Materials Horizons

ROYAL SOCIETY OF CHEMISTRY

REVIEW

Published on 13 July 2026

Cite this: DOI: 10.1039/d6mh01052k

Advancing extracellular vesicles as drug delivery systems: a conceptual and prospective outlook on microfluidic methods for scalable, GMP-compliant drug loading

Edoardo Bertania,[a] Elia Bari,*[a] Lorena Segale,[a] Marzio Sorlini[bc] and Maria Luisa Torre[ab]

Extracellular vesicles (EVs) are increasingly recognized as effective drug delivery systems because of their natural biocompatibility, ability to cross biological barriers, and inherent targeting of specific cell types. However, their clinical translation faces challenges related to scalability and the absence of reliable, efficient loading methods. Although direct studies of microfluidic EV drug loading are still limited, this paper leverages principles established in liposome engineering, nanoparticle manufacturing, and microfluidic bioprocessing to explore how microfluidics could revolutionize future EV loading strategies. Three pivotal aspects were considered: (i) precisely regulated passive loading, (ii) mechanically or electrically mediated soft active loading, and (iii) microfluidic disassembly and reassembly techniques that produce EV-mimetic vesicles. In this context, microfluidic control over flow, shear, mixing, and transport phenomena could address many limitations of current in-bulk techniques. This is thus a conceptual and prospective review: rather than providing a systematic or critical comparison of existing studies, this paper uses selected literature as a foundation to propose plausible microfluidic EV-loading strategies, discuss their mechanistic rationale, and identify the key technical constraints that must be addressed for practical and translational implementation. By exploring these emerging opportunities, the review posits that microfluidic platforms could enable the transition of EV drug loading from a heterogeneous, lab-scale process to a scalable and automated process that is compatible with good manufacturing practice (GMP) requirements, provided that appropriate materials, validation strategies, and scale-out solutions are implemented, guiding the development of the next generation of EV-based drug delivery systems.

Received 25th May 2026,
Accepted 13th July 2026

DOI: 10.1039/d6mh01052k

rsc.li/materials-horizons

Wider impact

This review discusses emerging microfluidic strategies for loading therapeutic cargo into extracellular vesicles (EVs), focusing on controlled passive incubation, soft active membrane permeabilization, and disassembly–reassembly approaches for EV-mimetic engineering. By integrating concepts from fluid dynamics, membrane biophysics, and micro/nanofluidic engineering, the work highlights how microscale control of shear stress, extensional flow, electric fields, temperature, and chemical gradients can improve loading efficiency while preserving EV integrity and enabling reproducible, scalable, and potentially GMP-compatible manufacturing processes. This area is of broad interest because EVs are increasingly recognized as highly promising biological nanocarriers for precision medicine, capable of transporting nucleic acids, proteins, and small molecules with intrinsic biocompatibility and targeting capabilities that are difficult to reproduce with synthetic nanoparticles. However, current loading methods remain inefficient, poorly standardized, and difficult to translate clinically. The future of the field will likely involve fully integrated microfluidic platforms combining EV isolation, loading, purification, and real-time quality control into automated continuous-flow systems. By establishing a conceptual framework that connects microscale transport phenomena with EV engineering, this review helps position microfluidics as a foundational technology for the next generation of clinically translatable and manufacturing-ready EV therapeutics.

[a] *Department of Pharmaceutical Sciences, University of Piemonte Orientale, Largo Donegani 2, 28100, Novara, Italy. E-mail: elia.bari@uniupo.it*
[b] *PharmaExceed S.r.l, Piazza Castello 19, 27100, Pavia, Italy*
[c] *Department of Innovative Technologies, University of Applied Sciences and Arts of Southern Switzerland, SUPSI, Lugano University Center, Campus Est, Via la Santa 1, CH-6962, Viganello, Switzerland*

1. Introduction

Extracellular vesicles (EVs) have recently attracted significant attention as highly promising drug delivery systems because of

Table 1 Comparison between some properties of EVs and synthetic nanocarriers used as drug delivery systems

Feature	EVs	Liposomes	Polymeric nanoparticles	Metallic nanoparticles
Origin	Natural	Synthetic	Synthetic	Synthetic
Structure	Vesicular	Vesicular	Matrix, capsular, micellar	Metallic core with functionalized surface
Composition	Complex lipid–protein membrane, with surface ligands and receptors	Simple lipid bilayer, often PEGylated for improved stability	Biodegradable or hydrophilic, natural or synthetic origin (*e.g.*, PLGA, chitosan, PEG)	Inorganic metals (*e.g.*, Au, Ag) or metal oxides (*e.g.*, Fe_3O_4)
Biocompatibility	Excellent; fully biological composition	Generally high, depends on lipid formulation	Variable: changes with polymer type and degradation products	Variable: some metals induce oxidative stress or toxicity
Immunogenicity	Low, due to endogenous origin	Low to moderate; PEGylation reduces recognition by the immune system	Variable: may trigger immune responses depending on polymer chemistry	Often significant due to reactive surfaces
Targeting ability	Intrinsic tropism and cell-specific recognition *via* membrane proteins and glycans	Targeting *via* chemical ligands, surface modification, or antibody functionalization	Targeting *via* chemical ligands, surface modification, or antibody functionalization	Targeting *via* magnetic, photothermal, or antibody conjugation
Cargo versatility	Proteins, nucleic acids, peptides, small molecules	Proteins, nucleic acids, peptides, small molecules	Proteins, nucleic acids, peptides, small molecules	Mainly small molecules or contrast agents
Cargo protection	High: membrane shields cargo from enzymatic degradation	Moderate: possible leakage under stress	High: dense polymer matrix provides protection	High: metal core provides strong stability
Cellular uptake	Receptor-mediated endocytosis, membrane fusion	Endocytosis, fusion (limited targeting)	Endocytosis (non-specific)	Endocytosis, often non-specific
Circulation stability	Long circulation; can evade immune clearance (CD47 effect)	Moderate; improved by PEGylation	Tunable *via* surface chemistry	High, but may accumulate in organs (liver, spleen)
Drug loading capacity	Low to moderate; often heterogeneous	High for amphiphilic drugs	High; tunable by polymer ratio and processing	Limited to surface adsorption or encapsulation
Scalability/GMP compliance	Limited: new scalable isolation and loading methods under development	Established, scalable processes	Well-established, GMP-compatible	Scalable but requires strict safety validation
Safety profile	Excellent, but donor and batch variability remain	Good: potential for complement activation	Generally safe; depends on degradation products	Potential cytotoxicity, oxidative damage
Clinical status	Early-stage, mostly preclinical	Several FDA-approved formulations	Multiple approved or in trials	Few approved; primarily diagnostic or theranostic
Ref.	2	8 and 9	10 and 11	12

their natural biocompatibility, inherent targeting capabilities, and ability to cross biological barriers.[1–3] These nanosized vesicles, secreted by nearly all cell types, serve as endogenous carriers of biological information and offer distinct advantages over synthetic nanosized delivery systems (Table 1).[2,4–12] Specifically, their low immunogenicity, efficient cellular uptake, and capacity to protect encapsulated molecules from enzymatic degradation in biological fluids make them especially attractive for therapeutic applications.[1,2,13,14] Additionally, their capacity to transport various classes of bioactive compounds – such as small molecules, nucleic acids, peptides, and proteins – has generated increasing interest in both academic research and pharmaceutical development.[6,15]

Beyond their general versatility, EVs' natural composition confers remarkable biological specificity, reflecting the identity and function of their parent cells.[16] Through unique combinations of proteins, lipids, and glycans on their lipidic layers, EVs can selectively recognize and interact with target cells – a feature that remains difficult to replicate synthetically.[16–22] This innate homing ability makes them highly promising for targeted delivery of cytotoxic therapeutic agents, reducing off-target effects and improving treatment accuracy.[23] Additionally, EVs can evade phagocytosis, circulate longer, and potentially bypass the endosomal–lysosomal pathway to deliver their cargo directly into the cytoplasm.[5,24]

Despite these exceptional qualities and the increasing body of literature supporting their effectiveness as drug delivery systems,[25,26] the translation of EV-based drug delivery systems from research into clinical practice remains limited by two significant challenges: (i) the need for scalable, good manufaturing practice (GMP)-compliant manufacturing processes for EV isolation and purification, and (ii) the lack of efficient, reproducible, and controlled methods for loading drugs into EVs.[27–29]

Regarding the first challenge, most conventional EV isolation techniques – such as ultracentrifugation and precipitation – are not easily scalable and often yield heterogeneous results, limiting reproducibility and industrial feasibility.[30] In recent years, advances in filtration- and chromatography-based manufacturing processes have begun to address these issues by enhancing scalability, purity, and process consistency.[31] For example, a scalable, GMP-compliant manufacturing process for EV-containing products by combining ultrafiltration with lyophilization was developed by Bari *et al.* and Mocchi *et al.*[32–34] In pilot GMP runs, mesenchymal stem cell culture supernatants were concentrated and purified by ultrafiltration,

Table 2 Comparison of passive and active drug-loading methods for EVs

Loading method	Mechanism	Advantages	Limitations/drawbacks	Impact on EV integrity	Typical applications/cargo	Ref.
Passive incubation	Diffusion of drug molecules across the EV membrane during co-incubation	Simple, non-destructive; preserves EV surface markers; compatible with most drug types	Very low encapsulation efficiency; poor control over drug amount per EV; long incubation times; unsuitable for hydrophilic compounds	Maintains structure and surface proteins	Hydrophobic small molecules (*e.g.*, paclitaxel, curcumin)	38, 40 and 43
Electroporation	Application of short electric pulses to transiently permeabilize EV membranes	Higher encapsulation efficiency for nucleic acids; widely used; easily tunable	EV aggregation; risk of RNA degradation; variable efficiency; poor reproducibility	Membrane disruption, aggregation, and possible cargo leakage	siRNA, miRNA, DNA	38 and 40
Sonication	Acoustic cavitation induces temporary membrane disruption	Improved loading efficiency; suitable for hydrophobic and hydrophilic drugs	Irreversible structural alteration; loss of functional membrane proteins; possible cytotoxicity	Partial membrane denaturation; altered zeta potential	Doxorubicin, paclitaxel	38,40,43,44
Freeze–thaw cycles	Repeated freezing and thawing increases membrane permeability *via* phase transition	Simple and low-cost; no need for specialized equipment	Produces heterogeneous EV populations; low reproducibility; potential vesicle aggregation	Structural instability; variable size distribution	Proteins, small molecules	38, 40, 43 and 45
Transfection	Chemical or lipid-based reagents promote cargo incorporation	Effective for nucleic acids; widely established protocols	Possible reagent residues; altered surface properties; reduced biocompatibility and uptake	Surface chemistry modification; reduced targeting ability	mRNA, plasmids, siRNA	38, 40 and 46
Extrusion	Physical mixing of EVs and cargo through polycarbonate membranes	Produces uniform vesicles; scalable	Alteration of membrane composition; reduced flexibility; potential loss of bioactivity	Partial denaturation; loss of surface receptors	Small molecules, nanoparticles	38, 40 and 43

cryoprotectants were added, and the preparations were freeze-dried to create a stable ''lyo-secretome'' containing EVs; the resulting powders maintained EV characteristics and *in vitro* bioactivity after reconstitution.[35–37] These studies demonstrate that combining ultrafiltration and lyophilization is a practical, industry-compatible approach to producing storable EV formulations suitable for even drug delivery applications.

The second major limitation involves drug loading efficiency and process control, where current loading methods face significant technical and regulatory challenges, as summarized in Table 2.[38–46]

Beyond technical inefficiencies, a more fundamental obstacle arises from strict regulatory standards that oversee pharmaceutical manufacturing. Large-scale production requires processes that are validated, reliable, and reproducible, consistently producing batches with well-defined critical quality attributes (CQAs) even in the face of minor variations in raw materials, operators, or environmental conditions.[27,47–51] This is especially crucial given the inherent variability of EVs, which depends on cell source and physiological state. Validation demonstrates that a process can reliably and consistently produce a product that meets its defined CQAs, while robustness ensures consistent performance across different batches and manufacturing environments. Unfortunately, current EV loading methods do not meet these standards: as noted earlier, they show significant batch-to-batch differences and limited process control; additionally, most existing EV-loading approaches are not yet compliant with GMP principles, making them fundamentally incompatible with established pharmaceutical regulatory frameworks.[52,53]

To address these limitations, microfluidic technology may be a highly promising alternative platform: microfluidic systems enable precise control of fluids at the microscale, enabling tight regulation of process parameters such as shear stress, incubation time, and concentration gradients.[54,55] This can facilitate reproducible and efficient EV loading while maintaining their structural integrity. Additionally, microfluidic approaches can be integrated into continuous and automated workflows that are, in principle, compatible with GMP requirements, offering high throughput, reduced reagent consumption, and improved batch-to-batch reproducibility, provided that appropriate device materials, process validation strategies, and quality-control frameworks are implemented.[56–59]

The goal of this review is to examine the emerging frontier of microfluidic technologies for loading drugs into EVs through a conceptual and prospective lens. Rather than offering a systematic or critical review of existing experimental evidence – a function served by recent device-oriented surveys of the broader EV microfluidic field[60] – this work is intentionally framework-driven: it leverages selected studies from EV research, liposome engineering, nanoparticle manufacturing, and microfluidic bioprocessing to articulate plausible microfluidic loading strategies and their underlying physical mechanisms. In this context, the cited literature is not used to perform an exhaustive comparison of reported efficiencies or outcomes, but to

support a positioning that microfluidics can provide the level of control, reproducibility, and scalability required for clinically viable EV drug loading. The central question addressed is therefore not whether individual microfluidic approaches outperform current bulk methods in isolated cases, but whether microfluidic platforms, by design, can enable a paradigm shift from empirical, lab-scale loading to standardized, GMP-compatible manufacturing processes.

2. Microfluidic strategies for loading drugs into EVs

This section explores microfluidic strategies for EV drug loading, categorizing them into three main approaches: (i) controlled passive incubation, (ii) soft active loading, and (iii) disassembly and reassembly techniques for EV-mimetic synthesis. Each strategy is summarized in Table 3 and discussed below in terms of its mechanistic principles, advantages, limitations, and supporting literature, with figures illustrating conceptual workflows and device designs. A comparative synthesis across the three strategies – covering cargo compatibility, EV structural risk, hardware complexity, and GMP-relevant manufacturing considerations – is presented in Section 2.4.

Before examining these strategies in detail, however, it is important to recognize that their underlying mechanisms cannot be directly extrapolated from the extensive literature on synthetic nanocarriers. Unlike liposomes or polymeric nanoparticles, which are compositionally defined and structurally homogeneous, EVs possess biologically assembled

Table 3 Microfluidic strategies for drug loading into EVs: mechanisms, advantages, limitations, and quantitative performance metrics reported in the literature. For each strategy, reported ranges for loading efficiency, vesicle recovery/integrity, and particle size change are drawn from representative experimental studies employing microfluidic platforms, including cases directly benchmarked against bulk/conventional loading methods. Because the underlying experimental literature on microfluidic EV drug loading remains limited and methodologically heterogeneous, these values should be interpreted as illustrative ranges from individual studies rather than as systematic, cross-comparable benchmarks; differences in EV source, cargo type, and characterization method preclude direct quantitative comparison across strategies

	Controlled passive incubation	Soft active loading	Disassembly and reassembly (EV-mimetic synthesis)
Mechanism	Laminar microfluidic co-flow enables precise control of drug diffusion into EVs, with flow, temperature, pH, and residence time under precise control	Localized shear, extensional flow, or micro-electroporation temporarily increases membrane permeability, making it reversible and tunable	Controlled membrane disruption in microchannels, mixing with cargo, followed by reassembly into uniform hybrid vesicles
Advantages	– Reproducible – GMP-compatible – Preservation of EV integrity – Scalable – Supports inline monitoring	– High loading while maintaining EV structure – Uniform stress – Continuous and controllable – Inline quality control possible	– Very high encapsulation – Tunable vesicle composition and size – Reproducible and scalable – Suitable for large or hydrophilic drugs
Limitations	– Limited to passively permeable drugs – Low efficiency for large, hydrophilic, or charged molecules	– Necessity of careful optimization – Risk of partial EV damage, aggregation, or device fouling – More complex than bulk methods	– Loss of native EV identity – Altered surface proteins – Regulatory challenges – More invasive manipulation
Loading efficiency (literature range)	11–38% depending on cargo and residence time[65,66]; up to ~11-fold improvement over bulk passive incubation of equal duration[66]	Mechanical nanoporation achieved loading efficiencies of 31–37% for macromolecular dextrans (3–10 kDa)[67]; a microfluidic droplet-based electroporation system achieved a 10-fold enhancement in loading efficiency and a >1000-fold increase in processing throughput for gRNA:Cas9 RNP complexes compared with conventional bulk electroporation[68]	90.5% core–shell assembly yield vs 47.3% without microfluidic sonication[69]
Vesicle recovery/ integrity	Minimal EV loss reported[66]	Nanoporation preserved 94.4% recovery (particle concentration from 9.0×10^8 to 8.5×10^8 particles mL^{-1})[67]; integrated surface acoustic wave (SAW)-isolation/electroporation platforms achieved 85% recovery with reduced aggregation relative to ultracentrifugation[70]	Narrower size distribution and improved batch consistency vs bulk[69]
Particle size change	Modest shifts (*e.g.*, 114 → 127 nm)[66]	Nanoporation: 130 → 170 nm shift attributed to membrane permeabilization and cargo loading[67]; SAW-isolation/electroporation: 102 ± 6 → 135 ± 8 nm (cell-derived EVs)[70]	177 nm (PDI 0.19) *vs.* 238 nm (PDI 0.47) for bulk-assembled controls[69]
Compatible cargo types	Small, membrane-permeant hydrophobic molecules (*e.g.*, verteporfin[66]); limited efficiency for hydrophilic or charged macromolecules such as siRNA	Broad range, from small molecules and macromolecular dextrans (mechanical nanoporation[67]) to nucleic acids such as mRNA and siRNA (electroporation-based platforms[71,72]), reflecting the tunable intensity of membrane permeabilization	Large proteins, nucleic acids, and hydrophilic drug molecules that cannot passively cross an intact EV membrane

membranes enriched in transmembrane proteins, glycoconjugates, and asymmetric lipid domains inherited from their parent cells. Consequently, EV populations display substantial heterogeneity in size, membrane composition, surface charge distribution, and mechanical properties, even within the same preparation. For example, combined atomic force and fluorescence microscopy analyses have revealed marked variability in EV diameter, Young's modulus, and membrane protein distribution at the single-vesicle level,[61] while single-vesicle profiling studies have demonstrated extensive molecular heterogeneity beyond traditional biogenetic classifications.[62] Furthermore, EV membranes retain complex lipid asymmetry and a glycan-rich surface chemistry that contribute to their characteristic charge distribution and interfacial behavior.[63,64] These features are expected to influence membrane deformability, permeability, and responsiveness to hydrodynamic, electrical, and thermal stimuli. Therefore, microfluidic loading strategies originally developed for liposomes and other synthetic nanocarriers should be regarded primarily as mechanistic frameworks rather than directly transferable solutions. Their successful implementation in EV-based systems will require consideration of EV-specific membrane biology and biophysical behavior.

2.1. Controlled passive incubation: precision through laminar flow

Conventional passive loading depends on the spontaneous diffusion of drugs into EVs during co-incubation. The main factor driving uptake is the drug's partition coefficient between the surrounding medium and the EV membrane, reflecting its lipophilicity and affinity for the lipid bilayer.[43,73] For weak acids or bases, the pH of the medium can also influence their ionization, affecting their partitioning into EV lipids.[74] Although the drug solution is homogeneous under typical incubation conditions, passive loading in static batch mode remains limited by slow diffusion-driven transport and by factors that affect the spatial distribution and accessibility of EVs. Indeed, EVs may sediment, aggregate, or adhere to container surfaces, reducing uniform drug-membrane contact and lowering the frequency of effective collisions (Fig. 1A). These physical constraints, despite the user's ability to control parameters such as drug concentration, incubation time,

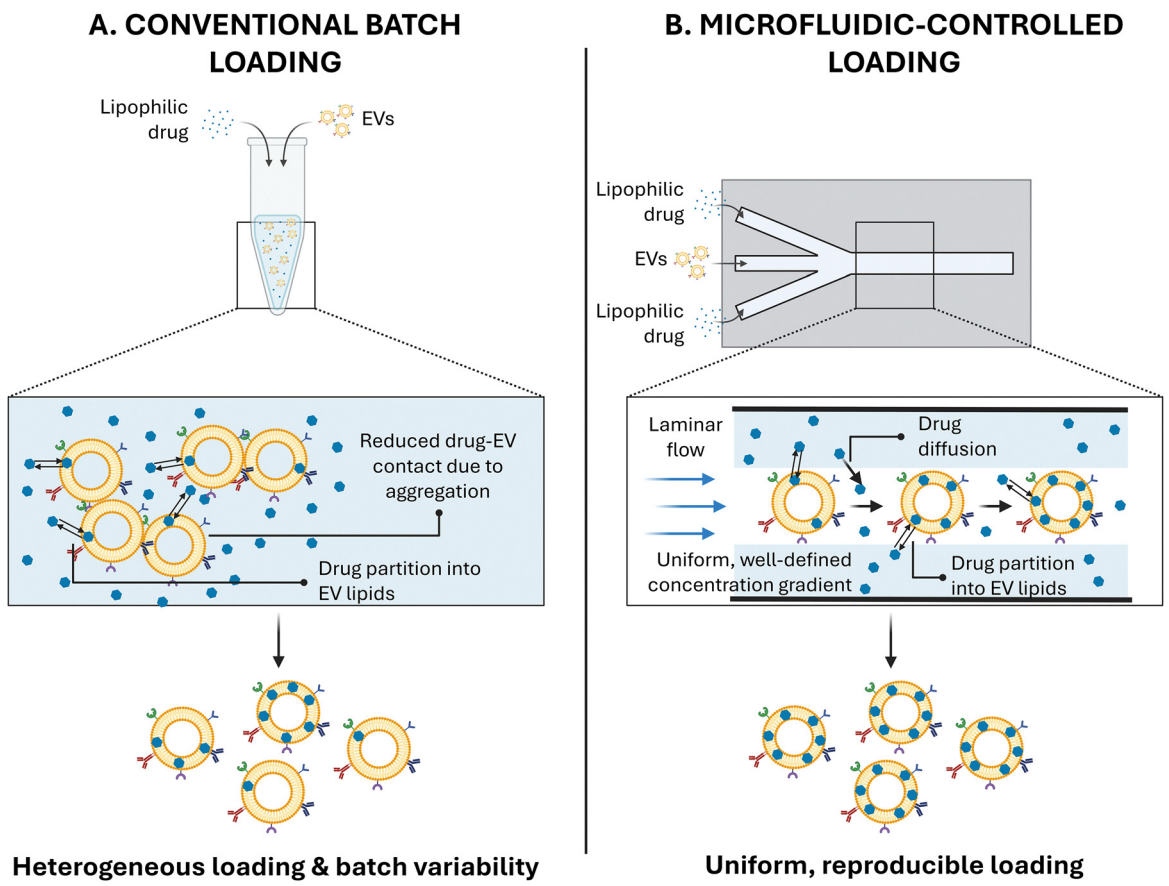

Fig. 1 (A) Conventional batch loading. Although the drug solution is homogeneous, EVs may sediment, aggregate, or adhere to container surfaces, which reduces their accessibility to drug molecules. As shown in the inset, these limitations result in heterogeneous and inefficient loading across the EV population. (B) Microfluidic loading based on laminar flow and diffusion. EVs and drug molecules flow in parallel streams through microchannels, enabling controlled, uniform mixing *via* diffusion while minimizing aggregation. The inset illustrates the resulting homogeneous and reproducible loading, underscoring the enhanced precision of microfluidic systems compared with batch-mode mixing. Created with Biorender.

temperature, and pH, result in low encapsulation efficiency, heterogeneous loading, and significant batch-to-batch variability.[28,75,76] As such, while non-disruptive for EVs and straightforward, this method is challenging to implement in GMP-compliant production; although some studies have reported GMP-grade EV production for therapeutic applications (such as mesenchymal stem cell-derived EVs loaded with paclitaxel[77]) with fully standardized co-incubation protocols, such protocols therefore remain rare. Using approaches that pre-load drugs into parent cells can help address some of these issues, but introduce additional regulatory and technical challenges.[78]

In microfluidic systems, passive diffusion remains the primary mode of transport, but precise control over physicochemical parameters enables more efficient and reproducible drug partitioning into EV lipids[67,79,80] (Fig. 1B). This precision stems from the underlying fluid dynamics: fluids flow at low Reynolds numbers ($Re < 1$), meaning viscous forces dominate over inertial forces and turbulence is effectively eliminated.[81–83] As a result, laminar flow in microchannels allows streams of EVs and drug solutions to travel in parallel layers, creating stable, well-defined concentration gradients at the microscale. This controlled environment ensures that drug molecules are continuously available at the EV membrane interface, reducing local depletion and enhancing diffusion-driven uptake. Furthermore, the gentle, continuous flow minimizes EV sedimentation, aggregation, and adsorption to surfaces, addressing key limitations of static batch incubations. Consequently, each EV experiences the same drug concentration for an identical residence time as it moves through the channel, ensuring uniform exposure and reducing heterogeneity in drug loading across the vesicle population. In contrast, manual batch incubation exposes EVs to highly variable local concentrations, with some vesicles encountering excessive drug and others receiving little or no drug, leading to inconsistent loading efficiency and potential structural damage.

Beyond enabling predictable laminar flow, microfluidic platforms allow precise control over the physicochemical environment experienced by EVs during drug loading, a feature that bulk methods cannot match.[82,84,85] In a microchannel, the incubation or residence time is determined by the channel length, geometry, and flow rates, allowing contact times ranging from less than a second to several minutes to be finely tuned, thereby avoiding uncontrolled mixing delays common in batch incubation.[86–88] Temperature, a crucial factor influencing membrane fluidity and drug solubility, can be quickly and evenly adjusted using integrated microheaters or external Peltier controllers, leveraging the low thermal mass and high surface-area-to-volume ratio of microfluidic chips.[41,89] This precise thermal regulation enables short heating pulses to enhance membrane fluidity and drug solubility without prolonged exposure that might damage surface proteins or compromise EV integrity.[90,91] Similarly, pH and buffer composition – key variables for drugs in which ionization affects lipid solubility – can be rapidly modified on-chip using mixing structures such as herringbone or serpentine mixers, providing a uniform and stable chemical environment that supports passive and pH-dependent loading strategies.[92–94]

Collectively, these features convert time, temperature, and pH into tightly engineered design parameters, enabling reproducible, finely adjustable drug-loading conditions that are difficult to achieve with traditional bulk methods. One of the main advantages of microfluidic-controlled passive incubation is therefore its reproducibility, which reduces batch-to-batch variability, enables high-throughput workflows, and can facilitate GMP validation when combined with appropriate process control, documentation, and quality-assurance strategies.[55,80,84,95] The precise, controllable management of environmental and process parameters, indeed, allows inline monitoring and documentation, which are essential for meeting regulatory standards in clinical manufacturing. Parameters such as flow rates, temperature, and pH can be continuously recorded and adjusted in real time, supporting robust quality assurance protocols.[96] Another key benefit is the low risk of membrane disruption: in this case, the structural integrity and surface proteins of EVs are maintained because loading depends solely on diffusion under carefully controlled conditions, unlike active loading methods such as sonication or electroporation, which can cause permanent damage (see Section 2.2). Keeping the native membrane structure is essential for preserving EV functionality, including cellular targeting properties. Moreover, the ongoing operational potential of microfluidics enables scalable production. Finally, unlike batch methods, which are constrained by manual handling and mixing, microfluidic platforms can sustain a continuous flow of EVs and drugs, supporting high-throughput processing with minimal human intervention.[97] This not only enhances efficiency but also decreases contamination risk and simplifies integration into industrial manufacturing workflows.

Despite the benefits of microfluidic-controlled passive incubation, several inherent limitations still exist. The method is primarily limited to drugs that can passively cross the EV membrane; highly hydrophilic or large-molecular-weight molecules face substantial barriers to diffusion and are unlikely to be effectively encapsulated. Even with precise control over flow, temperature, and pH, the lipid bilayer's intrinsic permeability limits loading efficiency. Moreover, passive diffusion cannot actively transport cargo against concentration gradients or cause membrane permeabilization, meaning some drugs – especially charged or bulky ones – may require alternative techniques, such as active loading or vesicle reassembly, to reach therapeutically effective concentrations within EVs. Therefore, while microfluidics improves reproducibility and quantitative control, it does not fundamentally overcome the biophysical limitations of passive loading.

An early demonstration of microfluidic passive loading is provided by Itakura *et al.*, who used a pressure-driven microfluidic device to load siRNA into grapefruit-derived EVs.[65] The device acted primarily as an efficient mixing tool, increasing membrane-siRNA contact without disruptive forces; however, this gentle processing achieved only a modest loading efficiency ($\sim$11%), constrained by the short residence time of the EV–cargo mixture within the microchannel. This residence-time limitation

is addressed in the device subsequently reported by Piunti *et al.*[66] for loading the hydrophobic drug verteporfin into mesenchymal stem cell-derived EVs, which combines a micromixer with V-shaped obstacles (122° angle, 240/200 μm arms) for rapid EV–drug contact with a downstream delay-line incubation unit (700 μm × 264 mm) providing a tunable, precisely controlled residence time of up to 10 minutes. In this design, the obstacle-induced chaotic advection increases the contact surface between drug solution and EVs without generating damaging turbulent shear forces, while the delay-line ensures sufficient diffusive interaction time without resorting to harsh permeabilization. This combination resulted in a loading efficiency of 37.9 ± 10.4%, significantly higher than the 3.5 ± 2.6% obtained with standard off-chip incubation, with negligible EV loss. Importantly, this gentle microfluidic approach preserved the EVs' structural integrity and surface protein markers (*e.g.*, CD63, CD81), unlike harsh methods such as electroporation, which led to vesicle aggregation and protein loss.

2.2. Soft active loading: controlled permeabilization without destruction

Within soft active loading, the central challenge for microfluidics is to reproduce the beneficial aspects of transient membrane permeabilization – *via* mechanical, thermal, or electrical stress – while overcoming the limitations associated with conventional active loading methods. Electroporation, sonication, freeze–thaw cycling, chemical transfection, and extrusion are commonly used to enhance drug encapsulation into EVs, but each technique introduces membrane stressors that can compromise vesicle integrity and loading reproducibility.[76] As summarized in Fig. 2, these approaches share a common limitation: the lack of precise control over stress intensity, exposure duration, and spatial distribution, which can result in heterogeneous and often irreversible membrane damage, ranging from pore overstabilization and protein denaturation (electroporation[13,90,98–102]) to cavitation-induced rupture and lipid peroxidation (sonication[103–105]), aggregation and fusion (freeze–thaw[43,45,75,106]), surface chemistry alteration (chemical transfection[46,107,108]), and loss of native biochemical complexity (extrusion[109–111]). At the microscale, however, flow conditions, device geometry, and stimulus exposure can be precisely confined in space and time, rather than being uniformly and uncontrollably applied across an entire bulk sample, enabling membrane permeabilization to occur briefly, reversibly, and uniformly across a population of vesicles while preserving EV structure and functionality[80,112] – the defining features of the strategies discussed below.

Shear stress-mediated perturbation. The primary mechanism in these systems is shear stress-mediated perturbation. Microfluidic channels create predictable laminar shear fields where the magnitude of the stress (τ) can be precisely adjusted by changing flow rate, channel height, and fluid viscosity.[113,114] At moderate shear rates, usually between 10^2 and 10^4 s^{-1}, fluid dynamics cause lateral membrane tension on the vesicle surface. This tension causes temporary lipid packing defects – as transient "holes" in the bilayer – that let small molecules and nucleic acids diffuse into the lumen without damaging the entire EV.[115] Importantly, as EVs leave the high-shear region of the microchannel, mechanical stress drops almost instantly, allowing the lipid bilayer to reseal quickly and spontaneously.[116] This predictable and reversible process contrasts sharply with bulk sonication, where uncontrolled cavitation generates highly heterogeneous shear fields that often cause irreversible structural damage (Fig. 3).

In this regard, recent advances in nanofluidic engineering demonstrate how precisely calibrated shear and confinement can enable efficient EV loading while preserving vesicle integrity. A representative example is the "exosome nanoporator", a nanofluidic platform that drives EVs through tens of thousands of nanochannels with heights (~130 nm) comparable to EV diameter. As EVs are compressed within these confined geometries, they experience a combination of uniform mechanical deformation and well-defined shear gradients that generate transient nanopores, permitting convective influx of macromolecules without compromising membrane topology or protein composition. The exosome nanoporator achieves loading efficiencies of ~30–40% for macromolecular dextrans and supports the efficient incorporation of small-molecule therapeutics, such as doxorubicin, while maintaining >90% vesicle recovery and structural integrity. Importantly, numerical modeling confirms that both membrane permeabilization and loading efficiency scale with predictable velocity gradients within the nanochannels, enabling mechanical stress tuning without the uncontrolled damage associated with bulk electroporation or sonication.[67] Notably, this mechanism arises from shear and nanoconfinement, rather than the whole-vesicle elongational deformation characteristic of extensional-flow systems. Together, these findings highlight that micro- and nanofluidic confinement can provide a gentle yet effective means of producing functional, cargo-bearing EVs at throughputs suitable for translational use.

Extensional flow-induced membrane dilation. Beyond simple shear, microfluidic geometries can be designed to harness extensional flow-induced membrane dilation, a distinct mechanical regime. Elements such as constrictions, ridges, cross-slots, or hydrodynamic focusing regions create zones where the fluid velocity gradient changes suddenly, exposing EVs to extensional rather than purely shear stresses.[117,118] In this flow regime, the fluid stretches the vesicle along the principal flow direction while compressing it along the orthogonal axis.[119] This axisymmetric elongation leads to membrane thinning, which increases permeability to external cargo without causing rupture (Fig. 4). Specifically, the resulting transient membrane dilation creates short-lived permeability "holes" that enable therapeutic molecules in the surrounding medium to diffuse more readily into the EV lumen, thereby enhancing drug-loading efficiency while maintaining vesicle structural integrity. The extent and duration of this extensional strain depend on the channel shape and sheath-flow configuration, offering a level of control impossible with bulk extrusion, where EVs are forced through rigid polycarbonate membranes that often cause permanent deformation or clogging: by relying on

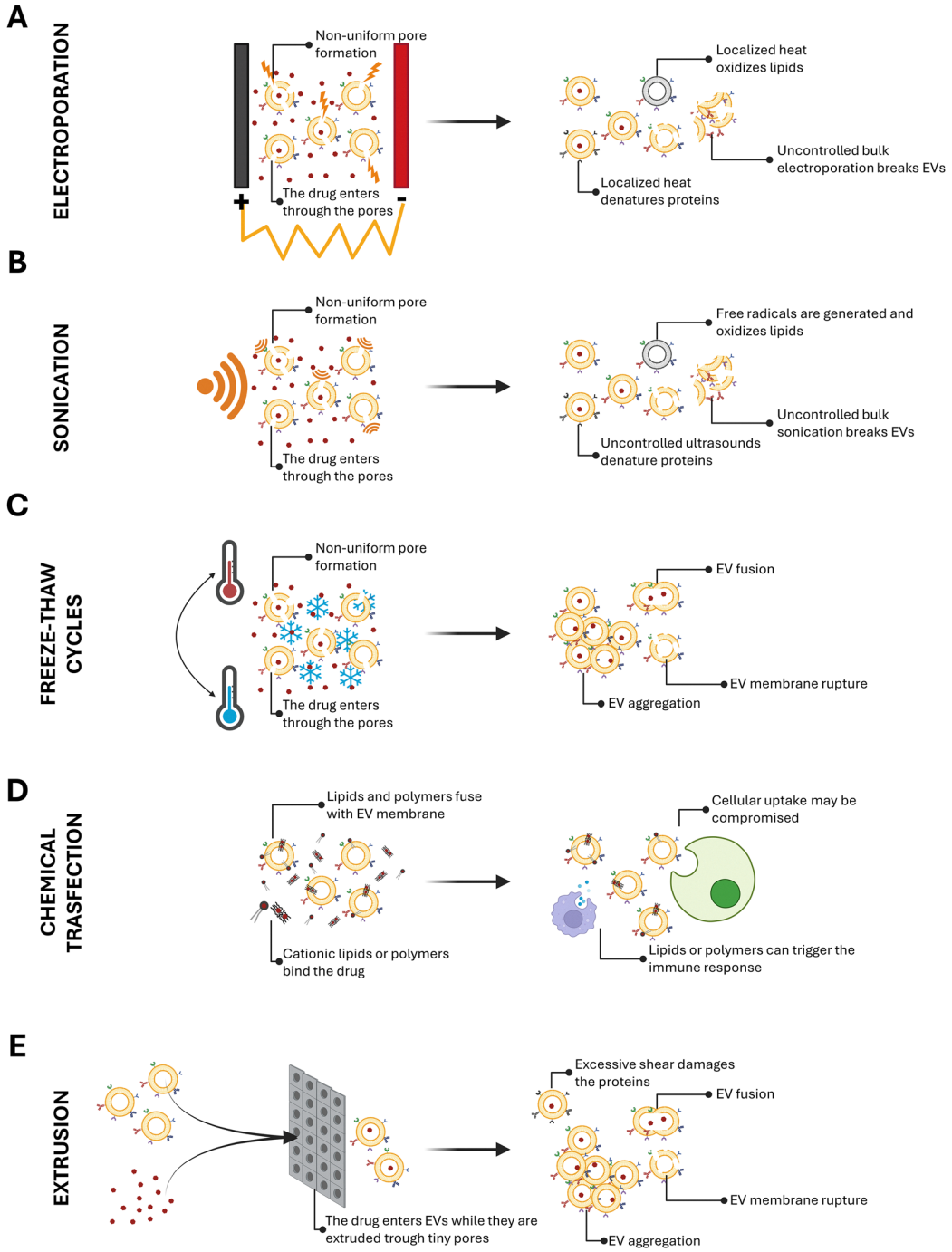

Fig. 2 Schematic representation of the active EV-loading methods. (A) Electroporation creates transient membrane pores but can destabilize or rupture EVs when poorly controlled; localized heating can also induce protein denaturation, lipid oxidation, and nucleic acid aggregation. (B) Sonication generates cavitation-driven shear stresses that increase membrane permeability but can also rupture EVs, denature membrane proteins, and produce free radicals that oxidize lipids. (C) Freeze–thaw cycling forms transient defects that permit cargo entry but promote EV aggregation, fusion, and membrane rupture due to repeated thermal stress. (D) Chemical transfection relies on cationic lipids or polymers to bind and deliver cargo, yet these foreign components can alter EV surface properties, reduce uptake specificity, and trigger immunogenic responses. (E) Extrusion forces EVs and cargo through defined pores, enabling loading but subjecting vesicles to mechanical shear that reshapes membranes, damages surface proteins, and induces EV fusion or aggregation. Created with Biorender.

fluid-based deformation rather than physical barriers, microfluidic extensional flow enables reversible mechanical deformation while maintaining EV integrity.[120]

Localized micro-electroporation. For applications that require electrical permeabilization, microfluidic devices offer a better alternative to bulk cuvettes, enabling gentle, electric field-based

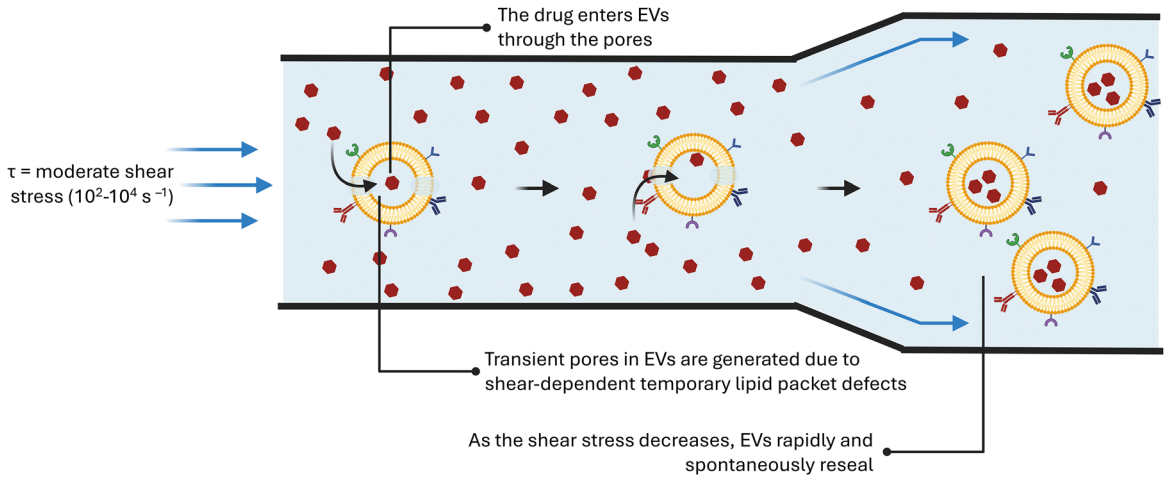

Fig. 3 Shear stress-mediated perturbation of EVs in microfluidic channels to load drugs. In the microfluidic channel, moderate shear stress ($\tau = 10^2$–10^4 s^{-1}) induces transient membrane perturbations, including lipid packing defects, lateral membrane tension, and temporary "holes" that allow molecular diffusion of cargo into EVs. These perturbations are reversible, and vesicles rapidly reseal under low shear conditions, resulting in controlled loading without permanent damage. Created with Biorender.

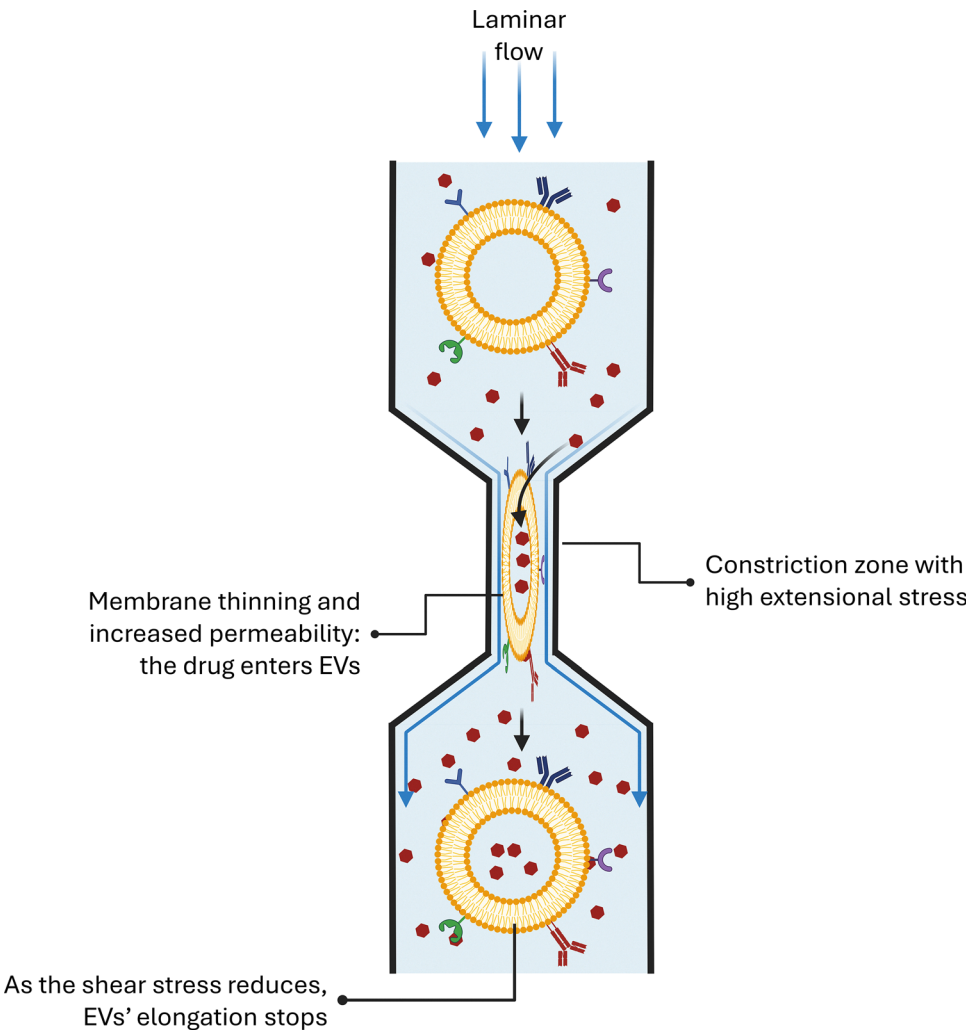

Fig. 4 Extensional flow-induced membrane dilation in microfluidics. EVs experience high-velocity elongation and axisymmetric stretching in the constriction zone, leading to reversible membrane thinning, increased permeability, and the development of extensional stress. The microfluidic approach allows controlled, gentle deformation, minimizing EV damage. Created with Biorender.

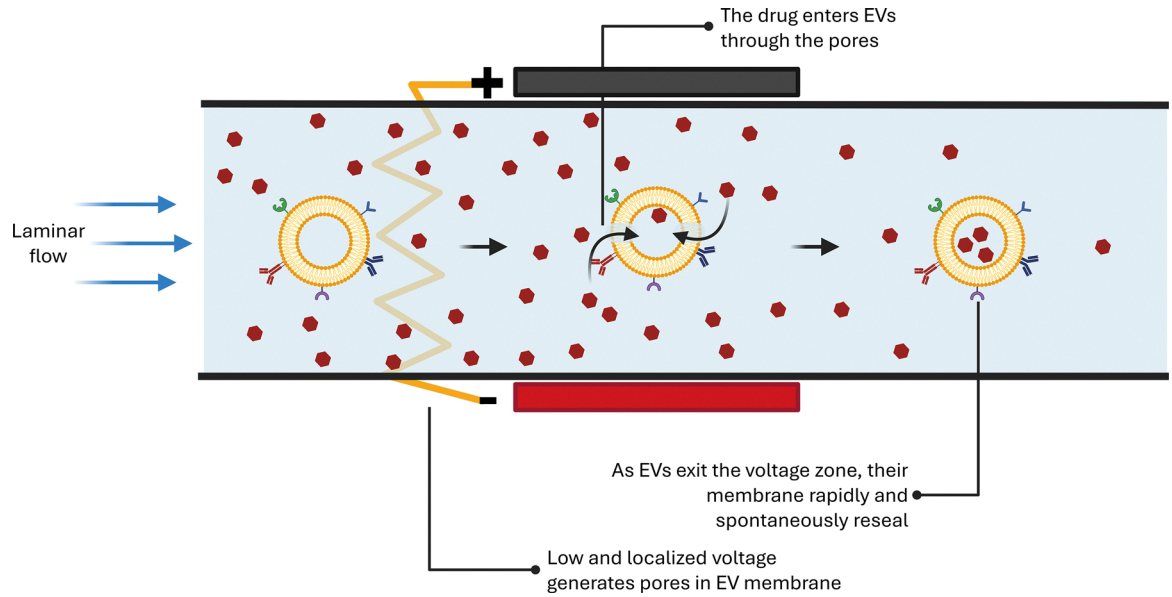

Fig. 5 Microfluidic electroporation of EVs. By using microscale electrodes, localized, low-voltage electric fields that gently and precisely permeabilize EVs are generated. Flow-controlled residence times ensure brief, well-defined pulses that minimize heating and prevent molecular aggregation. As EVs move out of the field, they rapidly reseal, improving loading efficiency compared to bulk electroporation. Created with Biorender.

permeabilization.[121] These systems incorporate microelectrodes to create electric fields or gradients within tightly confined volumes. Because the electrodes are positioned only micrometers from the flowing EVs, sufficient transmembrane potentials can be generated with much lower voltages than those needed in macro-scale systems.[122] Additionally, the residence time of EVs within the electric field is controlled by the flow rate, ensuring vesicles are exposed to pulses for precisely the time needed to produce nanopores (often on the order of milliseconds or microseconds). This spatial confinement reduces heating, thereby preventing the thermal aggregation of nucleic acids and proteins that typically occurs during bulk electroporation. As EVs are quickly removed from the active field area, they enter a relaxation zone that promotes rapid resealing, thereby increasing loading efficiency[123] (Fig. 5).

Environmental and thermal control. Complementing these physical forces is the ability to exchange local buffers and stabilize the environment quickly. Microfluidic systems can incorporate diffusive mixers or parallel laminar streams to alter the chemical environment around EVs rapidly.[124] This allows for rapid exposure to hypotonic, hypertonic, or pH-modified buffers that temporarily modulate membrane curvature to support cargo entry, followed by immediate normalization of the buffer composition downstream.[125] This enables controlled "on–off" cycles of membrane permeability that are difficult to achieve in bulk processing, where mixing is slow and spatial gradients are poorly defined (Fig. 6A). Lastly, the design of microfluidic devices naturally supports membrane self-assembly through on-chip thermal management. The high surface-to-volume ratio of microchannels allows heat (whether generated by electrodes or external sources) to dissipate quickly, preventing thermal hotspots that could damage EV proteins. Downstream temperature-controlled zones can be added to cool the EVs, thermodynamically stabilizing the lipid bilayer and preserving native functionalities such as surface protein targeting[126] (Fig. 6B).

Ultimately, microfluidic perturbation differs fundamentally from bulk energy delivery because of energy localization and precise control. Forces act on EVs only when they pass through specific channel regions, rather than exposing the entire sample to disruptive energy fields. With the ability to vary flow rates, pressures, and field strengths with sub-millisecond precision, microfluidics has the potential to transform EV loading from a chaotic, batch-based process into a continuous and highly reproducible operation that can be adapted to manufacturing environments, provided that scalability, robustness, and regulatory constraints are appropriately addressed. A key benefit of this method is the ability to adjust stress intensity and duration. Since shear, extensional strain, or electric fields can be fine-tuned by changing microchannel geometry, flow speed, or pulse timing, the user can apply just enough disturbance to temporarily loosen lipid packing or create nanoscale pores without reaching levels that cause permanent damage. This adjustability allows the lipidic layer of EVs to open briefly for cargo uptake and then reseal downstream, maintaining both the EV's structural integrity and its surface protein profile. The reversible aspect of microfluidic permeabilization is advantageous when keeping EV bioactivity, targeting ligands, or immunomodulatory properties intact is crucial. Another key benefit is the ability for inline quality control: since microfluidic systems naturally integrate with optical detectors, impedance sensors, or nanoparticle counters, EVs can be monitored in real time as they experience mechanical or electrical disturbances.[127] Parameters such as size distribution,

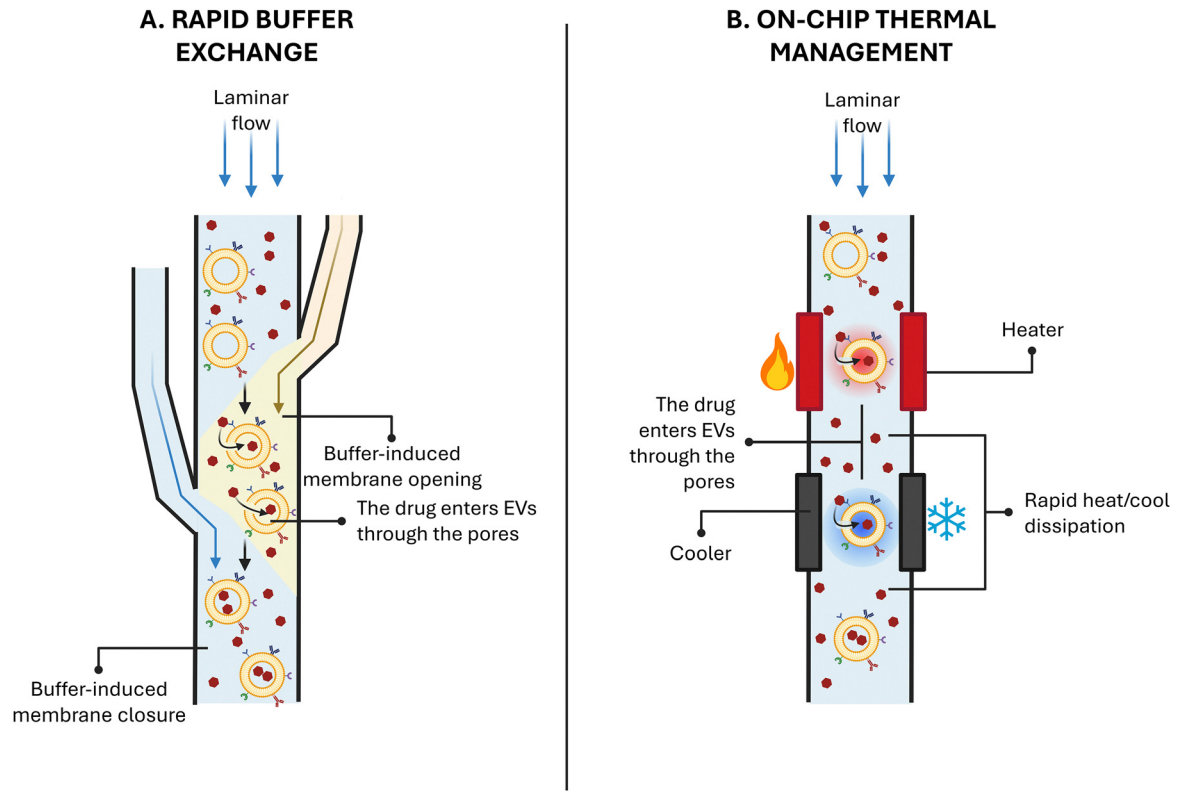

Fig. 6 Microfluidic environmental and thermal control. (A) Rapid buffer exchange: utilizing parallel laminar streams, the local chemical environment around EVs is rapidly altered, inducing membrane opening for cargo entry, followed by immediate buffer normalization downstream to reseal the membrane. (B) On-chip thermal management: a heating (or cooling) zone facilitates drug entry through EV membrane permeabilization, while the high surface-to-volume ratio of microchannels ensures rapid heat (or cooling) dissipation for precise temperature control. Created with Biorender.

concentration, zeta potential, or deformability can be continuously measured, enabling feedback-controlled adjustments to flow rate or field strength. This is very different from bulk methods, where quality is usually assessed only at the end of the process, making it hard to prevent or fix damage as it happens.

Despite these strengths, the approach also has notable limitations. Microfluidic perturbation requires careful optimization of flow conditions, channel geometries, and stress thresholds, as even minor deviations can shift the system from reversible to damaging.[128] Although the forces are adjustable, overstressing the EVs (through excessive shear, overly narrow constrictions, or extended field exposure) can lead to partial rupture, changes in membrane mechanics, or loss of surface markers.[115] Device materials and channel surfaces may also foul when exposed to protein-rich EV preparations, complicating long-term operation and potentially altering the stress distribution within the device. Additionally, designing, fabricating, and maintaining microfluidic systems is more complex than using simple tube-based bulk techniques, requiring specialized equipment and expertise.[129,130]

Although most mechanisms discussed above remain conceptual or have only been demonstrated in model vesicles, a small but growing number of experimental studies provide proof-of-principle that microfluidics can enable controlled, reversible membrane permeabilization of EVs. In detail, the benefits of soft active loading are best demonstrated through microfluidic electroporation platforms. An example is the recent "plug-and-play" microfluidic electroporation system for cancer immunotherapy.[71] By applying a specific sequence of nano- and millisecond pulses, researchers successfully loaded EVs with IFN-γ mRNA and simultaneously promoted surface expression of CD64. This made the EVs act as "docking stations" for anti-PD-L1 antibodies, creating a powerful, multifunctional therapeutic targeting tool for glioblastoma. Recent innovations also include "all-in-one" electrochemical devices that automate the complete process.[72] These portable systems use microstructured electrodes to capture EVs from biofluids with surface antibodies, load them with siRNA through localized electric fields, and release them electrochemically. This integration reduces a complex, multi-step, multi-day process into a quick, automated operation on a single chip, supporting the idea that microfluidic confinement reduces sample loss and processing time.

2.3. Disassembly and reassembly: engineering EV-mimetic carriers

The disassembly and reassembly approach is the most structurally invasive yet highly engineerable method for EV cargo loading. In this strategy, native EVs are intentionally destabilized and reconstructed, effectively transitioning from authentic EVs to

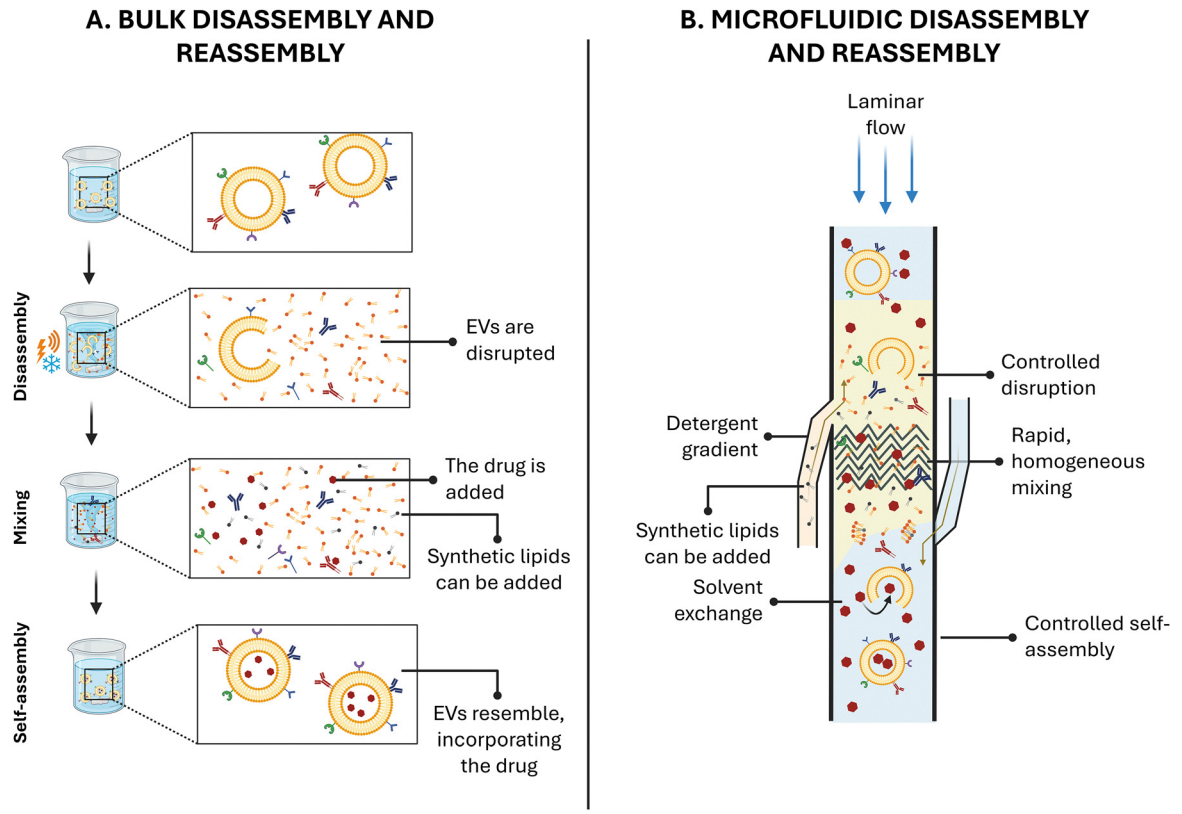

Fig. 7 Bulk *versus* microfluidic disassembly and reassembly method for loading EVs. (A) Bulk processing relies on detergent- or shear-induced disruption of native EV membranes, followed by mixing with therapeutic cargo and optional synthetic lipids. As membrane fragments reassemble, hybrid vesicles form with tunable size, composition, and drug content. (B) Microfluidic disassembly and reassembly offers a more controlled and reproducible alternative. EVs undergo finely regulated membrane destabilization within microchannels, rapid homogeneous cargo mixing, and solvent-exchange-driven self-assembly, yielding uniform vesicles with precise lipid–protein ratios and consistent loading efficiencies. Created with Biorender.

EV-mimetic or hybrid vesicles (Fig. 7A). Here, EV membranes serve as modular biological building blocks, providing the structural basis for engineered vesicles that retain some but not all features of their original EVs. Therefore, in this method, EV membranes are intentionally destabilized, leading to lipid bilayer disruption into smaller membrane fragments or mixed micellar phases.[45,131] During this disassembly process, membrane-associated proteins may detach or redistribute, and the EV's three-dimensional structure is essentially lost, creating an opportunity to combine EV-derived lipids with the desired therapeutic cargo in a homogeneous environment. Once the cargo is introduced, the system is gently guided back toward conditions that promote membrane self-assembly.[45,131,132] As the membrane components reassemble, the cargo becomes encapsulated within the reforming lumen or incorporated into the membrane, depending on its physicochemical properties. The resulting vesicles are not exact replicas of the original EVs; instead, they are EV-mimetic or hybrid constructs whose size, lipid composition, and surface protein levels can be intentionally adjusted.[133] For instance, the final vesicle size can be controlled, while the protein-to-lipid ratio can be modified by varying the extent of membrane solubilization during disassembly.

This approach offers a level of engineering control rarely possible when working with intact EVs. By dissolving the vesicle structure and allowing it to reform around the cargo, the method bypasses inherent membrane permeability limits and achieves high encapsulation efficiency even for large proteins, nucleic acids, or hydrophilic drug molecules that typically have difficulty crossing the intact bilayer.[45,75] It also enables targeted enrichment or removal of specific membrane components. Surface proteins responsible for targeting can be preserved by limiting the extent of solubilization, while non-essential or variable contaminants can be eliminated, thus reducing biological variability.[134] This opens the door to producing semi-synthetic EV-like particles with greater batch-to-batch consistency, addressing a significant challenge in the clinical translation of native EVs. At the same time, this approach falls between natural EVs and entirely artificial liposomes, conceptually. It leverages the biological complexity of EV-derived lipids and proteins – elements that enhance cellular uptake, immune compatibility, and biodistribution – while incorporating the manufacturing controls found in synthetic nanocarriers.[135,136] Because size, composition, and encapsulation efficiency can be systematically adjusted, the disassembly and reassembly technique helps overcome the variability of native EVs, and supports the standardization needed for consistent therapeutic products. However, the reconstruction process inevitably changes the EV's native identity: protein orientation may shift,

some membrane-associated molecules may be lost, and the final vesicle might lack certain subtle, cell type-specific features that define authentic EVs. Therefore, while this method provides unmatched engineering flexibility, it does so at the expense of biological authenticity. For applications where exact molecular composition, reproducibility, and tunable loading are more critical than preserving native EV features, disassembly and reassembly offer a robust and scalable approach.

Microfluidic systems make the disassembly and reassembly process much more controlled, modular, and reproducible than when done in bulk. In a microfluidic format, each step (membrane destabilization, cargo mixing, vesicle reconstruction, and purification) can be performed with spatial and temporal precision that is not achievable in traditional becher-based workflows (Fig. 7B). The process usually starts with a disassembly phase, where EVs are exposed to precisely controlled shear forces, detergent levels, or solvent gradients within microchannels. Unlike bulk detergent treatment, which exposes the entire suspension to uniform but often excessive solubilization, microfluidics allows the detergent or solvent concentration to increase gradually along the flow path. This results in partial membrane disruption rather than complete solubilization, helping prevent protein denaturation or lipid loss.[131] In systems that rely on shear-based destabilization, channel constrictions or extensional flow segments can be designed to deliver short, repeated pulses of mechanical stress that break the membrane into nanoscale patches without entirely dissolving the lipid and protein matrix. Because each EV encounters nearly identical stress patterns, the level of disassembly remains much more consistent than in bulk processes, where vesicle fragmentation tends to be random and uneven. Once the membrane has been destabilized, the fragmented EV components enter a controlled-mixing region, where they come into contact with the drug payload. Microfluidic mixers (whether based on hydrodynamic focusing, serpentine channels, staggered herringbone structures, or diffusive laminar interfaces) ensure that cargo molecules rapidly spread throughout the lipid and protein fragments.[137] This removes concentration gradients and incomplete mixing, which often lowers encapsulation efficiency in bulk methods. Importantly, because the microfluidic environment maintains a specific ratio of membrane fragments to cargo, the final vesicle population can be designed to contain precise amounts of therapeutic material per particle. Reassembly occurs as the mixture moves into a solvent-exchange zone or a hydrodynamic focusing area. In solvent-exchange systems, organic solvents or detergents are slowly removed through buffer dilution or by diffusive laminar contact with a detergent-free stream. As the local solvent composition changes, lipid fragments naturally self-assemble into bilayers and form vesicles.[138] Hydrodynamic focusing can speed up this process by squeezing the disassembling mixture into a narrow stream, changing interfacial tension, and encouraging vesicle formation.[139] Because the reassembly conditions are carefully controlled, the resulting vesicles have narrow size distributions, consistent membrane composition, and reliable encapsulation efficiencies.[140] A final purification or size-control step can be integrated on-chip, using microfluidic filters, electrophoretic separation, or tangential-flow microfiltration.[141] This allows for the immediate removal of excess drug, solvent, or detergent, producing a clean preparation without the need for off-chip purification. The entire workflow (from disassembly to purification) can thus be performed in a continuous flow, making the process inherently scalable and suitable for automation.

The benefits of this microfluidic approach are significant. By enabling vesicles to reassemble around the therapeutic cargo, the method attains very high encapsulation efficiency, even for hydrophilic drugs or large biomolecules that cannot pass through intact EV membranes.[142] Since membrane solubilization can be adjusted, the lipid-to-protein ratio of the final vesicles can be precisely controlled, enabling the production of standardized EV-mimetic carriers rather than relying on biologically variable native preparations. However, it is important to note that the characteristics of the starting EV population – such as cell source, physiological state, and intrinsic molecular composition – can still influence the final vesicle properties, meaning that upstream variability may partially propagate through the microfluidic assembly process. The continuous microfluidic operation enables predictable, reproducible large-scale manufacturing, with the entire workflow monitored and controlled in real time. Combining loading, assembly, and purification into one platform reduces sample handling, lowers contamination risk, and simplifies GMP implementation.

These advantages, however, come with trade-offs. The process inevitably disrupts the native architecture of EVs, producing EV-mimetic or hybrid vesicles that retain some native features but are no longer authentic EVs, effectively bridging the space between natural EVs and fully synthetic nanocarriers. Surface biomarkers may be partially lost, reoriented, or redistributed during solubilization, thereby altering their biodistribution, targeting, and immunomodulatory function. Because the end products differ from the native EV phenotype, they present regulatory challenges: it is unclear whether they should be classified as engineered EVs, synthetic liposomes with biological components, or a new category of hybrid nanoparticles. This ambiguity impacts both safety evaluation and approval processes. Despite these limitations, the microfluidic disassembly and reassembly method offers a strong framework for producing highly uniform, fully customizable EV-mimetic vesicles with predictable therapeutic cargo loading. It is the most engineerable frontier of EV-based drug delivery, providing unprecedented control over structure and composition while enabling scalable, reproducible manufacturing.

Although the microfluidic disassembly and reassembly framework remains mostly conceptual for natural EVs, extensive evidence from microfluidic liposome and hybrid nanoparticle synthesis strongly supports its feasibility. For example, precise liposome size control and reduced polydispersity can be achieved *via* microfluidic hydrodynamic focusing.[143] The foundational work by Jahn *et al.* showed that microfluidic hydrodynamic flow focusing can enable highly controlled solvent exchange, producing monodisperse lipid vesicles whose size is

easily tunable simply by adjusting flow-rate ratios,[144] an essential mechanism behind the microfluidic reassembly strategies mentioned earlier. This principle was further systematized by Yu *et al.*, who outlined operational rules for reproducible vesicle formation *via* the gradual dilution of organic solvents within microchannels, directly reflecting the solvent exchange-driven reassembly step proposed for EV-derived membranes.[53] More recently, Hood *et al.* extended microfluidic hydrodynamic flow focusing to 3D concentric capillaries (vertical flow focusing), achieving high throughput while maintaining monodisperse size distributions.[145] Microfluidics further enabled large-scale liposome production with tight size control.[146] Although these studies were conducted on synthetic lipids rather than biological EV membranes, they experimentally confirm the core principles of controlled destabilization, uniform cargo mixing, and solvent-mediated vesicle reassembly in flow, exactly the steps needed for EV-mimetic construction. Further emerging research supports this trend: microfluidic droplet fusion has been used to combine EV fragments with lipid nanoparticles, creating hybrid vesicles with programmable membrane composition;[147] shear-induced vesiculation chips have produced EV-like vesicles from cell membranes with high uniformity;[148] and microfluidic lipid-insertion platforms have demonstrated that surface proteins and ligands can be incorporated into reassembling membranes under laminar flow.[149] Overall, this body of work shows that the physical principles underlying microfluidic disassembly and reassembly have already been well validated in related systems, making the transition to EV-derived vesicles theoretically feasible, though still in its early experimental stages.

2.4. Cross-strategy comparison: cargo compatibility, manufacturing complexity, and GMP readiness

Taken together, the three microfluidic strategies discussed above are not interchangeable, but rather complementary across several practically relevant dimensions, summarized in Table 3, and detailed here.

Cargo compatibility. Controlled passive incubation remains intrinsically constrained to small, membrane-permeant hydrophobic molecules, as loading relies solely on passive partitioning across an intact bilayer (*e.g.*, verteporfin[66]). Soft active loading strategies accommodate a substantially broader cargo range – from small molecules and macromolecular dextrans (mechanical nanoporation[67]) to nucleic acids such as mRNA and siRNA (electroporation-based platforms[71,72]) – because the intensity and duration of membrane permeabilization (shear, extensional, or electric field-based) can be tuned to the size and charge of the target cargo. Disassembly and reassembly approaches, by bypassing the permeability constraints of an intact membrane altogether, are best suited to large proteins, nucleic acids, and hydrophilic drugs that are otherwise poorly compatible with the other two strategies.[46,75]

Risk to EV structural integrity. The three strategies occupy distinct positions along a gradient of invasiveness. Passive incubation poses minimal risk, as no mechanical or electrical perturbation is applied. Soft active loading is reversible by design, but carries a risk of partial damage, aggregation, or loss of surface markers if shear, field strength, or exposure time are not carefully calibrated.[115,128] Disassembly and reassembly is, by definition, the most invasive of the three: it does not merely risk damage but deliberately and irreversibly dismantles the native EV architecture, trading biological authenticity for engineering control over the resulting vesicle composition.[131]

Hardware configuration, manufacturing cost, and technical complexity. Beyond the cargo compatibility and structural risk considerations discussed above, the three strategies differ substantially in the engineering specifications and fabrication effort required for implementation, broadly tracking their position along the invasiveness gradient just described: controlled passive incubation requires only laminar flow control and is the most straightforward to implement and validate under GMP frameworks; soft active loading requires more sophisticated channel geometries or integrated microelectrodes;[121,129,130] and disassembly and reassembly requires the greatest overall complexity, integrating sequential destabilization, mixing, reassembly, and purification stages within a single workflow.[131,140,141] Controlled passive incubation requires the simplest hardware: precise control of flow rate and channel geometry is sufficient to define residence time (*e.g.*, flow rates on the order of 1 μL min^{-1} with residence times of approximately 10 minutes have been reported for verteporfin loading into MSC-derived EVs[66]), without the need for integrated actuators, electrodes, or transducers. This translates into lower fabrication cost and simpler GMP qualification, though at the expense of the narrower cargo range discussed above. Soft active loading requires substantially more complex device architectures. Mechanical nanoporation devices, for instance, rely on nanochannel arrays with heights on the order of 130 nm – closely matched to EV diameter – fabricated with high-precision nanolithography, which increases manufacturing cost and limits throughput scalability relative to simpler microchannel geometries.[67] Microfluidic electroporation platforms require integrated microelectrodes and precise voltage control; reported configurations span from low-voltage droplet-based systems operating at 10–60 V DC[70] to continuous-flow serpentine electroporation channels with interdigitated electrodes operating at 6–22 V_PP under AC excitation.[70] The need for electrode microfabrication, electrical insulation, and prevention of Joule heating adds engineering complexity and cost compared to purely hydrodynamic (passive or shear-based) designs, and requires additional validation steps (*e.g.*, electrode degradation monitoring) for GMP-compliant manufacturing. Disassembly and reassembly platforms require the greatest hardware complexity among the three strategies, as they must sequentially integrate distinct functional zones for membrane destabilization, cargo mixing, vesicle reassembly, and on-chip purification within a single continuous-flow device.[131] This multi-stage architecture increases both fabrication cost and the number of process parameters that must be independently validated for regulatory qualification, representing the most demanding strategy in terms of manufacturing investment, even though it offers the highest engineering control over the final vesicle composition.

Process validation, critical quality attributes, and manufacturing considerations. Although specific regulatory guidance

for EV-based therapeutics is still evolving, current Food and Drug Administration expectations for biologics and advanced therapy medicinal products emphasize process validation, definition of critical quality attributes, aseptic manufacturing, traceability, and process analytical technologies enabling real-time monitoring. In this context, microfluidic platforms are particularly attractive because their closed-system architecture and precise control of process parameters facilitate implementation of these requirements, although strategy-specific validation frameworks remain to be established. As outlined in the Introduction, GMP-compliant manufacturing requires processes capable of consistently producing batches that meet pre-defined CQAs, supported by real-time process monitoring and validated, reproducible operating conditions.[28,48–52] Although a comprehensive regulatory analysis under specific national frameworks is beyond the conceptual scope of this review, the three microfluidic strategies discussed above differ meaningfully in how readily they support these requirements. Controlled passive incubation is the most directly amenable to process validation: because loading depends solely on diffusion under fixed flow, temperature, and pH conditions, key process parameters can be continuously recorded and adjusted in real time, supporting robust, well-defined CQAs (*e.g.*, residence time, drug concentration, EV/drug stream ratio) and facilitating inline documentation, as already discussed in Section 2.1.[56,80,84,95,96] Soft active loading strategies similarly support inline quality control, as microfluidic platforms can integrate optical detectors, impedance sensors, or nanoparticle counters to monitor size distribution, concentration, and surface charge in real time during shear-, extensional-, or field-based perturbation (Section 2.2);[127] however, defining robust CQAs for these strategies is comparatively more demanding, since the reversibility of membrane permeabilization is sensitive to small deviations in shear, voltage, or exposure time, requiring tighter process control windows than passive incubation.[115,128] Disassembly and reassembly strategies present the greatest challenge for CQA definition and process validation, as the resulting EV-mimetic vesicles are not standard EVs but hybrid constructs whose surface composition, lipid-to-protein ratio, and biological identity can vary depending on the extent of membrane solubilization; this ambiguity directly affects how such products would be classified and validated within existing pharmaceutical quality frameworks, even though the sequential, multi-stage nature of the process (destabilization, mixing, reassembly, purification) is, in principle, compatible with continuous, automatable, and aseptic operation.[131] Across all three strategies, the closed, continuous-flow nature of microfluidic processing is inherently favorable to aseptic manufacturing, as it limits manual handling and open-system exposure relative to bulk batch processing. However, translating this inherent advantage into a fully validated GMP process will require strategy-specific quantitative CQA definitions and dedicated process validation studies, which remain largely undeveloped for EV drug loading and represent an important direction for future translational work.

Universal engineering limitations. Beyond the strategy-specific trade-offs discussed above, microfluidic EV loading platforms share several inherent engineering limitations that are often underemphasized relative to their advantages in reproducibility and process control. Non-specific protein adsorption onto channel walls is a well-documented limitation of microfluidic devices handling protein-rich biological suspensions, including EV preparations; surface fouling progressively alters channel hydrodynamics and can compromise long-term reproducibility, an issue already noted for soft active loading devices in Section 2.2[128] but equally relevant to passive incubation and disassembly and reassembly platforms, where membrane fragments and reassembling vesicles can similarly adsorb to channel surfaces. Channel clogging represents a related and frequently reported concern in microfluidic systems handling biological particulates, where progressive accumulation of trapped material can unpredictably alter channel hydrodynamic resistance and ultimately halt operation altogether;[150,151] this is especially critical for strategies relying on sub-micron constrictions or nanochannels, such as mechanical nanoporation, where even minor particulate contamination can obstruct flow entirely. Throughput represents a further, frequently underemphasized limitation. While microfluidic systems are often described as scalable, a single microchannel operating under the low-Reynolds-number, laminar-flow conditions required for precise, reproducible loading (Section 2.1) inherently processes comparatively small sample volumes per unit time. Achieving clinical-batch throughput, therefore, typically requires parallelization across multiple channels or devices, as demonstrated for other microfluidic nanoparticle manufacturing applications,[58] which introduces additional engineering challenges in maintaining uniform flow distribution, pressure balancing, and consistent process parameters across parallel units – challenges that are rarely addressed in proof-of-concept, single-channel demonstrations. Finally, long-term continuous operation stability remains insufficiently characterized for most of the strategies discussed in this review. Microfluidic devices handling biological samples are frequently developed and validated only for short-term, single-use operation, since progressive fouling and clogging can severely limit performance over extended timescales unless specifically engineered against.[152,153] Systematic characterization of device performance, reproducibility, and material stability over the longer operational timescales required for industrial manufacturing remains, to our knowledge, largely unaddressed for the microfluidic EV-loading strategies discussed in this review. Taken together, this multidimensional positioning – spanning cargo compatibility, structural risk, hardware complexity, regulatory readiness, and shared engineering constraints – rather than a strict hierarchy of performance, should guide strategy selection in future translational efforts.

3. Future perspectives

While the conceptual framework outlined in this review establishes the physical plausibility of microfluidic EV drug loading, its translation into clinically deployable manufacturing

processes hinges on resolving several concrete scientific questions, emerging directly from the comparative trade-offs identified in Section 2.4.

First, can the cargo-compatibility boundaries identified for each strategy (Section 2.4) be extended – for example, through hybrid passive/active schemes – to access a broader range of therapeutic cargo without sacrificing the gentleness of passive incubation or fully committing to the structural invasiveness of disassembly and reassembly?

Second, what quantitative relationship links shear, voltage, or exposure-time parameters to the threshold separating reversible, structure-preserving permeabilization from irreversible damage in soft active loading, and can this threshold be defined with sufficient precision to support robust process design across EV sources?

Third, what scalable, parallel-channel architectures and fouling-resistant materials can sustain long-term, clinical-batch-compatible throughput across all three strategies, without disproportionately increasing the hardware complexity and manufacturing cost identified for the more invasive approaches?

Fourth, can strategy-specific, quantitatively defined CQA panels be established and experimentally validated for soft active loading and disassembly and reassembly – whose narrower process-control windows currently limit GMP readiness relative to passive incubation – to bring all three strategies to a comparable level of regulatory preparedness?

Addressing these questions will be essential to move microfluidic EV drug loading from a conceptually promising but still largely unvalidated framework toward standardized, automated, and clinically deployable manufacturing processes. Beyond the loading process itself, microfluidic platforms will also need closer integration with upstream biological workflows – EV sorting, concentration, and on-chip quality-control modules – to ensure consistent, well-characterized inputs across the entire manufacturing pipeline.

4. Conclusions

Microfluidic technologies are poised to transform EV drug loading by providing unprecedented control over processes that were once empirical, low-throughput, and inconsistent. By analyzing the fluid-mechanical regimes most relevant to EV membrane perturbation (from shear-stress-mediated defects to extensional flow-induced membrane dilation), this review highlighted how EVs can be loaded with therapeutic cargo through precise manipulation of microscale fluid dynamics, shear stress, residence time, temperature, pH, and concentration gradients. Conceptually, this review reframes microfluidic EV drug loading not as a scaled-down version of existing loading methods, but as a platform-level shift in control: physical parameters that are empirically managed in bulk processing – shear, residence time, temperature, and field exposure – become precisely defined, tunable design variables under microfluidic confinement. This shift is articulated through three mechanistically distinct strategies (controlled passive incubation, soft active loading, and disassembly and reassembly), each evaluated against the EV-specific biophysical constraints discussed above, rather than assumed directly transferable from synthetic vesicle systems, and further differentiated in terms of cargo compatibility, risk to EV integrity, hardware complexity, and amenability to GMP-relevant process validation. This shift in thinking opens the door to standardized and automated processes that could be developed toward GMP compatibility and, in the long term, replace current bulk loading techniques, provided the translational questions outlined above are systematically addressed.

Conflicts of interest

Maria Luisa Torre and Marzio Sorlini are co-founders and owners of PharmaExceed s.r.l. The company had no role in the design of the experiments, data collection and analysis, decision to publish, or preparation of the manuscript.

Data availability

No data were used.

Acknowledgements

This work is part of the project RESALE, which has received funding from the European Commission – NextGeneration EU and Compagnia di San Paolo.

References

1 S. El Andaloussi, I. Maeger, X. O. Breakefield and M. J. A. Wood, Extracellular vesicles: biology and emerging therapeutic opportunities, *Nat. Rev. Drug Discovery*, 2013, **12**, 348–358.
2 I. Herrmann, M. Wood and G. Fuhrmann, Extracellular vesicles as a next-generation drug delivery platform, *Nat. Nanotechnol.*, 2021, **16**, 748–759.
3 M. Kumar, S. Baba, H. Sadida, S. Marzooqi, J. Jerobin, F. Altemani, N. Algehainy, M. Alanazi, A. Abou-Samra, R. Kumar, A. Akil, M. Macha, R. Mir and A. Bhat, Extracellular vesicles as tools and targets in therapy for diseases, *Signal Transduction Targeted Ther.*, 2024, **9**(1), 27.
4 G. van Niel, G. D'Angelo and G. Raposo, Shedding light on the cell biology of extracellular vesicles, *Nat. Rev. Mol. Cell Biol.*, 2018, **19**, 213–228.
5 S. Kamerkar, V. LeBleu, H. Sugimoto, S. Yang, C. Ruivo, S. Melo, J. Lee and R. Kalluri, Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer, *Nature*, 2017, **546**(7659), 498–503.
6 O. Wiklander, M. Brennan, J. Lötval, X. Breakefield and S. E. L. Andaloussi, Advances in therapeutic applications of extracellular vesicles, *Sci. Transl. Med.*, 2019, **11**(492), 8521.

7 M. Mitchell, M. Billingsley, R. Haley, M. Wechsler, N. Peppas and R. Langer, Engineering precision nanoparticles for drug delivery, *Nat. Rev. Drug Discovery*, 2021, **20**, 101–124.

8 L. Sercombe, T. Veerati, F. Moheimani, S. Wu, A. Sood and S. Hua, Advances and Challenges of Liposome Assisted Drug Delivery, *Front. Pharmacol.*, 2015, **6**, 286.

9 U. Bulbake, S. Doppalapudi, N. Kommineni and W. Khan, Liposomal Formulations in Clinical Use: An Updated Review, *Pharmaceutics*, 2017, **9**(2), 12.

10 N. Kamaly, Z. Xiao, P. Valencia, A. Radovic-Moreno and O. Farokhzad, Targeted polymeric therapeutic nanoparticles: design, development and clinical translation, *Chem. Soc. Rev.*, 2012, **41**, 2971–3010.

11 J. Lü, X. Wang, C. Marin-Muller, H. Wang, P. Lin, Q. Yao and C. Chen, Current advances in research and clinical applications of PLGA-based nanotechnology, *Expert Rev. Mol. Diagn.*, 2009, **9**, 325–341.

12 R. Sperling, P. Rivera gil, F. Zhang, M. Zanella and W. Parak, Biological applications of gold nanoparticles, *Chem. Soc. Rev.*, 2008, **37**, 1896–1908.

13 L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhal and M. J. A. Wood, Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes, *Nat. Biotechnol.*, 2011, **29**, 341–345.

14 O. Elsharkasy, J. Nordin, D. Hagey, O. de Jong, R. Schiffelers, S. El Andaloussi and P. Vader, Extracellular vesicles as drug delivery systems: Why and how?, *Adv. Drug Delivery Rev.*, 2020, **159**, 332–343.

15 S. Shaffi, O. Hairuddin, S. Mansor, T. Syafiq and B. Yahaya, Unlocking the Potential of Extracellular Vesicles as the Next Generation Therapy: Challenges and Opportunities, *Tissue Eng. Regener. Med.*, 2024, **21**, 513–527.

16 M. Yanez-Mo, P. R. M. Siljander, Z. Andreu, A. B. Zavec, F. E. Borras, E. I. Buzas, K. Buzas, E. Casal, F. Cappello, J. Carvalho, E. Colas, A. Cordeiro-Da Silva, S. Fais, J. M. Falcon-Perez, I. M. Ghobrial, B. Giebel, M. Gimona, M. Graner, I. Gursel, M. Gursel, N. H. H. Heegaard, A. Hendrix, P. Kierulf, K. Kokubun, M. Kosanovic, V. Kralj-Iglic, E.-M. Kraemer-Albers, S. Laitinen, C. Lasser, T. Lener, E. Ligeti, A. Line, G. Lipps, A. Llorente, J. Lotvall, M. Mancek-Keber, A. Marcilla, M. Mittelbrunn, I. Nazarenko, E. N. M. Nolte-t' Hoen, T. A. Nyman, L. O'Driscoll, M. Olivan, C. Oliveira, E. Pallinger, H. A. del Portillo, J. Reventos, M. Rigau, E. Rohde, M. Sammar, F. Sanchez-Madrid, N. Santarem, K. Schallmoser, M. S. Ostenfeld, W. Stoorvogel, R. Stukelj, S. G. Van der Grein, M. Helena Vasconcelos, M. H. M. Wauben and O. De Wever, Biological properties of extracellular vesicles and their physiological functions, *J. Extracell. Vesicles*, 2015, **4**, 27066.

17 A. Hoshino, B. Costa-Silva, T. L. Shen, G. Rodrigues, A. Hashimoto, M. T. Mark, H. Molina, S. Kohsaka, A. Di Giannatale, S. Ceder, S. Singh, C. Williams, N. Soplop, K. Uryu, L. Pharmer, T. King, L. Bojmar, A. E. Davies, Y. Ararso, T. Zhang, H. Zhang, J. Hernandez, J. M. Weiss, V. D. Dumont-Cole, K. Kramer, L. H. Wexler, A. Narendran, G. K. Schwartz, J. H. Healey, P. Sandstrom, K. J. Labori, E. H. Kure, P. M. Grandgenett, M. A. Hollingsworth, M. de Sousa, S. Kaur, M. Jain, K. Mallya, S. K. Batra, W. R. Jarnagin, M. S. Brady, O. Fodstad, V. Muller, K. Pantel, A. J. Minn, M. J. Bissell, B. A. Garcia, Y. Kang, V. K. Rajasekhar, C. M. Ghajar, I. Matei, H. Peinado, J. Bromberg and D. Lyden, Tumour exosome integrins determine organotropic metastasis, *Nature*, 2015, **527**, 329–335.

18 J. Armstrong, M. Holme and M. Stevens, Re-Engineering Extracellular Vesicles as Smart Nanoscale Therapeutics, *ACS Nano*, 2017, **11**, 69–83.

19 P. Cheng, X. Wang, Q. Liu, T. Yang, H. Qu and H. Zhou, Extracellular vesicles mediate biological information delivery: A double-edged sword in cardiac remodeling after myocardial infarction, *Front. Pharmacol.*, 2023, **14**, 1067992.

20 J. Macedo-da-Silva, V. Santiago, L. Rosa-Fernandes, C. Marinho and G. Palmisano, Protein glycosylation in extracellular vesicles: Structural characterization and biological functions, *Mol. Immunol.*, 2021, **135**, 226–246.

21 G. Sánchez, K. Bunn, H. Pua and M. Rafat, Extracellular vesicles: mediators of intercellular communication in tissue injury and disease, *Cell Commun. Signaling*, 2021, **19**(1), 104.

22 Y. Liu and C. Wang, A review of the regulatory mechanisms of extracellular vesicles-mediated intercellular communication, *Cell Commun. Signaling*, 2023, **21**(1), 77.

23 D. Murphy, O. de Jong, M. Evers, M. Nurazizah, R. Schiffelers and P. Vader, Natural or Synthetic RNA Delivery: A Stoichiometric Comparison of Extracellular Vesicles and Synthetic Nanoparticles, *Nano Lett.*, 2021, **21**, 1888–1895.

24 D. Ha, N. Yang and V. Nadithe, Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges, *Acta Pharm. Sin. B*, 2016, **6**, 287–296.

25 S. Du, Y. Guan, A. Xie, Z. Yan, S. Gao, W. Li, L. Rao, X. Chen and T. Chen, Extracellular vesicles: a rising star for therapeutics and drug delivery, *J. Nanobiotechnol.*, 2023, **21**(1), 231.

26 P. de Castilla, L. Tong, C. Huang, A. Sofias, G. Pastorin, X. Chen, G. Storm, R. Schiffelers and J. Wang, Extracellular vesicles as a drug delivery system: A systematic review of preclinical studies, *Adv. Drug Delivery Rev.*, 2021, **175**, 113801.

27 W. Whitford and P. Guterstam, Exosome manufacturing status, *Future Med. Chem.*, 2019, **11**, 1225–1236.

28 M. Piffoux, J. Volatron, K. Cherukula, K. Aubertin, C. Wilhelm, A. Silva and F. Gazeau, Engineering and loading therapeutic extracellular vesicles for clinical translation: A data reporting frame for comparability, *Adv. Drug Delivery Rev.*, 2021, **178**, 113972.

29 K. Dave, P. Pinky and D. Manickam, Molecular engineering of extracellular vesicles for drug delivery: Strategies, challenges, and perspectives, *J. Controlled Release*, 2025, **386**, 114068.

30 M. Konoshenko, E. Lekchnov, A. Vlassov and P. Laktionov, Isolation of Extracellular Vesicles: General Methodologies and Latest Trends, *BioMed Res. Int.*, 2018, **2018**, 8545347.
31 B. Li, W. Wu, W. Xu, H. Qian and C. Ji, Advances of extracellular vesicles isolation and detection frontier technology: from heterogeneity analysis to clinical application, *J. Nanobiotechnol.*, 2025, **23**(1), 678.
32 E. Bari, S. Perteghella, D. Di Silvestre, M. Sorlini, L. Catenacci, M. Sorrenti, G. Marrubini, R. Rossi, G. Tripodo, P. Mauri, M. Marazzi and M. L. Torre, Pilot Production of Mesenchymal Stem/Stromal Freeze-Dried Secretome for Cell-Free Regenerative Nanomedicine: A Validated GMP-Compliant Process, *Cells*, 2018, **7**(11), 190.
33 E. Bari, S. Perteghella, L. Catenacci, M. Sorlini, S. Croce, M. Mantelli, M. A. Avanzini, M. Sorrenti and M. L. Torre, Freeze-dried and GMP-compliant pharmaceuticals containing exosomes for acellular mesenchymal stromal cell immunomodulant therapy, *Nanomedicine*, 2019, **14**(6), 753–765.
34 M. Mocchi, E. Bari, G. Marrubini, A. F. Bonda, S. Perteghella, F. Tartara, F. Cofano, G. di Perna, L. Giovannelli, D. Mandracchia, M. Sorlini, D. Garbossa, M. L. Torre and L. Segale, Freeze-Dried Mesenchymal Stem Cell-Secretome Pharmaceuticalization: Optimization of Formulation and Manufacturing Process Robustness, *Pharmaceutics*, 2021, **13**(8), 1129.
35 E. Bari, D. Di Silvestre, L. Mastracci, F. Grillo, P. Grisoli, G. Marrubini, M. Nardini, M. Mastrogiacomo, M. Sorlini, R. Rossi, M. L. Torre, P. Mauri, G. Sesana and S. Perteghella, GMP-compliant sponge-like dressing containing MSC lyosecretome: proteomic network of healing in a murine wound model, *Eur. J. Pharm. Biopharm.*, 2020, **155**, 37–48.
36 P. Berni, M. Del Bue, V. Conti, V. Andreoli, R. Ramoni, M. Angelone, G. Squassino, E. Bari, M. Torre, M. Rinaldi, S. Dotti, R. Rossi, I. Yusuf, P. Mauri, D. Di Silvestre and S. Grolli, Clinical evaluation of freeze-dried secretome (lyosecretome) for osteoarthritis: a controlled trial in dogs and preliminary safety assessment in horses, *Int. J. Pharm.*, 2025, **681**, 125864.
37 M. Mocchi, E. Bari, S. Dotti, R. Villa, P. Berni, V. Conti, M. Del Bue, G. P. Squassino, L. Segale, R. Ramoni, M. L. Torre, S. Perteghella and S. Grolli, Canine Mesenchymal Cell Lyosecretome Production and Safety Evaluation after Allogenic Intraarticular Injection in Osteoarthritic Dogs, *Animals*, 2021, **11**(11), 3271.
38 G. Melling, E. Carollo, R. Conlon, J. Simpson and D. Carter, The Challenges and Possibilities of Extracellular Vesicles as Therapeutic Vehicles, *Eur. J. Pharm. Biopharm.*, 2019, **144**, 50–56.
39 X. Lu, S. Fan, M. Cao, D. Liu, K. Xuan and A. Liu, Extracellular vesicles as drug delivery systems in therapeutics: current strategies and future challenges, *J. Pharm. Invest.*, 2024, **54**, 785–802.
40 B. Joshi, D. Ortiz and I. Zuhorn, Converting extracellular vesicles into nanomedicine: loading and unloading of cargo, *Mater. Today Nano*, 2021, **16**, 100148.
41 G. Fuhrmann, A. Serio, M. Mazo, R. Nair and M. Stevens, Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins, *J. Controlled Release*, 2015, **205**, 35–44.
42 H. Kim, J. Park, Y. Zhu, X. Wang, Y. Han and D. Zhang, Recent advances in extracellular vesicles for therapeutic cargo delivery, *Exp. Mol. Med.*, 2024, **56**, 836–849.
43 M. J. Haney, N. L. Klyachko, Y. Zhaoa, R. Gupta, E. G. Plotnikova, Z. He, T. Patel, A. Piroyan, M. Sokolsky, A. V. Kabanov and E. V. Batrakova, Exosomes as drug delivery vehicles for Parkinson's disease therapy, *J. Controlled Release*, 2015, **207**, 18–30.
44 M. S. Kim, M. J. Haney, Y. Zhao, V. Mahajan, I. Deygen, N. L. Klyachko, E. Inskoe, A. Piroyan, M. Sokolsky, O. Okolie, S. D. Hingtgen, A. V. Kabanov and E. V. Batrakova, Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells, *Nanomedicine*, 2016, **12**, 655–664.
45 Y. T. Sato, K. Umezaki, S. Sawada, S. Mukai, Y. Sasaki, N. Harada, H. Shiku and K. Akiyoshi, Engineering hybrid exosomes by membrane fusion with liposomes, *Sci. Rep.*, 2016, **6**, 21933.
46 R. McConnell, M. Youniss, B. Gnanasambandam, P. Shah, W. Zhang and J. Finn, Transfection reagent artefact likely accounts for some reports of extracellular vesicle function, *J. Extracell. Vesicles*, 2022, **11**(10), e12253.
47 M. Gimona, K. Pachler, S. Laner-Plamberger, K. Schallmoser and E. Rohde, Manufacturing of Human Extracellular Vesicle-Based Therapeutics for Clinical Use, *Int. J. Mol. Sci.*, 2017, **18**(6), 1190.
48 T. Lener, M. Gimona, L. Aigner, V. Boerger, E. Buzas, G. Camussi, N. Chaput, D. Chatterjee, F. A. Court, H. A. del Portillo, L. O'Driscoll, S. Fais, J. M. Falcon-Perez, U. Felderhoff-Mueser, L. Fraile, Y. S. Gho, A. Goergens, R. C. Gupta, A. Hendrix, D. M. Hermann, A. F. Hill, F. Hochberg, P. A. Horn, D. de Kleijn, L. Kordelas, B. W. Kramer, E.-M. Kraemer-Albers, S. Laner-Plamberger, S. Laitinen, T. Leonardi, M. J. Lorenowicz, S. K. Lim, J. Lotvall, C. A. Maguire, A. Marcilla, I. Nazarenko, T. Ochiya, T. Patel, S. Pedersen, G. Pocsfalvi, S. Pluchino, P. Quesenberry, I. G. Reischl, F. J. Rivera, R. Sanzenbacher, K. Schallmoser, I. Slaper-Cortenbach, D. Strunk, T. Tonn, P. Vader, B. W. M. van Balkom, M. Wauben, S. El Andaloussi, C. Thery, E. Rohde and B. Giebel, Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper, *J. Extracell. Vesicles*, 2015, **4**, 30087.
49 L. Giovannelli, E. Bari, C. Jommi, F. Tartara, D. Armocida, D. Garbossa, F. Cofano, M. L. Torre and L. Segale, Mesenchymal stem cell secretome and extracellular vesicles for neurodegenerative diseases: Risk-benefit profile and next steps for the market access, *Bioact. Mater.*, 2023, **29**, 16–35.
50 E. Bari, I. Ferrarotti, M. L. Torre, A. G. Corsico and S. Perteghella, Mesenchymal stem/stromal cell secretome for lung regeneration: The long way through "pharmaceuticalization" for the best formulation, *J. Controlled Release*, 2019, **309**, 11–24.

51 H. Aleem, Y. Zhao, S. Lord, T. McCarthy and P. Sharratt, Pharmaceutical process validation: an overview, *Proc. Inst. Mech. Eng., Part E*, 2003, **217**, 141–151.

52 C. Wang, T. Tsai and C. Lee, Regulation of exosomes as biologic medicines: Regulatory challenges faced in exosome development and manufacturing processes, *CTS: Clin. Transl. Sci.*, 2024, **17**(8), e13904.

53 J. Huang, H. Chen, N. Li, P. Liu, J. Yang and Y. Zhao, Emerging technologies towards extracellular vesicles large-scale production, *Bioact. Mater.*, 2025, **52**, 338–365.

54 P. Valencia, O. Farokhzad, R. Karnik and R. Langer, Microfluidic technologies for accelerating the clinical translation of nanoparticles, *Nat. Nanotechnol.*, 2012, **7**, 623–629.

55 F. Moreno-Sibaja, D. Zou, S. Liu, C. Zhao and L. Gerstweiler, Microfluidics in biomanufacturing process development, *Biotechnol. Adv.*, 2025, **85**, 108715.

56 W. Jo, D. Jeong, J. Kim, S. Cho, S. Jang, C. Han, J. Kang, Y. Gho and J. Park, Microfluidic fabrication of cell-derived nanovesicles as endogenous RNA carriers, *Lab Chip*, 2014, **14**, 1261–1269.

57 S. Shepherd, C. Warzecha, S. Yadavali, R. El-Mayta, M. Alameh, L. Wang, D. Weissman, J. Wilson, D. Issadore and M. Mitchell, Scalable mRNA and siRNA Lipid Nanoparticle Production Using a Parallelized Microfluidic Device, *Nano Lett.*, 2021, **21**, 5671–5680.

58 A. Bezelya, B. Küçüktürkmen, T. Böncü and A. Bozkir, Microfluidics-Based Nanoparticle Formulations: Preparation and Evaluation of Protein Delivery Systems, *Polym. Adv. Technol.*, 2025, **36**(7), e70250.

59 A. Kara, B. Ongoren, B. Anaya, A. Lalatsa and D. Serrano, Continuous manufacturing of nanomedicines using 3D-printed microfluidic devices, *Appl. Mater. Today*, 2025, **43**, 102672.

60 C. Wang, H. Zhang, Y. Yu, J. He, Y. Xing and Z. Meng, Microfluidic magnetic-field-assisted nanoparticle assembly for rapid separation and profiling of small extracellular vesicles, *Sep. Purif. Technol.*, 2026, **382**(5), 136108.

61 S. Cavallaro, F. Pevere, F. Stridfeldt, A. Görgens, C. Paba, S. Sahu, D. Mamand, D. Gupta, S. El Andaloussi, J. Linnros and A. Dev, Multiparametric Profiling of Single Nanoscale Extracellular Vesicles by Combined Atomic Force and Fluorescence Microscopy: Correlation and Heterogeneity in Their Molecular and Biophysical Features, *Small*, 2021, **17**(4), e2008155.

62 Y. Lee, S. Chae and D. Choi, Monitoring of single extracellular vesicle heterogeneity in cancer progression and therapy, *Front. Oncol.*, 2023, **13**, 1256585.

63 N. Seo and T. Ichiki, Negative Surface Charge and Membrane Lipid Composition Underlying Extracellular Vesicle Function, *ACS Nano Medicine*, 2026, **4**, 791–805.

64 L. van der Koog, T. B. Gandek and A. Nagelkerke, Liposomes and Extracellular Vesicles as Drug Delivery Systems: A Comparison of Composition, Pharmacokinetics, and Functionalization, *Adv. Healthcare Mater.*, 2022, **11**(5), e2100639.

65 S. Itakura, A. Shohji, S. Amagai, M. Kitamura, K. Takayama, K. Sugibayashi and H. Todo, Gene knockdown in HaCaT cells by small interfering RNAs entrapped in grapefruit-derived extracellular vesicles using a microfluidic device, *Sci. Rep.*, 2023, **13**, 3102.

66 C. Piunti, S. Micheli, S. Giancaterino, P. Fusco, C. Boi and E. Cimetta, Microfluidic loading of verteporfin into extracellular vesicles for neuroblastoma therapy, *Lab Chip*, 2025, **25**, 1718–1727.

67 R. Hao, Z. Yu, J. Du, S. Hu, C. Yuan, H. Guo, Y. Zhang and H. Yang, A High-Throughput Nanofluidic Device for Exosome Nanoporation to Develop Cargo Delivery Vehicles, *Small*, 2021, **17**(35), e2102150.

68 X. Pan, P. Huang, S. Ali, B. Renslo, Z. Greenberg, N. Erwin, Y. Li, Z. Ding, T. Hutchinson, A. Warnecke, N. Fernandez, H. Staecker and M. He, Extracellular vesicle-mediated gene editing for the treatment of nonsyndromic progressive hearing loss in adult mice, *Sci. Transl. Med.*, 2025, **17**(824), eadn3993.

69 C. Liu, W. Zhang, Y. Li, J. Chang, F. Tian, F. Zhao, Y. Ma and J. Sun, Microfluidic Sonication To Assemble Exosome Membrane-Coated Nanoparticles for Immune Evasion-Mediated Targeting, *Nano Lett.*, 2019, **19**, 7836–7844.

70 A. Ozcelik, G. Demirbolat, O. Erdogan, B. Kozan, F. Akkoyun, E. Cevik, E. Gumus, I. Gul, L. Trabzon and O. Cevik, Lab-on-a-chip system for small extracellular vesicle isolation and drug loading for small extracellular vesicle-mediated drug delivery for treatment of cancer patients, *Emergent Mater.*, 2025, **8**, 1153–1169.

71 S. Dong, X. Liu, Y. Bi, Y. Wang, A. Antony, D. Lee, K. Huntoon, S. Jeong, Y. Ma, X. Li, W. Deng, B. Schrank, A. Grippin, J. Ha, M. Kang, M. Chang, Y. Zhao, R. Sun, X. Sun, J. Yang, J. Chen, S. Tang, L. Lee, A. Lee, L. Teng, S. Wang, L. Teng, B. Kim, Z. Yang and W. Jiang, Adaptive design of mRNA-loaded extracellular vesicles for targeted immunotherapy of cancer, *Nat. Commun.*, 2023, **14**, 6610.

72 V. Krivitsky, A. Krivitsky, V. Mantella, M. Greenwald, D. Sankar, J. Betschmann, J. Bader, N. Zoratto, K. Schreier, S. Feiss, D. Walker, J. Dengjel, S. Werner and J. Leroux, Ultrafast and Controlled Capturing, Loading, and Release of Extracellular Vesicles by a Portable Microstructured Electrochemical Fluidic Device, *Adv. Mater.*, 2023, **35**(44), 2212000.

73 I. Moriguchi, S. Hirono, Q. Liu, I. Nakagome and Y. Matsushita, Simple Method of Calculating Octanol Water Partition-Coefficient, *Chem. Pharm. Bull.*, 1992, **40**, 127–130.

74 M. Gabba, J. Frallicciardi, J. van't Klooster, R. Henderson, L. Syga, R. Mans, A. van Maris and B. Poolman, Weak Acid Permeation in Synthetic Lipid Vesicles and Across the Yeast Plasma Membrane, *Biophys. J.*, 2020, **118**, 422–434.

75 M. Piffoux, A. Silva, C. Wilhelm, F. Gazeau and D. Tareste, Modification of Extracellular Vesicles by Fusion with Liposomes for the Design of Personalized Biogenic Drug Delivery Systems, *ACS Nano*, 2018, **12**, 6830–6842.

76 H. Zeng, S. Guo, X. Ren, Z. Wu, S. Liu and X. Yao, Current Strategies for Exosome Cargo Loading and Targeting Delivery, *Cells*, 2023, **12**(10), 1416.

77 A. Marcianti, E. Spampinato, S. Nava, G. Stella, P. Perego, S. Pogliani, S. Frigerio, L. Mirra, P. Gagni, F. Moda, F. Cazzaniga, G. Beretta, G. Maronati, G. Paglia, A. Corsico, C. Traversari and D. Lisini, Extracellular vesicles isolated from adipose tissue-derived mesenchymal stromal cells as carriers for Paclitaxel delivery, *Stem Cell Res. Ther.*, 2025, **16**, 307.
78 L. Pascucci, V. Cocce, A. Bonomi, D. Ami, P. Ceccarelli, E. Ciusani, L. Vigano, A. Locatelli, F. Sisto, S. M. Doglia, E. Parati, M. E. Bernardo, M. Muraca, G. Alessandri, G. Bondiolotti and A. Pessina, Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: A new approach for drug delivery, *J. Controlled Release*, 2014, **192**, 262–270.
79 C. Chen, J. Skog, C. Hsu, R. Lessard, L. Balaj, T. Wurdinger, B. Carter, X. Breakefield, M. Toner and D. Irimia, Microfluidic isolation and transcriptome analysis of serum microvesicles, *Lab Chip*, 2010, **10**, 505–511.
80 A. Hassanzadeh-Barforoushi, X. Sango, E. Johnston, D. Haylock and Y. Wang, Microfluidic Devices for Manufacture of Therapeutic Extracellular Vesicles: Advances and Opportunities, *J. Extracell. Vesicles*, 2025, **14**(7), e70132.
81 T. Squires and S. Quake, Microfluidics: Fluid physics at the nanoliter scale, *Rev. Mod. Phys.*, 2005, **77**, 977–1026.
82 G. Whitesides, The origins and the future of microfluidics, *Nature*, 2006, **442**, 368–373.
83 A. Mudugamuwa, U. Roshan, S. Hettiarachchi, H. Cha, H. Musharaf, X. Kang, Q. Trinh, H. Xia, N. Nguyen and J. Zhang, Periodic Flows in Microfluidics, *Small*, 2024, **20**(50), 2404685.
84 S. Gimondi, H. Ferreira, R. Reis and N. Neves, Microfluidic Devices: A Tool for Nanoparticle Synthesis and Performance Evaluation, *ACS Nano*, 2023, **17**, 14205–14228.
85 S. Dertinger, D. Chiu, N. Jeon and G. Whitesides, Generation of gradients having complex shapes using microfluidic networks, *Anal. Chem.*, 2001, **73**, 1240–1246.
86 B. Yu, R. Lee and L. Lee, Microfluidic Methods for Production of Liposomes, in *Methods in Enzymology Liposomes*, 2009, PT G, pp. 129–141.
87 P. Valencia, P. Basto, L. Zhang, M. Rhee, R. Langer, O. Farokhzad and R. Karnik, Single-Step Assembly of Homogenous Lipid - Polymeric and Lipid - Quantum Dot Nanoparticles Enabled by Microfluidic Rapid Mixing, *ACS Nano*, 2010, **4**, 1671–1679.
88 S. Chen, Q. Lin, N. Pan, M. Hao, Y. Jiang, Y. Xie, Y. Ba, X. Bian and K. Liu, Residence time distributions in microchannels with assistant flow inlets and assistant flow outlets, *Phys. Fluids*, 2023, **35**, 083609.
89 V. Miralles, A. Huerre, F. Malloggi and M.-C. Jullien, A Review of Heating and Temperature Control in Microfluidic Systems: Techniques and Applications, *Diagnostics*, 2013, **3**, 33–67.
90 J. Hood, Post isolation modification of exosomes for nanomedicine applications, *Nanomedicine*, 2016, **11**, 1745–1756.
91 W. Fan and R. Evans, Turning Up the Heat on Membrane Fluidity, *Cell*, 2015, **161**, 962–963.
92 N. Belliveau, J. Huft, P. Lin, S. Chen, A. Leung, T. Leaver, A. Wild, J. Lee, R. Taylor, Y. Tam, C. Hansen and P. Cullis, Microfluidic Synthesis of Highly Potent Limit-size Lipid Nanoparticles for In Vivo Delivery of siRNA, *Mol. Ther. – Nucleic Acids*, 2012, **1**(8), e37.
93 I. Zhigaltsev, Y. Tam, A. Leung and P. Cullis, Production of limit size nanoliposomal systems with potential utility as ultra-small drug delivery agents, *J. Liposome Res.*, 2016, **26**, 96–102.
94 G. Nunziata, A. Borroni and F. Rossi, Advanced microfluidic strategies for core-shell nanoparticles: the next-generation of polymeric and lipid-based drug nanocarriers, *Chem. Eng. J. Adv.*, 2025, **22**, 100759.
95 C. Roces, G. Lou, N. Jain, S. Abraham, A. Thomas, G. Halbert and Y. Perrie, Manufacturing Considerations for the Development of Lipid Nanoparticles Using Microfluidics, *Pharmaceutics*, 2020, **12**(11), 1095.
96 F. Sarbaland, M. Kobayashi, D. Tanaka, R. Fujita, N. Tanaka and M. Furuya, Temperature Control in Microfluidic Devices: Approaches, Challenges, and Future Directions, *Appl. Sci.*, 2025, **15**(18), 9902.
97 V. Sharma, A. Mottafegh, J. Joo, J. Kang, L. Wang and D. Kim, Toward microfluidic continuous-flow and intelligent downstream processing of biopharmaceuticals, *Lab Chip*, 2024, **24**, 2861–2882.
98 C. Luft and R. Ketteler, Electroporation Knows No Boundaries: The Use of Electrostimulation for siRNA Delivery in Cells and Tissues, *J. Biomol. Screening*, 2015, **20**, 932–942.
99 P. Mueller and D. O. Rudin, Bimolecular Lipid Membranes: Techniques of Formation, Study of Electrical Properties, and Induction of Ionic Gating Phenomena, in *Laboratory Techniques in Membrane Biophysics: An Introductory Course*, ed. W. M. Armstrong, K. Baumann, P. C. Caldwell, T. W. Clarkson, J. Dudel, B. Frankenhaeuser, E. Frömter, G. Gardos, B. Z. Gilzburg, J. F. Hoffman, A. Kepes, R. D. Keynes, A. Kleinzeller, P. G. Kostyuk, A. Kotyk, A. A. Lev, B. Lindemann, P. Mueller, H. Muth, W. Nonner, E. Oberhausen, H. Passow, A. Rothstein, D. O. Rudin, R. Stämpfli, T. Teorell, K. J. Ullrich, N. A. Walker and W. Wilbrandt, Springer Berlin Heidelberg, Berlin, Heidelberg, 1969, pp. 141–156.
100 R. Mathieu, O. Briat, P. Gyan and J. Vinassa, Comparison of the impact of fast charging on the cycle life of three lithium-ion cells under several parameters of charge protocol and temperatures, *Appl. Energy*, 2021, **283**, 116344.
101 S. Kooijmans, S. Stremersch, K. Braeckmans, S. de Smedt, A. Hendrix, M. Wood, R. Schiffelers, K. Raemdonck and P. Vader, Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles, *J. Controlled Release*, 2013, **172**, 229–238.
102 K. Johnsen, J. Gudbergsson, M. Skov, G. Christiansen, L. Gurevich, T. Moos and M. Duroux, Evaluation of electroporation-induced adverse effects on adipose-derived stem cell exosomes, *Cytotechnology*, 2016, **68**, 2125–2138.
103 Y. Hu, J. Wei, Y. Shen, S. Chen and X. Chen, Barrier-breaking effects of ultrasonic cavitation for drug delivery

and biomarker release, *Ultrason. Sonochem.*, 2023, **94**, 106346.
104 H. Fu, J. Comer, W. Cai and C. Chipot, Sonoporation at Small and Large Length Scales: Effect of Cavitation Bubble Collapse on Membranes, *J. Phys. Chem. Lett.*, 2015, **6**, 413–418.
105 M. Ganguly, D. Debraj, N. Mazumder, J. Carpenter, S. Manickam and A. Pandit, Impact of Ultrasonication on the Oxidative Stability of Oil-in-Water Nanoemulsions: Investigations into Kinetics and Strategies to Control Lipid Oxidation, *Ind. Eng. Chem. Res.*, 2024, **63**, 10212–10225.
106 J. Teissie, M. Golzio and M. Rols, Mechanisms of cell membrane electropermeabilization: A minireview of our present (lack of?) knowledge, *Biochim. Biophys. Acta, Gen. Subj.*, 1724, **2005**, 270–280.
107 T. Shtam, R. Kovalev and M. Filatov, Exosomes are natural carriers of exogenous siRNA to human cells in vitro, *FEBS J.*, 2013, **280**, 83.
108 L. Zhu and R. Mahato, Lipid and polymeric carrier-mediated nucleic acid delivery, *Expert Opin. Drug Delivery*, 2010, **7**, 1209–1226.
109 Y. J. Li, J. Y. Wu, J. H. Liu, W. J. Xu, X. H. Qiu, S. Huang, X. B. Hu and D. X. Xiang, Artificial exosomes for translational nanomedicine, *J. Nanobiotechnol.*, 2021, **19**(1), 242.
110 S. Antimisiaris, S. Mourtas and A. Marazioti, Exosomes and Exosome-Inspired Vesicles for Targeted Drug Delivery, *Pharmaceutics*, 2018, **10**(4), 218.
111 Y. Sato, W. Zhang, T. Baba, U. Chung and Y. Teramura, Extracellular vesicle-liposome hybrids *via* membrane fusion using cell-penetrating peptide-conjugated lipids, *Regener. Ther.*, 2024, **26**, 533–540.
112 A. Sharei, N. Cho, S. Mao, E. Jackson, R. Poceviciute, A. Adamo, J. Zoldan, R. Langer and K. Jensen, Cell Squeezing as a Robust, Microfluidic Intracellular Delivery Platform, *J. Visualized Exp.*, 2013, **7**(81), e50980.
113 D. Wibowo, C.-X. Zhao and Y. He, Fluid properties and hydrodynamics of microfluidic systems, in: *Microfluidics for Pharmaceutical Applications*, ed. H. A. Santos, D. Liu and H. Zhang, William Andrew Publishing, 2019, ch. 2, pp. 37–77.
114 R. Dores, M. Oliveira and L. Bimbo, Microfluidic Manufacture of Composite Fibers for Biomedical Applications, *Adv. Mater. Technol.*, 2025, **10**(5), 2400976.
115 K. Islam, M. Razizadeh and Y. Liu, Coarse-grained molecular simulation of extracellular vesicle squeezing for drug loading, *Phys. Chem. Chem. Phys.*, 2023, **25**, 12308–12321.
116 K. Mediratta, M. Diab, P. Han, H. Hu and L. Wang, Emerging Strategies for Cargo Loading and Engineering of Extracellular Vesicles for Breast Cancer Treatment, *Nanomaterials*, 2025, **15**(18), 1418.
117 A. Uvizl, R. Goswami, S. Gandhi, M. Augsburg, F. Buchholz, J. Guck, J. Mansfeld and S. Girardo, Efficient and gentle delivery of molecules into cells with different elasticity *via* Progressive Mechanoporation, *Lab Chip*, 2021, **21**, 2437–2452.
118 J. Hur and A. Chung, Microfluidic and Nanofluidic Intracellular Delivery, *Adv. Sci.*, 2021, **8**(15), 2004595.
119 D. Abreu, M. Levant, V. Steinberg and U. Seifert, Fluid vesicles in flow, *Adv. Colloid Interface Sci.*, 2014, **208**, 129–141.
120 Y. Bae, H. Jang, T. Shin, G. Phukan, T. Tran, G. Lee, W. Hwang and J. Kim, Microfluidic assessment of mechanical cell damage by extensional stress, *Lab Chip*, 2016, **16**, 96–103.
121 S. Choi, H. Khoo and S. Hur, Recent Advances in Microscale Electroporation, *Chem. Rev.*, 2022, **122**(13), 11247–11286.
122 M. Wu, Z. Liu and Y. Gao, Design and Fabrication of Microelectrodes for Dielectrophoresis and Electroosmosis in Microsystems for Bio-Applications, *Micromachines*, 2025, **16**(2), 190.
123 T. Geng and C. Lu, Microfluidic electroporation for cellular analysis and delivery, *Lab Chip*, 2013, **13**, 3803–3821.
124 J. Kuo and D. Chiu, Controlling Mass Transport in Microfluidic Devices, in *Annual Review of Analytical Chemistry*, 2011, Vol. 4, pp. 275–296.
125 E. Grigorov, B. Kirov, M. Marinov and V. Galabov, Review of Microfluidic Methods for Cellular Lysis, *Micromachines*, 2021, **12**(5), 498.
126 J. Peng, C. Fang, S. Ren, J. Pan, Y. Jia, Z. Shu and D. Gao, Development of a microfluidic device with precise on-chip temperature control by integrated cooling and heating components for single updates cell-based analysis, *Int. J. Heat Mass Transfer*, 2019, **130**, 660–667.
127 M. Buttkewitz, C. Heuer and J. Bahnemann, Sensor integration into microfluidic systems: trends and challenges, *Curr. Opin. Biotechnol*, 2023, **83**, 102978.
128 P. Chakrabarty, P. Gupta, K. Illath, S. Kar, M. Nagai, F. Tseng and T. Santra, Microfluidic mechanoporation for cellular delivery and analysis, *Mater. Today Bio*, 2022, **13**, 100193.
129 K. Lei, Microfluidic Systems for Diagnostic Applications: A Review, *JALA*, 2012, **17**, 330–347.
130 D. Bodas, Fundamentals of microfluidics, in *Microfluidics-Aided Technologies*, ed. D. Bodas and V. Gajbhiye, Academic Press, 2025, ch. 1, pp. 1–31.
131 R. Molinaro, C. Corbo, J. Martinez, F. Taraballi, M. Evangelopoulos, S. Minardi, I. Yazdi, P. Zhao, E. De Rosa, M. Sherman, A. De Vita, N. Furman, X. Wang, A. Parodi and E. Tasciotti, Biomimetic proteolipid vesicles for targeting inflamed tissues, *Nat. Mater.*, 2016, **15**, 1037–1046.
132 C. Ducrot, S. Loiseau, C. Wong, E. Madec, J. Volatron and M. Piffoux, Hybrid extracellular vesicles for drug delivery, *Cancer Lett.*, 2023, **558**, 216107.
133 S. C. Jang, O. Y. Kim, C. M. Yoon, D. S. Choi, T. Y. Roh, J. Park, J. Nilsson, J. Lotvall, Y. K. Kim and Y. S. Gho, Bioinspired Exosome-Mimetic Nanovesicles for Targeted Delivery of Chemotherapeutics to Malignant Tumors, *ACS Nano*, 2013, **7**, 7698–7710.
134 B. J. Ryan, G. K. Kinsella and G. T. Henehan, Protein Extraction and Purification by Differential Solubilization, *Methods Mol. Biol.*, 2023, **2699**, 349–368.
135 C. Zhu, Y. Pu and L. Liang, Learn from nature: Biomimetic nanocarriers for intracellular protein delivery, *Bioact. Mater.*, 2026, **55**, 658–678.

136 S. Chennamsetty, M. Keerikkadu, A. Shetty, P. D. Bangera, V. K. Tippavajhala and M. Rathnanand, Biohybrid nanocarriers for targeted drug delivery by integrating biological interfaces with synthetic platforms for site-specific therapeutics, *Int. J. Pharm.*, 2025, **687**, 126400.
137 A. Vogelaar, S. Marcotte, J. Q. Cheng, B. Oluoch and J. Zaro, Use of Microfluidics to Prepare Lipid-Based Nanocarriers, *Pharmaceutics*, 2023, **15**(4), 1053.
138 Y. Cheng, C. Hay, S. Mahuttanatan, J. Hindley, O. Ces and Y. Elani, Microfluidic technologies for lipid vesicle generation, *Lab Chip*, 2024, **24**, 4679–4716.
139 L. Peng, M. Yang, S. Guo, W. Liu and X. Zhao, The effect of interfacial tension on droplet formation in flow-focusing microfluidic device, *Biomed. Microdevices*, 2011, **13**, 559–564.
140 A. Pittiu, M. Pannuzzo, L. Casula, R. Pireddu, D. Valenti, M. Cardia, F. Lai, A. Rosa, C. Sinico and M. Schlich, Production of liposomes by microfluidics: The impact of post-manufacturing dilution on drug encapsulation and lipid loss, *Int. J. Pharm.*, 2024, **664**, 124641.
141 S. Choe, B. Kim and M. Kim, Progress of Microfluidic Continuous Separation Techniques for Micro-/Nanoscale Bioparticles, *Biosensors*, 2021, **11**(11), 464.
142 A. Danilushkina, C. Emene, N. Barlev and M. Gomzikova, Strategies for Engineering of Extracellular Vesicles, *Int. J. Mol. Sci.*, 2023, **24**(17), 13247.
143 T. Trantidou, M. Friddin, Y. Elani, N. Brooks, R. Law, J. Seddon and O. Ces, Engineering Compartmentalized Biomimetic Micro- and Nanocontainers, *ACS Nano*, 2017, **11**, 6549–6565.
144 A. Jahn, W. Vreeland, M. Gaitan and L. Locascio, Controlled vesicle self-assembly in microfluidic channels with hydrodynamic focusing, *J. Am. Chem. Soc.*, 2004, **126**, 2674–2675.
145 J. Han, J. La Fiandra and D. DeVoe, Microfluidic vortex focusing for high throughput synthesis of size-tunable liposomes, *Nat. Commun.*, 2022, **13**, 6887.
146 H. Lin, J. Leng, P. Fan, Z. Xu and G. Ruan, Scalable production of microscopic particles for biological delivery, *Mater. Adv.*, 2023, **4**, 2885–2908.
147 U. Chintapula, S. Liu, A. Del Castillo, J. Lim, Y. Roh, S. Mageswaran, X. Zhang, R. Morales, M. Sellmyer, Y. Chang, X. Xu and J. Ko, Droplet Squeeze Microfluidic Platform for Generating Extracellular Vesicle Hybrids for Drug Delivery, *Small*, 2025, **21**(37), e2503807.
148 J. Yoon, W. Jo, D. Jeong, J. Kim, H. Jeong and J. Park, Generation of nanovesicles with sliced cellular membrane fragments for exogenous material delivery, *Biomaterials*, 2015, **59**, 12–20.
149 J. Wade, J. Jones, I. Lenov, C. Riordan, S. Sligar and R. Bailey, Microfluidic platform for efficient Nanodisc assembly, membrane protein incorporation, and purification, *Lab Chip*, 2017, **17**, 2951–2959.
150 Y. Yoon, S. Kim, J. Lee, J. Choi, R. Kim, S. Lee, O. Sul and S. Lee, Clogging-free microfluidics for continuous size-based separation of microparticles, *Sci. Rep.*, 2016, **6**, 26531.
151 A. Bakhtiari and C. Kaehler, A method to prevent clogging and clustering in microfluidic systems using microbubble streaming, *Biomicrofluidics*, 2024, **18**(4), 044101.
152 D. Kang, K. Kim and Y. Kim, An anti-clogging method for improving the performance and lifespan of blood plasma separation devices in real-time and continuous microfluidic systems, *Sci. Rep.*, 2018, **8**, 17015.
153 N. Mehendale, O. Sharma, S. Pandey and D. Paul, Clogging-free continuous operation with whole blood in a radial pillar device (RAPID), *Biomed. Microdevices*, 2018, **20**(3), 75.

