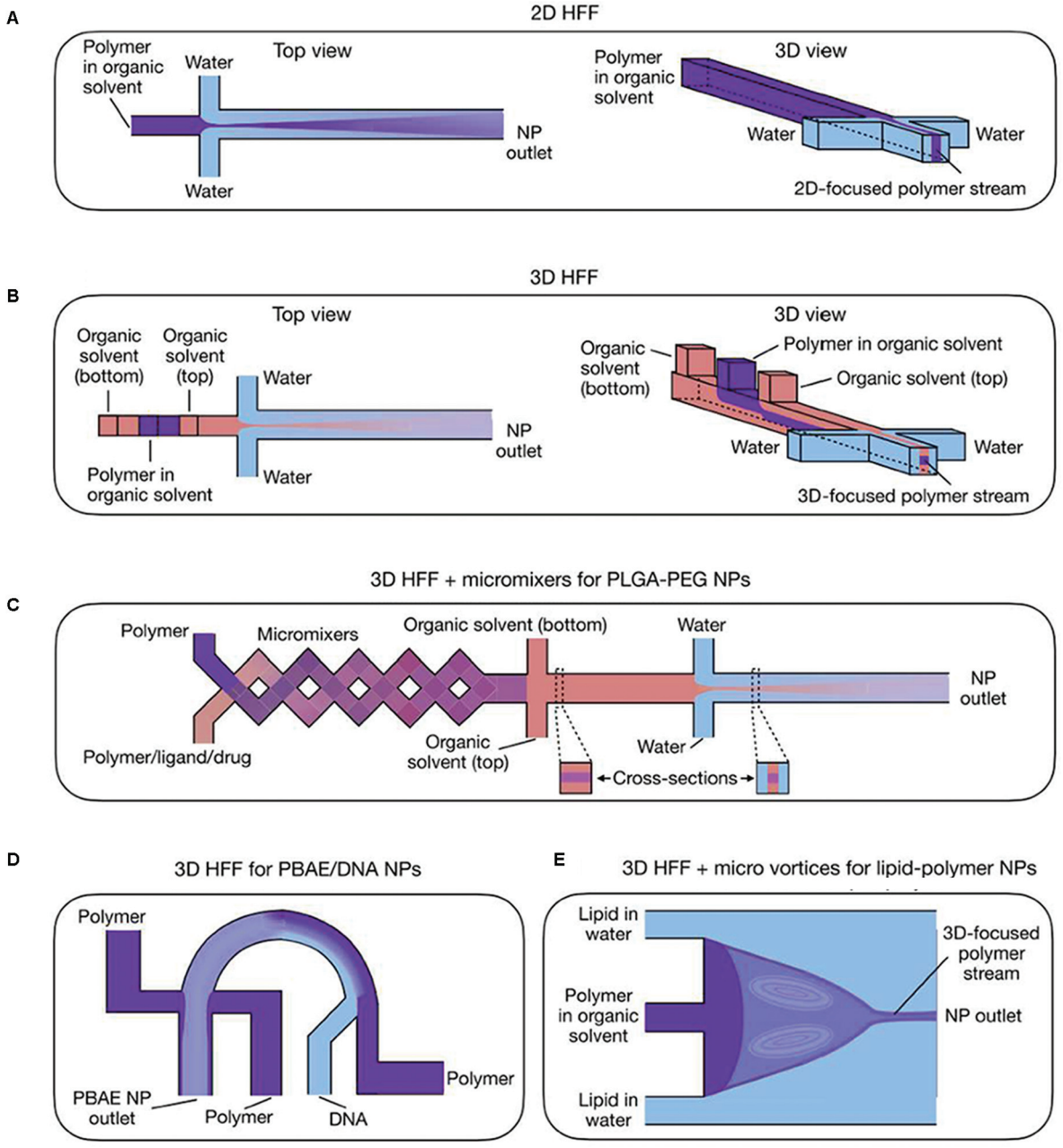

Fig. 5 (**A–E**) Various 2D and 3D HFF microchannels employed for the fabrication of nanoparticles. The image is reproduced with permission from Ref. [54], copyright 2021 Elsevier Science Ltd. HFF, hydrodynamic flow focusing.

DDSs.[22,60–67] It has found applications in producing liposomes,[18,68–70] polymer NPs,[71] and related nanomedicines.[72–74]

Researchers employed conventional methods and SHM-based microfluidic techniques to prepare PLGA (poly(lactic-co-glycolic acid)) NPs loaded with rutin. The results demonstrated that NPs fabricated using the microfluidic approach exhibited smaller and more uniform diameters, as well as superior encapsulation and release behaviors.[75]

Furthermore, researchers have developed the reverse-SHM, and fluid dynamics simulations indicate that its mixing performance is essentially consistent with that of the SHM.[76]

iLiNP Based on Baffle Obstacle Design

In 2018, Tokeshi et al introduced the iLiNP mixing device, and subsequent studies have explored its diverse applications. For instance, iLiNP enables precise tuning of LNP sizes within the range of 10 nm,[77] introduces a posttreatment process for size-controlled LNPs for siRNA delivery,[78] facilitates one-step production of biologically compatible noncationic exosome-like NPs for siRNA delivery,[79] and involves a 3D-iLiNP microfluidic device for LNP production.[80] Further applications include the production of LNP-based CRISPR/Cas ribonucleoprotein (RNP) delivery using iLiNP,[81] mass production of LNPs enabled by iLiNP,[82,83] and the control of liposome

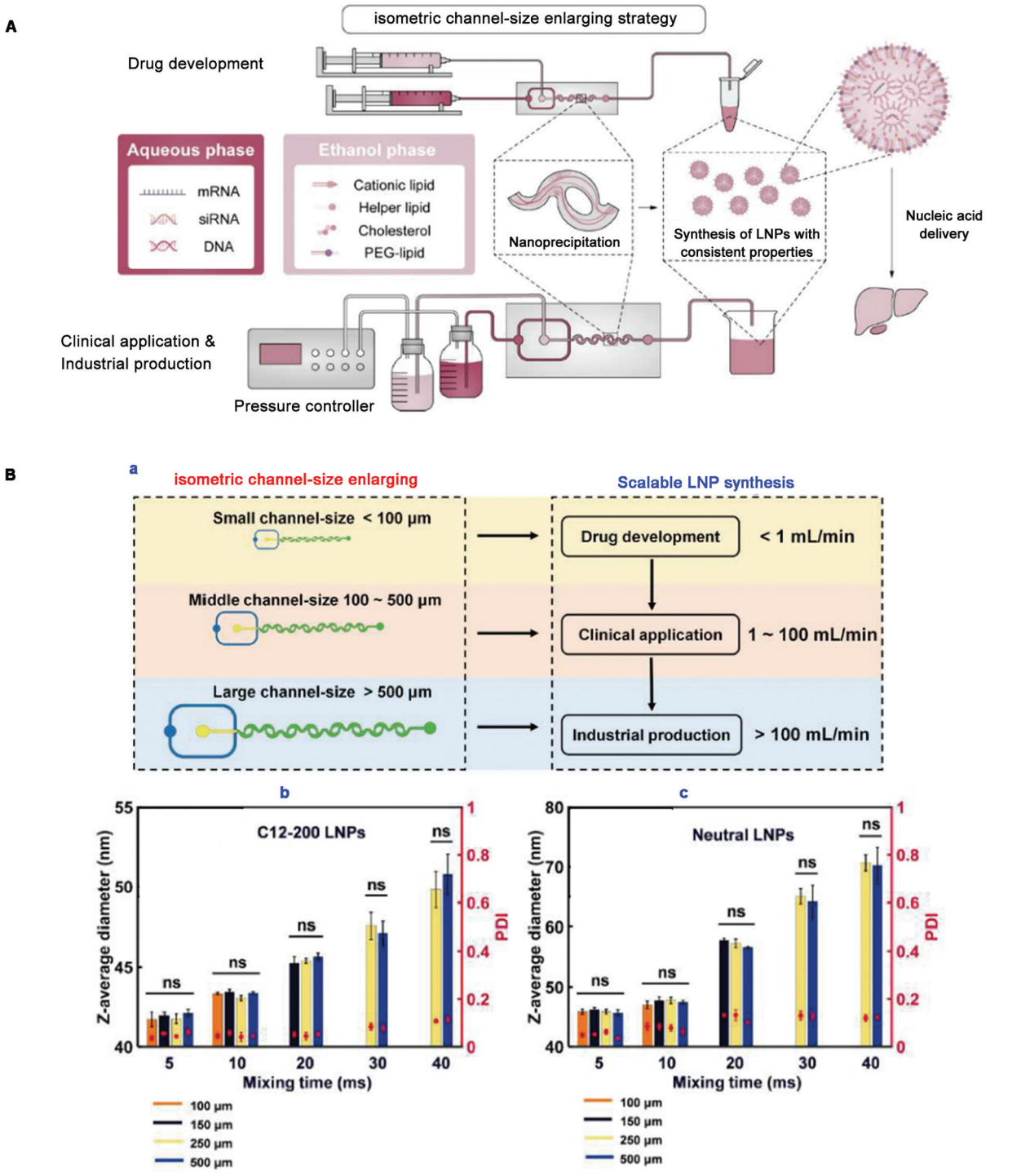

Fig. 6 (A) Schematic of the isometric channel-size enlarging strategy (as a SAR mixer) for the synthesis of LNPs. The image is reproduced with permission from Ref. [58], copyright 2023 Tsinghua University Press. (B) Synthesis of LNPs with consistent particle size and PDI at the same mixing times using different channel-size mixers. (**a**) Four channel-size mixers to satisfy different production requirements. (**b**, **c**) Two LNP formulations to prove the universality of the prediction method. The image is reproduced with permission from Ref. [58], copyright 2023, Tsinghua University Press. LNPs, lipid nanoparticles; SAR, split-and-recombine.

lamellarity using iLiNP.[84] ►**Fig. 8** presents related research on applying iLiNP in micro/nanoparticle DDSs.

In summary, iLiNP has demonstrated substantial potential in LNP fabrication. Beyond size control, lamellarity, and physicochemical properties of liposomes, it also offers the capability to integrate postprocessing steps such as online mixing and dilution for continuous production. Moreover, using eight parallel glass-iLiNP units, the total flow rate (TFR) for LNP preparation can reach up to 160 mL/min.

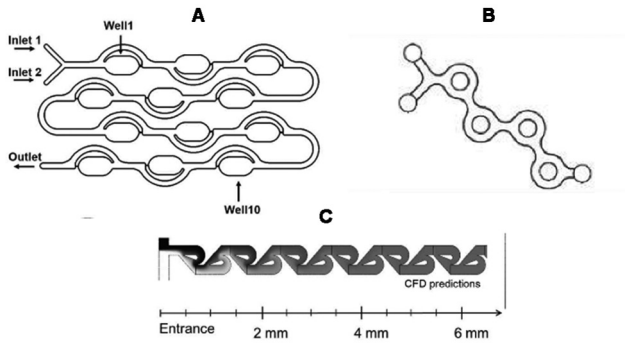

Fig. 7 Schematic of typical SAR microchannels. (**A**) Fluidic trap mixer. The image is reproduced with permission from Ref. [56], copyright 2021 Elsevier. (**B**) TrM. The image is reproduced with permission from Ref. [57], copyright 2020 Elsevier. (**C**) Tesla mixer. The image is reproduced with permission from Ref. [55], copyright 2015 Elsevier Science SA. SAR, split-and-recombine.

Capillary-Based Coaxial-Flow Mixer

Capillary-based coaxial-flow mixers (CFMs) are commonly employed for constructing droplet-based segmented-flow systems, facilitating the preparation of microparticulate DDSs like microspheres and microcapsules. Employing CFM, complex-structured microparticles can be manufactured with diverse configurations, facilitating co-encapsulation, sustained release, or controlled release of pharmaceuticals.[85] ►**Fig. 9** presents related research on CFM in preparing micro/nanoparticle DDSs.

Zhong et al utilized a CFM to create multicomponent microspheres (MCMs) featuring a "Particle in Particle" structure composed of sodium alginate (ALG) shells and gelatin methacrylate cores. These MCMs demonstrate the potential for encapsulating DOX and augmenter of liver regeneration within the shell and the cores, respectively (►**Fig. 9**).[86]

High-Speed Vortex/Jet Mixer Based on Flash

High-speed jet/vortex mixers, primarily utilizing flash technology, serve as a pivotal catalyst for the clinical translation of nanomedicines, encompassing two main principles: flash nanocomplexation (FNC) and flash nanoprecipitation (FNP). FNC primarily involves the production of nanomedicines through electrostatic interactions, hydrogen bonding, or metal coordination, leading to polyelectrolyte complexation. Conversely, FNP primarily operates through solvent supersaturation to produce polymer or inorganic NPs.[87] ►**Fig. 10** illustrates the operational principles of FNC and FNP.

Mixers based on the flash technology include the coaxial jet mixer (CJM),[88,89] CIJMs,[90] MIVM,[90] CFMs,[91] impingement jet mixer (IJM), swirl mixer,[92,93] etc. ►**Fig. 11** illustrates the standalone application of various high-speed vortex/jet mixers.

Tibbitt and colleagues introduced a CJM to produce polymeric NPs across a broad spectrum of solvents. It was revealed that the properties of the polymer NPs produced by CJM were similar to those obtained through the batch nanoprecipitation method. Yet, the production efficiency of CJM surpassed the batch method by 30-fold. Additionally, enhanced gene silencing effects were observed when using CJM to prepare complexes loaded with siRNA.[88]

The Pfizer COVID-19 vaccine employs an IJM obtained from the KNAUER company (Germany) (►**Fig. 12**).[94]

High-speed vortex/jet mixers facilitate the formation of nanocomposites or nanoprecipitates in a cost-effective, large-scale, and controllable manner.[87]

Microchannel for High-Throughput

Although investigators have achieved significant accomplishments in utilizing micro/nanoparticles as vectors for DDSs, unlocking the full potential of these systems requires large-scale production of micro/nanodrugs with precise control over particle properties. It is crucial to enhance production scale without compromising CQAs. To meet industrial demands, researchers currently employ three primary strategies to boost productivity: (1) a parallel strategy with multiple mixing units in micrometer-scale channels (lower TFR); (2) an independent use strategy of millimeter-scale channel mixing units exhibiting microfluidic size characteristics (higher TFR); and (3) a parallel strategy with multiple mixing units in millimeter-scale channels operating at higher TFR.[57,95,96]

Parallel Strategy with Multiple Mixing Units in the Micrometer-Scale Channel (Lower TFR)

The lower TFR parallelization strategy was initially proposed in chemical synthesis,[97] and later extended to NP fabrication.[98] This strategy, utilizing a parallel design of "*n*" microchannel mixing units, enables the TFR to be "*n*" times the TFR of each mixing unit. Parallel schemes for HFF,[99–101] SHM,[102] and iLiNP[103] have been employed to enhance the batch production of NPs. ►**Fig. 13** illustrates various applications of lower TFR in parallel configurations. ►**Table 2**–[104] lists reported instances of enhancing the productivity of microfluidic techniques based on the lower TFR parallelization strategy in the past 5 years.

Shepherd et al reported a highly integrated parallel scheme for SHM, incorporating 128 SHM mixing units, achieving a production rate of 18.4 L/h.[102]

One significant advantage of microchannel parallelization is the ability to scale up to large batches without altering the inherent characteristics of the individual unit. However, a drawback of this strategy is that the performance of the entire mixing system is contingent upon the robustness of the fluidic infrastructure and system operation. A malfunction in a single mixer could compromise the entire platform. Additionally, owing to minor perturbations that typically occur along parallel fluidic networks, the size and distribution of NPs may be affected.

Independent Use Strategy of Millimeter-Scale Channel Mixing Unit (Higher TFR)

The higher TFR millimeter-scale single mixing unit standalone strategy involves the modulation of fluids through millimeter-scale geometries, aiming to enhance throughput while preserving the microscale characteristics of microfluidics (uniform mixing/precipitation environments to support the formation of uniform particles). Examples of such schemes include CFM,[91] CJM,[88] IJM,[90] MIVM,[90] and swirl mixer[92,105] (►**Fig. 11**). ►**Table 3** lists reported instances of

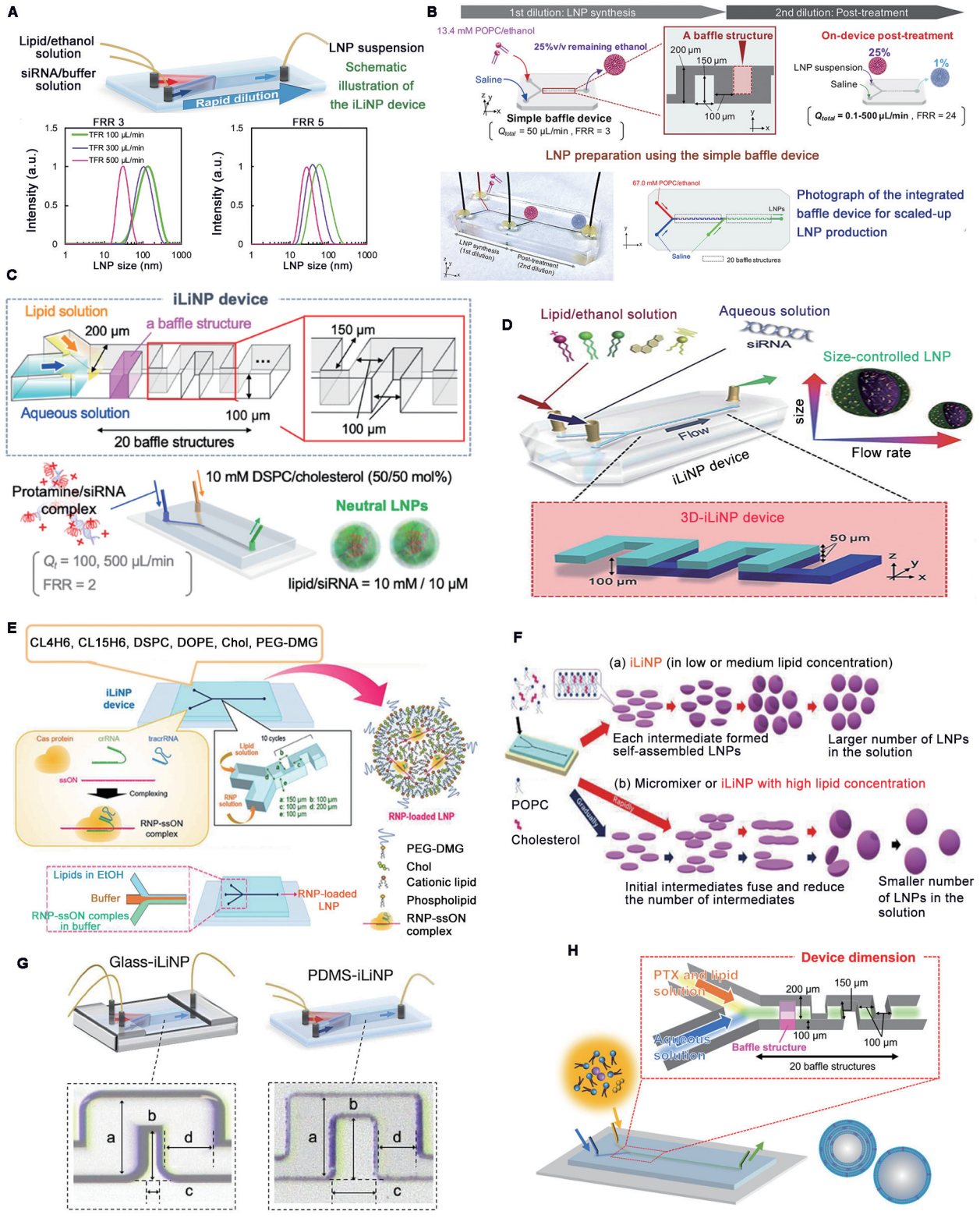

Fig. 8 Applications of iLiNP in micro/nanoparticle DDSs. (**A**) Tunable particle size within 10 nm. The image is reproduced with permission from Ref. [148], copyright Journal of visualized experiments. (**B**) On-device posttreatment process for size-controlled LNP to deliver siRNA. The image is reproduced with permission from Ref. [78], copyright 2020 American Chemical Society. (**C**) One-step production of biologically compatible exosome-like nanoparticles to deliver siRNA. The image is reproduced with permission from Ref. [79], copyright 2021 American Chemical Society. (**D**) 3D-iLiNP microfluidic device for LNP production. The image is reproduced with permission from Ref. [80], copyright 2021 Royal Society of Chemistry. (**E**) Production of an NP-based CRISPR/Cas RNP delivery using iLiNP. The image is reproduced with permission from Ref. [81], copyright 2021 Elsevier. (**F**) iLiNP enabled uniform and small LNP with high lipid concentration. The image is reproduced with permission from Ref. [82], copyright 2022 American Chemical Society. (**G**) Glass-iLiNP enabled mass production of LNP. The image is reproduced with permission from Ref. [83], copyright 2023 Elsevier. (**H**) iLiNP-enabled controlling size, lamellarity, and physicochemical properties of liposomes. The image is reproduced with permission from Ref. [84], copyright 2023 Royal Society of Chemistry. iLiNP, invasive lipid nanoparticle production; DDSs, drug delivery systems; LNP, lipid nanoparticle.

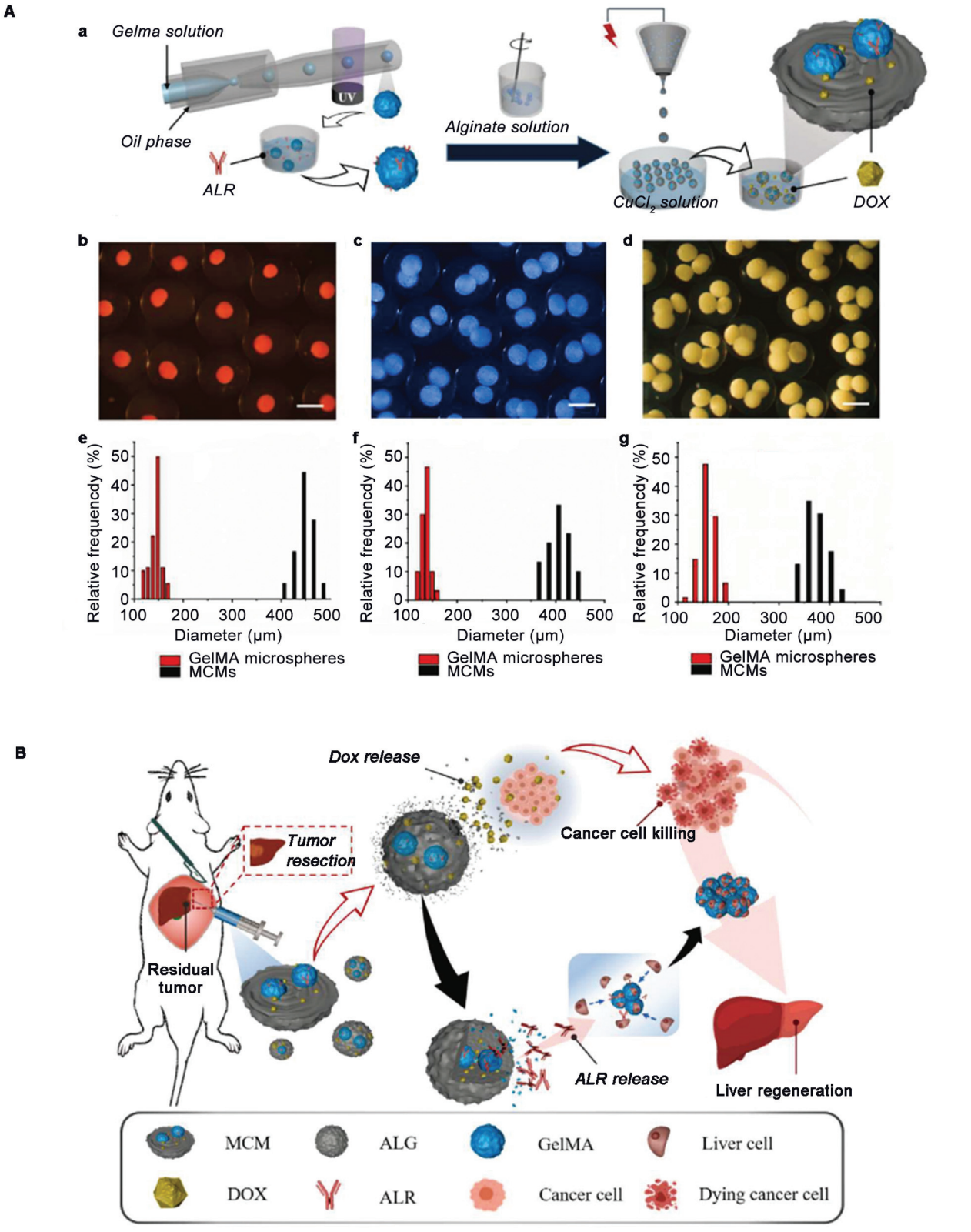

Fig. 9 (A) MCMs preparation using CFM. (**a**) Preparation process of the MCMs. (**b–d**) Optical microscopy images of MCMs with (b) single, (c) double, and (d) triple GelMA microspheres embedded, respectively. (**e–g**) Size distribution of the GelMA microspheres and the entire MCMs. (B) Schematic of the MCMs composed of DOX-loaded ALG shell and ALR-loaded GelMA cores for postsurgical liver cancer treatment and liver regeneration. The image is reproduced with permission from Ref. [86], copyright 2023 Elsevier Science SA. ALG, alginate; ALR, augmenter of liver regeneration; CFM, capillary-based coaxial-flow mixers; DOX, doxorubicin; GelMA, gelatin methacylate; MCMs, multicomponent microspheres.

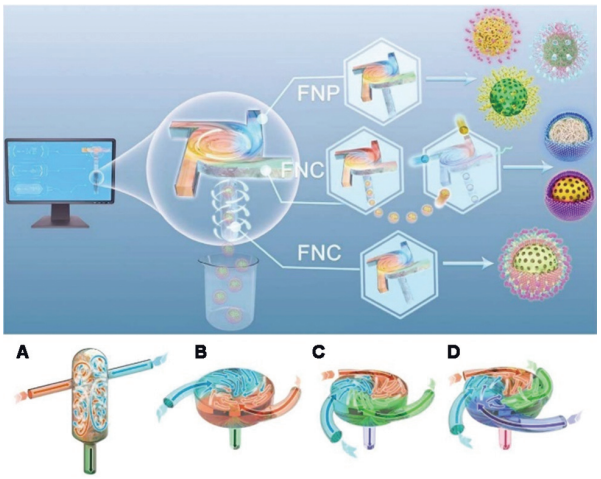

Fig. 10 Operational principles and structures of FNC and FNP. (**A**) CIJM. (**B**) Two-inlet MIVM. (**C**) Three-inlet MIVM. (**D**) Four-inlet MIVM. The image is reproduced with permission from Ref. [87], copyright 2021 Elsevier Science Ltd. CIJM, confined impingement jet mixers; FNC, flash nanocomplexation; FNP, flash nanoprecipitation; MIVM, multiple inlet vortex mixers.

enhancing the productivity of microfluidic techniques based on the independent use strategy of millimeter-scale channel mixing units (higher TFR) in the last 5 years.

Lim et at reported a CJM capable of NP reshaping and controlled synthesis at high production rates.[89] It has also been utilized to produce liposomes[106] and polymer NPs.[88] Xu et al achieved a production yield of curcumin liposomes using the swirl mixer, reaching up to 320 mL/min.[93] According to Lumiere Tech Ltd. (LumTech), the production of IJM can reach up to 60 L/h.[94]

The advantage of the standalone single mixing unit strategy lies in its ability to achieve batch scale up using simple, cost-effective microfluidic devices with minimal instrument operation. However, the design of such channels involves fine-tuning fluid dynamic states, necessitating professionals to bridge the gap for industrial translation. Another drawback is that during the initial phase of developing new formulations, the system operates at a higher TFR, leading to relatively greater reagent consumption.

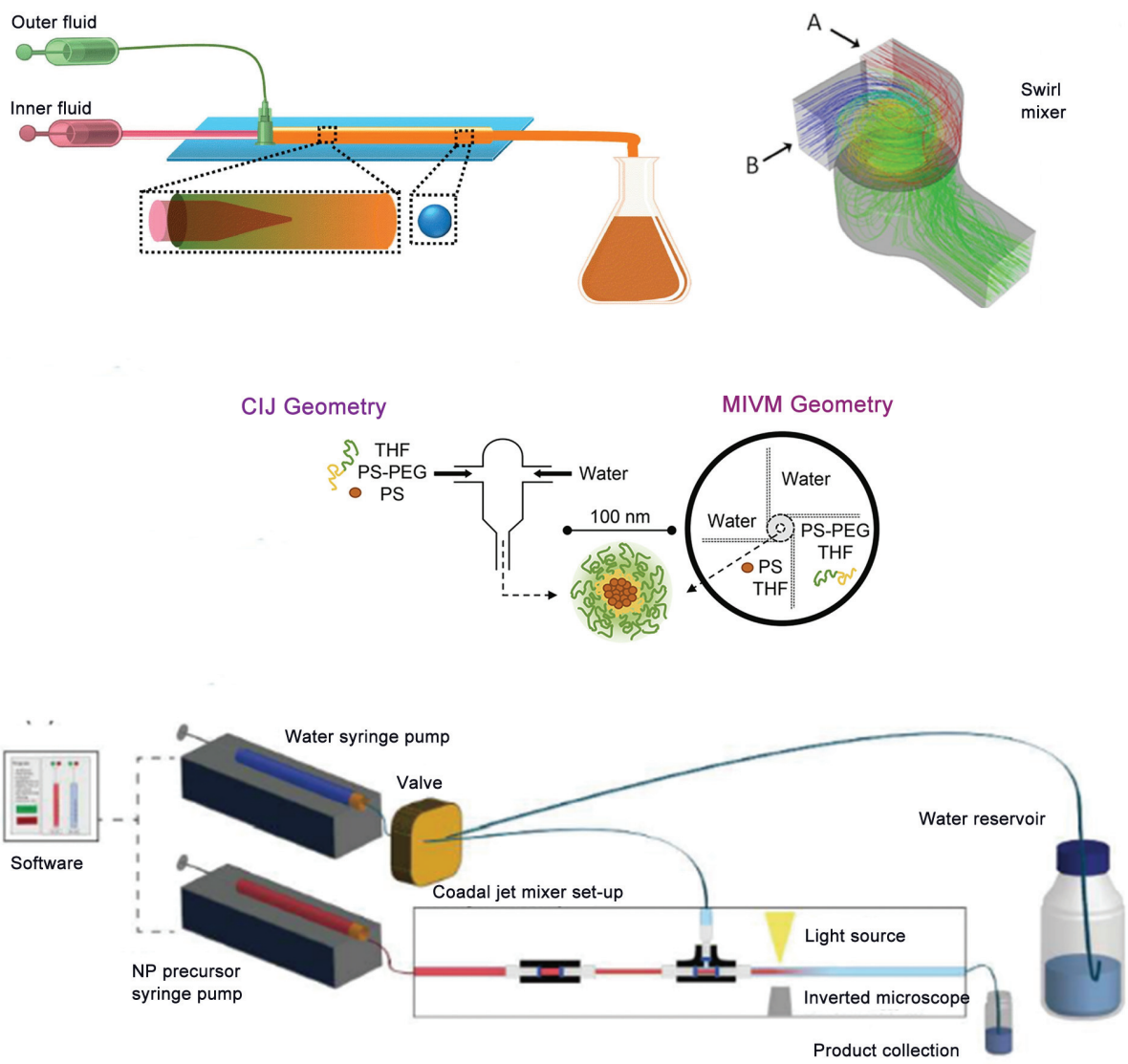

Fig. 11 Various high-speed vortex/jet mixers. The image is reproduced with permission from Ref. [90], copyright 2018 Elsevier Science Inc.; Ref. [91], copyright 2019 Elsevier; Ref. [88], copyright 2019 Wiley; Ref. [92], copyright 2022 Elsevier.

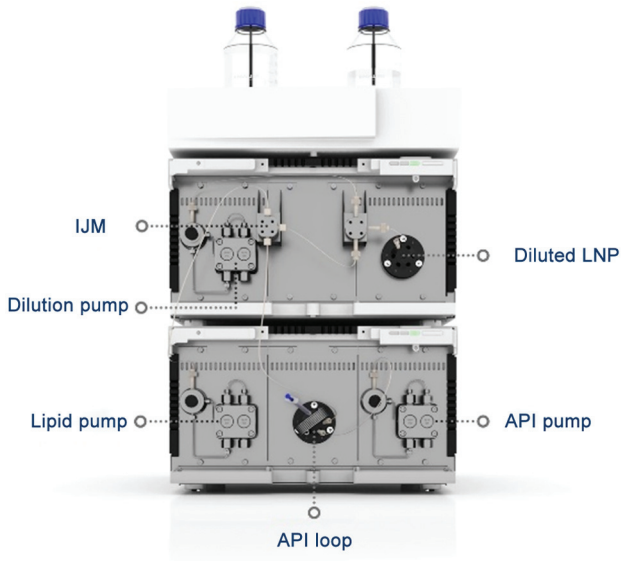

Fig. 12 IJM by the Pfizer COVID-19 vaccine.[94]

Table 2 Examples of increased productivity in microfluidic techniques based on lower TFR parallelization strategy in the last 5 years

DDSs	Lower TFR parallelization strategy	TFR (L/h)	Ref.
LNP	SHM ($n = 128$)	18.4	102
LNP	SHM ($n = 6$)	4.32	104
LNP	Glass-iLiNP ($n = 8$)	9.6	83

Abbreviations: DDSs, drug delivery systems; LNP, lipid nanoparticle; SHM, staggered herringbone mixer; TFR, total flow rate.

Parallel Strategy with Multiple Mixing Units in Millimeter-Scale Channels (Higher TFR)

In industrial applications, the abovementioned parallel lower TFR mixing units and higher TFR millimeter-scale single mixing units still encounter limitations regarding production rate enhancement. Therefore, researcher has proposed another constructive strategy derived from combining the abovementioned strategies: (1) parallelized swirl mixer[92,107]; (2) parallelized IJM[108]; and (3) parallelized fluid trap type mixers.[56] These

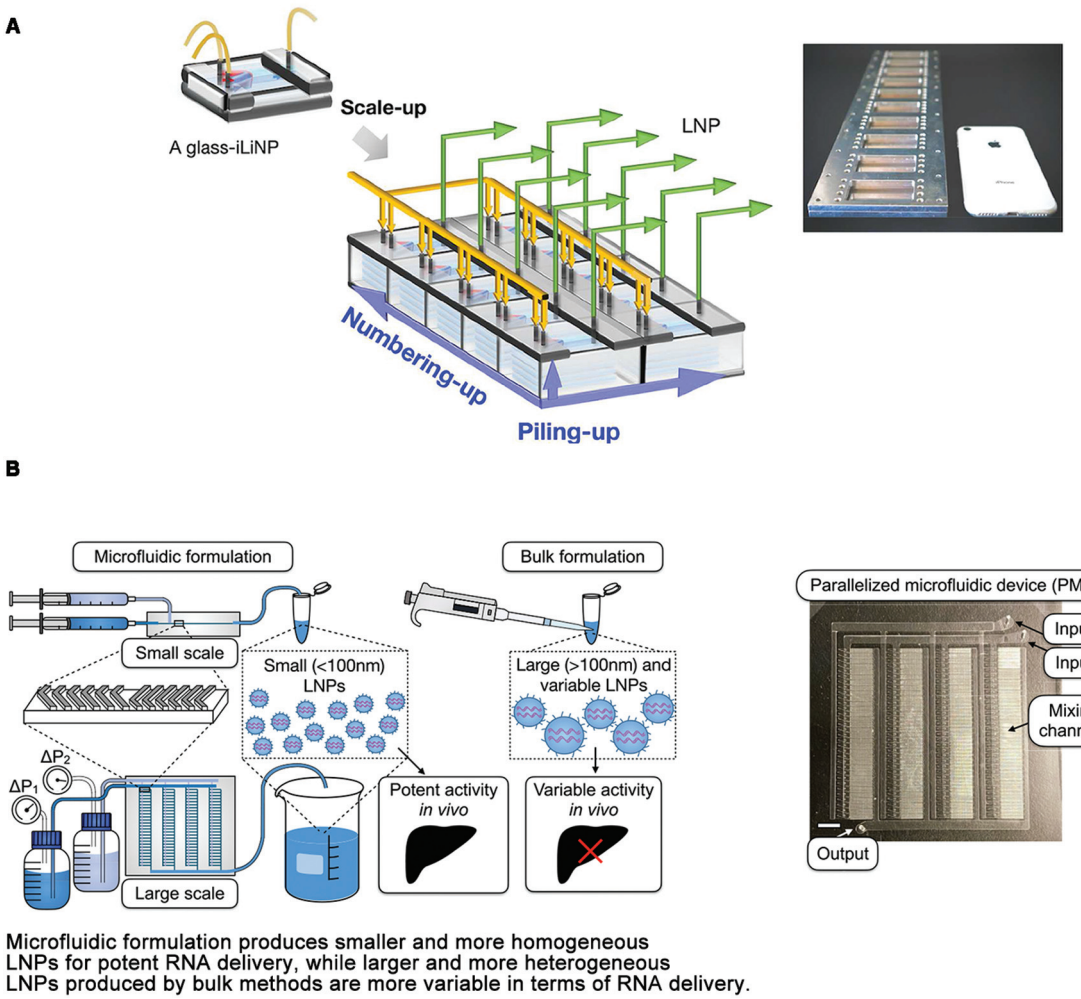

Fig. 13 Application examples of lower TFR in parallel configurations. (**A**) glass-iLiNP ($n = 8$). The image is reproduced with permission from Ref. [83], copyright 2023 Elsevier. (**B**) SHM ($n = 128$). The image is reproduced with permission from Ref.[102], copyright 2021 American Chemical Society. SHM, staggered herringbone mixer.

Table 3 Examples of increased productivity in microfluidic techniques based on independent use strategy of millimeter-scale channel mixing unit (higher TFR) in the last 5 years

DDSs	Higher TFR millimeter-scale single mixing unit standalone strategy	TFR (L/h)	Ref.
Liposome	Swirl mixer	20	93
Polymeric nanoparticles	CJM	2.1	88
LNP	IJM (Knauer)	60	94
LNP	SAR	>6	58

Abbreviations: CJM, coaxial jet mixer; DDSs, drug delivery systems; IJM, impingement jet mixer; LNP, lipid nanoparticles; SAR, split-and-recombine mixer; TFR, total flow rate.

Table 4 Examples of increased productivity in microfluidic techniques based on parallel strategy with multiple mixing units in millimeter-scale channels in the last 5 years

DDSs	Parallel strategy with multiple mixing units in millimeter-scale channels	TFR (L/h)	Ref.
LNP	Swirling mixer ($n=4$)	>18	92
Liposome, polymeric nanoparticles	Fluid trap parallel type mixers ($n=5$)	6	56
LNP	IJM (Knauer, $n=2$)	120	94
LNP	IJM NanoScaler ($n=5$)	6	94

Abbreviations: DDSs, drug delivery systems; IJM, impingement jet mixer; LNP, lipid nanoparticles; TFR, total flow rate.

strategies have emerged as the fastest routes for the industrial production of nanocarriers. ►**Fig. 14** illustrates a schematic diagram of the combined use of various parallelized millimeter-scale mixing units. ►**Table 4** lists reported instances of enhancing the productivity of microfluidic techniques based on the parallel strategy with multiple mixing units in millimeter-scale channels in the past 5 years.

In producing the COVID-19 vaccine (COMIRNATY), the parallelization of 100 IJMs is utilized to mix the lipid solution and mRNA solution under a pressure of 400 pounds, facilitating the scalable production of LNPs. This approach ultimately boosts the vaccine output to 100 million doses per month. Currently, in China, Lumiere Tech Ltd. (LumTech) represents KNAUER in offering IJM equipment for LNP preparation. Additionally, their commercially available IJM NanoScaler, by paralleling IJMs ($n=5$), employs the same technology at a laboratory scale for preclinical studies (6 L/h).[94]

Microchannel Employed for Continuous Manufacturing, Online Monitoring, and Intelligent Feedback

Because the production of micro/nanoparticles through traditional methods typically involves extensive postprocessing steps (such as dilution, overnight dialysis, etc.), which are numerous and complex, and multiple rounds of processing may induce detrimental effects such as damage or aggregation to the particles, researchers have been actively exploring approaches for the continuous production of micro/nanoparticles based on microfluidic technology in recent years. Kimura et al have achieved the integration of two steps—generation and dilution of LNPs—on one iLiNP device (►**Fig. 15**).[78]

Forbes et al have implemented a microfluidic device featuring a SHM structure, integrated with tangential flow filtration and Zetasizer Nano ZS, to accomplish a continuous liposome production process encompassing preparation, purification, and online particle size analysis. The application of this

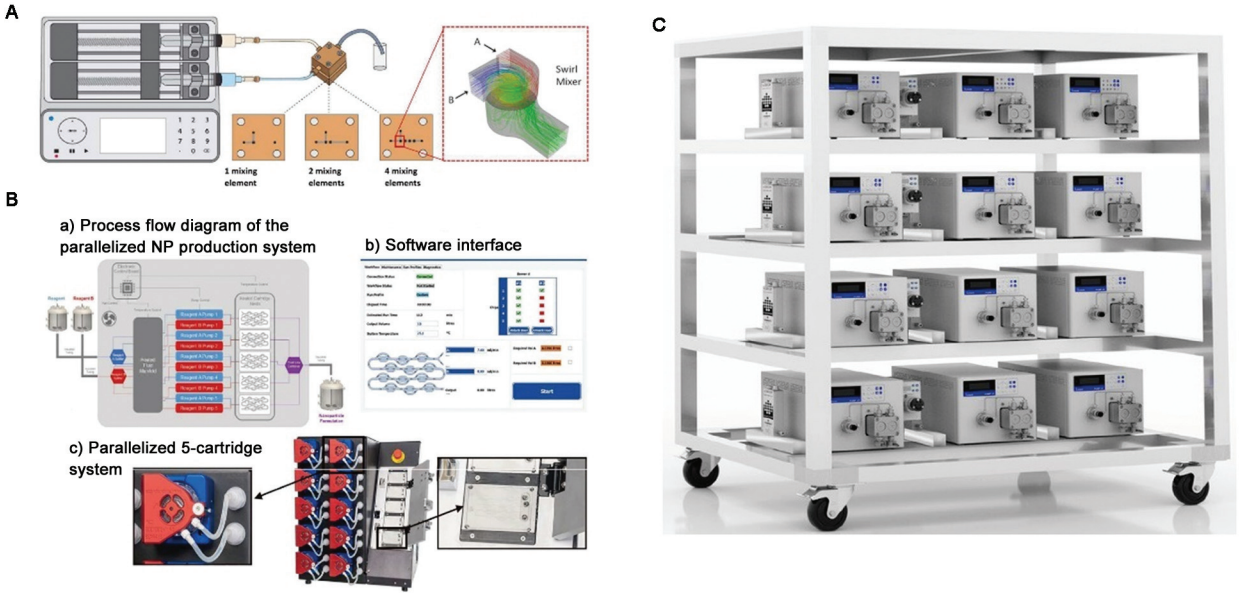

Fig. 14 Combination of various parallelized millimeter-scale mixing units. (**A**) Parallelized Swirl Mixer. The image is reproduced with permission from Ref. [92], copyright 2022 Elsevier. (**B**) Parallelized fluid trap-type mixers. The image is reproduced with permission from Ref. [56], copyright 2021 Elsevier. (**C**) Parallelized IJM.[94,108]

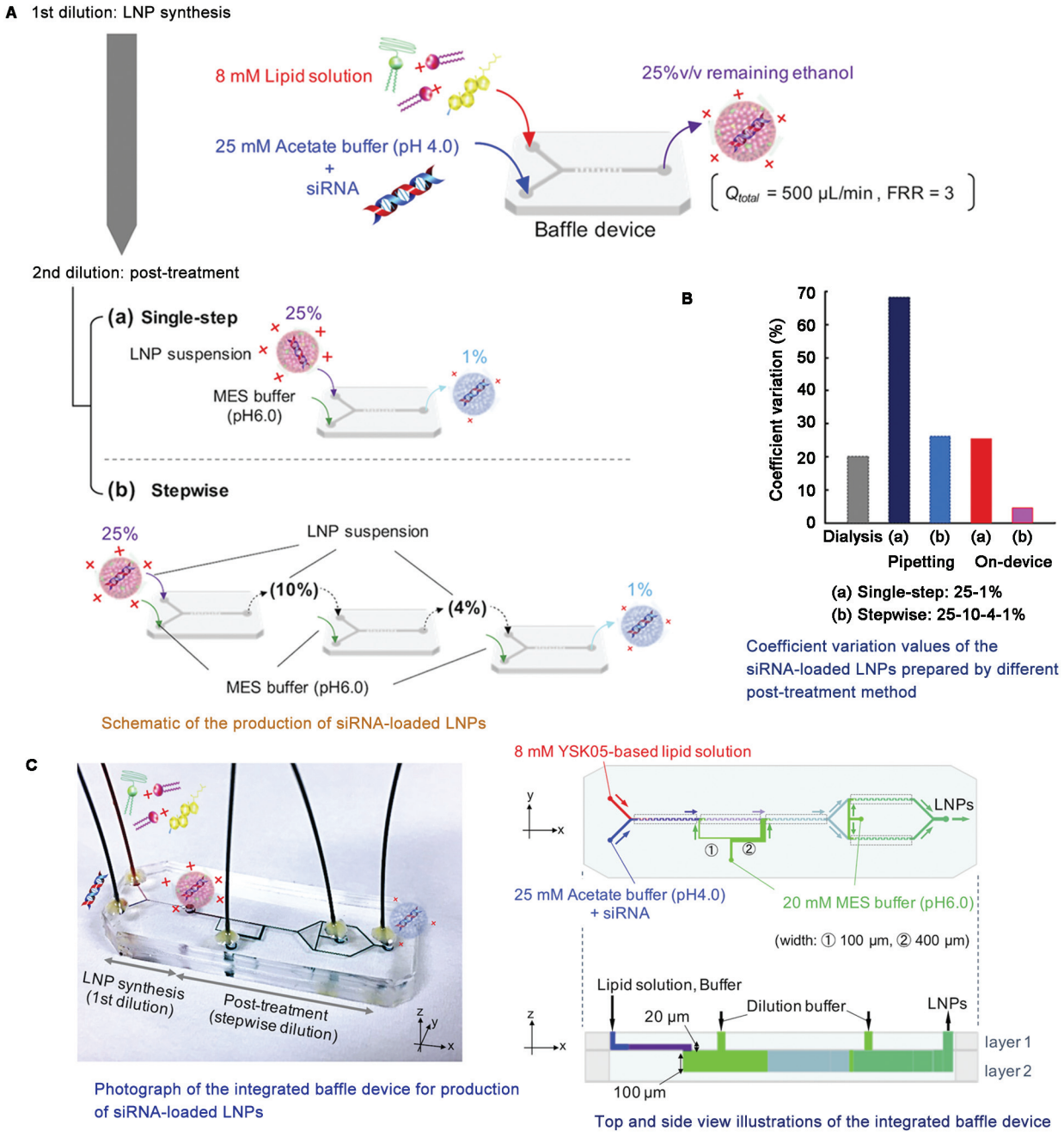

Fig. 15 (A–C) Integration of two steps (generation and dilution of siRNA-loaded LNPs) on one iLiNP device. The image is reproduced with permission from Ref. [78], copyright 2020 American Chemical Society. LNPs, lipid nanoparticles.

technology ensures a rapid and efficient transition of lipid nanocarriers from the laboratory to production while concurrently mitigating the risks associated with large-scale manufacturing.[61]

Strategies for Using Microfluidics to Manipulate Micro/Nanoparticle DDSs Precisely

Microchannels' high specific surface area allows for the precise construction of micro/nanoparticle DDSs. Furthermore, precisely designed microchannels facilitate uniform and rapid heat and mass transfer, shorten particle preparation time, and rapidly produce uniform particles.[17,109] ►**Fig. 16** compares NPs' particle size and morphology produced by conventional nanoprecipitation and microfluidic techniques.[110]

With the new demands and challenges of personalized therapies, including gene and cell therapies, it becomes vital to design nanodrugs precisely.[5] The capacity of microfluidics to manipulate and control the formation of micro/nanoparticles is mainly a function of the control of their fundamental construction, including size, morphology and structure, stiffness, surface characteristics, and elasticity. By shaping the abovementioned fundamental construction, precisely controlled CQAs of micro/nanoparticle DDSs with predictable particle size and distribution, zeta potential, stability, release

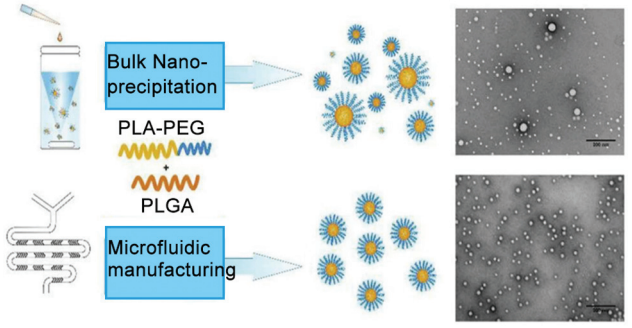

Fig. 16 Comparison of the size and morphology of nanoparticles prepared by the conventional nanoprecipitation method and microfluidic techniques. The image is reproduced with permission from Ref. [110], copyright 2022 Wiley.

characteristics, targeting capacity, and cellular uptake of the final production can be achieved.[111–116]

Size

Microfluidics enables the continuous preparation of various monodisperse particles with controllable sizes.[117–119] Microfluidic technology has been employed in the production of particles of different sizes, including ALG microgels with diameters of 8 to 28 μm,[120] hybrid microgels with diameters of 70 to 90 μm,[121] liposomes with diameters of 100 to 300 nm,[122] ginseng polysaccharide NPs with diameters of approximately 20 nm,[123] LNPs with diameters of approximately 20 nm,[104] and gold NPs with diameters of 2 to 4 nm.[124] ►**Fig. 17** shows examples of using microfluidics to control micro/nanoparticle size.

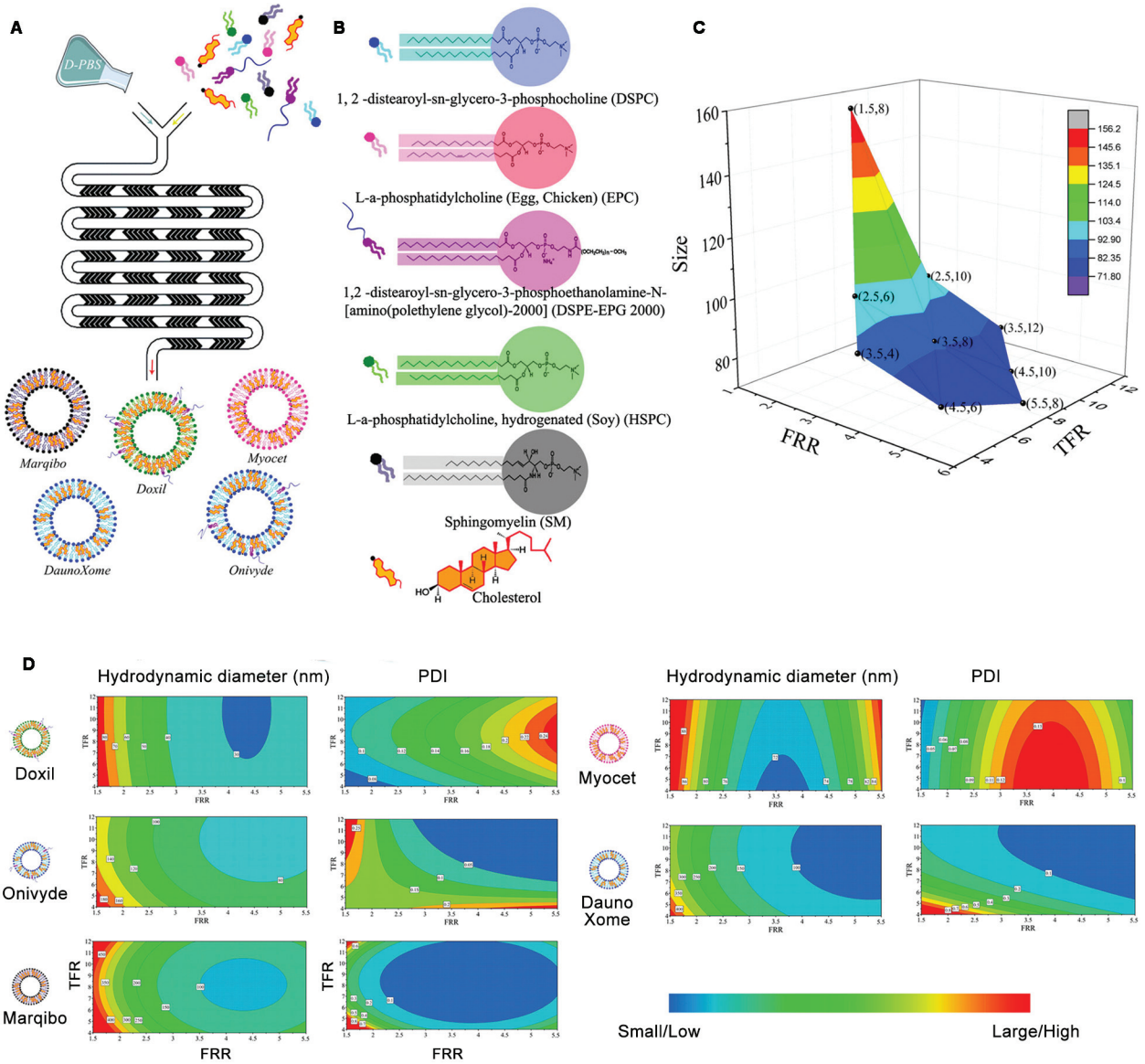

Fig. 17 Examples of controlling the size of micro/nanoparticle by microfluidics.[125] (**A**) Liposomes were formulated from clinically approved lipid compositions and synthesized using an SHM. (**B**) Chemical structures of different lipids used in clinical liposomes. (**C**) Schematic representation of the design space studied by the microfluidic-based full factorial DoE approach. (**D**) TFR or FRR was modulated to assess their effect on the diameter and PDI of the resultant liposomes. The image is reproduced with permission from Ref. [125], copyright 2019 Springer Heidelberg. FRR, flow rate ratio; SHM, staggered herringbone mixer; TFR, total flow rate.

Sedighi et al prepared liposomes with well-defined sizes and robust qualities, with the size controllable by adjusting the TFR and other parameters.[125] Kimura et al utilized an iLiNP device to achieve controlled preparation of LNPs with sizes at 10 nm intervals in the 20 to 100 nm range. NPs loaded with siRNA and prepared using this device exhibited effective gene silencing efficacy *in vivo*.[77]

Morphology and Structure

Microfluidics presents the capability for controlled preparation of functional micro/nanoparticles with specific shapes tailored for diverse applications. For instance, it can control the densities and void sizes of polymeric materials, influencing the characteristics of DDSs, such as stability and degradation rates.[126,127] ►**Fig. 18** provides examples of how microfluidics can control the morphology and structure of micro/nanoparticles.

Chen et al employed a microfluidics-enabled serial assembly to achieve the co-encapsulation of siRNA and Sorafenib. This microfluidics platform facilitated the regulation of fast and slow processes on a single chip, enabling the sequential self-assembly of sorafenib, siRNA, and lipids. The resulting lipid-siRNA-sorafenib NPs demonstrated outstanding anticancer effects *in vivo*.[128]

Using microfluidics, Hao et al synthesized mesoporous silica nanofibers with varying aspect ratios and diameters.[129] Additionally, microfluidics can modulate microspheres' shell size and thickness, thereby adjusting their release profiles.[130]

Stiffness

The stiffness of drug-loaded micro/nanoparticles plays a crucial role in determining their deformability, influencing circulation profiles, cellular uptake, and aggregation and penetration behavior.[131] Microfluidics is a valuable tool for preparing NPs with varying stiffness, enabling the exploration of how particle stiffness impacts drug release. ►**Fig. 19** shows examples of microfluidics employed to control micro/nanoparticle stiffness.

Lozano Vigario et al utilized an SHM to produce highly rigid anionic liposomes, offering a rapid, size-tunable, and scalable method for generating rigid tolerogenic liposomes.[132]

Liu et al employed the multifunctional microfluidic ultrasonic cavitation method to synthesize NPs in a controlled, nonclogging, and scalable manner. Leveraging its cavitation mode, this approach enabled the synthesis of soft and fragile mRNA-LNPs. Additionally, compared with conventional microfluidic techniques, the method yielded smaller particles with a more uniform distribution and demonstrated robust, scalable production capacity, achieving a throughput of up to 1.6 g/h.[47]

Surface Characteristics

Surface modification is a highly effective strategy for enhancing targeting efficiency and extending the circulation time of micro/nanoparticle DDSs. Microfluidics enables precise manipulation of particle surface characteristics, as illustrated in ►**Fig. 20**.[133]

Using a one-step methodology, Li et al employed microfluidics to prepare complex multifunctional nanoliposomes with highly controllable surface characteristics, particle sizes, and photodynamic and chemodynamical effects. This study fully exploited the capabilities of microfluidics for precise manipulation, laying the foundation for constructing customized functional NPs.[134]

Gao et al demonstrated the fabrication of structurally complex CUR@ZIF-SF-PDA NPs using the swirl mixer. This channel sequentially achieved the formation of ZIF-8 metal–organic framework (MOF), encapsulation of curcumin, and coating with silk fibroin (SF) and polydopamine (PDA). Microfluidic rapid mixing exhibited a unique advantage over conventional methods in constructing NPs with appropriate charge density and controllable particle size.[135]

Microfluidics has also been instrumental in surface modification or ligand conjugation of LNPs to achieve targeted delivery to specific cells/organs. In addition to Glu-urea-Lys ligand-conjugated siRNA-LNPs,[136] transferrin-conjugated siRNA-TfLNP,[137] peptides, and antibody-conjugated LNPs have been successfully prepared using the microfluidic technology.[138]

Elasticity

When considering embolic drug delivery, the intrinsic elasticity and deformability of micro/nanoparticles play a pivotal role in their function as embolic DDSs. Microfluidics presents an avenue for constructing micro/nanoparticles with tunable elasticity, controllable swelling, and adjustable release properties, as shown in ►**Fig. 21**.

Yang et al introduced an innovative strategy for the facile fabrication of monodisperse poly(vinyl alcohol) (PVA) microspheres with controllable elastic properties for embolization using a glass microcapillary. PVA microspheres' elasticity and swelling ratios become adjustable through precise control of microfluidic parameters. These microspheres exhibit high DL owing to their significant swelling and negatively charged properties. This study provides valuable insights for the easy fabrication of monodisperse PVA microspheres with controllable elasticity, swelling ratio, and DL, facilitating efficient embolization therapy and drug delivery applications.[139]

Application of Microfluidic Technology in DDSs

Microfluidic technology has played a significant role in the preparation of a wide range of micro/nanoparticle DDSs, encompassing liposomes, micro/nanocrystals, NPs, and microspheres/microcapsules, as well as biomimetic particles.[54]

►**Tables 5** and **6** present instances of the preparation of NPs and microparticles, respectively, using microfluidic technology in recent years.

Liposome

As microfluidic technology continues to advance, it is anticipated that it will revolutionize traditional industrial processes for liposomes. ►**Fig. 22** shows the conventional large-scale liposome production process alongside the transformative impact of microfluidic technology on existing production processes.

Numerous examples of preparing liposomes based on microchannels such as T-type, Y-type,[140] iLiNP,[84] SHM,[57,60] TrM,[57] etc. are shown in ►**Tables 5** and **6**.[141–168]

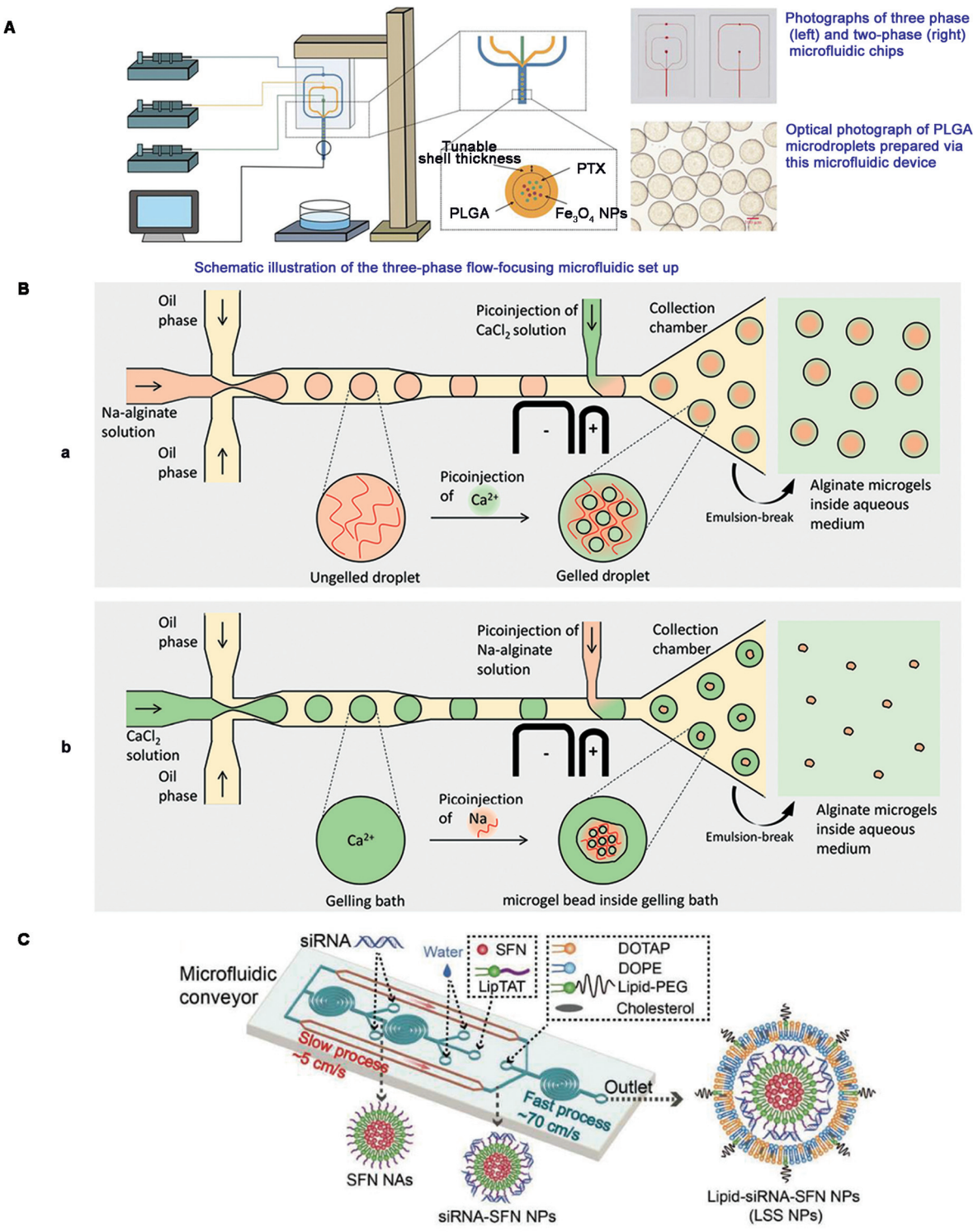

Fig. 18 Microfluidics in controlling the morphology and structure of micro/nanoparticles. (**A**) An emulsion droplet generation microfluidic device in preparing PLGA microspheres with tunable shell thickness. The image is reproduced with permission from Ref. [130], copyright 2021 Taylor & Francis Ltd. (**B**) Two kinds of Microgel beads by (**a**) the picoinjection of chelate-free aqueous $CaCl_2$ in emulsion droplets of aqueous Na-alginate; and (**b**) the picoinjection of Na-alginate solution in $CaCl_2$-emulsified droplets. The image is reproduced with permission from Ref. [120], copyright 2021 Royal Society of Chemistry. (**C**) Preparation of lipid-siRNA-sorafenib nanoparticles (LSS NPs) that successively encapsulate the sorafenib and siRNA by controlling the fast and slow processes on one chip. The image is reproduced with permission from Ref. [128], copyright 2023 Wiley-VCH Verlag GmbH.

Micro/Nanocrystals

In conventional methods for micro/nanocrystals, issues such as broad particle distribution and inconsistent reproducibility are prevalent. This can be particularly problematic for sustained-release micro/nanocrystals, where a wider particle distribution may impact release characteristics, leading

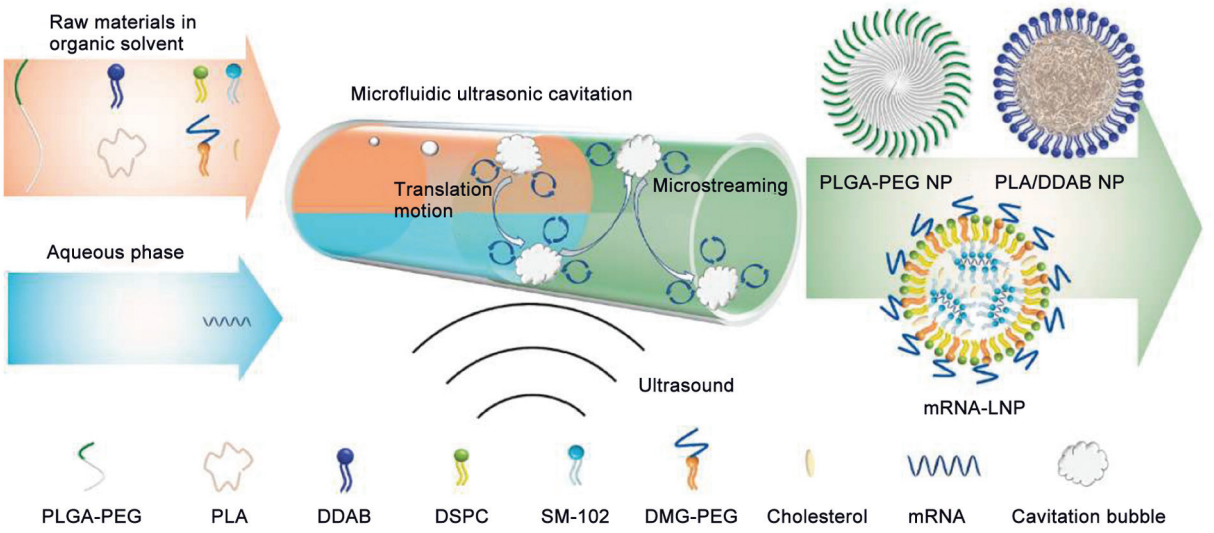

Fig. 19 The synthesis of soft and fragile mRNA-LNPs by microfluidic ultrasonic cavitation. The image is reproduced with permission from Ref. [47], copyright 2023 Pergamon-Elsevier Science Ltd.

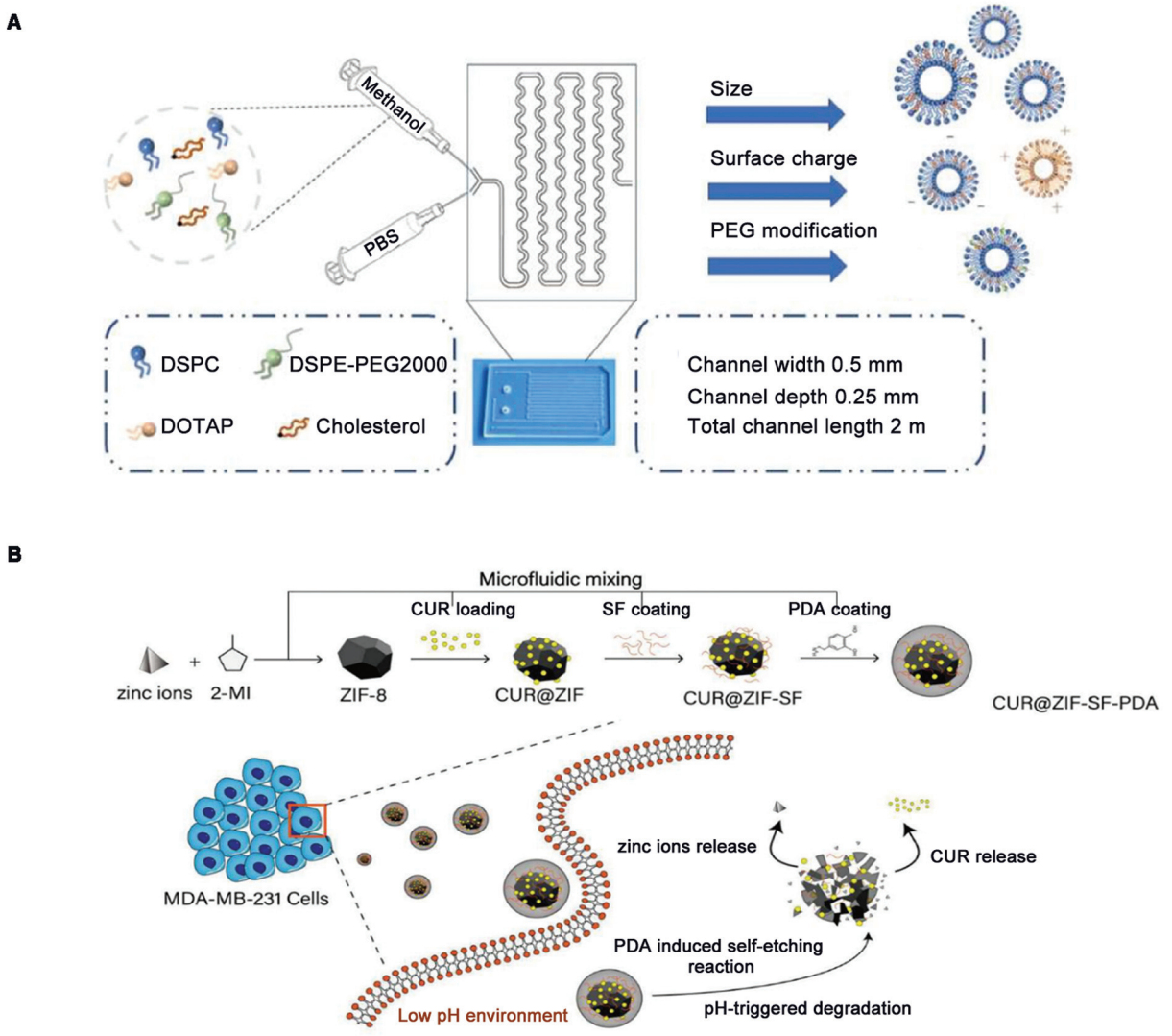

Fig. 20 (A) Microfluidic technology in a one-step process of multifunctional nanoliposomes with highly controllable surface characteristics and particle sizes. The image is reproduced with permission from Ref. [134], copyright 2022 American Chemical Society. (**B**) The preparation of structurally complex CUR@ZIF-SF-PDA nanoparticles utilizing the Swirl mixer. The image is reproduced with permission from Ref. [135], copyright 2023 MDPI.

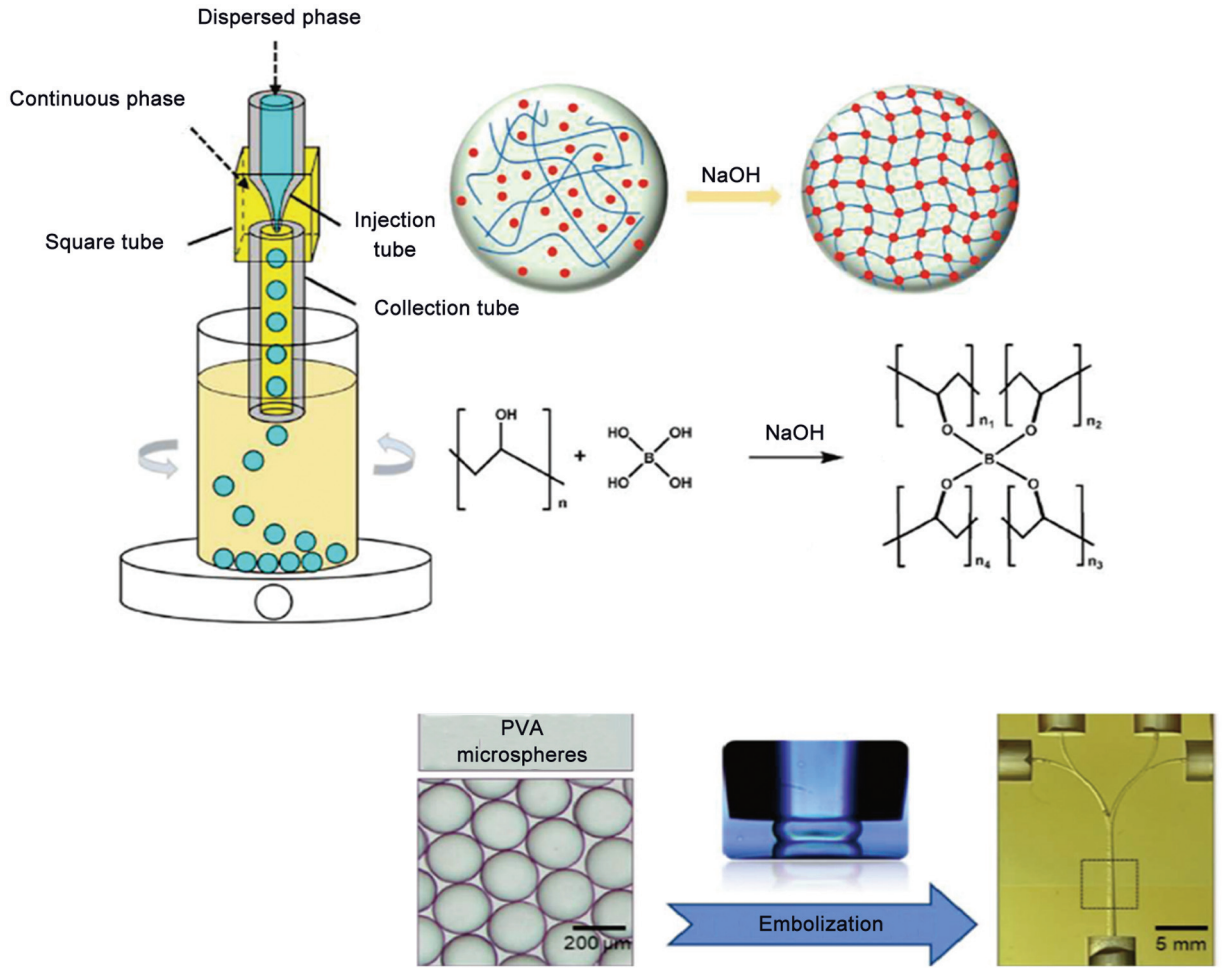

Fig. 21 Glass microcapillary in preparing PVA microspheres with controllable elastic. The image is reproduced with permission from Ref. [139], copyright 2022 American Chemical Society. PVA, poly(vinyl alcohol).

to issues like burst release effects and potential adverse reactions, thereby presenting challenges in clinical safety.

Until now, researchers have increasingly attempted to prepare micro/nanocrystals by using the microfluidic technology based on Y-type and S-type[164] and other approaches.[164,169] For instance, Liu et al employed microfluidic technology, integrating a Y-type mixer and an S-type channel, to produce microcrystals of dolutegravir sodium. This approach minimized the risks of channel blockage during continuous crystallization by segregating crystal nucleation from subsequent crystal growth. The resulting microcrystals exhibited small particle sizes, a narrow distribution, and a distinctive prismatic shape.[164]

Ahmad utilized a Y-type mixer to fabricate Repaglinide nanocrystals, achieving a particle size of 71.31 ± 11 nm and a PDI of 0.072, thereby enhancing efficacy and safety profiles.[145]

In another innovative approach, Coliaie et al harnessed microfluidic technology to modulate the crystallization kinetics of *O*-aminobenzoic acid, identifying distinct crystal forms through various microfluidic channels. These novel designs play a pivotal role in supporting the Food and Drug Administration's (FDA's) initiative to promote innovation in continuous manufacturing, significantly contributing to advancements in drug development.[169]

Micelles

In contrast to traditional methods of micelle fabrication, microfluidics demonstrates a significant advantage in the production of micelles. In this process, polymeric precursors dissolved in a water-miscible organic solvent are introduced into a microchannel (HFF mixer[158] or Tesla mixer[161]) and mixed with water. This interaction induces the aggregation of the hydrophobic segments of the amphiphilic copolymers, leading to the self-assembly of micelles.[158]

Karimi-Soflou et al utilized the HFF mixer to create micelles designed for intracellular retinoic acid release on demand. These micelles offer adjustable sizes and precisely controlled physio-biological properties, thereby facilitating efficient neuronal differentiation.[158]

Nanoparticle

The advantage of NPs as DDSs lies in their high structural malleability, enabling precise design and laying the foundation for targeted drug delivery.[5] NPs are categorized into two main types: organic and inorganic.

Table 5 Examples of nanoparticle preparation using microfluidic technology in recent years

DDSs	Microfluidic device	Loaded drug	Characterizes of DDSs					Advantages	Ref.
			Size (nm)	PDI	EE (%)	DL (%)	Others		
Liposome	Y-type HFF	Curcumin	106.22 ± 4.94	<0.3	99.33 ± 1.05	4.21 ± 0.12	/	Enhanced stability and skin penetration	140
Liquid crystal nanoparticles	SHM	Calcein	191–274	0.02–0.11	65.9–81.0	0.2–1.0	/	High-throughput and high EE	141
LNP	iLiNP	mRNA	179.2 ± 9.0	0.22 ± 0.06	85.2 ± 9.2	/	/	Excellent cell uptake	142
MOF	Y-type	miRNA	≈ 121	0.32	60.35 ± 4.61	/	/	Low cytotoxicity, high loading efficiency, improved cell uptake, and endosomal escape	143
Liposome	Co-flow combined with coaxial electrostatic spray	PTX and DOX	179.5 ± 0.3	0.119 ± 0.027	PTX: 99.15 ± 0.43; DOX: 90.47 ± 0.31	PTX: 8.27 ± 0.31; DOX: 7.54 ± 0.03	/	Excellent targeting biodistribution and higher tumor growth inhibition rate	144
Liposome	iLiNP	PTX	<120	<0.3	≈ 100	0.5–3.5	/	Controllable lamellarity and physicochemical properties	84
LNP	iLiNP	mRNA	80–100	<0.2	>85%	/	/	Scalable LNP production system	83
LNP	Inertial flow mixer (isometric channel-size enlarging)	siRNA	46.31–47.48	<0.1	>90%	/	/	Scalable LNP production system	58
MOF nanoparticles	Swirl mixer	Zinc ions, curcumin	≈ 170	≈ 0.08	/	/	/	High biocompatibility and low cytotoxicity	135
Nanocrystals	Y-type	Repaglinide	71.31 ± 11.00	≈ 0.072	/	/	/	Improved antidiabetic properties and safety profiles	145
Lipid nanoparticle	T-type	siRNA	≈ 100	/	/	/	/	Effectively silence the *HMGB1* gene	146
Lipid/polymer hybrid nanoparticle	TrM	pDNA	110–120	<0.07	≈ 65	/	/	Steric stability effectively silences gene	147
Lipid nanoparticle	iLiNP	siRNA	20–40	/	≈ 95	/	20–30 mV	A precise LNP size control technique	148
Gelatin nanoparticle	Swirl mixer	CuS, Fe_3O_4, curcumin	180 ± 15	<0.1	77	25		Enhanced the cellular uptake and achieved the targeted delivery	149
Nanocapsules	HFF	PTX, curcumin, and vitamin D	100–200	/	/	40–70	70 mg/h throughput	Scaled-up capability and continuous synthesis	150
Nanoparticle	The nozzle chip	Curcumin	241.91 ± 1.46	0.097 ± 0.017	64.29 ± 0.29	3.06 ± 0.01	86.4 g/d throughput	High production	151

Table 5 (*Continued*)

DDSs	Microfluidic device	Loaded drug	Characterizes of DDSs					Advantages	Ref.
			Size (nm)	PDI	EE (%)	DL (%)	Others		
Lipid nanoparticle	Turbulent jet mixer	ASO	<100	<0.2	>80	/	/	Smaller size and narrower distribution, higher EE	152
Chitosan nanoparticle	Fluidic 186	mRNA and siRNA	75–105	0.15–0.22	>80%	/	Zeta potential: 6–17 mV	Increased cellular internalization	153
Lipid nanoparticle	Microfluidics-enabled serial assembly	Sorafenib and siRNA	108	/	100 (sorafenib) and 95 (siRNA)	/	Zeta potential: 24.7 mV	Combination therapy, high gene silencing efficiency, and extended survival period	128
Liposome	Flow focusing	Bisdemethoxycurcumin	49.86 ± 0.91	0.210 ± 0.020	89.67 ± 0.01	5.460 ± 0.001	/	Small size, homogenous size distribution, enhanced antitumor effect	154
MOFs	Y-type S-shaped	Diclofenac sodium	500–200	/	/	/	Cubic symmetry	Sustained drug release without sudden release within the first day	155
Lipid nanoparticles	SHM	pDNA	<190	≈ 0.1	76–92	/	/	Uniformly sized and homogeneous dispersion	156
Chitosan-coated magnetic nanoparticles	2D HFF	Cisplatin	104 ± 15	≈ 0.029	≈ 91	/	/	Higher EE, controlled release, and lower IC_{50}	53
Liposome	SHM and TrM	DOX	<100	<0.1	/	/	12 L/h throughput	Scalable production	57
Liposome	SHM	DOX	≈ 100	<0.2	>80	/	/	High EE	60
Liposome	SHM	Ovalbumin	60–100	<0.2	30–40	/	/	Incorporated in-line purification and at-line monitoring	61
Liposome	SHM	Curcumin	≈ 120	<0.2	/	17	/	Superior loading capacity, enhanced efficacy, and reduced toxicity	67
Liposome	SHM	Ovalbumin	≈ 150	<0.2	41 ± 4	/	/	Good physicochemical characteristics, no toxicity, protein integrity, and effective uptake by endocytosis	69
Liposome	Swirl mixer	Curcumin	120	$<0.15 \pm 0.02$	70 ± 4	/	320 mL/min throughput	Scalable production	93
Liposome	SHM	1-α,25-dihydroxyvitamin D3	190–210	0.2–0.3	/	/	/	Highly rigid tolerogenic liposomes	132
Lipid nanoparticles	Swirl mixer	DOX	100–200	≈ 0.2	79.7 ± 4.0	/	/	Precise controllable and scalable	92

(*Continued*)

Table 5 (*Continued*)

DDSs	Microfluidic device	Loaded drug	Characterizes of DDSs					Advantages	Ref.
			Size (nm)	PDI	EE (%)	DL (%)	Others		
Superparamagnetic iron oxide nanoparticles	T-type	Fe_3O_4	90.5 ± 26.2	/	/	/		Enable labeling human platelets and excellent compatibility	157
Micelles	HFF	Retinoic acid	96.37 ± 8.70	<0.3	54.25 ± 3.10	9.79 ± 1.12	Zeta potential: 15–21 mV	Size-adjustable	158
Liposome	Y-type	Paclitaxel	<200	<0.2	88–91	/	/	Excellent EE and sustained drug release	159
Lipid nanoparticles	iLiNP	Sorafenib and siRNA	60.47 ± 6.90	0.10 ± 0.01	94.5 ± 6.5 (siRNA) and 96.5 ± 4.8 (sorafenib)	/	Zeta potential: −17.4 ± 5 mV	Tunable particle size and high tumor penetration efficiency	160
Small unilamellar vesicles	SHM	Chlorpromazine	54–147	<0.2	/	/	/	Temperature-dependent size tunability	74
Micelle	Tesla mixer	DOX	19.4 ± 0.2	<0.25	92.4 ± 0.5	33.4 ± 0.3	Zeta potential: −43.7 ± 2.4 mV	Higher antitumor activity	161

Abbreviations: DDSs, drug delivery systems; DL, drug loading; DOX, doxorubicin hydrochloride; EE, encapsulation efficiency; HFF, hydrodynamic flow focusing; LNP, lipid nanoparticle; MOFs, metal–organic frameworks; PDI, polydispersity index; PTX, paclitaxel; SHM, staggered herringbone micromixer.

Organic Nanoparticles

Lipid Nanoparticles

The microfluidic mixing method has been recognized as the most successful manufacturing technology for preparing LNPs at a good manufacturing practices (GMP) scale. Onpattro, Comirnaty, and Spikevax are produced using microfluidic technology.[57] As an excellent carrier for nucleic acid delivery, LNP has been efficiently prepared using SHM,[62–65,170–172] IJM,[108] TrM,[147] and iLiNP.[77,78,80–82,148] The LNPs loaded with nucleic acids, prepared using the abovementioned microchannels, exhibit a small size, uniform distribution, high EE, and high gene delivery efficiency.

RNPs as active components can mitigate the off-target effects present in conventional nucleotide molecules. However, the denaturation of RNPs in the presence of ethanol and the challenges associated with their encapsulation complicate the incorporation of RNPs within LNPs. Suzuki et al prepared LNPs loaded with RNPs using a tri-inlet iLiNP microfluidic device. By rapidly diluting the ethanol phase, the denaturation of RNPs was avoided. The results demonstrated that the RNP-loaded LNPs achieved gene editing with commendable gene knockout efficiency (97%) and base substitution rate (23%).[81]

Polymeric Nanoparticles

There is a growing focus on preparing polymeric NPs using microfluidic technology. Employing microfluidic techniques for synthesizing polymeric NPs can significantly enhance DL and EE. Furthermore, this approach provides finer control over drug release compared with traditional methods. Researchers have employed various microfluidic devices, including HFF,[23,173,174] SHF,[71,175] and two-phase droplet microfluidics,[176] to fabricate synthetic polymeric NPs.

Martins et al developed PLGA NPs loaded with Efavirenz using conventional and microfluidic techniques. The results demonstrated that NPs prepared using microfluidic technology exhibited a smaller size (73 versus 133 nm), higher EE (80.7% versus 32.7%), and increased DL (10.8% versus 3.2%) compared with the conventional method.[177]

Sequential nanoprecipitation is a method to enhance the DL of NPs. Leveraging the unique design of microfluidic devices and the theory of sequential precipitation, achieving high DL and responsive release is feasible under specific conditions.[178]

Lipid–Polymer Hybrid Nanoparticles

Lipid–polymer hybrid NPs combine the advantages of biodegradable polymer NPs with the biomimetic benefits of lipid materials, addressing certain limitations associated with lipid and biodegradable polymer NPs.[179,180]

Researchers have recently utilized microfluidic technology to fabricate various hybrid NPs. The synthesis of hybrid NPs typically involves multistage one-step methods or multi-level microchannel platforms to achieve NP precipitation and lipid self-assembly encapsulation sequentially. Several microfluidic devices have been employed for the preparation of hybrid NPs (PLGA–lipid hybrid NPs[42] and polyethyleneimine hybrid

Table 6 Examples of microparticle preparation using microfluidic technology in recent years

DDSs	Microfluidic device	Drug loaded	Characterizes of DDSs				Advantages	Ref.
			Size (μm)	CV (%)	EE (%)	DL (%)		
Microspheres	CFM	DOX and liver regeneration	300–450	/	63.9 and 38.1	20.2 and 1.18	Co-encapsulating two drugs, synergistic and gradient antitumor effects	86
Microparticles	The parallelized microfluidic step emulsification device	Basic fibroblast growth factor (bFGF)	76	/	/	/	High-throughput, monodisperse, and controlled release system	162
Microspheres (droplet microfluidics)	CFM	DOX	158–470	/	/	/	Controllable size, elasticity, swelling ratio, and drug-loading capacity	139
Gelatin methacryloyl microgel	CFM	Dexamethasone sodium phosphate	≈ 31	/	≈ 90	25–35	Finely tunable size	85
Microsphere	Three-phase flow-focusing mixer	Bicalutamide	51.33	4.43	/	/	Uniform size and sustained release without any initial burst release	163
Microcrystals	Y-type and S-type	Dolutegravir sodium	≈ 10	/	/	/	Lower risks of channel blockage, small particle size, and narrow crystal size distribution	164
Microsphere	CFM	Rhodamine B	56	4.91	/	/	Finely tune the drug release	165
Microparticles	CFM	Amoxicillin	220 ± 17	/	/	/	Uniform size distribution, adjustable diameter	166
Microsphere	Micromixer with a prismatic pillar array and flow-focusing droplet generator	Curcumin	≈ 40	30	10	/	Controllable porosity, higher EE, and drug-loading capacity	167
Microspheres	CFM	Paclitaxel	60 ± 2	/	/	6	Tunable size and shell thickness	130
Microspheres	CFM	DOX	95.9 ± 3.4	/	89.7–95.9	15.8 ± 0.2	Strong ability to inhibit tumor growth	168

Abbreviations: CFM, capillary-based coaxial-flow mixer; CV, the coefficient of variation; DDSs, drug delivery systems; DL, drug loading; DOX, doxorubicin hydrochloride; EE, encapsulation efficiency.

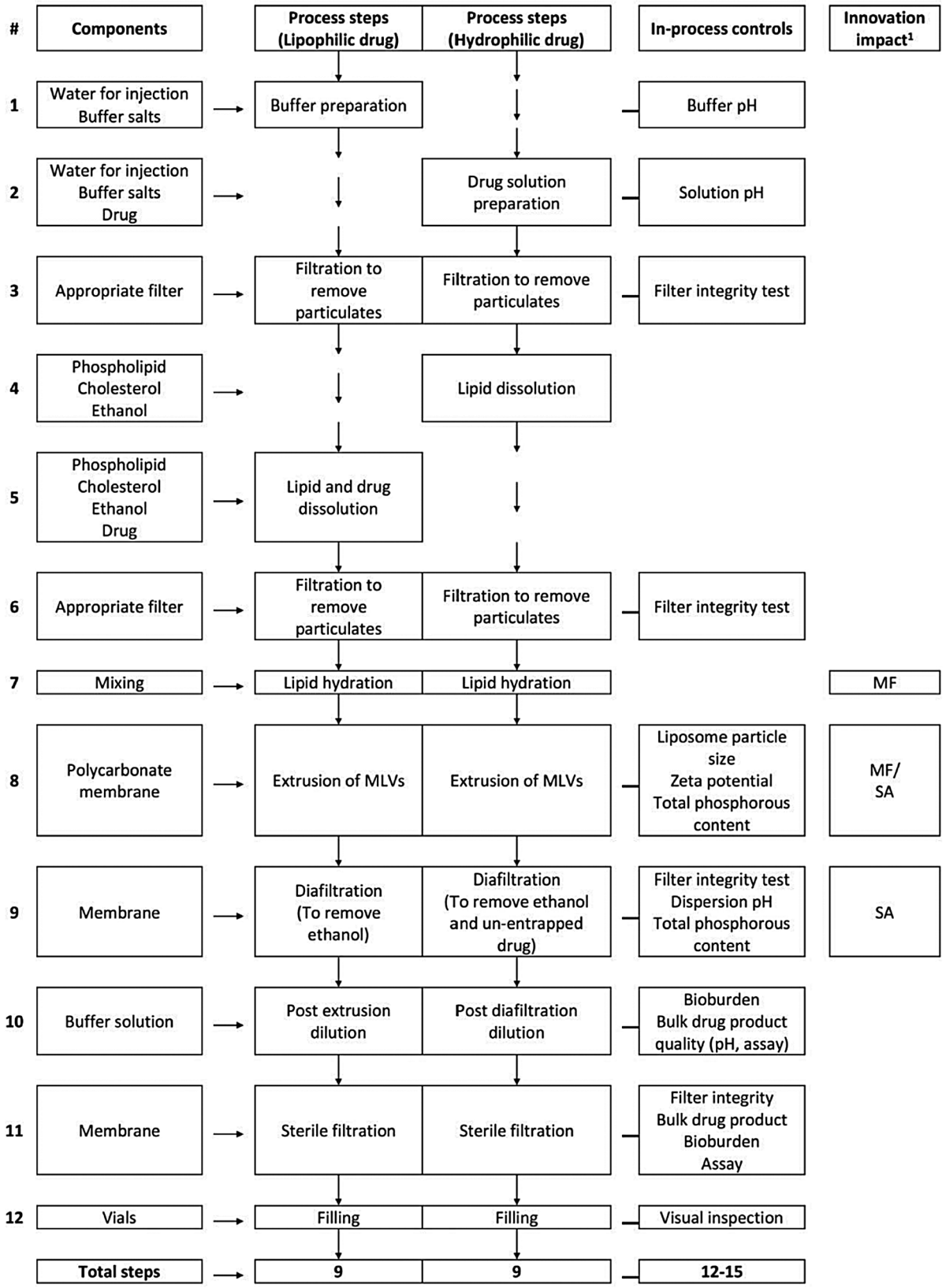

Fig. 22 A typical large-scale liposome production process. MF is microfluidics technology and SA is self-assembled vesicular DDSs. The image is reproduced with permission from Ref. [18], copyright 2020 Elsevier.

NPs[181]), such as HFF device,[42] multistage microfluidic chip devices,[128] microfluidic devices based on glass capillaries,[182] TrM,[147] and three-inlet microfluidic chip devices.[183]

Zeng et al combined HFF with electrospray to fabricate PLGA/DPPG (1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol) hybrid NPs. In their study, a mixed solution of DPPG and PLGA was transferred into the microchannel, and subsequently, DPPG spontaneously covered the PLGA surface through electrostatic repulsion, forming an outer shell to enhance the stability of the hybrid NPs.[42]

Lipid–polymer hybrid NPs based on microfluidic technology also exhibit prominent advantages in delivering nucleic acid-based drugs.[181,184,185] Wei et al developed a special lipid/polymer hybrid NP-encapsulated siRNA. Unlike traditional methods, the microfluidic technology offers enhanced protection for siRNA enveloped within the core, exhibiting superior circulation time. Additionally, hybrid NPs based on this microfluidic approach demonstrated a significant down-regulation of corresponding gene and protein expression and a notable inhibitory effect on tumor growth.[181]

Biomimetic Nanoparticles

Drug-loaded NPs, "camouflaged" with the natural biomembrane of cells or exosomes, exhibit reduced immune clearance and enhanced targeting, showcasing significant potential in drug delivery.[186,187] Currently, biomimetic NPs primarily rely on active mixing systems. In recent years, ultrasonic microfluidics[32] and microfluidic electroporation[40,188] have been developed to fabricate biomimetic NPs.

Liu et al utilized an ultrasonic microfluidic method to prepare exosome membrane-coated PLGA NPs. The first stage involved an HFF in an ultrasonic bath to form monodispersed PLGA NPs. In the second stage, a double helical channel was employed, extending the residence time to 30 milliseconds, facilitating effective rupture of the exosome membrane under strong ultrasonic stress (≈100 KPa). Simultaneously, the exosome membrane reassembled on the outside of the PLGA NPs. Utilizing this microfluidic approach, the exosome membrane achieved an EE exceeding 90%, with its surface proteins remaining intact and correctly oriented. Ultimately, the circulation half-life increased sevenfold compared with conventional lipid PLGA NPs.[32]

An alternative approach to creating a membrane mimicking the natural one involves integrating lipid nanostructures with molecules found in mammalian membranes or extracellular vesicles. Molinaro et al employed a microfluidic method to incorporate leukocyte membrane proteins composed of DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine), DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine), and cholesterol into nanovesicles. This nanostructure exhibited biological functions similar to those of the donor cells.[70]

Metal–Organic Frameworks

MOFs are novel crystalline nanoporous materials developed in recent years.[189] High DL and controlled release can be achieved by adjusting the size, functionality, and geometric structure of the porous framework in metal–organic NPs. Microfluidic technology enables the continuous fabrication of MOFs with controllable morphologies.[190,191]

Balachandran et al employed an integrated multistage microchannel microfluidic platform to synthesize lymph node and tumor-targeting aptamer-modified biozeolitic imidazolate framework (BioZIF-8) in a one-step approach. ZIF-8 loaded with different molecules (doxorubicin, siRNA, and bovine serum albumin) and aptamer modification on the BioZIF-8 surface were accomplished by two stages on one chip. This strategy shortens the overall time from 15 hours (traditional approach) to approximately 10 minutes and enables the loading of various biomolecules.[192]

Hu et al utilized microfluidic strategies to incorporate natural enzymes into MOFs. The dynamic alteration in the concentration of MOF precursors within the microfluidic resulted in structural cavities within the MOFs. These cavities were instrumental in the proficient encapsulation and safeguarding of the enzymes. Compared with traditional methodologies, the enzyme–MOF hybrids synthesized via this method exhibited enhanced enzymatic activity.[191] Consequently, it is plausible to anticipate that this innovative modality offers substantial potential in the encapsulation and conveyance of protein-centric therapeutics.

Inorganic Nanoparticles

Inorganic NPs generally feature a larger specific surface area than organic NPs.[193,194] Many inorganic NPs exhibit unique optical, photothermal, and magnetic characteristics, rendering them well-suited for therapeutic applications, hyperthermia, and magnetically induced tumor targeting. These distinct characteristics position inorganic NPs as promising candidates for drug delivery.

Segmented droplet microfluidic techniques are predominantly employed in producing inorganic NPs, resulting in finer particle sizes.

Hao et al employed two spiral laminar flow microreactors to synthesize triangular silver core-silica shell nanocomposites with tunable shell thicknesses. This approach can sequentially combine individual reaction processes (such as nucleation, growth, and coating) within a single system. The internalization efficiency of triangular particles by PANC-1 (human pancreatic cancer cells) and MCF-7 (human breast cancer cells) was higher than that of spherical particles. This discovery unveils a new direction in rationally designing functional nanostructures to enhance their biological functionalities.[195]

Microfluidic technology is acknowledged as an emerging field for synthesizing metal NPs. To date, researchers have used multistage microfluidic techniques to prepare Cu NPs,[196] Pd NPs based on the T-type method,[197] and Gd NPs.[198]

Bemetz et al employed a 3D HFF device to produce magnetic iron oxide NPs, eliminating channel fouling and demonstrating precise control over diameter.[199] Furthermore, microfluidic technology has been applied to generate various metal oxide NPs,[157] such as TiO_2 NPs[200] and CeO_2 NPs.[201]

Schemberg et al have synthesized superparamagnetic iron oxide NPs using three distinct systems: batch system, continuous-flow regime (T-type), and segmented-flow regime (T-type). The findings indicate that the continuous-flow system can produce NPs with a smaller size and better stability

than the segmented-flow system. Both flow systems enhance the characteristics of the NPs relative to the batch system.[157]

Microsphere

Microfluidic technology offers notable advantages in rapidly and efficiently preparing single-phase and multiple emulsions.[202] As a result, it is recognized as an innovative technique for creating highly controllable and monodisperse microspheres.[130,165,203,204]

Researchers typically employ multiphase HFF[163] and CFM[166] for microsphere fabrication. Su et al employed multiphase HFF to prepare sustained-release PLGA microspheres, showcasing a sustained-release profile without an initial burst release.[163]

In another study, He et al used a droplet-based microfluidic device to manufacture monodisperse PLGA magnetic arterial chemoembolization microspheres loaded with paclitaxel. These microspheres, characterized by tunable size and shell thickness, displayed delayed and smooth release kinetics without burst release behavior. Moreover, they exhibited superior magnetic responsiveness.[130]

Giant Unilamellar Vesicles

Giant unilamellar vesicles (GUVs), characterized by diameters exceeding 1 μm and typically ranging between 10 and 30 μm, have analogous size to biological cells, making them effective individual microliter reactors. Using microfluidic technology for GUV production represents a groundbreaking paradigm in advancing artificial cells. Employing this method to create GUVs provides several advantages, including monodispersity, high-throughput production, elevated encapsulation efficiency, and the ability to form asymmetric lipid bilayers mirroring those commonly observed in biological cell membranes.[205]

Droplet-based microfluidic technology has the advantage of precisely controlling chamber dimensions and structures.[206] Various microfluidic channels have been used for the preparation of GUVs, including T-type,[207,208] HFF,[209] and CFM.[210,211]

Weiss et al successfully constructed monodisperse cell-like GUV chambers loaded with lipid membranes and cytoskeletal proteins by integrating microfluidic technology with pico-injection techniques. The resulting GUVs closely resemble the physiological environment.[207]

Yandrapalli et al introduced a high-throughput microfluidic technique based on a water-in-oil-in-water (W/O/W) approach, enabling controlled preparation of GUVs ranging from 10 to 130 μm. These designed cell-like vesicles effectively encapsulated various substances, including pDNA (96%), small unilamellar vesicles with a size of 50 nm (94%), and fibroblasts (75%).[212]

Michelon et al utilized a capillary-based CFM to fabricate monodisperse and stable W/O/W emulsion droplets, subsequently generating GUVs for delivering active drugs.[211] In summary, the high-throughput fabrication of biomimetic artificial cells through microfluidic technology is a feasible and promising avenue.

Others

Incorporating the aforementioned vehicles into microgels enhances the development of gel-based DDSs with superior designs. Notably, integrating liposomes into gels has emerged as an attractive strategy for minimizing adverse effects in drug delivery. Moreover, incorporating liposomes into the polymer matrix within gels shows significant potential in mitigating burst release effects attributed to lipid instability.[213,214] Microfluidic technology has been introduced to encapsulate proteins,[215] NPs,[121] viral carriers,[216] and nonviral carriers[217] within microgels.

Additionally, microfluidic technology can also be employed for the preparation of cubosomes.[218] Kim et al employed self-assembly on an SHM to fabricate a cubosome with a complex array of interlocking membranes. This cubosome was utilized for encapsulating a substantial quantity of siRNA. Additionally, the inherent fusion properties of cubosomes facilitated rapid escape from endosomes, ensuring efficient siRNA delivery into HeLa cells.[218]

Industrialization: Current Status and Prospects

As a novel paradigm for constructing micro/nanoparticle DDSs, microfluidic technology has undergone significant exploration in the literature. However, its full potential in the pharmaceutical industry remains to be fully realized, and several factors contribute to this delay. (1) Limitations in translation: the interdisciplinary nature of the field requires collaboration among biologists, microfluidics engineers, mechanical engineers, and other experts, making successful translation challenging yet essential. (2) Clogging and contamination: the small dimensions of microchannels can lead to clogging and the generation of impurities, affecting mixing efficiency and scalability. Managing and cleaning these contaminants are critical considerations. (3) Infrastructure improvement: effective microfluidic systems for micro/nanoparticle production often require enhancements in facility hardware. Meeting compliance with GMP and regulatory standards poses a significant bottleneck in integrating new technologies into producing and applying precision pharmaceutical formulations.

Fortunately, in 2020, FDA and European Medicines Agency approvals for COVID-19 vaccines based on LNPs acted as catalysts for expanding the microfluidic technique in the pharmaceutical industry. The market demand has accelerated the adoption of microfluidic technology in pharmaceutical processes by several manufacturing companies. Noteworthy international manufacturers including Coring, Ehrfeld, Dolomite, Knauer, PNI, and Chinese pharmaceutical companies focusing on DDSs are actively intensifying collaborations with mainstream microfluidic equipment suppliers such as Micro & Nano Technologies (Shanghai) Inc., Suzhou Aitesen Pharmaceutical Equipment Co. Ltd., and Lumiere Tech Ltd. (LumTech).

Conclusion

In conclusion, microfluidic technology exhibits the capability to precisely control various attributes such as size, morphology, structure, stiffness, surface properties, and elasticity of micro/nanoparticle DDSs, including liposomes, micro/nanocrystals, NPs, microspheres, GUVs, and others. As microfluidic technology advances more functionalized micro/nanoparticles as DDSs with novel structures and specific properties will emerge. This progression opens up new possibilities for personalized and precise disease diagnosis and treatment strategies.

Furthermore, a new trend is emerging in the field of drug delivery based on microfluidic technology as the transformation of laboratory research into industrial applications gains momentum. It is anticipated that pharmacists, leveraging disease characteristics, will significantly enhance their capacity to rapidly design and fabricate specific, innovative, and functionalized micro/nano formulation products. In summary, whether viewed through the lens of new trends in continuous manufacturing, personalized precision therapy, or considering the concepts of process control technology and process analytical technology advocated in current regulatory policies, the utilization of microfluidic technology is poised to create unprecedented opportunities for precise drug delivery.

Funding

This work was supported by the Science and Technology Commission of Shanghai Municipality (Grant No. 23S41900200).

Conflict of Interest

None declared.

Acknowledgments

We sincerely acknowledge Yulin Ye and other members from Suzhou Aitesen Pharmaceutical Equipment Co., Ltd. for their suggestions in the prospects section of this work.

Reference

1 Ramezankhani R, Solhi R, Chai YC, Vosough M, Verfaillie C. Organoid and microfluidics-based platforms for drug screening in COVID-19. Drug Discov Today 2022;27(04):1062–1076
2 Maurya R, Gohil N, Bhattacharjee G, Alzahrani KJ, Ramakrishna S, Singh V. Microfluidics device for drug discovery, screening and delivery. Prog Mol Biol Transl Sci 2022;187(01):335–346
3 Fabozzi A, Della Sala F, di Gennaro M, et al. Design of functional nanoparticles by microfluidic platforms as advanced drug delivery systems for cancer therapy. Lab Chip 2023;23(05):1389–1409
4 Kashaninejad N, Moradi E, Moghadas H. Micro/nanofluidic devices for drug delivery. Prog Mol Biol Transl Sci 2022;187 (01):9–39
5 Mitchell MJ, Billingsley MM, Haley RM, Wechsler ME, Peppas NA, Langer R. Engineering precision nanoparticles for drug delivery. Nat Rev Drug Discov 2021;20(02):101–124
6 Maged A, Abdelbaset R, Mahmoud AA, Elkasabgy NA. Merits and advances of microfluidics in the pharmaceutical field: design technologies and future prospects. Drug Deliv 2022;29(01):1549–1570
7 Maeki M, Uno S, Niwa A, Okada Y, Tokeshi M. Microfluidic technologies and devices for lipid nanoparticle-based RNA delivery. J Control Release 2022;344:80–96
8 Liu Y, Yang G, Hui Y, Ranaweera S, Zhao CX. Microfluidic nanoparticles for drug delivery. Small 2022;18(36):e2106580
9 Zimmermann R, Leal BBJ, Braghirolli DI, Pranke P. Production of nanostructured systems: main and innovative techniques. Drug Discov Today 2023;28(02):103454
10 Torkay G, Oeztuerk AB. A stem cell and tissue engineering perspective on microfluidic chips. J Polytechnic 2024; published online ahead of print on March, 2024. DOI: 10.2339/politeknik.1094010
11 Kopp MRG, Linsenmeier M, Hettich B, et al. microfluidic shrinking droplet concentrator for analyte detection and phase separation of protein solutions. Anal Chem 2020;92(08):5803–5812
12 Utharala R, Tseng Q, Furlong EEM, Merten CAA. A versatile, low-cost, multiway microfluidic sorter for droplets, cells, and embryos. Anal Chem 2018;90(10):5982–5988
13 Yu F, Choudhury D. Microfluidic bioprinting for organ-on-a-chip models. Drug Discov Today 2019;24(06):1248–1257
14 Wu H, Shi S, Liu Y, et al. Recent progress of organ-on-a-chip towards cardiovascular diseases: advanced design, fabrication, and applications. Biofabrication 2023;15(04):042001
15 Marre S, Jensen KF. Synthesis of micro and nanostructures in microfluidic systems. Chem Soc Rev 2010;39(03):1183–1202
16 Yaghmur A, Ghazal A, Ghazal R, Dimaki M, Svendsen WE. A hydrodynamic flow focusing microfluidic device for the continuous production of hexosomes based on docosahexaenoic acid monoglyceride. Phys Chem Chem Phys 2019;21(24): 13005–13013
17 Zhang L, Chen Q, Ma Y, Sun J. Microfluidic methods for fabrication and engineering of nanoparticle drug delivery systems. ACS Appl Bio Mater 2020;3(01):107–120
18 Shah S, Dhawan V, Holm R, Nagarsenker MS, Perrie Y. Liposomes: advancements and innovation in the manufacturing process. Adv Drug Deliv Rev 2020;154–155:102–122
19 Aboelela SS, Ibrahim M, Badruddoza AZM, Tran V, Ferri JK, Roper TD. Encapsulation of a highly hydrophilic drug in polymeric particles: a comparative study of batch and microfluidic processes. Int J Pharm 2021;606:120906
20 Chung MT, Kurabayashi K, Cai D. Single-cell RT-LAMP mRNA detection by integrated droplet sorting and merging. Lab Chip 2019;19(14):2425–2434
21 Shrimal P, Jadeja G, Patel S. A review on novel methodologies for drug nanoparticle preparation: microfluidic approach. Chem Eng Res Des 2020;153:728–756
22 Abstiens K, Goepferich AM. Microfluidic manufacturing improves polydispersity of multicomponent polymeric nanoparticles. J Drug Deliv Sci Technol 2019;49:433–439
23 Amoyav B, Benny O. Controlled and tunable polymer particles' production using a single microfluidic device. Appl Nanosci 2018;8(04):905–914
24 Yuan Z, Liu J, Yang Y, He J. Recent advances and application prospects of microfluidic technology for preparation of nanoformulations [in Chinese]. Zhongguo Yiyao Gongye Zazhi 2021; 52(04):440–450
25 Valencia PM, Farokhzad OC, Karnik R, Langer R. Microfluidic technologies for accelerating the clinical translation of nanoparticles. Nat Nanotechnol 2012;7(10):623–629
26 Ding Y, Howes PD, deMello AJ. Recent advances in droplet microfluidics. Anal Chem 2020;92(01):132–149
27 Tian F, Cai L, Liu C, Sun J. Microfluidic technologies for nanoparticle formation. Lab Chip 2022;22(03):512–529
28 Carvalho BG, Ceccato BT, Michelon M, Han SW, de la Torre LG. Advanced microfluidic technologies for lipid nano-microsystems from synthesis to biological application. Pharmaceutics 2022;14(01):141

29 Gonidec M, Puigmartí-Luis J. Continuous- versus segmented-flow microfluidic synthesis in materials science. Crystals (Basel) 2018;9(01):12
30 Bayareh M, Ashani MN, Usefian A. Active and passive micromixers: a comprehensive review. Chem Eng Process 2020;147:107771
31 An Le NH, Deng H, Devendran C, et al. Ultrafast star-shaped acoustic micromixer for high throughput nanoparticle synthesis. Lab Chip 2020;20(03):582–591
32 Liu C, Zhang W, Li Y, et al. Microfluidic sonication to assemble exosome membrane-coated nanoparticles for immune evasion-mediated targeting. Nano Lett 2019;19(11):7836–7844
33 Shrimal P, Jadeja G, Patel S. Ultrasonic enhanced emulsification process in 3D printed microfluidic device to encapsulate active pharmaceutical ingredients. Int J Pharm 2022;620:121754
34 Huang PH, Zhao S, Bachman H, et al. Acoustofluidic synthesis of particulate nanomaterials. Adv Sci (Weinh) 2019;6(19):1900913
35 Le NHA, Van Phan H, Yu J, Chan HK, Neild A, Alan T. Acoustically enhanced microfluidic mixer to synthesize highly uniform nanodrugs without the addition of stabilizers. Int J Nanomedicine 2018;13:1353–1359
36 Pourabed A, Brenker J, Younas T, He L, Alan T. A Lotus shaped acoustofluidic mixer: high throughput homogenisation of liquids in 2 ms using hydrodynamically coupled resonators. Ultrason Sonochem 2022;83:105936
37 Endaylalu SA, Tien WH. A numerical investigation of the mixing performance in a Y-junction microchannel induced by acoustic streaming. Micromachines (Basel) 2022;13(02):338
38 Pourabed A, Younas T, Liu C, Shanbhag BK, He L, Alan T. High throughput acoustic microfluidic mixer controls self-assembly of protein nanoparticles with tuneable sizes. J Colloid Interface Sci 2021;585:229–236
39 Bolze H, Riewe J, Bunjes H, Dietzel A, Burg TP. Continuous production of lipid nanoparticles by ultrasound-assisted microfluidic antisolvent precipitation. Chem Eng Technol 2021;44 (09):1641–1650
40 Rao L, Cai B, Bu LL, et al. Microfluidic electroporation-facilitated synthesis of erythrocyte membrane-coated magnetic nanoparticles for enhanced imaging-guided cancer therapy. ACS Nano 2017;11(04):3496–3505
41 Hou L, Ren Y, Jia Y, et al. Continuously electrotriggered core coalescence of double-emulsion drops for microreactions. ACS Appl Mater Interfaces 2017;9(14):12282–12289
42 Zeng W, Guo P, Jiang P, Liu W, Hong T, Chen C. Combination of microfluidic chip and electrostatic atomization for the preparation of drug-loaded core-shell nanoparticles. Nanotechnology 2020;31(14):145301
43 Al-Hetlani E, Amin MO. Continuous magnetic droplets and microfluidics: generation, manipulation, synthesis and detection. Mikrochim Acta 2019;186(02):55
44 Kahkeshani S, Di Carlo D. Drop formation using ferrofluids driven magnetically in a step emulsification device. Lab Chip 2016;16 (13):2474–2480
45 Choi YH, Hwang JS, Han SH, Lee CS, Jeon SJ, Kim SH. Thermoresponsive microcapsules with tunable molecular permeability for controlled encapsulation and release. Adv Funct Mater 2021; 31(24):2100782
46 Paulsen KS, Deng Y, Chung AJ. DIY 3D microparticle generation from next generation optofluidic fabrication. Adv Sci (Weinh) 2018;5(07):1800252
47 Liu Z, Yang M, Yao W, Wang T, Chen G. Microfluidic ultrasonic cavitation enables versatile and scalable synthesis of monodisperse nanoparticles for biomedical application. Chem Eng Sci 2023;280:119052
48 Kotnik T, Rems L, Tarek M, Miklavčič D Membrane electroporation and electropermeabilization: mechanisms and models. Annu Rev Biophys 2019;48:63–91
49 Lin L, Wang M, Peng X, et al. Opto-thermoelectric nanotweezers. Nat Photonics 2018;12(04):195–201
50 Tian F, Han Z, Deng J, Liu C, Sun J. Thermomicrofluidics for biosensing applications. VIEW 2021;2:20200148
51 de Hemptinne A, Gelin P, Ziemecka I, De Malsche W. Microfluidic device for multilayer coating of magnetic microparticles. Powder Technol 2023;416:118223
52 Zheng H, Zhao C, Lu Y, et al. Celastrol-encapsulated microspheres prepared by microfluidic electrospray for alleviating inflammatory pain. Biomater Adv 2023;149:213398
53 Siavashy S, Soltani M, Ghorbani-Bidkorbeh F, et al. Microfluidic platform for synthesis and optimization of chitosan-coated magnetic nanoparticles in cisplatin delivery. Carbohydr Polym 2021;265:118027
54 Shepherd SJ, Issadore D, Mitchell MJ. Microfluidic formulation of nanoparticles for biomedical applications. Biomaterials 2021; 274:120826
55 Yang AS, Chuang FC, Chen CK, et al. A high-performance micromixer using three-dimensional Tesla structures for bio-applications. Chem Eng J 2015;263:444–451
56 Desai D, Guerrero YA, Balachandran V, et al. Towards a microfluidics platform for the continuous manufacture of organic and inorganic nanoparticles. Nanomedicine 2021;35:102402
57 Webb C, Forbes N, Roces CB, 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
58 Ma Z, Tong H, Lin S, et al. Scalable synthesis of lipid nanoparticles for nucleic acid drug delivery using an isometric channel-size enlarging strategy. Nano Res 2023;17(04):2899–2907
59 Stroock AD, Dertinger SK, Ajdari A, Mezic I, Stone HA, Whitesides GM. Chaotic mixer for microchannels. Science 2002;295 (5555):647–651
60 Cheung CCL, Al-Jamal WT. Sterically stabilized liposomes production using staggered herringbone micromixer: effect of lipid composition and PEG-lipid content. Int J Pharm 2019; 566:687–696
61 Forbes N, Hussain MT, Briuglia ML, 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
62 Roces CB, Lou G, Jain N, et al. Manufacturing considerations for the development of lipid nanoparticles using microfluidics. Pharmaceutics 2020;12(11):1095
63 Sago CD, Lokugamage MP, Paunovska K, et al. High-throughput *in vivo* screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing. Proc Natl Acad Sci U S A 2018; 115(42):E9944–E9952
64 Lou G, Anderluzzi G, Schmidt ST, et al. Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: the impact of cationic lipid selection. J Control Release 2020;325:370–379
65 Evers MJW, Kulkarni JA, van der Meel R, Cullis PR, Vader P, Schiffelers RM. State-of-the-art design and rapid-mixing production techniques of lipid nanoparticles for nucleic acid delivery. Small Methods 2018;2:1700375
66 Dimov N, Kastner E, Hussain M, Perrie Y, Szita N. Formation and purification of tailored liposomes for drug delivery using a module-based micro continuous-flow system. Sci Rep 2017;7 (01):12045
67 Hamano N, Böttger R, Lee SE, et al. Robust microfluidic technology and new lipid composition for fabrication of curcumin-loaded liposomes: effect on the anticancer activity and safety of cisplatin. Mol Pharm 2019;16(09):3957–3967
68 Shah VM, Nguyen DX, Patel P, et al. Liposomes produced by microfluidics and extrusion: a comparison for scale-up purposes. Nanomedicine 2019;18:146–156

69 Hussain MT, Tiboni M, Perrie Y, Casettari L. Microfluidic production of protein loaded chimeric stealth liposomes. Int J Pharm 2020;590:119955

70 Molinaro R, Evangelopoulos M, Hoffman JR, et al. Design and development of biomimetic nanovesicles using a microfluidic approach. Adv Mater 2018;30(15):e1702749

71 Morikawa Y, Tagami T, Hoshikawa A, Ozeki T. The use of an efficient microfluidic mixing system for generating stabilized polymeric nanoparticles for controlled drug release. Biol Pharm Bull 2018;41(06):899–907

72 Leung AWY, Amador C, Wang LC, Mody UV, Bally MB. What drives innovation: the Canadian touch on liposomal therapeutics. Pharmaceutics 2019;11(03):124

73 Van de Vyver T, Bogaert B, De Backer L, et al. Cationic amphiphilic drugs boost the lysosomal escape of small nucleic acid therapeutics in a nanocarrier-dependent manner. ACS Nano 2020;14 (04):4774–4791

74 Vogler J, Böttger R, Al Fayez N, et al. Altering the intra-liver distribution of phospholipid-free small unilamellar vesicles using temperature-dependent size-tunability. J Control Release 2021;333:151–161

75 Vu HTH, Streck S, Hook SM, McDowell A. Utilization of microfluidics for the preparation of polymeric nanoparticles for the antioxidant rutin: a comparison with bulk production. Pharm Nanotechnol 2019;7(06):469–483

76 Hama B, Mahajan G, Fodor PS, Kaufman M, Kothapalli CR. Evolution of mixing in a microfluidic reverse-staggered herringbone micromixer. Microfluid Nanofluidics 2018;22:54

77 Kimura N, Maeki M, Sato Y, et al. Development of the iLiNP device: fine tuning the lipid nanoparticle size within 10 nm for drug delivery. ACS Omega 2018;3(05):5044–5051

78 Kimura N, Maeki M, Sato Y, 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

79 Kimura N, Maeki M, Ishida A, Tani H, Tokeshi M. One-step production using a microfluidic device of highly biocompatible size-controlled noncationic exosome-like nanoparticles for RNA delivery. ACS Appl Bio Mater 2021;4(02):1783–1793

80 Kimura N, Maeki M, Sasaki K, et al. Three-dimensional, symmetrically assembled microfluidic device for lipid nanoparticle production. RSC Advances 2021;11(03):1430–1439

81 Suzuki Y, Onuma H, Sato R, et al. Lipid nanoparticles loaded with ribonucleoprotein-oligonucleotide complexes synthesized using a microfluidic device exhibit robust genome editing and hepatitis B virus inhibition. J Control Release 2021;330:61–71

82 Matsuura-Sawada Y, Maeki M, Nishioka T, et al. Microfluidic device-enabled mass production of lipid-based nanoparticles for applications in nanomedicine and cosmetics. ACS Appl Nano Mater 2022;5(06):7867–7876

83 Maeki M, Okada Y, Uno S, et al. Mass production system for RNA-loaded lipid nanoparticles using piling up microfluidic devices. Appl Mater Today 2023;31:101754

84 Matsuura-Sawada Y, Maeki M, Uno S, Wada K, Tokeshi M. Controlling lamellarity and physicochemical properties of liposomes prepared using a microfluidic device. Biomater Sci 2023; 11(07):2419–2426

85 Wang J, Wang C, Wang Q, et al. Microfluidic preparation of gelatin methacryloyl microgels as local drug delivery vehicles for hearing loss therapy. ACS Appl Mater Interfaces 2022;14(41): 46212–46223

86 Zhong J, Zhang Q, Kuang G, Xia J, Shang L. Multicomponent microspheres with spatiotemporal drug release for post-surgical liver cancer treatment and liver regeneration. Chem Eng J 2023; 455:140585

87 Hu H, Yang C, Li M, Shao D, Mao HQ, Leong KW. Flash technology-based self-assembly in nanoformulation: fabrication to biomedical applications. Mater Today 2021;42:99–116

88 Bovone G, Guzzi EA, Tibbitt MW. Flow-based reactor design for the continuous production of polymeric nanoparticles. AIChE J 2019;65(12):e16840

89 Lim JM, Swami A, Gilson LM, et al. Ultra-high throughput synthesis of nanoparticles with homogeneous size distribution using a coaxial turbulent jet mixer. ACS Nano 2014;8(06): 6056–6065

90 Markwalter CE, Prud'homme RK. Design of a small-scale multi-inlet vortex mixer for scalable nanoparticle production and application to the encapsulation of biologics by inverse flash nanoprecipitation. J Pharm Sci 2018;107(09):2465–2471

91 Di D, Qu X, Liu C, Fang L, Quan P. Continuous production of celecoxib nanoparticles using a three-dimensional-coaxial-flow microfluidic platform. Int J Pharm 2019;572:118831

92 Tomeh MA, Mansor MH, Hadianamrei R, Sun W, Zhao X. Optimization of large-scale manufacturing of biopolymeric and lipid nanoparticles using microfluidic swirl mixers. Int J Pharm 2022; 620:121762–121762

93 Xu R, Tomeh MA, Ye S, et al. Novel microfluidic swirl mixers for scalable formulation of curcumin loaded liposomes for cancer therapy. Int J Pharm 2022;622:121857

94 (LumTech) LTL Homepage. Accessed April 5, 2024 at: http://www.lumtech.com.cn

95 Han T, Zhang L, Xu H, Xuan J. Factory-on-chip: modularised microfluidic reactors for continuous mass production of functional materials. Chem Eng J 2017;326:765–773

96 Jeong HH, Issadore D, Lee D. Recent developments in scale-up of microfluidic emulsion generation via parallelization. Korean J Chem Eng 2016;33(06):1757–1766

97 DeMello AJ. Control and detection of chemical reactions in microfluidic systems. Nature 2006;442(7101):394–402

98 Karnik R, Gu FX, Basto P, et al. Microfluidic synthesis of organic nanoparticles. U.S. Patent 9381477. July, 2016

99 Carugo D, Bottaro E, Owen J, Stride E, Nastruzzi C. Liposome production by microfluidics: potential and limiting factors. Sci Rep 2016;6:25876

100 Lim JM, Bertrand N, Valencia PM, et al. Parallel microfluidic synthesis of size-tunable polymeric nanoparticles using 3D flow focusing towards *in vivo* study. Nanomedicine 2014;10(02):401–409

101 Min KI, Im DJ, Lee HJ, Kim DP. Three-dimensional flash flow microreactor for scale-up production of monodisperse PEG-PLGA nanoparticles. Lab Chip 2014;14(20):3987–3992

102 Shepherd SJ, Warzecha CC, Yadavali S, et al. Scalable mRNA and siRNA lipid nanoparticle production using a parallelized microfluidic device. Nano Lett 2021;21(13):5671–5680

103 Okada Y, Maeki M, Sato Y, et al. Development of an integrated glass-based microfluidic system for mass production of RNA-loaded lipid nanoparticles. Paper presented at: 25th International Conference on Miniaturized Systems for Chemistry and life Sciences; October 10-14, 2021; CA, US

104 Belliveau NM, Huft J, Lin PJ, et al. Microfluidic synthesis of highly potent limit-size lipid nanoparticles for *in vivo* delivery of siRNA. Mol Ther Nucleic Acids 2012;1(08):e37

105 Ou Z, Zhang Q, Hu S, Dang Y. Microfluidic system for particle manipulation based on swirl. Appl Phys Lett 2023;123(01): 013508

106 Costa AP, Xu X, Khan MA, Burgess DJ. Liposome formation using a coaxial turbulent jet in co-flow. Pharm Res 2016;33(02):404–416

107 Toth MJ, Kim T, Kim Y. Robust manufacturing of lipid-polymer nanoparticles through feedback control of parallelized swirling microvortices. Lab Chip 2017;17(16):2805–2813

108 Sealy A. Manufacturing moonshot: how Pfizer makes its millions of Covid-19 vaccine doses. Accessed April 2, 2021 at: https://edition.cnn.com/2021/03/31/health/pfizer-vaccine-manufacturing/index.html

109 van der Woerd M, Ferree D, Pusey M. The promise of macromolecular crystallization in microfluidic chips. J Struct Biol 2003; 142(01):180–187

110 Khizar S, Zine N, Errachid A, Jaffrezic-Renault N, Elaissari A. Microfluidic-based nanoparticle synthesis and their potential applications. Electrophoresis 2022;43(7–8):819–838

111 Di J, Gao X, Du Y, Zhang H, Gao J, Zheng A. Size, shape, charge and "stealthy" surface: carrier properties affect the drug circulation time *in vivo*. Asian J Pharm Sci 2021;16(04):444–458

112 Andar AU, Hood RR, Vreeland WN, Devoe DL, Swaan PW. Microfluidic preparation of liposomes to determine particle size influence on cellular uptake mechanisms. Pharm Res 2014;31 (02):401–413

113 Lee JS, Hwang SY, Lee EK. Imaging-based analysis of liposome internalization to macrophage cells: effects of liposome size and surface modification with PEG moiety. Colloids Surf B Biointerfaces 2015;136:786–790

114 Feng Q, Zhang L, Liu C, et al. Microfluidic based high throughput synthesis of lipid-polymer hybrid nanoparticles with tunable diameters. Biomicrofluidics 2015;9(05):052604

115 Chen S, Tam YYC, Lin PJC, Sung MMH, Tam YK, Cullis PR. Influence of particle size on the *in vivo* potency of lipid nanoparticle formulations of siRNA. J Control Release 2016;235:236–244

116 Liu D, Zhang H, Fontana F, Hirvonen JT, Santos HA. Microfluidic-assisted fabrication of carriers for controlled drug delivery. Lab Chip 2017;17(11):1856–1883

117 Maeki M, Kimura N, Sato Y, Harashima H, Tokeshi M. Advances in microfluidics for lipid nanoparticles and extracellular vesicles and applications in drug delivery systems. Adv Drug Deliv Rev 2018;128:84–100

118 Okuda K, Sato Y, Iwakawa K, et al. On the size-regulation of RNA-loaded lipid nanoparticles synthesized by microfluidic device. J Control Release 2022;348:648–659

119 Gimondi S, Guimarães CF, Vieira SF, et al. Microfluidic mixing system for precise PLGA-PEG nanoparticles size control. Nanomedicine 2022;40:102482

120 Ahmed H, Stokke BT. Fabrication of monodisperse alginate microgel beads by microfluidic picoinjection: a chelate free approach. Lab Chip 2021;21(11):2232–2243

121 de Carvalho BG, Taketa TB, Garcia BBM, Han SW, de la Torre LG. Hybrid microgels produced via droplet microfluidics for sustainable delivery of hydrophobic and hydrophilic model nanocarriers. Mater Sci Eng C 2021;118:111467

122 Balbino TA, Aoki NT, Gasperini AAM, et al. Continuous flow production of cationic liposomes at high lipid concentration in microfluidic devices for gene delivery applications. Chem Eng J 2013;226:423–433

123 Akhter KF, Mumin MA, Lui EMK, Charpentier PA. Immunoengineering with ginseng polysaccharide nanobiomaterials through oral administration in mice. ACS Biomater Sci Eng 2019;5(06): 2916–2925

124 Al-Ahmady ZS, Donno R, Gennari A, et al. Enhanced intraliposomal metallic nanoparticle payload capacity using microfluidic-assisted self-assembly. Langmuir 2019;35(41):13318–13331

125 Sedighi M, Sieber S, Rahimi F, et al. Rapid optimization of liposome characteristics using a combined microfluidics and design-of-experiment approach. Drug Deliv Transl Res 2019;9 (01):404–413

126 Sommonte F, Arduino I, Iacobazzi RM, et al. Microfluidic assembly of "Turtle-Like" shaped solid lipid nanoparticles for lysozyme delivery. Int J Pharm 2023;631:122479

127 Lengert EV, Trushina DB, Soldatov M, Ermakov AV. Microfluidic synthesis and analysis of bioinspired structures based on $CaCO_3$ for potential applications as drug delivery carriers. Pharmaceutics 2022;14(01):139

128 Chen Y, Zhao D, Xiao F, et al. Microfluidics-enabled serial assembly of lipid-siRNA-sorafenib nanoparticles for synergetic hepatocellular carcinoma therapy. Adv Mater 2023;35(13): e2209672

129 Hao N, Nie Y, Zhang JXJ. Microfluidic flow synthesis of functional mesoporous silica nanofibers with tunable aspect ratios. ACS Sustain Chem& Eng 2018;6(02):1522–1526

130 He C, Zeng W, Su Y, et al. Microfluidic-based fabrication and characterization of drug-loaded PLGA magnetic microspheres with tunable shell thickness. Drug Deliv 2021;28(01):692–699

131 Hui Y, Yi X, Hou F, et al. Role of nanoparticle mechanical properties in cancer drug delivery. ACS Nano 2019;13(07):7410–7424

132 Lozano Vigario F, Nagy NA, The MH, et al. The use of a staggered herringbone micromixer for the preparation of rigid liposomal formulations allows efficient encapsulation of antigen and adjuvant. J Pharm Sci 2022;111(04):1050–1057

133 Visaveliya NR, Leishman CW, Ng K, et al. Surface wrinkling and porosity of polymer particles toward biological and biomedical applications. Adv Mater Interfaces 2017;4(24):1700929

134 Li M, Liu C, Yin J, Liu G, Chen D. Single-step synthesis of highly tunable multifunctional nanoliposomes for synergistic cancer therapy. ACS Appl Mater Interfaces 2022;14(18):21301–21309

135 Gao Z, Mansor MH, Winder N, et al. Microfluidic-assisted ZIF-silk-polydopamine nanoparticles as promising drug carriers for breast cancer therapy. Pharmaceutics 2023;15(07):1811

136 Lee JB, Zhang K, Tam YY, et al. A Glu-urea-Lys ligand-conjugated lipid nanoparticle/siRNA system inhibits androgen receptor expression *in vivo*. Mol Ther Nucleic Acids 2016;5(08):e348

137 Li Y, Lee RJ, Huang X, et al. Single-step microfluidic synthesis of transferrin-conjugated lipid nanoparticles for siRNA delivery. Nanomedicine 2017;13(02):371–381

138 Jia Z, Gong Y, Pi Y, et al. pPB peptide-mediated siRNA-loaded stable nucleic acid lipid nanoparticles on targeting therapy of hepatic fibrosis. Mol Pharm 2018;15(01):53–62

139 Yang SH, Ju XJ, Deng CF, et al. Controllable fabrication of monodisperse poly(vinyl alcohol) microspheres with droplet microfluidics for embolization. Ind Eng Chem Res 2022;61 (34):12619–12631

140 Zhu Y, Xiao W, Zhong W, et al. Study of the skin-penetration promoting effect and mechanism of combined system of curcumin liposomes prepared by microfluidic chip and skin penetrating peptides TD-1 for topical treatment of primary melanoma. Int J Pharm 2023;643:123256

141 Yu H, Dyett BP, Zhai J, Strachan JB, Drummond CJ, Conn CE. Formation of particulate lipid lyotropic liquid crystalline nanocarriers using a microfluidic platform. J Colloid Interface Sci 2023;634:279–289

142 Younis MA, Sato Y, Elewa YHA, Kon Y, Harashima H. Self-homing nanocarriers for mRNA delivery to the activated hepatic stellate cells in liver fibrosis. J Control Release 2023;353:685–698

143 Yang K, Ni M, Xu C, et al. Microfluidic one-step synthesis of a metal-organic framework for osteoarthritis therapeutic microRNAs delivery. Front Bioeng Biotechnol 2023;11:1239364

144 Wang Q, Xu W, Li Q, et al. Coaxial electrostatic spray-based preparation of localization missile liposomes on a microfluidic chip for targeted treatment of triple-negative breast cancer. Int J Pharm 2023;643:123220

145 Ahmad M, Khan S, Shah SMH, et al. Formulation and optimization of repaglinide nanoparticles using microfluidics for enhanced bioavailability and management of diabetes. Biomedicines 2023; 11(04):1064

146 Zhou JE, Sun L, Liu L, et al. Hepatic macrophage targeted siRNA lipid nanoparticles treat non-alcoholic steatohepatitis. J Control Release 2022;343:175–186

147 Santhanes D, Wilkins A, Zhang H, John Aitken R, Liang M. Microfluidic formulation of lipid/polymer hybrid nanoparticles for plasmid DNA (pDNA) delivery. Int J Pharm 2022;627:122223

148 Maeki M, Okada Y, Uno S, et al. Production of siRNA-loaded lipid nanoparticles using a microfluidic device. J Vis Exp 2022;(181): e62999

149 Xia Y, Xu R, Ye S, et al. Microfluidic formulation of curcumin-loaded multiresponsive gelatin nanoparticles for anticancer therapy. ACS Biomater Sci Eng 2023;9(06):3402–3413

150 Shen Y, Yuk SA, Kwon S, Tamam H, Yeo Y, Han B. A timescale-guided microfluidic synthesis of tannic acid-Fe^{III} network nanocapsules of hydrophobic drugs. J Control Release 2023; 357:484–497

151 Lei Y, Kilker S, Lee Y. A nozzle simulation chip toward high-throughput formation of curcumin-loaded zein nanoparticles with tunable properties. J Food Sci 2023;88(08):3524–3537

152 O'Brien Laramy MN, Costa AP, Cebrero YM, et al. Process robustness in lipid nanoparticle production: a comparison of microfluidic and turbulent jet mixing. Mol Pharm 2023;20(08): 4285–4296

153 Greco A, Gabold B, Chen S, et al. Microfluidic mixing as platform technology for production of chitosan nanoparticles loaded with different macromolecules. Eur J Pharm Biopharm 2023; 188:170–181

154 Cao X, Liu Q, Shi W, et al. Microfluidic fabricated bisdemethoxycurcumin thermosensitive liposome with enhanced antitumor effect. Int J Pharm 2023;641:123039

155 Bendre A, Hegde V, Ajeya KV, et al. Microfluidic-assisted synthesis of metal-organic framework -alginate micro-particles for sustained drug delivery. Biosensors (Basel) 2023;13(07):737

156 Alam SB, Wang F, Qian H, Kulka M. Apolipoprotein C3 facilitates internalization of cationic lipid nanoparticles into bone marrow-derived mouse mast cells. Sci Rep 2023;13(01):431

157 Schemberg J, Abbassi AE, Lindenbauer A, et al. Synthesis of biocompatible superparamagnetic iron oxide nanoparticles (SPION) under different microfluidic regimes. ACS Appl Mater Interfaces 2022;14(42):48011–48028

158 Karimi-Soflou R, Karkhaneh A, Shabani I. Size-adjustable self-assembled nanoparticles through microfluidic platform promotes neuronal differentiation of mouse embryonic stem cells. Biomater Adv 2022;140:213056

159 Jaradat E, Weaver E, Meziane A, Lamprou DA. Microfluidic paclitaxel-loaded lipid nanoparticle formulations for chemotherapy. Int J Pharm 2022;628:122320

160 Younis MA, Khalil IA, Elewa YHA, Kon Y, Harashima H. Ultrasmall lipid nanoparticles encapsulating sorafenib and midkine-siRNA selectively-eradicate sorafenib-resistant hepatocellular carcinoma *in vivo*. J Control Release 2021;331:335–349

161 Yang YN, Ge C, He J, Lu WG. Novel worm-like micelles for hydrochloride doxorubicin delivery: preparation, characterization, and *in vitro* evaluation. Pharmaceutical Fronts 2022;4(04): e284–e294

162 Zheng Y, Chen H, Lin X, Li M, Zhao Y, Shang L. Scalable production of biomedical microparticles via high-throughput microfluidic step emulsification. Small 2023;19(17):e2206007

163 Su Y, Liu J, Tan S, Liu W, Wang R, Chen C. PLGA sustained-release microspheres loaded with an insoluble small-molecule drug: microfluidic-based preparation, optimization, characterization, and evaluation *in vitro* and *in vivo*. Drug Deliv 2022;29(01): 1437–1446

164 Liu F, Luo W, Qiu J, Guo Y, Zhao S, Bao B. Continuous antisolvent crystallization of dolutegravir sodium using microfluidics. Ind Eng Chem Res 2022;61(19):6693–6702

165 Zhou J, Zhai Y, Xu J, Zhou T, Cen L. Microfluidic preparation of PLGA composite microspheres with mesoporous silica nanoparticles for finely manipulated drug release. Int J Pharm 2021; 593:120173

166 Yang D, Gao K, Bai Y, et al. Microfluidic synthesis of chitosan-coated magnetic alginate microparticles for controlled and sustained drug delivery. Int J Biol Macromol 2021;182:639–647

167 Yeh SI, Fu CY, Sung CY, Kao SC. Microfluidic fabrication of porous PLGA microspheres without pre-emulsification step. Microfluid Nanofluidics 2023;27:47

168 Chen M, Guo X, Shen L, et al. Monodisperse $CaCO_3$-loaded gelatin microspheres for reversing lactic acid-induced chemotherapy resistance during TACE treatment. Int J Biol Macromol 2023; 231:123160

169 Coliaie P, Kelkar MS, Nere NK, Singh MR. Continuous-flow, well-mixed, microfluidic crystallization device for screening of polymorphs, morphology, and crystallization kinetics at controlled supersaturation. Lab Chip 2019;19(14):2373–2382

170 Quagliarini E, Renzi S, Digiacomo L, et al. Microfluidic formulation of DNA-loaded multicomponent lipid nanoparticles for gene delivery. Pharmaceutics 2021;13(08):1292

171 Sato Y, Okabe N, Note Y, et al. Hydrophobic scaffolds of pH-sensitive cationic lipids contribute to miscibility with phospholipids and improve the efficiency of delivering short interfering RNA by small-sized lipid nanoparticles. Acta Biomater 2020; 102:341–350

172 Mucker EM, Karmali PP, Vega J, et al. Lipid nanoparticle formulation increases efficiency of DNA-vectored vaccines/immunoprophylaxis in animals including transchromosomic bovines. Sci Rep 2020;10 (01):8764

173 Lari AS, Zahedi P, Ghourchian H, Khatibi A. Microfluidic-based synthesized carboxymethyl chitosan nanoparticles containing metformin for diabetes therapy: *in vitro* and *in vivo* assessments. Carbohydr Polym 2021;261:117889

174 Huang KS, Yang CH, Wang YC, Wang WT, Lu YY. Microfluidic synthesis of vinblastine-loaded multifunctional particles for magnetically responsive controlled drug release. Pharmaceutics 2019;11(05):212

175 Chiesa E, Greco A, Riva F, et al. Staggered herringbone microfluid device for the manufacturing of chitosan/TPP nanoparticles: systematic optimization and preliminary biological evaluation. Int J Mol Sci 2019;20(24):6212

176 Huang Y, Jazani AM, Howell EP, Reynolds LA, Oh JK, Moffitt MG. Microfluidic shear processing control of biological reduction stimuli-responsive polymer nanoparticles for drug delivery. ACS Biomater Sci Eng 2020;6(09):5069–5083

177 Martins C, Araújo F, Gomes MJ, et al. Using microfluidic platforms to develop CNS-targeted polymeric nanoparticles for HIV therapy. Eur J Pharm Biopharm 2019;138:111–124

178 Liu D, Zhang H, Cito S, et al. Core/shell nanocomposites produced by superfast sequential microfluidic nanoprecipitation. Nano Lett 2017;17(02):606–614

179 Mandal B, Bhattacharjee H, Mittal N, et al. Core-shell-type lipid-polymer hybrid nanoparticles as a drug delivery platform. Nanomedicine 2013;9(04):474–491

180 Scopel R, Falcao MA, Cappellari AR, et al. Lipid-polymer hybrid nanoparticles as a targeted drug delivery system for melanoma treatment. Int J Polym Mater 2022;71(02):127–138

181 Wei W, Sun J, Guo XY, et al. Microfluidic-based holonomic constraints of siRNA in the kernel of lipid/polymer hybrid nanoassemblies for improving stable and safe *in vivo* delivery. ACS Appl Mater Interfaces 2020;12(13):14839–14854

182 Tahir N, Madni A, Li W, et al. Microfluidic fabrication and characterization of Sorafenib-loaded lipid-polymer hybrid nanoparticles for controlled drug delivery. Int J Pharm 2020; 581:119275

183 Wan F, Bohr SS, Kłodzińska SN, et al. Ultrasmall TPGS-PLGA hybrid nanoparticles for site-specific delivery of antibiotics into *Pseudomonas aeruginosa* biofilms in lungs. ACS Appl Mater Interfaces 2020;12(01):380–389

184 Yang R, Deng Y, Huang B, et al. A core-shell structured COVID-19 mRNA vaccine with favorable biodistribution pattern and promising immunity. Signal Transduct Target Ther 2021;6(01):213

185 Nie T, He Z, Zhou Y, et al. Surface coating approach to overcome mucosal entrapment of DNA nanoparticles for oral gene delivery of glucagon-like peptide 1. ACS Appl Mater Interfaces 2019;11 (33):29593–29603

186 Bose RJC, Paulmurugan R, Moon J, Lee SH, Park H. Cell membrane-coated nanocarriers: the emerging targeted delivery system for cancer theranostics. Drug Discov Today 2018;23(04): 891–899
187 Herrmann IK, Wood MJA, Fuhrmann G. Extracellular vesicles as a next-generation drug delivery platform. Nat Nanotechnol 2021; 16(07):748–759
188 Yang L, Sun L, Zhang H, Bian F, Zhao Y. Ice-inspired lubricated drug delivery particles from microfluidic electrospray for osteoarthritis treatment. ACS Nano 2021;15(12):20600–20606
189 Zou D, Yu L, Sun Q, et al. A general approach for biomimetic mineralization of MOF particles using biomolecules. Colloids Surf B Biointerfaces 2020;193:111108
190 Cui J, Gao N, Yin X, et al. Microfluidic synthesis of uniform single-crystalline MOF microcubes with a hierarchical porous structure. Nanoscale 2018;10(19):9192–9198
191 Hu C, Bai Y, Hou M, et al. Defect-induced activity enhancement of enzyme-encapsulated metal-organic frameworks revealed in microfluidic gradient mixing synthesis. Sci Adv 2020;6(05): eaax5785
192 Balachandran YL, Li X, Jiang X. Integrated microfluidic synthesis of aptamer functionalized biozeolitic imidazolate framework (BioZIF-8) targeting lymph node and tumor. Nano Lett 2021; 21(03):1335–1344
193 Yang G, Liu Y, Jin S, Zhao CX. Development of core-shell nanoparticle drug delivery systems based on biomimetic mineralization. ChemBioChem 2020;21(20):2871–2879
194 Tengjisi HY, Hui Y, Yang G, Fu C, Liu Y, Zhao CX. Biomimetic core-shell silica nanoparticles using a dual-functional peptide. J Colloid Interface Sci 2021;581(Pt A):185–194
195 Hao N, Nie Y, Xu Z, Zhang JXJ. Ultrafast microfluidic synthesis of hierarchical triangular silver core-silica shell nanoplatelet toward enhanced cellular internalization. J Colloid Interface Sci 2019;542:370–378
196 Xu L, Peng J, Srinivasakannan C, et al. Synthesis of copper nanoparticles by a T-shaped microfluidic device. RSC Advances 2014; 4(48):25155–25159
197 Pekkari A, Say Z, Susarrey-Arce A, et al. Continuous microfluidic synthesis of Pd nanocubes and PdPt core-shell nanoparticles and their catalysis of NO_2 reduction. ACS Appl Mater Interfaces 2019; 11(39):36196–36204
198 Li X, Feng Q, Jiang X. Microfluidic synthesis of Gd-based nanoparticles for fast and ultralong MRI signals in the solid tumor. Adv Healthc Mater 2019;8(20):e1900672
199 Bemetz J, Wegemann A, Saatchi K, et al. Microfluidic-based synthesis of magnetic nanoparticles coupled with miniaturized NMR for online relaxation studies. Anal Chem 2018;90(16): 9975–9982
200 Deng N, Wang Y, Luo G. A novel method for fast and continuous preparation of superfine titanium dioxide nanoparticles in microfluidic system. Particuology 2022;60:61–67
201 Yao H, Wang Y, Jing Y, Luo G. Ultrafast, continuous and shape-controlled preparation of CeO2 nanostructures: nanorods and nanocubes in a microfluidic system. Ind Eng Chem Res 2018;57 (22):7525–7532
202 Mahdavi Z, Rezvani H, Keshavarz Moraveji M. Core-shell nanoparticles used in drug delivery-microfluidics: a review. RSC Advances 2020;10(31):18280–18295
203 Su Y, Zhang B, Sun R, et al. PLGA-based biodegradable microspheres in drug delivery: recent advances in research and application. Drug Deliv 2021;28(01):1397–1418
204 Zhu C, Yang H, Shen L, et al. Microfluidic preparation of PLGA microspheres as cell carriers with sustainable Rapa release. J Biomater Sci Polym Ed 2019;30(09):737–755
205 Kamiya K, Takeuchi S. Giant liposome formation toward the synthesis of well-defined artificial cells. J Mater Chem B Mater Biol Med 2017;5(30):5911–5923
206 Seo H, Lee H. Recent developments in microfluidic synthesis of artificial cell-like polymersomes and liposomes for functional bioreactors. Biomicrofluidics 2021;15(02):021301
207 Weiss M, Frohnmayer JP, Benk LT, et al. Sequential bottom-up assembly of mechanically stabilized synthetic cells by microfluidics. Nat Mater 2018;17(01):89–96
208 Schaich M, Cama J, Al Nahas K, et al. An integrated microfluidic platform for quantifying drug permeation across biomimetic vesicle membranes. Mol Pharm 2019;16(06):2494–2501
209 Deshpande S, Dekker C. On-chip microfluidic production of cell-sized liposomes. Nat Protoc 2018;13(05):856–874
210 Arriaga LR, Huang Y, Kim SH, et al. Single-step assembly of asymmetric vesicles. Lab Chip 2019;19(05):749–756
211 Michelon M, Huang YT, de la Torre LG, Weitz DA, Cunha RL. Single-step microfluidic production of W/O/W double emulsions as templates for beta-carotene-loaded giant liposomes formation. Chem Eng J 2019;366:27–32
212 Yandrapalli N, Petit J, Bäumchen O, Robinson T. Surfactant-free production of biomimetic giant unilamellar vesicles using PDMS-based microfluidics. Commun Chem 2021;4(01):100
213 Costa C, Liu Z, Martins JP, et al. All-in-one microfluidic assembly of insulin-loaded pH-responsive nano-in-microparticles for oral insulin delivery. Biomater Sci 2020;8(12):3270–3277
214 Yang JL, Zhu Y, Wang F, Deng LF, Xu XY, Cui WG. Microfluidic liposomes-anchored microgels as extended delivery platform for treatment of osteoarthritis. Chem Eng J 2020;400:126004
215 Gikanga B, Maa YF. A review on mixing-induced protein particle formation: the puzzle of bottom-mounted mixers. J Pharm Sci 2020;109(08):2363–2374
216 Madrigal JL, Sharma SN, Campbell KT, Stilhano RS, Gijsbers R, Silva EA. Microgels produced using microfluidic on-chip polymer blending for controlled released of VEGF encoding lentivectors. Acta Biomater 2018;69:265–276
217 Deveza L, Ashoken J, Castaneda G, et al. Microfluidic synthesis of biodegradable polyethylene-glycol microspheres for controlled delivery of proteins and DNA nanoparticles. ACS Biomater Sci Eng 2015;1(03):157–165
218 Kim H, Sung J, Chang Y, Alfeche A, Leal C. Microfluidics synthesis of gene silencing cubosomes. ACS Nano 2018;12(09):9196–9205

