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Current developments and applications of microfluidic technology toward clinical translation of nanomedicines

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Abstract

Nanoparticulate drug delivery systems hold great potential for the therapy of many diseases, especially cancer. However, the translation of nanoparticulate drug delivery systems from academic research to the industrial and clinical practice has been slow. This slow translation can be ascribed to the high batch-to-batch variations and insufficient production rate of the conventional methods, and the lack of technologies for rapid screening of nanoparticulate drug delivery systems with high correlation to the *in vivo* tests. These issues can be addressed by the microfluidic technologies. For example, microfluidics can not only produce nanoparticles in a well-controlled, reproducible, and high-throughput manner, but also create 3D environments with continuous flow to mimic the physiological and/or pathological processes. This review provides an overview of the microfluidic devices developed to prepare nanoparticulate drug delivery systems, including drug nanosuspensions, polymer nanoparticles, polyplexes, structured nanoparticles and theranostic nanoparticles. We also highlight the recent advances of microfluidic systems in fabricating the increasingly realistic models of the *in vivo* milieu for rapid screening of nanoparticles. Overall, the microfluidic technologies offer a promise approach to accelerate the clinical translation of nanoparticulate drug delivery systems.

Keywords: High throughput preparation; lab-on-a-chip; nanomedicines; drug delivery; organ-on-a-chip; formulation screening

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1 Introduction

Nanomedicine is defined as “the medical application of nanotechnology” [1]. The detailed characteristics of nanomedicine have been reported by the European Science Foundation and the European Technology Platform on Nanomedicines, specifying the favorable features offered by the nanoscale structures and the aims of the administration of such materials [2-5]. The features of nanoscale materials, such as the smaller size, high surface-to-volume ratio, tunable surface chemistry, and the capacity to load drugs, are positively affecting nanomedicines [6]. The applications of nanomedicines have been studied in different areas, from controlled drug delivery to the prevention and diagnosis of diseases [2, 4, 7]. The delivery of drugs to treat different diseases is of particular interest, as exemplified by the great number of related publications and clinical trials in this area [8].

The application of nanoparticulate drug delivery systems in diseases therapy has been widely investigated, since the first idea of Ehrlich’s “magic bullet” [9, 10]. Several different categories of nanoparticles have been developed, such as drug nanocrystals, lipid-based systems (*e.g.*, liposomes, solid lipid nanoparticles), polymeric-based systems (*e.g.*, nanospheres, nanocapsules, polymer-drug conjugates, and dendrimers), and inorganic nanoparticles (*e.g.*, gold, porous silica, porous silicon, carbon nanotubes, and fullerenes) [11]. The wide applications of nanoparticles in disease treatment can be ascribed to their favorable features [12, 13]. For example, the improvement of the physicochemical characteristics of a drug is particularly useful in case of potent therapeutics with limited aqueous solubility, like paclitaxel (PTX) [14]. Meanwhile the formation of drug nanocrystals can increase the dissolution rate of poorly water-soluble drugs. The loading or conjugation of therapeutics into nanoparticle precursors can protect the loaded therapeutics from degradation in the body fluids, and alter their pharmacokinetics and biodistribution. The release of payloads from nanoparticles can be tailored in a stimuli-responsive manner. Nanoparticles can even interfere with the drug resistance mechanisms developed by cancer cells [12, 13]. Regarding the combinatorial therapy, a single type of nanoparticle can not only deliver multiple molecules to the same site, but also enable

the timely release of drug molecules to achieve the synergistic effect and overcome the drug resistance in cancer cells [15].

The successful translation of nanoparticle formulations still faces great challenges, for example, the low production efficacy of formulations, high batch-to-batch variations, and poor scale-up feasibility of the manufacturing process. Therefore, an advanced approach that can prepare nanoparticles with desired physicochemical characteristics in a high-throughput and reproducible manner is strongly desirable [16-18]. This challenging task is not easily addressed by the conventional batch approaches, such as bulk mixing, because of their macroscale and random nature. The reasons for this slow clinical translation of nanomedicines also include the difficulties to precisely predict the behavior of engineered nanoparticles in a system as complex as the human body. Moreover, the *in vitro* and *in vivo* evaluations of drug delivery systems are expensive and resource intensive, which further slows down the translation of nanomedicines. The human body is a multi-level, highly dynamic and interconnected system, characterized by the structural organization and feedback-regulation. It is a challenge task to mimic the human body or even a specific tissue with an artificial system. This system must possess a hierarchy of structures with multifunctional dimensions that differ by many orders of magnitude.

Microfluidics involves “the manipulation of fluids in channels with dimensions of tens of micrometers” [19], where the fluid behavior is controlled by the viscous forces rather than the inertial ones [20]. Nanoparticulate drug delivery systems are usually prepared by nanoprecipitation in the microfluidic channels with continuous flows [21]. The continuous flows ensure the same quality over time for the obtained nanoparticles, avoiding the issues of batch-to-batch variability. By utilizing the geometries of microfluidic channels, different types of fast mixing patterns formed, resulting in a reliable and controlled production of nanoparticles [22, 23]. Moreover, microfluidic devices can mimic the important aspects of the *in vitro* and *in vivo* situations, enabling the fast evaluation of the interactions between nanoparticles and biological systems, and facilitating the correlation between the

in vitro and *in vivo* studies. The volume of fluids flowing inside the channels is in the order of nanoliters, which can dramatically reduce the consumption of reagents [21].

Microfluidics is versatile technology in the production of micro/nano-sized drug delivery systems, analysis of compounds, and evaluation of the nanoparticles produced, as shown in **Figure 1** [24, 25]. In this review, we first discuss the formation mechanism of nanoparticles prepared by microfluidic mixing. Next, we discuss the versatility of microfluidics for combinatorial preparation of nanoparticles, and the optimization of the features that nanoparticles should possess for targeted drug therapy. The microfluidic mixing strategies used for preparing on-demand nanoparticles, including drug nanosuspensions, polymeric nanoparticles, ployplexes, structured nanoparticles, and theranostic nanoparticles, are reviewed in detail. Finally, we also highlight the on-chip evaluation of nanoparticulate drug delivery systems.

Figure 1. Microfluidic methods are proposed as alternatives to the current drug development technologies. In the fabrication of nanoparticles, microfluidics allows the precise control over the producing conditions and easily scale-up of production. Continuous flows in microfluidic device result

in conditions closer to those *in vivo* evaluation in large animals. Reproduced with permission from ref. [26], © 2012 NPG.

2 Engineering and evaluating nanoparticles by microfluidics

2.1 Microfluidics as an enabling technology for nanoparticles production

When the precursors, such as block copolymers, experience a change in solvent quality, these polymers can assemble into nanoparticles. As shown in **Figure 2a**, the process of assembling block copolymers into nanoparticles can be divided in three stages, including (I) polymer nucleation, (II) nucleus growth, and (III) nanoparticle formation [27]. At the first stage, several block copolymers form a nucleus during a solvent change. In the second stage, the size of the nucleus continually grows by adding more block copolymers until the formation of a polymer brush layer on the surface of a nanoparticle. The formed brush layer inhibits the addition of more block copolymers. In the third stage, the equilibrium between the free and assembled block copolymers is achieved, and thus, the size of the nanoparticles remains stable [28].

For nanoparticle preparation, an efficient way to accomplish the change in solvent quality is mixing the solvent (*e.g.*, organic solvents) with non-solvent (*e.g.*, aqueous solutions). The mixing time directly affects the average particle size and size distribution of the obtained nanoparticles [17, 18, 29]. When the mixing time is faster than the duration of nucleation, the nucleation of block copolymers and the growth of nuclei both occur in a homogeneous solvent environment. In conventional mixing methods, the gradually changed ratio between solvent and non-solvent forms a heterogeneous solvent environment. Moreover, the slow mixing rate implies that the nucleation of block copolymers primarily occurs when mixing is incomplete (**Figure 2b**). Because of the heterogeneous solvent environment and the incomplete mixing condition in bulk mixing methods, polymers easily assemble to the polymer aggregates, forming of larger nanoparticles with wide size distribution [26].

The manipulation of liquids at the picoliter (or less) scale by microfluidics is superior over bulk mixing methods. The mixing timescale for microfluidic approach can be tuned to be faster than the characteristic timescale for nucleation of nanoparticle precursors [18, 30]. After complete mixing, the nucleation of nanoparticle precursors occurs in the environment with the lowest fraction of organic solvents (**Figure 2b**). This homogenous solvent environment facilitates the stabilization of nanoparticles by amphiphilic compounds, forming smaller nanoparticles with narrower size distribution [31]. The accurate manipulation of fluids in microchannels results in a precisely controlled mixing process as well as the in a fine modulation of formation conditions. These favorable features offered by microfluidics enable the accurate control over the physicochemical properties of the prepared nanoparticles [22, 30, 32]. Microfluidic devices are usually operated with continuous flows, supporting the production of nanoparticles with the same quality over time, which is important for the pharmaceutical industry [16, 33].

Figure 2. Assembly of nanoparticle precursors. (a) The staged assembly process of block copolymers to form nanoparticles. (b) The impact of slow and rapid mixing on the features of obtained nanoparticles. Reproduced with permission from ref. [27], © 2008 ACS.

The physicochemical features of the prepared nanoparticles can be controlled by simply adjusting the microfluidic formulation parameters, such as the concentrations and types of particle precursors in solvent, the flow ratios between the solvents and non-solvents, and their total flow rates. By tuning these microfluidic formulation parameters, the average size, size distribution, compositions, surface properties, and payload release profiles of the obtained nanoparticles can be precisely controlled [32, 34]. The one-step preparation of nanoparticles with complex structures, such as the core/shell structure, has been achieved by assembling a variety of producing procedures into a single microfluidic device [35].

2.2 Combinatorial preparation of nanoparticulate drug delivery systems

Targeted drug delivery refers to the strategy of delivering therapeutics in a manner that increases the drug concentration in some districts of the body compared to others. To facilitate targeting drug delivery, it is necessary to optimize the physicochemical parameters for the fabricated nanoparticles, such as shape, average size, surface charge, and surface composition [36, 37]. These physicochemical parameters simultaneously confer the behavior of nanoparticles, including molecular targeting, immune evasion, and controlled drug release. Cancer targeted drug delivery is one of most popular research hotpots in nanomedicine. The effects of physicochemical features of nanoparticulate drug delivery system on targeted cancer therapy are listed in **Table 1**.

The traditional dogma of cancer treatment in solid tumors has been the passive targeting of nanoparticulate drug delivery systems to the tumor tissue due to the enhanced permeability and retention effect [38]. The passive accumulation of the nanoparticles is dependent on their circulation time and on their features (*e.g.*, size, surface charge, surface characteristics, and shape), as well as on the characteristics of the tumor [6, 39]. Regarding the nanoparticle surface, it is commonly modified with stealth moieties, like polyethylene glycol (PEG), to reduce the opsonization of nanoparticles and to prevent the uptake of nanoparticles by the mononuclear phagocyte system (MPS) [22]. Nanoparticles can also be actively targeted to cancer cells by modifying the surface of the particles with targeting moieties (*e.g.*, antibodies, peptides, and biological molecules) against molecules (proteins) or receptors overexpressed by the cancer cells [6, 39]. The efficacy of these formulations depends on the conjugation reaction used to bind the targeting moiety, as well as on the ligand density, charge and orientation [39]. To achieve a prolonged circulation, the surface charge of the nanoparticles should be negative or neutral [22].

The shape of nanosystem affects their circulation time, the extravasation and uptake by cancer cells. Specifically, nanoparticles with elongated structures, like filomicelles, exhibit prolonged circulation time when compared to spherical particles, unlike spherical and discoidal particles that present

enhanced accumulation in the tumor tissue [21]. The circulation time and cell uptake of nanoparticles can be even affected by the stiffness of nanoparticles. Softer particles exhibit prolonged circulation time, while harder particles present enhanced uptake in both cancer and immune cells [40, 41].

Targeting delivery efficiency is affected by the physicochemical features of nanoparticles. For a specific disease, we need to find the optimal parameters for every nanoparticle and every loaded drug, which require multiple optimization cycles to gradually improve the performance of the developed nanoparticulate drug delivery systems. The formulation optimization is time-intensive and a huge challenge for the conventional engineering technology, not to mention the combination therapy. Therefore, there is an urgent need to develop platforms for rapid fabrication of a combinatorial library of nanoparticles with a wide range of properties in a reproducible manner.

Microfluidics is an ideal approach for rapid and combinatorial preparation of nanoparticles. Nanoparticle size can be manipulated by adjusting the flow rates and therefore mixing times, device geometries, polarity of the solvents, and concentration and composition of the precursors. The addition of reagents during the mixing process and high throughput production of formulations make microfluidics even more attractive for nanoparticle formulation screening. The microfluidic device integrated with a multi-inlet micromixer enables the programmable mixing of a large variety of precursors before nanoprecipitation. By simply changing the flow rate of each precursor, the composition of the formed nanoparticles can be easily tuned. Therefore, the fully integrated microfluidic devices are amenable to high throughput screening of nanoparticle formulations. Under the help of an integrated microfluidic platform, Valencia *et al.* successfully synthesized 45 types of nanoparticles with a wide range of distinct properties by combining > 15 different nanoparticle precursors with different ratios [42]. With automated sampling, dilution, metering, and mixing of precursors, a library of 648 different nanoparticles was prepared within only 2.5 h by a microfluidic device [43].

Table 1. The impact of physicochemical properties of nanoparticulate drug delivery systems on their biodistribution and tumor accumulation.

Features	Specific characteristics	Effect on biodistribution	Effect on tumor accumulation
Shape	Elongated particles	Prolonged circulation time [44]	Lower tumor accumulation [45]
	Spherical particles	Short circulation time [44]	High uptake by tumor cells [45]
Size	100-200 nm	Stable particles, prolonged circulation [46, 47]	Extravasation, but accumulation on the tumor margins [48]
	ca. 20 nm	Shorter circulation [48, 49]	Enhanced penetration within the tumor tissue [48, 50]
Surface modification	Stealth (PEG)	Enhanced circulation, decreased interaction with the MPS [51, 52]	Lower tumor accumulation and uptake by cancer cells [53]
	Targeting moieties	Formation of the plasma corona may interfere with the targeting [54]	Enhanced accumulation in the tumor [53, 55]
Surface charge	Negative/ neutral	Prolonged circulation, lower interaction with the MPS [56, 57]	Low uptake by the cancer cells, but deep penetration within the tissue [58]
	Positive	Reduced circulation for highly positively charged particles [59]	Enhanced uptake in the cancer cells [58]
Stiffness of the particle	Soft	Prolonged circulation due to lower interaction with the MPS and the spleen [60]	Low uptake [40]
	Hard	High interaction with the MPS [61]	Enhanced uptake [40]

2.3 Microfluidics for evaluation of nanoparticulate drug delivery systems

Nanoparticles hold great potential in the diagnosis and treatment of different diseases, especially cancer. However, due to the complexity of human body, the *in vitro*–*in vivo* correlations of nanoparticles are in general low level, which greatly hinder the clinical translation of nanoparticles [62]. There is a demand to develop biomimetic *in vitro* models for rapid screening of nanoparticles. Microfluidics can drive small sample volumes and process reactions at a biologically length scale. Both micrometer structures and well-controlled fluid flows are the distinct aspects of biological systems. For example, the gases, nutrients, and metabolites are exchanged through microvasculature. The fluidic flows of biological systems include not only the flows of blood and lymph, but also the interstitial flow in almost all the soft tissues.

Microfluidic devices exhibit several advantages in mimicking biological systems, including the biologically relevant length scales, a highly-controlled device and channel geometry, precisely controlled fluidics and microenvironment, and the real-time monitoring of the process inside the device. At first, through soft lithography, flexible microfluidic channels with a high degree control of geometry has been designed to create complexed 3D environments in a reliable and reproducible manner. In these channels, characterized by continuous flow, different types of interactions, reactions and screenings can be carried out, mimicking the physiological and pathological processes [26]. Some of these devices can also create spatiotemporal dynamics. Second, many important biological structures are in micrometer scale, a dimension similar to that of microfluidic channels. Third, microfluidics enables the precise control of small volumes of fluids, which can modulate the microenvironment of cells. We summarize the advantages and challenges of microfluidic technologies for preparation and *in vitro* screening of nanoparticles in **Table 2**, which are likely to make an impact on drug delivery in the near future.

Table 2. The impact of microfluidics on the clinical translation of nanoparticles, including the nanoparticle preparation and *in vitro* screening.

Advantages		Challenges	
Preparation	Ü Tunable particle size	∩	Polydimethylsiloxane is sensitive to organic solvents
	Ü Narrow size distribution	∩	Glass, silicon, or polytetrafluoroethylene are difficult to be engineered with high cost
	Ü Combinatorial preparation a library of distinct nanoparticles		
	Ü Continuous preparation with low batch-to-batch variations	∩	Requiring specialized equipment, microfabrication facilities, and specialized skills
	Ü High scale-up feasibility from bench to clinic	∩	Complexed designs are required for parallelization of microfluidic channels
<i>In vitro</i>	Ü Biological conditions close to <i>in vivo</i> environments	∩	High cost to fabricate the <i>in vitro</i> evaluation microdevices
	Ü Low variations of features for the organs engineered on the chip	∩	The single use of the microdevices further increased the cost of <i>in vitro</i> tests
		∩	The poor correlation between the <i>in vitro</i> and <i>in vivo</i> tests

3 Microfluidic mixing and devices

3.1 Mixing by microfluidics

Mixing is a transport process for species, temperature and phases to reduce inhomogeneity [63]. During the mixing process, secondary effects occur, such as reaction and change in properties. Microfluidics enables a thorough and rapid mixing of several mutually soluble fluids in microscale channels or devices. In such devices, the diminutive dimension (tens to hundreds of micrometers) of the channels enhances the surface to volume ratio, and consequently increases the effect of surface tension, as well as the fluid viscous forces. Reynolds (Re) number is a dimensionless parameter in fluid mechanics used to predict flow patterns [64]. Reynolds number can be calculated by **Eq. 1**.

Eq. 1
$$Re = \frac{\rho v L}{\mu}$$

where ρ is the density of the fluid, v is the velocity of the fluid, L is the characteristic liner dimension, and μ is the viscosity of the fluid [64]. Laminar flow occurs when a fluid flows in parallel layers without disruption. In contrast to laminar flow, the turbulent one is a type of fluid flow in which the fluid undergoes irregular fluctuations or mixing [65]. Usually, the onset of transition between laminar and turbulent flows occurs at Reynolds numbers of ~ 1800 – 2000 .

The microflows fall in the low Reynolds number (< 100) laminar regime, in which the mixing depends on the slow and insufficient molecular diffusion. The rapid mixing of nanoparticle precursors with non-solvent is of crucial importance to form nanoparticles with small particle size and narrow size distribution. As a solution to this problem, appropriate micromixers are usually applied to enhance the mixing efficiency. Ideally, a thorough mixing performance achieved within shorter mixing channels can reduce the characteristic size of microfluidic devices, contributing to the concept of lab-on-a-chip systems. Microfluidic mixing schemes can be categorized as either “active” or “passive”. In

active microfluidic mixing, an external energy force is applied to perturb the sample species. In contrast, specially-designed microchannel configurations are employed to enhance the contact area and contact time of the species samples for “passive” microfluidic mixing. In this review, we primarily focus on the nanoparticles prepared by “passive” microfluidic mixing.

As mentioned above, microfluidic devices used in nanoparticle preparation have been widely studied. The developed microfluidic devices can be divided into two categories: the single-phase continuous flow and the segmented flow system. By introducing a gas phase, called gas–liquid segmented flow systems, the mixing of two miscible streams has been enhanced. The Y- and T-shaped microfluidic schemes are usually employed in the preparation of drug nanosuspensions. One of the most common continuous flow mixing techniques is hydrodynamic flow focusing (HFF). Rapid processes, such as the nucleation of block copolymers, require a rapid mixing. To produce high-performance mixing, a variety of schemes have been designed, such as the Tesla structured channel [66, 67], and the herringbone mixer [64]. Apart from the two-dimensional (2D) HFF, the three-dimensional (3D) HFF forms a 3D-coaxial flow and facilitates a more rapid and uniform mass transfer [68]. The other continuous flow microfluidic schemes used for fast mixing includes multi-inlet vortex mixer (MIVM) and confined impinging jets (CIJM).

In addition to the mixing of miscible fluids, nanoparticles can also be prepared by droplet microfluidics [69]. For example, the formed microdroplets can shrink hundreds of times to nanoscale, when the concentration of particle precursors is low enough. Rondeau *et al.* [69] fabricated alginate nanogels with the average size ranging from 10 to 300 nm through the water-in-oil droplet microfluidics. Due to the low concentrations of particle precursors and the formation of droplets one-by-one, the production rate of nanoparticles by droplet microfluidics is usually much lower than that of microfluidic mixing. In interdigital multilamination micromixer, both oil (ethyl acetate) and aqueous fluids diverged into dozens of slits follow a trajectory of 90° with high pressure to exert high

shear stress [70]. Under the high shear stress, poly lactic-co-glycolic acid (PLGA) nanoparticles have been successfully obtained by droplet microfluidics.

3.2 Y- and T-shaped microfluidic devices

One of the easiest approach to achieve passive mixing is using Y-shaped microfluidic devices (**Figure 3a**), consisting of two inlets and one outlet [71]. When the angle between two inlets is 90 or 180 °C (**Figure 3b**), the microfluidic device is T-shaped [72]. In the case of Y-shaped device, the solution of nanoparticle precursors and the corresponding non-solvent flow through the two inlets and mixed with each other. The mixing occurs in the contact surface between the two inlet fluids. The diffusion rate at the interface between the two inlet fluids determines the rate of mixing (mixing time). At the low Reynolds numbers in microscale channels, the mixing of laminar flows is dominated by molecular diffusion. Therefore, the mixing time of fluids in this type of device is quite long: a distinct interface can be even observed in the collecting chamber. Moreover, the laminar flow in a microchannel brings about a broad distribution of residence-time, resulting in a wide distribution of particle size. The mixing efficiency has been enhanced by increasing the flow rates of fluids and introducing extra obstacles in the mixing channel, which can generate perturbations.

By parallelizing the T-shaped junctions, a digital microfluidic droplet generator (DMDG; **Figure 3c**) has been developed to fabricate uniform-sized supramolecular nanoparticles [43, 73]. Without consuming significant amounts of reagents, DMDG can rapidly scan a variety of preparation conditions by digitally adjusting the mixing ratios of the nanoparticle precursors. A highly scalable and closed-loop diafiltration technique (**Figure 3d**) has been developed to improve the production rate of layer-by-layer (LbL) assembled nanoparticles [74]. The samples were driven by a peristaltic pump through a circuit containing a filter membrane. The excess polyelectrolytes exited the circuit through the pores of the filter membrane, avoiding further purification of the obtained samples [74].

Figure 3. Y- and T-shaped schemes toward microfluidic mixing. (a) Schematic diagram of a typical Y-shaped microfluidic setup. (b) Schematic view of a typical T-shaped microfluidic device. (c) Schematic illustration of a digital microfluidic droplet generator. (d) The tangential flow filtration purification schematic depicting continuous diafiltration through a porous membrane. Figures are reproduced with permissions from: (a) ref. [71], © 2003 NPG; (b) ref. [72], © 2006 NPG; (c) ref. [43], © 2010 ACS; (d) ref. [74], © 2016 WILEY-VCH.

3.3 HFF microfluidic systems for nanoparticle preparation

The HFF, usually classified as two-dimensional (2D-HFF) and three-dimensional (3D-HFF), is one of the most common fluids mixing approach in microfluidics. Because of the planar feature of most microfluidic setups, 2D-HFF is usually focused in the horizontal direction (**Figure 4a**) [75]. Instead of one inlet for the non-solvent in a Y- or T-shaped device, 2D-HFF is achieved by introducing two inlets of non-solvent as the sheath liquid to horizontally confine the fluid containing nanoparticle precursors

from the central inlet. The confinement of the solution containing nanoparticle precursors by the side sheath flows significantly shortens the diffusion length and therefore reduces the mixing time [18]. Under typical operating conditions, fluids in microfluidic channels are laminar. Although the molecular diffusion across the fluids and the fluids homogenization is relatively slow[64], the estimated solvent mixing time could be < 0.4 ms for 2D-HFF [27]. Consequently, the nanoparticles form in a rapidly generated highly supersaturated aqueous solution of the nanoparticle precursors. To further enhance the mixing efficiency of HFF devices, a variety of delicate designs for the mixing channel have been developed, such as the Tesla structured channel (**Figure 4b**) [66, 67] , and the herringbone mixer (**Figure 4c**) [64].

In the 3D-HFF, also called co-axial flow focusing, the solution containing nanoparticle precursors is both horizontally and vertically focused into a small fluid filament. In addition to the horizontal focusing by two inlets for side sheath flows, three sequential inlets were introduced into the microfluidic device for vertical focusing the organic solution containing nanoparticle precursors, achieving the 3D-HFF in polydimethylsiloxane (PDMS) devices (**Figure 4d**). Alternatively, a “microfluidic drifting” technique has been developed to achieve 3D-HF in a single-layer, planar microfluidic structure (**Figure 4e**) [76]. The formation of 3D-coaxial flows is crucial for a rapid and uniform mass transfer [77]. A microfluidic co-flow capillary device has been employed to achieve the 3D-HFF, and therefore, 3D-coaxial flows (**Figure 4f**). The microcapillary device is fabricated by coaxial aligning a tapered capillary in a cylindrical one [78, 79].

Figure 4. HFF schemes of microfluidic devices for nanoparticles preparation. (a) Representative 2D-HFF microfluidic device. (b) Schematic of a 2D-HFF microfluidic setup with Tesla structures (scale bar = 100 μm). (c) Schematic view of the herringbone mixer (channel with ridges). (d) A 3D-HFF device consists of three sequential inlets for vertical focusing and a separate inlet for side sheath flows. (e) Schematic of the polydimethylsiloxane microfluidic device for nanoparticle prepared by 3D-HFF. (f) Schematic diagram of a 3D-HFF microfluidic device made of capillaries. Figures are reproduced with permissions from: (a) ref. [80], © 2010 PNAS; (b) ref. [81], © 2010 ACS. (c) ref. [64], © 2002 AAAS; (d) ref. [77], © 2011 WILEY-VCH; (e) ref. [76], © 2013 ACS; (f) ref. [68], © 2015 WILEY-VCH.

3.4 Confined impinging jets mixer

The characteristic mixing time in a confined impinging jets mixer (CIJM; **Figure 5a**) scales as the inverse of the jet velocity to the three halves power [82]. The CIJM is very efficient in controlling the supersaturation, a driving force of nanoprecipitation, allowing the precise control of the final particle size and surface properties [83]. The geometrical characteristics of the CIJM and the mixing process significantly affect the average particle size and size distribution. The initial nanoparticle precursors concentration and the inlet jet velocity turn out to be the most important operating parameters in CIJM

[84]. The CIJM enables the stream homogenization of nanoparticle precursors in milliseconds range, which alter the organic nucleation and growth, and provide steric stabilization for the particles [85]. The CIJM can efficiently control the supersaturation of the nanoparticle precursors, allowing the fine tuning of the final particle size and surface properties of the nanoparticles [83].

3.5 Multi-inlet vortex mixer

Recently, a scaled-up multi-inlet vortex mixer (MIVM; **Figure 5b**) was developed to achieve the rapid precipitation of organic compounds and the high throughput production of polymeric nanoparticles [86]. With respect to the four-stream MIVM, it allowed a precise control over the final solvent quality, and consequently the supersaturation of nanoparticle precursors, by simply varying stream velocities. When $Re > 1600$, an adequate mixing was obtained [86]. The large Re , from 3290 to 8225, resulted in a turbulent swirling flow within the mixer [87]. In turbulent flows, the spontaneous fluctuations of the fluid velocity facilitate the homogenization of fluids. The measured turbulence kinetic energy and Reynolds stresses are sensitive to the vortex wandering. The swirl ratio and micromixing time of the flow increases as the MIVM is scaled-up, indicating a flow with stronger swirl yet less mixing effectiveness in the scaled-up reactor [87].

3.6 Gas–liquid segmented microfluidics

Besides the single-phase continuous flow microfluidic systems, the gas–liquid segmented flow systems (**Figure 5c**), in which the introduction of a gas phase enhances the mixing of two miscible streams, have been developed for polymeric nanoparticle preparation [88, 89]. The introduced gas phase forms a segmented gas–liquid flow, and completely separates the mixed liquid and gas streams. Without requiring additional fabrication steps, the self-assembly of nanoparticle precursors is initiated by the fast mixing of water with polymer constituents by chaotic advection, when liquid plugs containing reactants move through a sinusoidal mixing channel. The fluid velocities in the direction normal to the channel amount to *ca.* 30% of the bulk liquid velocity inside a liquid segment, slightly

depending on the length of a liquid segment. Compared to the chaotic micromixers using 3D microchannel networks or patterned walls, the mixing length is reduced 2–3-folds for the segmented flow systems. With the help of segmented gas–liquid microflows, the mixing time of fluids can be varied over several orders of magnitude from several milliseconds to seconds [88, 89].

Figure 5. Schemes of microfluidic mixers for nanoparticles preparation. (a) Representative schematic of multi-inlet vortex mixer (MIVM). (b) Schematic view of a confined impinging jets mixer (CIJM). (c) Schematic diagram of a gas–liquid segmented flow systems. Figures are reproduced with

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4 Nanoparticulate drug delivery systems synthesized by microfluidics

Nanoprecipitation by microfluidic mixing has been used to synthesize a variety of nanoparticles, such as drug nanosuspensions, polymer nanoparticles and micelles, liposomes, and lipid nanoparticles. Precipitation requires two miscible fluids, whereas the nanoparticle precursors and active ingredients (*e.g.*, drugs) must be soluble in only one of the fluids (solvent). The fluids in which nanoparticle precursors and drug molecules precipitate are called non-solvent. During the precipitation process, nanoparticle precursors assemble rapidly, resulting in the immediate drug entrapment. Alternatively, complexes formed between oppositely charged nucleic acids and polycations (polyplexes) can also be prepared by microfluidic mixing. Both nanoprecipitation and formation of polyplexes and lipoplexes are a single-step technique that allows production of nanoparticles from a wide range of excipients. To simplify the comparison, we define the period of times to release 50% and 100% of payloads as the release half-life (t_{50}) and complete release (t_{100}), respectively. The polydispersity index (PDI) of the particle size is a measure of broadness of the size distribution. Acceptable values of PDI are lower than 0.3, corresponding to good quality of colloidal suspensions; while poor quality samples give values close to 1.

4.1 Drug nanosuspensions

Poor solubility and bioavailability are the reasons for the failure of more than 40% of active pharmaceutical ingredients in the drug development process [93]. According to the Noyes-Whitney equation [94], the dissolution rate is proportional to the surface area of the particles exposed to the medium. Consequently, reducing the size of poorly water-soluble drugs to enlarge the surface area is an effective strategy to speed-up the drug dissolution. The bioavailability of drugs, which have been classified as Class II (high permeability and low solubility), according to the Biopharmaceutics

Classification System, can be increased by nanocrystallization. Nanocrystallization, reducing the drug particle size to $< 1\ \mu\text{m}$, is more effective in expediting the dissolution. By formulating these so-called “brickdust” active pharmaceutical ingredients into crystalline nanosuspensions, these compounds can be rescued during the drug development process. Microfluidic nanoprecipitation offers a simple and low energy demanding process for preparing drug nanosuspensions.

Poorly water solubility and slow dissolution rate are responsible for the poor oral bioavailability of fenofibrate. By integrating antisolvent precipitation-in-microfluidics and spray drying, a fenofibrate nanosuspension (196–296 nm) was obtained [95]. The particle size of fenofibrate was reduced by increasing the volume ratio of the non-solvent (water) to drug ethanol solution, the total flow rates, or the drug concentrations. As expected, fenofibrate nanocrystals reached the complete dissolution within 30 min, exhibiting faster dissolution rate than their bulk powder counterparts (ca. 40% released in 120 min) [95]. After formulating the raw danazol powders into nanocrystals (ca. 364 nm), the drug dissolution rate within 5 min was enhanced from ca. 35% to 100% [96].

Recently, hydrocortisone nanosuspensions have been synthesized by nanoprecipitation in a Y-junction microfluidic device (**Figure 6a**) [97, 98]. As show in **Figure 6b**, the particle size and size distribution of hydrocortisone nanosuspension prepared by microfluidic nanoprecipitation ($295 \pm 32\ \text{nm}$, $\text{PDI} \approx 0.18$) were close to their counterparts prepared by wet milling (ca. 300 nm with a $\text{PDI} \approx 0.18$) [97]. The obtained nanosuspensions showed ca. 1.8-fold sustained action compared to the hydrocortisone solution. Concerning the area under the curve of percentage changes in intraocular pressure (**Figure 6c**), the precipitated and milled nanosuspension achieved the similar values of 28 ± 4 and 30 ± 2 , which were significantly higher than that of a hydrocortisone solution (16 ± 3) [97]. By prolonging the therapeutic effects and significantly improving the ocular bioavailability, the drug nanosuspensions can reduce the administration frequency, and therefore, improving the patient compliance.

Figure 6. Drug nanosuspensions prepared by microfluidic nanoprecipitation. (a) Preparation of hydrocortisone nanosuspension by the Y-shaped microfluidic device. (b) SEM images of different milled and precipitated hydrocortisone particles. (c) Changes in intraocular pressure (IOP) of after administration of hydrocortisone nanosuspensions. (d) Schematics of the rotating tube processor. (e) Electron microscopy images of freshly prepared uncoated meloxicam nanoparticles and after 3 h. Figures are reproduced with permissions from: (a-c) ref. [97], © 2011 Elsevier; (d and e) ref. [99], © 2013 RSC.

Instead of water, organic solvents can also serve as the non-solvent. For example, cefuroxime axetil nanosuspensions were prepared by precipitating the acetone solution of the drug in isopropyl ether in a Y-junction microfluidic device [100]. The dissolution rate of the obtained cefuroxime axetil amorphous nanoparticles ($t_{50} < 5$ min; 450 nm) was faster than the corresponding raw drug powders ($t_{50} \approx 30$ min) [100]. The amorphous cefuroxime axetil nanoparticles can be synthesized at high throughput up to 6 L/min in a microporous tube-in-tube reactor [101]. This reactor offers a general and facile pathway for mass production of the nanoparticles. Isopropanol served as the non-solvent for the atorvastatin calcium nanoparticles, which were synthesized in a Y-junction microfluidic setup [102]. The drug concentrations and the flow rates of the solvent and non-solvent efficiently controlled the particle size of atorvastatin calcium nanoparticles. By increasing the flow rate of the atorvastatin

calcium methanol solution from 2.7 to 16 mL/min, the size of obtained nanoparticles significantly increased from 430 to 2500 nm. Interestingly, the size of atorvastatin calcium nanoparticles decreased from 760 to 300 nm with an increase in the drug concentration from 30 to 60 mg/mL. The atorvastatin calcium particles were amorphous, showing enhanced dissolution rate ($t_{100} \approx 10$ min) compared with the corresponding powders ($t_{100} > 60$ min) [102].

Besides the Y-junction, a rotating tube processor (**Figure 6d**) was also developed to synthesize meloxicam [99, 103] and curcumin [104] nanoparticles. The size of meloxicam nanoparticles can be controlled from 20 to 200 nm by varying the process parameters, such as the concentrations (0-2%, w/v) of the stabilizer poloxamer 188 and rotational speed of the tube (500–1500 rpm). Curcumin nanoparticles with the particle size < 50 nm were obtained by the continuous flow microfluidic rotating tube processor [104]. The formed meloxicam and curcumin nanoparticles significantly enhanced the dissolution of the corresponding drug molecules. The complete drug dissolution (t_{100}) decreased from > 60 min for raw powders to ca. 20 min for drug nanoparticles [103]. Alternatively, Dev *et al.* [99] used the layer-by-layer polyelectrolyte encapsulation to improve the stability of meloxicam nanoparticles prepared by the rotating tube processor. The paired electrolytes, polyallylamine hydrochloride and polyprotomine sulfate, effectively suppressed the growth of macrocrystals (**Figure 6e**). Without using any organic solvents, meloxicam nanoparticles with an average particle size < 100 nm were obtained. For the polyelectrolyte coated (four layers) nanoparticles, ca. 80% of payloads was released within 6 h with a t_{50} close to 180 min. In contrast, the micron sized powders of meloxicam released only 16% of therapeutics after 6 h [99].

By introducing high pressure into the microfluidic device, the jet velocities and energy dissipation level can be several orders of magnitude higher than those devices without pressurization [105]. The average size of norfloxacin particles was ca. 320 nm, when prepared by the PureNanoTM continuous crystallizer with a pressure in chamber at 60 MPa (**Figure 7a**). After increasing the chamber pressure to 140 MPa, the diameter of norfloxacin particles decreased to ca. 200 nm. Moreover, phenytoin,

bezafibrate, flurbiprofen, and miconazole nanocrystals have also been successfully prepared by the microfluidic impinging jet technology [106]. The size of drug nanoparticles prepared by the microfluidic impinging jet technology was smaller than those prepared by the conventional batch method. Moreover, no miconazole nanoparticles could be obtained with the conventional batch method. As expected, the smaller particle size led to the faster dissolution rates of drug molecules (**Figure 7b**), and therefore, to higher plasma concentrations after oral administration (**Figure 7c**) [106]. The microfluidic impinging jet technology is scalable to process dozens of liters per minute.

Figure 7. Drug nanosuspensions prepared by microfluidic nanoprecipitation. (a) Schematic illustration of the process to prepare drug nanosuspensions by the pressurized microfluidic device. (b) *In vitro* dissolution profiles of flurbiprofen nanoparticles prepared by different methods. (c) Rat plasma concentration profile following oral administration of flurbiprofen nanoparticles prepared by different methods. PM, physical mixture; BC, batch crystallization; PCC, PureNanoTM continuous crystallizer. (d) Overview (left column) and close-up (right column) of a microfluidic nebulator. Liquids and air are injected through blue and white inlets, respectively. (e) Size distribution of obtained drug nanoparticles, including fenofibrate (■), clotrimazole (●), danazol (▼), and estradiol (▽). (f) Scanning electron micrograph of spray-dried fenofibrate nanoparticles. Figures are reproduced with permissions from: (a-c) ref. [106], © 2016 Springer; (d-f) ref. [107], © 2015 AAAS.

Recently, the preparation of amorphous drug nanoparticles by supersonic drying the ethanol-in-air droplets (**Figure 7d**) was developed by Weitz's group [107]. There were six pairs of air inlets in the fabricated nebulator. Benefiting from the supersonic air flow and the small diameter, the freshly formed drops dried in a superfast manner before the formation of crystal nuclei. The amorphous drug nanoparticles, including fenofibrate, estradiol, clotrimazole and danazol, with an average diameter ca. 14 nm were always obtained (**Figure 7e and f**). Under ambient conditions at room temperature, no crystal nucleation was detected in any of the obtained amorphous nanoparticles within 7 months [107]. To further improve their stability, amorphous nanoparticles were coated with a polymer layer by co-spraying drug molecules with poly(vinylpyrrolidone) [108]. Alternatively, the amorphous drug nanoparticles can be directly sprayed into a polymeric matrix, such as poly(vinylpyrrolidone), and this enables a much wider choice of stabilizing polymers [108].

According to the microfluidic schemes, a summary of the drug nanoparticles prepared by microfluidics is presented in **Table 3**. Although a variety of drug nanosuspensions was prepared by microfluidic nanoprecipitation, most studies only performed the formulations optimization and characterization. Although the *in vitro* interactions with cells can be found in several studies, the *in vivo* therapeutic efficiency of drug nanosuspensions is lacking in most studies. This phenomenon can be ascribed to the distinct feature of drug nanocrystals: the fast dissolution rates of poorly water-soluble drugs. The outcome of oral administration of drug nanosuspensions is predictable. Specifically, the drug nanosuspension can improve the bioavailability for BCS II drugs, which has been repeatedly proved before [109, 110]. Since the *in vivo* tests will not change the conclusions of these works, the *in vivo* tests for drug nanosuspensions were waived according to the guidance released by the US Food and Drug Administration [111]. The waiver of *in vivo* tests also minimizes the expensive animal studies, as well put the principle animal 3Rs (Replacement, Reduction and Refinement) into practice.

Table 3. The physicochemical properties of the drug nanoparticles synthesized by microfluidic platforms.

Microfluidic devices	Precursors	Solvent/ non-solvent	Stabilizers	Average size	PDI	Estimated t_{50} and t_{100}	Ref.
Y-junction	Hydrocortisone (12.5 mg/mL)	Ethanol/ water	Polyvinylpyrrolidone (0.2%, w/v), HPMC (0.5%, w/v) and Tween 80 (0.1%, w/v)	295 ± 32 nm	≈ 0.18	—	[97]
Y-junction	Fenofibrate (10 mg/mL) and polyvinylpyrrolidone (2%, w/v)	Ethanol/ water	Polyvinylpyrrolidone (2%, w/v) in ethanol	212 ± 4 nm	0.11 ± 0.08	$t_{50} < 5$ min, $t_{100} \approx 30$ min	[95]
Y-junction	Cefuroxime axetil (50 mg/mL)	Acetone/ isopropyl ether	No stabilizers	≈ 450 nm	—	$t_{50} < 5$ min, $t_{100} \approx 45$ min	[100]
Y-junction	Danazol, —	Ethanol/ water	No stabilizers	≈ 364 nm	—	$t_{50} \approx 2$ min, $t_{100} \approx 5$ min	[96]
Y-junction	Atorvastatin calcium (40 mg/mL)	Methanol/ isopropano	No stabilizers	≈ 260 nm	—	$t_{50} \approx 2$ min, $t_{100} \approx 5$ min	[102]
Pressurized T-junction	Phenytoin (10 mg/mL)	Ethanol/ water	PVA (0.1%, w/w)	≈ 432 nm	≈ 0.16	$t_{50} \approx 1$ min, $t_{100} \approx 10$ min	[106]
Pressurized T-junction	Bezafibrate (10 mg/mL)	Ethanol/ water	PVA (0.1%, w/w)	≈ 413 nm	≈ 0.10	$t_{50} \approx 2$ min, $t_{100} \approx 10$ min	[106]
Pressurized T-junction	Flurbiprofen (10 mg/mL)	Ethanol/ water	PVA (0.1%, w/w)	≈ 488 nm	≈ 0.12	$t_{50} \approx 2$ min, $t_{100} \approx 60$ min	[106]
Pressurized T-junction	Miconazole (10 mg/mL)	Ethanol/ water	PVA (0.1%, w/w)	≈ 526 nm	≈ 0.30	$\approx 15\%$ release in 60 min, $t_{50} > 60$ min	[106]
Microporous tube-in-tube reactor	Cefuroxime axetil (50 mg/mL)	Acetone/ isopropyl ether	No stabilizers	≈ 400 nm	—	$t_{50} \approx 20$ min, $t_{100} \approx 100$ min	[101]
Rotating tube consists of hollow cylinder	Meloxicam (3 mg/mL)	Benzyl alcohol/ water	Poloxamer (2%, w/v)	180 ± 18 nm	≈ 0.22	$t_{50} \approx 3$ min, $t_{100} \approx 20$ min	[103]
Rotating tube consists of hollow cylinder	Meloxicam (5.6 mM)	Alkaline solution/ citric acid solution (0.01 M)	Polyallylamine hydrochloride and polyprotomine sulfate	90 ± 32 nm	≈ 0.28	$t_{50} \approx 180$ min, $\approx 80\%$ released in 360 min	[99]
Rotating tube consists of hollow cylinder	Curcumin (500 μ M)	Sodium hydroxide solution (0.1 M)/ citric acid and tri-sodium citrate solution	Didodecyl-dimethylammonium bromide and Poloxamer 407	50 ± 15 nm	≈ 0.03	$t_{50} \approx 5$ min, $t_{100} \approx 60$ min	[104]
Microfluidic nebulator	Fenofibrate (5 mg/mL)	Ethanol-in-air droplets	No stabilizers	≈ 14 nm	—	—	[107]
Microfluidic nebulator	Estradiol (5 mg/mL)	Ethanol-in-air droplets	No stabilizers	≈ 14 nm	—	—	[107]
Microfluidic nebulator	Clotrimazole (5 mg/mL)	Ethanol-in-air droplets	No stabilizers	≈ 14 nm	—	—	[107]
Microfluidic nebulator	Danazol (5 mg/mL)	Ethanol-in-air droplets	No stabilizers	≈ 14 nm	—	—	[107]

4.2 Polymeric nanoparticles

The utilization of biodegradable polymeric nanoparticles for controlled drug delivery has shown significant therapeutic potential [112]. The polymer nanoparticles prepared by microfluidics,

according to the microfluidic schemes, are summarized in **Table 4**. The precipitation of PLGA-*b*-PEG diblock copolymers has been controlled by a tunable mixing through 2D-HFF [27]. The formulation of PLGA-*b*-PEG nanoparticles was optimized by varying the flow ratios between the solvent (acetonitrile) and non-solvent (water), and the composition of the particle precursors. The incorporation of PLGA into PLGA-*b*-PEG nanoparticles not only increased the encapsulation efficiency (from *ca.* 28% to 51%) and loading degree (from *ca.* 4.1% to 6.8%) of docetaxel, but also prolonged the release of docetaxel. For the PLGA-*b*-PEG nanoparticles prepared by bulk and 2D-HFF, their t_{50} of docetaxel was *ca.* 5-6 h. The t_{50} of docetaxel increased to *ca.* 11 h, upon the incorporation of PLGA into PLGA-*b*-PEG nanoparticles prepared by conventional bulk method. In terms of PLGA-*b*-PEG and PLGA hybrid nanoparticles prepared by 2D-HFF, the t_{50} of docetaxel further increased to *ca.* 19 h. By incorporating the Pt(IV) conjugated polylactic acid (PLA-Pt(IV)), the loading of water-soluble cisplatin in PLGA-*b*-PEG nanoparticles was enhanced from *ca.* 0.5% to 5.0% and the co-delivery of Pt(IV) and docetaxel with different water solubility was achieved (**Figure 8a**) [80]. The obtained hybrid nanoparticles provided the controlled release of Pt(IV) and docetaxel over a period of 70 h. After cell uptake, the release of the active platinum(II) center was achieved by reduction of the PLA-Pt(IV) prodrugs, which was confirmed by the formation of Pt-1,2,-d(GpG) intrastrand cross-links in the nuclear DNA of cells (**Figure 8b**).

In addition to PLGA-*b*-PEG nanoparticles, poloxamer [113, 114], hydrophobically modified chitosan [115], hyaluronic acid [116, 117], poly-2-vinylpyridine-*b*-poly(ethylene oxide) [118], and alginate [119] nanoparticles have also been successfully prepared by the 2D-HFF. The poloxamer block-copolymer micelles were designed for the co-delivery of dexamethasone and ascorbyl-palmitate for the combined induction of osteogenic differentiation [120]. Both drugs can be efficiently co-encapsulated in polymeric micelles and the different production parameters adjusted to modulate the characteristics of polymeric micelles. The hydrophobic modified chitosan can form nanoparticles in an organic solvent free system by simply mixing the polymer solution (pH = 5.5) with alkaline water

(pH 9.0). The precipitation of hydrophobic modified chitosan was driven by pH changes in a water environment, which was environment friendly by avoiding the use of organic solvent [115].

The 2D-HFF microfluidic devices were equipped with the Tesla structured channel to achieve high-performance mixing [67]. In this device, the solvent and non-solvent mixed completely after passing only five mixing cell-pairs with Re ranging from 0.1 to 100. The antibody recognition test further proved the high mixing efficiency of the Tesla structured channel [66]. In the Tesla structured channel, the hydrophobic modified chitosan (HMCS) nanoparticles were encapsulated by a pH-sensitive copolymer (Eudragit FS 30D) [121]. This pH-sensitive outer polymer layer helped the HMCS nanoparticles to bypass the harsh gastric fluid and to release the majority of the loaded PTX in the colorectal section [121].

The 3D-coaxial flows formed in the 3D-HFF microfluidic devices are critical for the rapid and uniform mass transfer [77]. A pressure-tolerant (up to 16 MPa) 3D-parallel polyimide film device was fabricated to prepare PLGA-*b*-PEG nanoparticles (**Figure 8c**) [122]. The PLGA-*b*-PEG acetonitrile solution was focused in both horizontal and vertical directions by 4 water inlets to achieve the 3D-HFF (**Figure 8d**). The size of PLGA-*b*-PEG nanoparticles under various conditions, such as molecular weight and concentration of nanoparticle precursors, as well as the total flow rates and flow ratios between solvents and non-solvents, are summarized in **Figure 8e**. By simply varying the above mentioned parameters, the nanoparticle size can be reproducibly controlled between 50 and 150 nm, an ideal size range for passive targeting in the treatment of solid tumors. The obtained device consisted of 8 sets of microchannels with a production rate of ca. 311 g/day [122]. The nanoparticles prepared by 3D-HFF were smaller than the counterparts prepared by bulk method and 2D-HFF (**Figure 8f**). Moreover, the longer the block of PLGA, the larger the size of nanoparticles. A microcapillary device constituted of coaxial aligned capillaries offers the distinct capability to form truly 3D coaxial flows [123]. For this microfluidic device, three types of polymeric nanoparticles, including PLGA, HMCS and acetalated dextran (AcDX), were fabricated with a production rate up to ca. 243 g/day [68]. Until

now, the highest production rate of nanoparticles achieved was 3 kg/day by a coaxial turbulent jet mixer [124].

Figure 8. Polymer nanoparticles prepared by HFF. (a) Schematic illustration of the design and fabrication of PLGA-*b*-PEG nanoparticles co-loaded with docetaxel and Pt(IV). (b) Visualization of Pt-1,2,-d(GpG) cross-links with nuclear DNA after treatment with Pt(IV)-prodrug encapsulated nanoparticles with and without docetaxel. (c) Schematic illustration of 3D-HFF for mass production of nanoparticles in a parallel manner with 8 sets of microchannels. (d) Schematics of the direct 3D flow focusing. (e) The effect of flow rate, flow ratio, molecular weight, and concentration of polymers on the size of produced nanoparticles. (f) Transmission electron microscope (TEM) images of nanoparticles prepared by bulk method (1), 2D-HFF (2) and 3D-HFF (3, PLGA_{20k}-*b*-PEG_{5k}; 4, PLGA_{55k}-*b*-PEG_{5k}). The scale bars are 200 nm. Figures are reproduced with permissions from: (a and b) ref. [80], © 2010 PNAS; (c-f) ref. [122], © 2014 RSC.

The rapid mixing in MIVM is driven by both the collision of fluids under a high shear rate and instant molecular diffusion among fluids [125]. The MIVM is used to efficiently produce poly(methyl methacrylate) (PMMA) nanoparticles, with a particle size *ca.* 100 nm and a narrow size distribution

(PDI < 0.2) [126]. The physicochemical and encapsulating/release properties of the obtained nanoparticles can be adjusted by changing the solvent and non-solvent flow rates [126]. A small hydrophobic molecule (β -carotene) and a cationic macromolecule (polyethyleneimine, PEI) were encapsulated into polymer nanoparticles by MIVM [127]. When $Re > 1400$, the size of nanoparticles became independent of mixing in the MIVM. The fabrication of MIVM using computational design and 3D-printing methods enables a continuous and industrial scale production of nanoparticles formed by electrostatic complexation between poly(diallyldimethylammonium chloride) and poly(sodium 4-styrenesulfonate) [128].

The CIJM technique was utilized to prepare bioactive nanoparticles made of hydrophobic core solutes, such as PLA and mucic acid modified with lauroyl groups, 2,3,4,5-tetrakis-dodecanoyloxy-hexanedioic acid (**Figure 9a**) [129]. These hydrophobic core solutes were stabilized by the PEG-based amphiphilic macromolecules with either a carboxylic acid (AM 1) or propyl amide (AM 2) terminal. The release of AM 1 from nanoparticles prepared by CIJM (NP 1) was significantly lower than that from the AM 1 micelles (**Figure 9b**), showing the high stability of nanoparticles prepared by the CIJM technique. In contrast to the analogous micelle assemblies, nanoparticles fabricated by CIJM inhibited the uptake of oxidized low-density lipoproteins and enhanced the formation of foam cells (**Figure 9c** and **d**). Lewis *et al.* [130] synthesized a library of sugar-based amphiphilic macromolecules, among which the strongest atheroprotective profile was identified for M₁₂PEG (**Figure 9e**). This amphiphilic macromolecule was fabricated into serum-stable nanoparticles by CIJM. When intravenously administered to a mouse model of cardiovascular disease, these nanoparticles showed a pronounced increase in lesion association, causing a significant reduction in neointimal hyperplasia, lipid burden, cholesterol clefts, and overall plaque occlusion (**Figure 9f** and **g**) [130].

The nanoparticle formed by turbulent precipitation through solvent displacement of polycaprolactone (PCL) with different CIJMs has been investigated [84]. In this case, the reactor geometry (notably the diameter of the inlet jets) affects the final mean particle size and size

distribution, suggesting that a CIJM with adjustable inlet jets can be more conveniently used to produce polymeric nanoparticles with specific characteristics. Nanoparticles (including both nanospheres and nanocapsules) constituted by a PEGylated derivative of cyanoacrylate have also been prepared by CIJM [83]. Reynolds numbers can be used to correlate the particle size at different conditions, such as the initial co-polymer concentration and flow rate. The use of quenching reduced the particle size and allowed the retrieval of nanospheres and nanocapsules in a reproducible way. At the same inlet jet velocity, a larger inlet diameter gives better results for nanocapsules, while the opposite is true for nanosphere precipitation. The most important operating parameters include the initial concentration of the nanoparticle precursors and the inlet jet velocity [83]. Taking advantage of an adjustable CIJM, the precise control over the particle size and size distribution of PCL nanoparticles was demonstrated as a proof-of-concept [84].

Figure 9. Polymer nanoparticles prepared by CIJM for atherosclerosis therapy. (a) Fast precipitation process is the requisite to form table nanoparticles. τ_{mix} , the duration for complete mixing; τ_{flash} , the duration of precipitation of nanoparticle precursors. (b) The release of AM 1 from nanoparticles prepared by CIJM (NP 1) and thermodynamically assembled AM 1 micelles. (c) Quantification of foam cell phenotype after 48 h incubation with human monocyte-derived

macrophages. (d) Representative bright field images of foam cell phenotype, which were stained by OilRed O (red). (e) The inhibition of atherosclerotic plaque development by repressing the lipid-scavenging receptors at the lesion-based macrophages. (f) The fluorescence images of en face aorta at 24 h post-injection. (g) *Ex vivo* fluorescence images show the accumulation of nanoparticles in the aortic arch at 60 d after injection. Figures are reproduced with permissions from: (a-d) ref. [129], © 2012 WILEY-VCH; (e-g) ref. [130], © 2015 PNAS.

Table 4. The physicochemical properties of the polymer nanoparticles synthesized by microfluidic platforms.

Microfluidic devices	Particle precursors	Drug loading degree	Solvent/non-solvent	Stabilizers	Average size	PDI	Estimated t_{50} and t_{100}	Ref.
2D-HFF	PLGA- <i>b</i> -PEG	Docetaxel 4.1%	Acetonitrile/ water	–	20–35 nm	< that of bulk method	In PBS, $t_{50} \approx 5$ h and <i>ca.</i> 90% released in 72 h	[27]
2D-HFF	PLGA- <i>b</i> -PEG and PLGA	Docetaxel, 6.8%	Acetonitrile/ water	–	30–110 nm	< that of bulk method	In PBS, $t_{50} \approx 20$ h and <i>ca.</i> 90% released in 72 h	[27]
2D-HFF	PLGA- <i>b</i> -PEG and PLA-Pt(IV)	Docetaxel, 1 %	Acetonitrile/ water	–	≈ 100 nm	0.06–0.17	In PBS, $t_{50} \approx 12$ h and $t_{100} \approx 72$ h	[80]
2D-HFF	PLGA- <i>b</i> -PEG and PLA-Pt(IV)	Pt(IV), 5%	Acetonitrile/ water	–	≈ 100 nm	0.06–0.17	In PBS, $t_{50} \approx 40$ h and 90% released in 80 h	[80]
2D-HFF	Poly[9,90-di(2-ethylhexyl)fluorene ethynylene]/PEG	Perylene diimide dye, -	Tetrahydrofuran/ water	–	30–80 nm	0.2	–	[131]
2D-HFF	Dendritic polyethylene and Poloxamer 407	PTX, –	Tetrahydrofuran/ water, mixing time 2.5 ms	Poloxamer 407	60–200 nm	close to 0.1	In PBS, $t_{50} \approx 35$ h and <i>ca.</i> 80% released in 14 day	[132]
2D-HFF	Poloxamer 407	Mithramycin, –	Dimethyl sulfoxide/ water	–	60–75 nm	< that of bulk method	–	[113]
2D-HFF	HMCS	PTX, <i>ca.</i> 8.7%	1% aqueous acetic acid/ basic water, flow ratio of 0.031	–	74 nm	<0.16	In PBS, $t_{50} \approx 22$ h and <i>ca.</i> 85% released in 390 h	[133]
3D-HFF	PLGA	PTX, <i>ca.</i> 6%	Acetonitrile/ 4% ethanol	Phospholipid (50 μ g/mL)	< that of bulk method	close to 0.1	–	[68]
3D-HFF	HMCS	PTX, <i>ca.</i> 4%	Acetonitrile and 1% (v/v) acetic acid/ 4% ethanol, pH 9	Phospholipid (50 μ g/mL)	< that of bulk method	close to 0.1	–	[68]
3D-HFF	PLGA	PTX, <i>ca.</i> 6%	Acetonitrile/ 4% ethanol, pH 8	Phospholipid (50 μ g/mL)	< that of bulk method	close to 0.1	–	[68]
3D-HFF	PLGA	Dexamethasone, < 2%	Acetonitrile/ water	–	35–350 nm	–	At pH 7.4, $t_{50} \approx 10$ min and $t_{100} \approx 30$ min	[123]
3D-HFF	PLGA- <i>b</i> -PEG	–	Acetonitrile/ water	–	30–230 nm	< that of 2D-HFF or bulk method	–	[77]

3D-HFF, 8 parallel sets	PLGA- <i>b</i> -PEG	Docetaxel, –	Acetonitrile/ water	–	20 and 35 nm	< that of 2D-HFF or bulk method	At pH 7.4, $t_{50} \approx 8$ h and $t_{100} \approx 24$ h	[134]
Coaxial turbulent jet mixer	PLGA- <i>b</i> -PEG	Docetaxel, <i>ca.</i> 1.7%	Acetonitrile/ water	–	< 100 nm	–	–	[124]
Coaxial turbulent jet mixer	PLGA- <i>b</i> -PEG	Insulin, <i>ca.</i> 1.6%	Dimethyl sulfoxide/ water	–	< 100 nm	–	–	[124]

4.3 Polyplexes

Among the materials for the delivery of nucleic acids, PEI, chitosan [135] and poly(beta-amino esters) (PBAE) [136] are well-established as non-viral vectors in gene therapy applications. The formed polyelectrolyte complexes, so-called polyplexes, are formed by the electrostatic interactions of oppositely charged polycations and nucleic acids. To facilitate the cell uptake, the obtained polyplexes are positively charged. The polyplexes prepared by microfluidics are summarized in **Table 5**, according to the microfluidic designs.

The spontaneously formed polyplexes are usually prepared by mixing solutions through pipetting in lab scale. In contrast, microfluidics offers an optimized preparation procedure, enabling the scale-up of well-defined polyplexes. Debus *et al.* [137] compared the characteristics of polyplexes between PEI and plasmid DNA or small interfere RNA (siRNA), which were prepared either by microfluidics or pipetting. Among all the factors, the ratio of PEI to DNA was one of the most important ones affecting the formation of polyplexes. The size of polyplexes prepared by microfluidic mixing was relatively constant between 140 and 160 nm over a wide range of complex concentrations (12–600 $\mu\text{g/mL}$). In comparison, the size of polyplexes prepared by pipetting varied considerably (ca. 90 nm to 160 nm) [137]. Instead of drug release, the levels of expression or inhibition of specific genes was used to evaluate the delivery of nucleic acids. The microfluidic assembled PBAE/DNA nanoparticles showed higher *in vitro* transfection efficacy in GB319, B16 and MDA-MB-231 cancer cell lines than the counterparts prepared by the conventional pipetting. Moreover, the microfluidic mixing resulted in fewer DNA-free polymeric nanoparticles compared to those produced by bulk mixing [138].

DMDG is a feasible method for production of nanoparticles and provides a great means for the preparation of size-controlled nanoparticles with desired surface ligand coverage [73]. Nanoparticles composed of β -cyclodextrin (β -CD)-grafted branched PEI (β -CD-PEI), adamantanamine (Ad) grafted polyamidoamine dendrimer (Ad-PAMAM) and Ad grafted PEG (PEG-Ad) were prepared by DMDG (**Figure 10a**) [43]. The surface chemistry of the obtained nanoparticles was easily tailored by incorporating additional building blocks functionalized with specific ligands, such as arginylglycylaspartic acid (RGD) and/or a peptide derived from the transactivator of transcription (TAT) of human immunodeficiency virus. The incorporation of RGD (RGD-PEG-Ad) and/or TAT (TAT-PEG-Ad) functionalized PEG-Ad improved the transfection efficiency of plasmids. Within 2.5 h, a library of 648 nanoparticle formulations were prepared by DMDG with automated sampling, dilution, metering, and mixing of precursors. These formulations filled up seven 96-well plates. After full-scale screening of these 648 formulations, the optimal plasmid transfection performance was achieved at concentrations of β -CD-PEI of 0.6-0.8 μ M, a RGD-PEG-Ad of 0.24-0.48 μ M, and TAT-PEG-Ad of 0.4-0.8 μ M (**Figure 10c-d**).

Figure 10. Polyplexes prepared by microfluidics for plasmid and insulin delivery. (a) Schematic of the self-assembly approach for producing a combinatorial library of plasmid encapsulated nanoparticles. (b and c) The transfection performance of RGD (b), TAT (c) functionalized plasmid-encapsulated nanoparticles. (d and e) The transfection performance of RGD and TAT co-functionalized enhanced green fluorescent protein (EGFP)- (d) and luciferase- (e) encoded plasmid-encapsulated nanoparticles. (f) Schematic of the MIVM system for preparing insulin-loaded chitosan nanoparticles. (g) The impact of mixing method on the average size and PDI of chitosan nanoparticles. (h) Blood glucose level of diabetic rats after the administration of different insulin formulations. p.o., oral administration; s.c., subcutaneous administration. Figures are reproduced with permissions from: (a-e) ref. [43], © 2010 ACS; (f-h) ref. [139], © 2017 Elsevier.

A convergence of quantum-dot-mediated fluorescence resonance energy transfer and microfluidics has been employed to study the kinetics of self-assembled DNA polyplexes under laminar flow in real time with millisecond resolution. For the polycation/DNA polyplexes, the self-assembly process can be divided in two distinct stages. At first, the DNA and polycation rapidly interacted at the laminar interface, which can be attributed to ‘fast cationic binding’ in the millisecond range. The growth of DNA polyplexes was found to be linearly dependent on the square root of time intervals, suggesting the diffusion-limited feature of the first stage process. The polyplex formation entered a secondary ‘nano-assembly flocculation’ stage, which was a diffusion-reaction limited process [140]. The integration of nanophotonics and microfluidics offers a powerful tool for elucidating the formation of polyelectrolyte polyplexes; this combination is expected to provide better control and preparation of uniform and customizable polyplexes for future nucleic acid-based therapeutics.

In a MIVM, insulin-loaded nanoparticles were engineered by infusing aqueous solutions of chitosan, tripolyphosphate and insulin with a production rate of ca. 122 g/day, when the flow rate was 50 mL/min (**Figure 10f**) [139]. The insulin-loaded chitosan nanoparticles showed a smaller average particle size (down to 45 nm), narrower size distribution and higher encapsulation efficiency (up to

90%), compared with the counterparts prepared by the bulk-mixing method (**Figure 10g**). After oral administration to a Type I diabetes rat model, the smaller the particle size of the insulin-loaded chitosan nanoparticles resulted in a more effective control of the blood glucose level (**Figure 10h**) [139].

Supramolecular cyclodextrin (CD)-based nanoparticles, mediated by host-guest interactions, have gained increased popularity because of their versatility on the inclusion of therapeutics. Nanoparticles composed of poly-condensation of CD (PBCD) were prepared in a 2D-HFF microfluidic chamber by mixing two aqueous solutions of neutral polymers, in the absence of organic solvents. Despite their non-covalent nature, the spontaneously formed PBCD nanoparticles were remarkably stable in terms of concentration and size distribution ($PDI < 0.1$), even on extreme dilution [141].

The LbL self-assembly is a versatile approach to fabricate multi-component and stimuli-responsive nanoscale nanoparticles for controlled drug delivery. The conventional LbL synthetic approach of nanoparticles requires numerous purification steps that limit scale, yield and potential applications. Correa *et al.* [74] developed a highly scalable and closed-loop diafiltration technique to manage intermediate purification steps, and consequently, to improve the production rate of LbL assembled nanoparticles. The purification of cationic and anionic nanoparticles was separated to different purification loops to prevent nonspecific adsorption onto the filter membrane. The model nanomaterials, such as polystyrene (PS) nanoparticles, mesoporous silica nanoparticles and liposomal vesicles, were successfully coated in a controlled manner by the LbL closed-loop diafiltration technique. This technique showed great versatility for the deposition of a broad range of polyelectrolytes, including native polysaccharides, linear polypeptides, and synthetic polymers [74].

Table 5. The physicochemical properties of the polyplex synthesized by microfluidic platforms.

Microfluidic devices	Particle precursors	Drug loaded	Solvent/non-solvent	Average size	PDI	Ref.
2D-HFF	Poly- β -CD and dextran-benzophenone	Benzophenone	Host-guest interactions	50–102 nm	≤ 0.1	[141]
2D-HFF	PEI	Plasmid DNA	Polyelectrolyte coacervation	60–120 nm	0.2–0.3	[137]
3D-HFF microfluidic drifting	Poly(2-hydroxypropyleneimine)	Plasmid DNA	Polyelectrolyte coacervation	≈ 200 nm	≈ 0.07	[76]

3D-HFF microfluidic drifting	Poly(beta-amino ester)	Plasmid DNA	Polyelectrolyte coacervation	≈ 100 nm	–	[138]
MIVM	PEG- <i>b</i> -PAA and PEI	PEI	Polyelectrolyte coacervation	30–60 nm	–	[127]
3D-printed MIVM	Poly(diallyldimethylammoniumchloride) (PDDA), and poly(sodium 4-styrenesulfonate) (PSS)	–	Polyelectrolyte coacervation	≈ 140 nm	≈ 0.3	[128]
CIJM	Chitosan	Insulin, $27.5 \pm 0.4\%$	0.2% acetic acid (pH 5.3)/ tripolyphosphate in HEPES	46.2 ± 2.7 nm	$\approx 0.15 \pm 0.02$	[139]
DMDG	Ad-PAMAM, RGD-PEG-Ad	β -CD-PEI-Cy5	Polyelectrolyte coacervation	37–344 nm	–	[73]
DMDG	β -CD-PEI, Ad-PAMAM, RGD-PEG-Ad and TAT-PEG-Ad	EGFP-encoded plasmid DNA	Polyelectrolyte coacervation	42 ± 4 and 86 ± 9 nm	< 0.05	[43]

4.4 Structured nanoparticles

Microfluidics is also a versatile approach to encapsulate nanoparticles to form structured nanocomposites, such as core/shell. The structured nanocomposites prepared by microfluidics are summarized in **Table 6**, according to the microfluidic designs.

A nanocomposite consisting of an encapsulated-porous silicon (PSi) nanoparticle and a stimuli responsive polymer layer was fabricated by a 3D-HFF capillary device [31, 79]. The assembly of poly(L-histidine)-*b*-PEG (PHIS-*b*-PEG) micelles onto the surface PSi nanoparticles temporally sealed their pores and enhanced their stability in plasma [79]. Upon exposure to acidic conditions, PSi nanoparticles were uncovered to release sorafenib (SFN), which can be attributed to the pH-responsive property of PHIS-*b*-PEG micelles. Thanks to their high drug loading capacity, PSi nanoparticles can regulate the drug loading degree of the PSi nanoparticle-encapsulated nanocomposites. For example, PTX, SFN and methotrexate (MTX) with different solubility were simultaneously loaded into PSi@AcDX with a ratiometric control (**Figure 11a**) [31]. The decomposition of the outer AcDX polymer layer controlled the release of all the payloads (**Figure 11b**). The surface functionalization with a cell penetrating peptide improved cell uptake of nanocomposites, thus the proliferation of two breast cancer cell lines, MCF-7 (**Figure 11c**) and MDA-MB-231 (**Figure 11d**), was markedly inhibited. Our group has also encapsulated the PSi or PSi@AcDX nanoparticles inside cancer cell membrane vesicles [142]. The obtained PSi-based nanocomposites promoted the expression of co-

stimulatory signals and the secretion of pro-inflammatory cytokines [142], with promising results in cancer immunotherapy.

Besides P*Si* nanoparticles, drug nanocrystals-encapsulated core/shell nanocomposites were successfully engineered by either a two-separated-step (**Figure 11e**) [78] or a superfast sequential [35] microfluidic nanoprecipitation. Toward the superfast sequential approach, the time intervals between the two precipitation processes were extremely short (< 0.1 s at Re 1300). The outer polymer layer deposited onto the surface of freshly formed drug nanocrystals in a superfast manner, stabilizing the drug nanocrystals without the need for using any stabilizers [35]. The obtained nanocomposite consisted of a drug nanocrystal core (*e.g.*, SFN or PTX) and a polymer [*e.g.*, AcDX or hydroxypropylmethylcellulose acetate succinate (HPMCAS)] shell on a 1:1 ratio, showing clear core/shell structures. The prepared core/shell nanocomposites merged the favorable characteristics of both polymer nanoparticles, such as stimuli-responsive release of payloads and ease of surface functionalization (**Figure 11f**), and drug nanocrystals, including ultrahigh mass fraction of therapeutics and fast drug dissolution. As shown in **Figure 11g**, the half-maximal anti-proliferation concentration of the SFN nanocrystal encapsulated nanocomposites (loading degree *ca.* 58%) was *ca.* 54 times lower than their conventional counterpart (loading degree *ca.* 5%) [78]. As a result, a small number of nanocarriers is enough to deliver a clinically relevant therapeutic dose, and the treatment cost and potential side effects of nanocarriers can be greatly reduced.

Figure 11. Structured nanoparticles prepared by microfluidic nanoprecipitation. (a) Schematic of the process to synthesize cell penetrating peptide-functionalized multidrug-loaded PSi@AcDX. (b) Degradation behavior of PSi@AcDX at pH 7.4 and 5.0 as a function of time. (c and d) Proliferation profiles of MCF-7 (c) and MDA-MB-231 (d) cells incubated with multidrug-loaded nanocomposites. (e) Schematic of the two-step microfluidic nanoprecipitation to prepare drug nanoparticle encapsulated nanocomposites. (f) Fluorescent microscopy images of MDA-MB-231 breast cancer cells incubated with prepared nanoparticles (green). (g) The IC₅₀ values for the prepared nanomaterials. Figures are reproduced with permissions from: (a-d) ref. [31], © 2015 Elsevier; (e-g) ref. [78], © 2017 WILEY-VCH.

The structural features of nanoparticles could also be employed to address the drug resistance in cancer therapy. Prickly zinc-doped copper oxide (Zn-CuO) nanoparticles were encapsulated in a pH-responsive polymer (spermine functionalized AcDX, AcDX-SP) to form a nanocomposite. With the help of surface functionalization, the nanocomposites specifically targeted carbonic anhydrase IX, which is overexpressed on the membrane of a wide variety of cancer cells. After cell uptake, the pH-responsive polymer degraded and the prickly zinc-doped copper oxide (Zn-CuO) nanoparticles disintegrated into small pieces. The degradation of nanocomposites facilitated the endosome escape of zinc-doped copper oxide pieces. The zinc-doped copper oxide pieces induced severe damage to the endoplasmic reticulum and mitochondria of the cancer cells [143].

A three-stage microfluidic device was developed to prepare a PLGA nanocapsules to effectively deliver hydrophilic therapeutics [144]. The engineered nanocapsules are composed of a hollow water core, a PLGA shell and an outer lipid layer. At first, the hydrophilic drugs-loaded lipid reverse micelles formed by mixing the drug aqueous solution with an organic fluid containing lipids and PLGA. Next, the capsule-like PLGA shell formed after further mixing with water. At last, the capsule-like PLGA particles were coated with other lipids. Different hydrophilic therapeutics, such as calcein, rhodamine B and siRNA, were efficiently (encapsulation efficiency >90%) incorporated into the nanocapsules.

The nanocapsules co-delivery of siRNA and DOX improved the therapeutic efficacy in a multi-drug resistance tumor model, compared to the combination of bare drugs [144].

The morphologies of amphiphilic block copolymers self-assembled in a gas-liquid microfluidic reactor are entirely different from off-chip equilibrium structures [145]. Moffitt's group has intensively studied the fascinating effects of high-shear environments in segmented gas-liquid microfluidic reactors on the self-assembly of block copolymers (**Figure 12a**) [146-148]. For example, the polystyrene-*block*-poly(acrylic acid) (PS-*b*-PAA) copolymer formed exclusively spheres off-chip. In the segmented gas-liquid microfluidic device, this polymer formed kinetic cylinders, Y-junctions, bilayers and networks [149-151]. The flow-directed variable micellar morphologies were ascribed to the collision-coalescence effect, enabled by the strong and localized on-chip shear fields [149].

In a gas-liquid segmented reactor, the variation in flow rates and chemical conditions, including solvent composition, water content, polymer concentration and added salt, all affect the size and morphologies of flow-directed PS-*b*-PAA nanostructures (**Figure 12b and c**) [149-151]. Because of the coalescence mechanism enabled by high shear force, the chemical conditions that favor spherical micelles of PS-*b*-PAA in the bulk were found to yield a nearly pure population of vesicles on the gas-liquid segmented microfluidic reactors [151]. After off-chip relaxation of 28 days, only spherical micelles remained for the structures obtained with 5.2 wt-% water. The mechanism of on-chip morphology transformations of micelles has been proposed by Moffitt's group (**Figure 12d**) [149, 151]. The fast mixing with water generates spheres adjacent to the injector, because the global equilibrium favours spheres (State A; **Figure 12d**). In the corners of the liquid plugs, the local shear-induced coalescence of spheres increases aggregation numbers to form aggregates (State B; **Figure 12d**). Through the kinetically favoured intramolecular chain rearrangements, these transition structures relax and form the morphology with a minimum local free energy (State C; **Figure 12d**).

The flow-directed production of the nanoparticles composed of the photoresponsive block copolymer poly(*o*-nitrobenzyl acrylate)-*b*-polydimethylacrylamide (PNBA-*b*-PDMA) was also

achieved in a gas-liquid segmented reactor [152]. Like PCL-*b*-PEO nanoparticles, by simply changing the flow rates, PNBA-*b*-PDMA nanoparticles with a variety of morphologies, such as spheres, short cylinders, large compound micelles and unique spooled cylinders were synthesized (**Figure 12e** and **f**). Toward the light-triggered dissociation of flow-directed nanoparticles, the higher the flow rate is, the faster the disruption of the nanoparticles. The flow rate not only controlled the particle size and morphologies of the obtained polymeric nanoparticles, but also their release rate of payloads and even therapeutic effects [153]. As shown in **Figure 12g**, the release rate of PTX was sustained from *ca.* 3 days for nanoparticles prepared by conventional bulk method to up to 2 weeks for those prepared by microfluidics. The slow release rate of PTX from the microfluidic assembly nanoparticles can be ascribed to the fast mixing giving rise to the relative homogenous distribution of payloads inside the nanoparticles. The greatest antiproliferation effect against MCF-7 breast cancer cells were observed for nanoparticles prepared at an intermediate flow rate (200 $\mu\text{L}/\text{min}$), corresponding to the small particle size and narrow size distribution of nanoparticles obtained at this flow condition [153].

Figure 12. Nanocomposites prepared by the gas–liquid segmented flow systems. (a) Schematic of the gas–liquid segmented microfluidic reactor and the select fluorescence microscopy images of the reactor in operation. (b) TEM images of the PS-*b*-PAA micelles formed off-chip (left) and on-chip (right) in dimethylformamide/dioxane mixtures containing water (5.2 wt-%). (c) Off-chip relaxation kinetics of nonspherical micelles formed on-chip formed at 5.2 wt-% water. (d) Proposed energy diagram for on-chip shear-induced morphological transitions. (e) 2D TEM image (top) and 3D TEM tomography image (bottom) of a triangular-shaped spooled cylinder. (f) TEM images of the end-on (top) and the enlarged (bottom) view of a spiraling cylinder. Figures are reproduced with permissions from: (a) ref. [145], © 2008 ACS; (b-d) ref. [149], © 2011 ACS; (e and f) ref. [152], 2015 ACS; (g and h) ref. [153], © 2017 ACS.

Table 6. The physicochemical properties of the structured nanoparticles engineered by microfluidic platforms.

Microfluidic devices	Particle precursors	Drug loading degree	Solvent/non-solvent	Stabilizers	Average size	PDI	Estimated t_{50} and t_{100}	Ref.
Y-shaped mixer with herringbone	Gold nanoparticles and polydi(carboxylatophenoxy)phosphazene (PCPP)	Weight ratio of gold nanoparticles to PCPP, 5:1	PBS, pH 7.4/ water	Spermine and PEG-PLL	40-500 nm	< crosslinking method	< 15% of gold NPs released in 7 days	[154]
2D-HFF	Conjugation of PLGA, gold particles and cyclodextrin	SFN, <i>ca.</i> 1.35%	Dimethylformamide and acetonitrile/ 30% methanol	Lipid (40 µg/mL)	85 nm	0.103	In PBS, $t_{50} \approx 3$ h and $t_{100} \approx 500$ h	[155]
2D-HFF	Conjugation of PLGA, gold particles and cyclodextrin	DOX, <i>ca.</i> 0.52%	Dimethylformamide and acetonitrile/ 30% methanol	Lipid (40 µg/mL)	85 nm	0.103	In PBS, $t_{50} \approx 18$ h and $t_{100} < 150$ h	[155]
2D-HFF	PEO- <i>b</i> -PS functionalized Gold nanorods and free PEO- <i>b</i> -PS	Rhodamine B, –	Tetrahydrofuran/ water	–	0.3-2.2 µm	–	Linear increase of payloads as a function of near-infrared irradiation time	[156]
2D-HFF	PEO- <i>b</i> -PS functionalized Gold nanorods and free PEO- <i>b</i> -PS	Rhodamine B, –	Tetrahydrofuran/ water	–	1.86 ± 0.47 and 0.92 ± 0.68 µm	–	Linear increase of payloads as a function of near-infrared irradiation time	[157]
2D-HFF	Bisphosphonate-PLGA, superparamagnetic iron oxide nanoparticles	PTX, <i>ca.</i> 8%	Tetrahydrofuran/ water, flow ratio of 0.03	–	<i>ca.</i> 70 nm	<0.13	At pH 7.4, $t_{50} \approx 40$ h and <i>ca.</i> 80% released in 120 h	[158]
2D-HFF	Bisphosphonate-PLGA, superparamagnetic	PTX, <i>ca.</i> 5.8%	Tetrahydrofuran/ water, flow ratio of 0.2	–	<i>ca.</i> 105 nm	<0.13	At pH 7.4, $t_{50} \approx 20$ h and <i>ca.</i> 85% released in 120 h	[158]

	iron oxide nanoparticles							
2D-HFF with tesla mixer	HMCS and Eudragit FS 30D	PTX, –	Solution pH 5.5/ water pH 9.0; HMCS nanoparticles in pH 6.5/Eudragit FS 30D solution	–	40–220 nm of size and 0–65 nm shell	–	–	[121]
3D-HFF	Folic acid functionalized AcDX-SP	SFN, <i>ca.</i> 58.4%	Acetone/water; 50% (v/v) ethanol/water	–	≈ 233 nm	< 0.15	At pH 5.0, $t_{50} \approx 3$ h and $t_{100} \approx 8$ h	[78]
3D-HFF	HPMCAS	SFN, <i>ca.</i> 45.2%	Acetone/water (pH 10.5); water (pH 10.5)/water (pH 3.0)	–	70–550 nm	< 0.2	At pH 7.4, $t_{50} \approx 9$ min and $t_{100} \approx 90$ min	[35]
3D-HFF	HPMCAS	PTX, <i>ca.</i> 42.6%	Acetone/water (pH 10.5); water (pH 10.5)/water (pH 3.0)	–	60–450 nm	< 0.2	At pH 7.4, $t_{50} \approx 4$ min and $t_{100} \approx 30$ min	[35]
3D-HFF	AcDX and PSi nanoparticles	SFN, <i>ca.</i> 5.0%	Ethanol/water	Polyvinyl alcohol (2 mg/mL)	<i>ca.</i> 350 nm,	< 0.1	At pH 5.0, $t_{50} \approx 3.5$ h and $t_{100} \approx 16$ h	[31]
3D-HFF	AcDX and PSi nanoparticles	PTX, <i>ca.</i> 5.0%	Ethanol/ water	Polyvinyl alcohol (2 mg/mL)	<i>ca.</i> 350 nm,	< 0.1	At pH 5.0, $t_{50} \approx 2.5$ h and $t_{100} \approx 24$ h	[31]
3D-HFF	AcDX and PSi nanoparticles	Methotrexate, <i>ca.</i> 4.5%	Ethanol/ water	Polyvinyl alcohol (2 mg/mL)	<i>ca.</i> 350 nm,	< 0.1	At pH 5.0, $t_{50} \approx 2$ h and $t_{100} \approx 8$ h	[31]
3D-HFF	PHIS- <i>b</i> -PEG and PLA- <i>b</i> -PEG	SFN, <i>ca.</i> 4.9%	Ethanol and 0.1 M hydrochloric acid / pH 12.8 solution	Poloxamer 407 (2 mg/mL)	<i>ca.</i> 260 nm	< 0.2	At pH 6.8, and 5.5, $t_{50} < 10$ min and $t_{100} < 1$ h	[79]

4.5 Theranostic nanoparticles

Theranostic nanoparticles achieve the therapeutic and diagnostic applications simultaneously, holding great promise to improve current cancer treatments. However, producing theranostic nanoparticles with multiple components in a reproducible manner is a challenging task. Microfluidic 2D-HFF approach has been developed for the single-step preparation of high-density lipoprotein-mimicking nanomaterials for medical imaging and drug delivery [159]. Different payloads have been successfully incorporated into the high-density lipoprotein-mimicking nanoparticles, including a hydrophobic drug (simvastatin), a fluorescent hydrophobic agent (3,3'-dioctadecyloxacarbocyanine perchlorate), gold nanoparticles, quantum dots, and iron oxide nanoparticles. In terms of cell membrane-coated nanoparticles, microfluidic electroporation effectively promoted the entry of iron oxide nanoparticles into red blood cell membrane-derived vesicles [160]. Due to the long blood circulation feature of the

cell membrane shells, the obtained core/shell nanocomposites improved the tumor magnetic resonance imaging and photothermal therapy, in comparison to the bare iron oxide nanoparticles [160].

Liposomes simultaneously incorporated with mRNA and magnetic resonance imaging contrast agents were prepared by microfluidics (**Figure 13a**). The obtained theranostic liposomes expressed functional proteins (**Figure 13b**) and provided real-time visualization *in vivo* (**Figure 13c**) [161]. A static micromixer-coaxial electrospray method was developed to synthesize theranostic lipoplexes in a single step (**Figure 13d**) [162]. Compared with bulk mixing, the quantum dots (QD605) and Cy5-labeled antisense oligodeoxynucleotides (Cy5-G3139) co-loaded lipoplexes fabricated by the static micromixer-coaxial electrospray method were highly uniform (194 ± 15 nm, PDI ≈ 0.02). The static micromixer-coaxial electrospray method showed higher encapsulation efficiency of both the imaging reagents and the therapeutic drugs than the counterpart prepared by the conventional mixing. A strong Cy5 signal mediated by Förster resonance energy transfer (FRET) was observed at 4 h post-transfection. The Cy5 signal almost disappeared after 48 h, indicating the disassembly of the lipoplexes and the successful release of Cy5-G3139 into the cytoplasm (**Figure 13e**). As shown in **Figure 13f**, the obtained lipoplexes efficiently down-regulated the Bcl-2 gene expression by ca. $48 \pm 6\%$ in A549 cells (non-small cell lung cancer cell line) [162].

Gold nanoparticles were encapsulated into a biodegradable polydi(carboxylatophenoxy)phosphazene (PCPP) nanomatrix to form an inorganic-organic hybrid nanocomposites (Au@PCPP; **Figure 13g**) [154]. The size of Au@PCPP was controlled by the amount of PEG-*b*-polylysine co-polymer (PEG-PLL). After inclusion of variable amounts of gold nanoparticles, the UV-Vis absorption peak of Au@PCPP was tuned into the near-infrared (NIR) region. The amount of gold nanoparticles included also controlled the final size of the Au@PCPP nanocomposites [154]. Due to the red shift of their UV-Vis spectra, Au@PCPP produced a significantly higher photoacoustic signal than the same concentration of bare gold nanoparticles (**Figure 13h**). The photoacoustic intensity of Au@PCPP continually increased with the gold

nanoparticle increasing concentration until 10 mg/mL, after which the photoacoustic intensity reached a plateau. Furthermore, the gold nanoparticle-encapsulated PCPP (Au@PCPP) nanocomposites produced strong CT contrast, serving as contrast agents (**Figure 13i-k**). After administration, Au@PCPP broke down into harmless by-products and subsequently released the gold nanoparticles. The superparamagnetic iron oxide nanoparticles have also been encapsulated into bisphosphonate-functionalized PLGA (PLGA-BP) nanoparticles by a 2D-HFF approach [158]. The obtained nanoparticles showed strong affinity to the bone minerals, and suppressed the bone metastatic tumour [158].

Figure 13. Nanocomposites prepared by microfluidics for theranostic application. (a) Schematic of TT3 lipid-like theranostic nanoparticles. (b) TT3-Gd18 encapsulated lipid-like nanoparticles delivery of luciferase mRNA. (c) T1 weighted images of mouse legs after intramuscular injection of TT3-Gd18 lipid-like nanoparticles. (d) Schematic of the microfluidic-coaxial electrospray device used

for theranostic lipoplexes preparation. (e) Transfection performance of lipoplexes on A549 cells produced by microfluidic-coaxial electrospray device. (f) The obtained lipoplexes down-regulated the expression of Bcl-2. (g) Schematic depiction of the gold nanoparticles encapsulated nanocomposites. (h) Photoacoustic contrast arising from Au@PCPP injected in mice. (i) CT image of Au@PCPP as a function of gold concentration. (j) Linear correlation of CT attenuation and gold concentration in Au@PCPP. (i) CT contrast enhancement by Au@PCPP from intramuscular injection in mice. Figures are reproduced with permissions from: (a-c) ref. [161], © 2017 RSC; (d-f) ref. [162], © 2012 ACS; (g-k) ref. [154], © 2016 Elsevier.

5 Evaluation of nanoparticulate drug delivery systems by microfluidics

Clinical translation of therapeutics is arguably challenging, especially in the case of nanomedicine [163]. In the case of nanoparticles, through *in vitro* and *in vivo* evaluations are highly demanded. The long evaluation process and the related high costs tremendously hindered the development and launching of novel nanomedicines in the market. The conventional assays for *in vitro* evaluation of nanoparticles are done in well plates. However, these systems fall short in mimicking the complexity of the *in vivo* conditions, thus leading to poor *in vitro*–*in vivo* correlation [26]. The establishment of reliable *in vivo* mimetic models is technically challenging, due to the demands in spatial control and regularly arrangement of cells, and in some cases fluid conditions [26]. Microfluidics provides flexibility in channel design and acts as an emerging tool for creating complex and controllable environments for ideally mimicking the *in vivo* situations. Thus it diminishes the time and cost of the biomedical evaluations through microfluidics (for example drug screening) in comparison to the conventional *in vitro* and *in vivo* evaluation models. In addition, microfluidic handles tiny amount of sample and enables high throughput screening, thus significantly improve the cost effectiveness over the evaluation of nanomaterials. Particle–cell, particle–cancer, and particle–organ interaction assays have been transferred and carried out in microfluidic channels. In practical consequence, those systems

have greatly accelerated the process of drug and nanomaterials development, and simplified the clinical translation.

Microfluidic devices can create asymmetry conditions with spatial control. As shown in **Figure 14a**, a microfluidic device with an independent temperature controller from the two sides was designed to evaluate spatial and temporal regulation of the embryonic development [164]. In addition to spatial control, microfluidic devices can also generate concentration gradient, which is critical for high throughput screening of soluble compound interaction with cells (**Figure 14b**) [165]. Hamon *et al.* [166] summarized the microfluidic devices for cell environment studies, including chemical stimulation, mechanical stimulation, cell–cell, and cell–extracellular matrix (ECM) interactions. In addition, many organ-on-a-chip platforms have been invented to act as *in vitro* organ mimetic tools for biological studies [166]. Theoretically, a human-on-a-chip device can be developed by assembling several organ-on-a-chip devices according to the physiological interconnections (**Figure 14c**) [167]. These systems are fundamental and acting as potential and promising platforms for *in vitro* screening of nanoparticle–nanoparticle; nanoparticle–cell and nanoparticle–organ interactions. In addition, microfluidics can also be combined with imaging tools for real time imaging of particle–cell interactions with the transparent channel designs [168, 169].

Figure 14. Microfluidic devices for biomedical analysis. (a) Laminar flow-based device for mechanical stimulation of cells. Schematic representation (left) and photograph (right) of a *Drosophila* embryo submitted to different temperature. (b) The interaction of soluble factors in wide range of concentration gradients are screened in a microfluidic device. Step-by-step process (top) and schematic representation (bottom) of the microfluidic device is illustrated. Blue and red lines represent the fluidic and control channels, respectively. (c) Human-on-a-chip design. The microfluidic devices are built to mimetic different organs and those devices are inter connected to mimetic the human body. Figures are reprinted with permissions from: (a) ref. [164], © 2005 NPG; (b) ref. [165], © 2011 ACS; ref.

5.1 On-chip interaction with cells

One of the first microfluidic platform for studying particle–cell interactions was designed by Farokhzad *et al.* in 2005 [170]. They conjugated the aptamer that recognize the transmembrane prostate specific membrane antigen (PSMA) to the PLA-*b*-PEG copolymer particles (**Figure 15a**). The binding affinity of particles on the cells expressing (positive) or not expressing (negative) PSMA is evaluated under different shear stresses created by microfluidics. The results indicated that at low shear stress (*i.e.* $<1.1 \text{ dyn/cm}^2$, flow rate $0.25 \text{ }\mu\text{L/min}$), more than 10 aptamer-conjugated particles in average bind to PSMA positive cells, while the number of bound particles significantly decreased at shear stress of 4.5 dyn/cm^2 (Flow rate $4 \text{ }\mu\text{L/min}$) (**Figure 15b and c**). This method mimetics the shear stress in blood veins, which is normally between $1\text{--}6 \text{ dyn/cm}^2$, thus providing a high-throughput assay for cell–particle interactions prior to *in vivo* experiments [170]. In another study, Fillafer *et al.* [171] compared the binding affinity of plain or human serum albumin (HSA) conjugated PLGA nanoparticles on Caco-2 cells. Due to the unspecific binding, no distinguishable difference was observed in well plates. Under fluidic condition, HSA conjugated particles bound tightly to Caco-2 cells, while plain particles were washed away. The results confirmed the importance of using fluidic conditions for evaluating particle–cell interactions to better mimetic physiological conditions [171]. Instead of culturing cells on the microfluidic channels, the particle–cell interactions assay can also be performed by grafting the particles in the channels and flowing the cells or bacteria through the channels.

Figure 15. Microfluidic devices for studying the particle–cell adhesion. (a) Schematic illustration of the microfluidic device and nanoparticle-aptamer bioconjugation. The PSMA positive cell LNCaP (human prostate adenocarcinoma cell) and PSMA negative cell PC3 (human prostate cancer cell) are patterned on glass substrates in microfluidics for screening the particle–cell interactions. (b) The bindings of the aptamer conjugated particles (red) to LNCaP cells (green) under flow at 0.25, 1, or 4 $\mu\text{L}/\text{min}$ were tracked. (c) The number of free particles and aptamer conjugated particles that attach to LNCaP or PC3 cells under fluid flow conditions were counted under microscope (150 randomly selected cells per group). A dagger represents a significant difference comparing to 4 $\mu\text{L}/\text{min}$ group ($p < 0.05$). Figures are reprinted with permissions from ref. [170], © 2005 ACS.

Particle cytotoxicity is another important parameter to be studied. A microfluidic system was established to investigate the cytotoxicity of particulate matter 2.5 (PM_{2.5}) on human HaCaT (spontaneously transformed aneuploid immortal keratinocyte) cells. The microfluidic device was made of a cell culture chip and a protein microarray chip (**Figure 16a**). In this platform, the exposure to PM_{2.5} (150 µg/mL) for 24 h led to 53.4% of cell apoptosis, and the activation of NF-κB and NALP3 transductions. These findings indicated an inflammatory response of HaCaT cells toward PM_{2.5} [172]. In another work, a multi-compartmented device that can create the flow exposure conditions was developed for evaluating the toxicity of quantum dots (QDs) [173]. Interestingly, noticeable different results were observed for the cytotoxicity of QDs under flow exposure and static exposure condition. By exposing the cells to 40 pM QD for 12 h, *ca.* 30% and 75% of cells remained alive under static and flow conditions, respectively. This finding further indicated the importance of studying particle–cell interactions under flow exposure conditions (**Figure 16b**) [173]. The impact of silica nanoparticles on human lung cells (epithelial H441) was studied under acute and chronic exposure scenarios (**Figure 16c**). In addition to the intrinsic nature of nanoparticles, the outer environment, *e.g.* perfusion condition, also significantly affected the cell–particle interactions, and consequently the cell uptake and cytotoxicity of nanoparticles. The results obtained under static conditions are not fully translatable to reflect the *in vivo* situation due to the gravitational settling of nanoparticles [174]. Therefore, the microfluidic regeneration assay is more reliable for *in vivo* prediction.

Figure 16. Microfluidic devices for studying the cytotoxicity of nanoparticles. (a) The microfluidic device for studying the effect of PM_{2.5} on HaCaT cells. The HaCaT cell culture and microarray chips for protein detection are shown. The device contains sample inlet, regular valve, wash buffer/antibody inlet, reaction chamber and outlet. The regular valves can open and close to prevent cross contamination. (b) Schematic illustration of the particle distribution in well plate and in microfluidics. The microfluidic platform can avoid particle sedimentation, which allows the homogeneous distribution of nanoparticles to better mimetic the *in vivo* situation. (c) Lab-on-a-chip system for monitoring the cancer cell cytotoxicity towards continuous exposure of silicon nanoparticles. The acute (static) and perfused (chronic) scenarios are simulated. Figures are reprinted with permissions from: (a) ref. [172], © 2017 Elsevier; (b) ref. [173], © 2010 AIP; (b) ref. [174], © 2015 MDPI.

The patterning microfluidic devices also used for studying the cell–particle interactions. A microfluidic chip was designed based on the induced-charge electro-osmosis in a rotating electric field (ROT-ICEO). This device showed great efficiency in trapping single particle and cell at large scale

[175]. Theoretically, this platform also showed good potential to study the cell–particle interactions. In another work, the gold dots and fibronectin-coated regions were allayed on the same glass substrate to create specific surface pattern for investigating cavitation bubbles–cell interactions at a single cell resolution. This microfluidic platform is promising in different types of single cell analysis, including macromolecule uptake, localized membrane deformation and intracellular calcium response [176]. The engineered microfluidic platforms for studying the particle–cell interactions have been summarized in **Table 7**.

Table 7. Microfluidic platforms for studying the particle–cell interactions.

Applications	Platform	Particle	Cell	Advantages	Ref
Cell and particle adhesion assay	PSMA adhesion assay	PLA-PEG-aptamer	LNCaP (+) PC3 (-)	Fluid condition that mimetic the blood shear stress was generated and the results clearly showed that the particle adhesion had shear stress dependent manner	[170]
	HSA binding assay	HSA conjugated PLGA	Caco-2	Clear HSA selective binding was detected in fluid condition while no different was observed in static condition	[171]
	Detection of bacterial	Detection beads	Bacteria	The fluid system reduced unspecific binding due to bacterial sedimentation	[166, 177]
Cytotoxicity assay	Inflammation and NF- κ B and NALP3 transductions	PM _{2.5}	HaCaT	The system is powerful and high throughput.	[172]
	Live/dead assay, ROS	Quantum dots	BALB/3T3	Fluid condition avoided particle sedimentation and allowed homogeneous particle cell interaction	[173]
	Cell viability	Silica nanoparticles	H441	The device simulated acute (static) and perfused (chronic) scenarios. In perfused scenarios, particles did not sediment and more accurate toxicity results were achieved.	[174]
Patterning	ROT-ICEO	PS microbeads (Fluka) 5 and 20 μ m	Yeast cells	Single cell and single particle patterning with high throughput	[175]
	Bubble cell interaction	Gold dots	fibronectin-recognition cells	Single cell analysis, including macromolecule uptake, localized membrane deformation and intracellular calcium response	[176]

5.2 Organ-on-a-chip

The microfluidic platforms for particle–cell interactions have many advantages, however they can only mimetic simple *in vivo* conditions. In a step forward from the particles–cell interactions, microfluidic systems can also create *in vitro* organ-mimetic models through complex geometries design for evaluating the particle–organ interactions.

In 1995, an organ-on-a-chip device was designed by Frame *et al.* [178], using semi-circular channels on glass for endothelial cells culture [178]. After that, more complex devices have been designed to study the particle–organ interactions. For example, Doshi *et al.* [179] developed a synthetic microvascular networks (SMN) to mimic the animal vasculature; particles with different geometries were flowed through the SMN. In this study, three spherical particles (1, 3 and 6 μm) were stretched to elliptical disk, circular disk and rod-like structures by film-stretching method. The particle geometries showed remarkable impacts on their adhesion to the channels, especially with the presence of bifurcations (**Figure 17a**) [179]. In addition to the particle geometries, the flow rates were also accounted for the channel wall adhesion of particles. At a shear rate of 15 s^{-1} , the elliptical disk particles showed *ca.* 6.5-fold higher attachment at the junction than the corresponding sphere ones. The particle attachment decreased with the increasing shear rates, while the particle geometry effect was enlarged. At a shear rate of 250 s^{-1} , the attachment of elliptical disk particles was *ca.* 11-fold higher than the sphere counterparts [179]. This platform showed great potentials in mimicking the animal vasculature. Furthermore, this platform also served as a versatile tool for *in vitro* evaluation of drug delivery systems on their targeting efficiency and adhesion properties [179]. A more recent work found out that the angle of bifurcating channels had apparent effects on the particle adhesion (**Figure 17b**) [180]. At the low shear stress (7.5 s^{-1}), the number of particles (7 μm) adhered at a bifurcation angle of 120° was *ca.* 2 times more than that with a bifurcation angle of 30° [180]. Therefore, the precise mimetic of *in vivo* vascular geometries is important for an accurate analysis of particles' adhesion. Without the microfluidic devices that can map microvascular network, it is merely impossible to uncover the behaviors of those particles *in vitro*.

Besides blood vascular systems, the particle interaction with other organs can also be mimicked using microfluidic platforms. For example, an artificial lung was constructed by culturing alveolar epithelial cells and microvascular endothelial cells in bioinspired microchannel separated by perforated PDMS membrane (**Figure 17c**) [181]. Subsequently, the toxicity and uptake of silica nanoparticles

were evaluated. It was noticed that the transport of nanoparticles into the vascular compartment increased more than 4 times in the breathing motion condition created by the microdevice [181]. Moreover, many of the findings from the microdevice match the results obtained with *in vivo* mice model, proving that the mechanically active lung mimetic microdevices can act as reliable *in vitro* model and provide low-cost alternatives for animal and clinical studies [181].

Figure 17. Microfluidic organ-on-a-chip devices for particle organ interaction. (a and b) The particles with different geometry were analyzed in microfluidic channels that mimetic the blood vessels for their adhesion. The shear stress and angles of the bifurcations significantly influenced the binding of particles. (c) Biologically inspired design of a human breathing lung-on-a-chip platform. By assemble a thin, porous, flexible PDMS membrane coated with ECM, the device recreated human breath. Figures are reprinted with permissions from: (a and b) refs. [179], © 2010 Elsevier; (c) ref. [181], © 2010 AAAS.

Using a similar design approach of the lung on a chip platform, a gut mimetic chip was developed

by culturing two lines of human intestinal epithelial cells separately with extracellular matrix (**Figure 18a**) [182, 183]. Moreover, a recent review highlighted that this “gut-on-a-chip” device can be used for screening of theranostic nanoparticles [184]. Although these fabricated organ-on-a-chip microfluidic systems showed good potential in studying the particle–organ interactions, no particles are tested yet, probably due to the early stage of the device development, relatively high cost of the devices, or lack of awareness.

As a major organ for detoxification, liver is the organ where most nanoparticles tend to accumulate. The “liver-on-a-chip” platform based on multiplexed microfluidic channels is one platform of great potential for the screening nanoparticles [185]. Kidney is an important organ involved in nanoparticle elimination, recently a “kidney-on-a-chip” platform has been developed (**Figure 18b**). The kidney glomerular capillary wall was reconstituted by inducing pluripotent stem cell derived human podocytes [186]. The “heart-on-a-chip” platform has also been developed and the device was constructed by 3D-print (**Figure 18c**) [187]. Moreover, the human blood-brain-barrier (BBB) was engineered in a microfluidic chip by creating a cylindrical collagen gel with central hollow lumen in a microchannel. The primary human brain microvascular endothelial cells were cultured on the inner surface of the gel while medium flowed through the lumen (**Figure 18d**) [188].

As the ultimate goal, the “body-on-a-chip” system containing more than one organ has been proposed. Esch *et al.*, 2014 [189] developed a system to simulate gastrointestinal and liver organs in one microfluidic chip, represented by co-culture of Caco-2/HT29-MTX cells and HepG2/C3A cells. Through simulating the oral uptake of carboxylated polystyrene nanoparticles (50 nm), a liver injury was detected [189]. This study confirmed the importance of using highly biomimetic microfluidic device instead of simple cell culture plates for evaluating the interactions between nanoparticles and biological systems.

Furthermore, microfluidic systems have been developed to manipulate small organisms, such as zebrafish and *Caenorhabditis elegans* worms, to achieve the high-throughput screening of

nanoparticles [26]. “Fish-on-a-chip” microfluidic devices have the high-throughput imaging capability to study the cell division and migration, degeneration and aging [190, 191]. These systems are promising for real-time monitoring the interactions of fluorescent labeled nanoparticles with cells and trafficking these particles inside the cells. This would be an alternative to the “body-on-a-chip” system to perform the rapid, cheap, and reliable *in vitro* screening of nanoparticles.

Figure 18. Organ-on-a-chip microfluidic devices that are potentially important for the screening of nanoparticles. (a) Gut-on-chip platform. The device contained a villus epithelium layer seated on top of a porous ECM coated membrane. The formed intestinal epithelium was imaged under confocal, the villin and nuclei were stained in yellow and blue, scale bar is 10 μm . (b) Kidney-on-chip. The

device replicated the urinary and capillary compartments of the glomerulus. The hiPS-cell-derived podocytes and human glomerular endothelial cells were stained in green and magenta, scale bar is 100 μm . (c) Heart-on-chip. The device can generate a resistance change proportional to the contractile stress of heart. In the confocal image, the cell nuclei are stained in blue and α -actinin in white, scale bar is 10 μm . In the other insert image, cantilever deflecting upon tissue contraction was shown. (d) BBB-on-chip. The human brain microvascular endothelial cells, pericytes and astrocytes were co-cultured and the engineered brain microvessel viewed from different direction was shown. Scale bar is 200 μm . Figures are reprinted with permission from: (a) ref. [183], 2017 © PLOS; (b) ref. [186], © 2017 Macmillan; (c) ref. [187], © 2017 Macmillan; (d) ref. [188], © 2017 PLOS.

5.3 Cancer-on-a-chip

In comparison to the “organ-on-a-chip”, the “disease models-on-a-chip” devices are more valuable as *in vitro* models for nanoparticle screening. For example, the “cancer-on-a-chip” platforms have been developed as alternatives for effectively evaluation of nanomedicine [192]. Through co-culture phenotypically normal and diseased cells and tumor nodules, the “breast cancer-on-a-chip” system has been developed. The targeting potential of superparamagnetic submicron particles during migration within luminal breast mimetic environment were investigated (**Figure 19a**) [193]. The results indicated that those particles do not induce cytotoxicity even at high concentration (10 particles per cell). The superparamagnetic submicron particles bound to neoplastic cells, acting as a potential diagnostic tool for the neoplastic condition [193]. Chen’s group created a “cancer-on-a-chip” system by culturing a tumor-like spheroid in a microfluidic channel. The penetration of nanoparticles into tumor-like spheroids was evaluated (**Figure 19b**) [194]. Both physicochemical properties of nanoparticles and tumor microenvironment affect the interactions between nanoparticles and the tumor spheroids. The tissue penetration initialed from the accumulation of nanoparticles at tissue-fluid interface, subsequently the nanoparticles progressively diffused into the spheroid. The penetration rate of PEGylated gold nanoparticles into tumor-like spheroid was determined primarily by the particles size.

With a particle size of 40 nm, the penetration rate was 0.00400 min^{-1} , and 0.00396 min^{-1} for 70 nm. When that particle size increased to 110 and 150 nm, the penetration of gold nanoparticles was significantly inhibited.

Various other cancer-on-a-chip platforms have been developed, showing great potential for evaluating nanoparticles *in vitro* [192]. Portillo-Lara and Annabi [195] summarized the microfluidic devices for studying cancer cell invasion, cancer cell trans-endothelial migration, tumor angiogenesis, and tumor hypoxia elsewhere [195].

Figure 19. Cancer-on-a-chip models for the evaluation of nanoparticles. (a) The fabrication of “breast-cancer-on-a-chip” device for studying superparamagnetic submicron particles (SMPs). The SMPs are labelled with green fluorescent and cells are labeled with DAPI (nuclear) and Dil (membrane). (b) Microfluidic device that mimetic the tumor spheroid, for evaluation of nanoparticles’

penetration to tumor tissue. Figures are reprinted with permissions from: (a) ref. [193], © 2011 RSC; (b) ref. [194], 2013 NPG.

Besides cancer, many other disease models have also been engineered on the microfluidic devices. Wang *et al.* [196] established a disease model of diabetic nephropathy using a glomerulus-on-a-chip microdevice, which can reconstitute the kidney functions and reproduce the glomerular microenvironment [196]. Lee *et al.* [197] developed an alcoholic liver disease model on a microfluidic chip with a spheroid [197]. This platform can simulate both reversibly and irreversibly injured liver and has the potential to be used for screening nanoparticles *in vitro*. A human lung disease model was also engineered to mimic the pulmonary edema, as a model of interleukin-2 induced drug toxicity in cancer patients [198]. Yi *et al.* [199] summarized several microfluidic devices for modeling central nervous system (CNS) diseases, such as Alzheimer's disease and Parkinson's disease, by co-culturing different (CNS) cells on a chip, putting brain slices in microfluidics, establishing microelectrode arrays or directionality of neuronal networks [199].

Table 8. Organ-on-a-chip platforms for the screening of nanoparticles.

Tissue/organ	Cells utilized	Nanoparticles	Parameters to analyze	Ref.
Microvascular networks (SMN)	Endothelial cells	Polystyrene with different size and geometry	Effect of particle size, geometry, shear stress and bi bifurcation angle on particle attachment to the tube	[179, 180]
Lung	Alveolar epithelium and microvascular endothelium	Silica nanoparticles	Particle transportation, interaction, uptake and cytotoxicity	[181]
Human	Caco-2/HT29-MTX, HepG2/C3A and many other cells	Carboxylated polystyrene nanoparticles, 50 nm	Particle uptake, transportation, toxicity in multiple organs system	[189]
Breast cancer	Human mammary epithelial HMT-3522 S1 cells	superparamagnetic submicron particles	Particles toxicity and interaction with targeted cells	[193]
Tumor-like spheroids	MDA-MB-435 cells	PEGylated gold nanoparticles	Influence of the physiochemical properties of nanoparticles and the tumor microenvironment on the particles' penetration to tumor	[194]

6 Conclusions and future prospects

Despite the recent advances in the fabrication and evaluation of nanoparticulate drug delivery systems using microfluidics, the translation of this technology to the industrial practice is still challenge. The production rate of nanoparticles of kilograms or more per day is desirable for clinical studies and for industrial-scale production [124]. However, the daily production rate of nanoparticles by microfluidics is usually in the milligram range [33]. A nanoparticle production rate up to 3 kg/day was recently reported by developing a versatile coaxial turbulent jet mixer [124]. This turbulent jet mixer is suitable for industrial-scale production of nanoparticulate drug delivery systems. The daily nanoparticle production rate by microfluidics can be multiplied by parallelization of microfluidic channels. Most importantly, the obtained nanoparticles show the same properties as those prepared at the bench scale. The parallelization of microfluidic channels is one of the most promising approach to produce nanoparticles with an industrial-scale [200, 201].

Generally, the higher the flow rate of fluids of the microfluidic device, the higher is the production of nanoparticles. When the flow rate increases, the pressure inside a microfluidic device enhances dramatically [68]. The highest flow rates for a certain microfluidic device are dependent on the affordable force of the fluid controlled apparatus and the withstanding pressure of the microfluidic device. The industrial-scale fluid control apparatus should be developed and employed for the high throughput production of nanoparticulate drug delivery systems. Usually large amounts of water and organic solvents are used in microfluidic preparation of nanoparticles. During the preparation process, the presence of unencapsulated therapeutics is inevitable. Purification is highly demanded to remove the unencapsulated therapeutics and organic solvents from the obtained formulations. The ultrafiltration [202], diafiltration [203], and cross-flow microfiltration [204, 205] are the industrial-scale purification approaches to treat large volume fluids. Verifiable protocols are necessary for the purification of the obtained formulations.

Because most nanoparticle formulations are for parenteral use, the sterilization of the obtained formulations is indispensable. However, the sterilization process may pose challenges to the stability of the nanoparticle formulations. Taking the commonly used energy-inducing terminal sterilization methods (e.g., steam sterilization and γ -irradiation) for example, they may lead to the oxidation or degradation of the nanoparticle precursors and the payloads, as well as the leakage of encapsulated compounds [206, 207]. Moreover, steam sterilization may exert negative effects on the stability of the nanoparticle formulations. The recommended pharmacopoeial conditions for sterilization in an autoclave are 15 minutes at 121-124 °C (200 kPa), which have been considered acceptable for some nanoparticle formulations.

Terminal sterilization by heat or irradiation is not an established process in industrial scale production of nanocarriers [208]. In contrast, filtration through bacteria-retentive membranes, with the nominal pore size around 0.22 μm , is often applied as a part of an aseptic production process [209]. Membrane filtration is a rather gentle process to extrude nanoparticles without significant loss of encapsulated therapeutics, except a certain degree of mechanical impact. Furthermore, membrane filtration is the most preferable final step prior to the filling of nanoparticle formulations [208]. To keep the major component of the formulation, usually nanoparticles should have sufficiently smaller size than the pore size of membrane. Microfluidic devices offer superior control over the size of nanoparticles, which is usually smaller than those prepared by the conventional batch approach. Membrane filtration could be an ideal aseptic approach for the nanoparticle suspensions prepared by the microfluidics.

To prevent the potential physical and chemical instabilities issues, nanoparticle formulations are frequently transformed into the dry state by freeze-drying [210]. Freeze-drying usually requires the addition of cryoprotectants, whose types and concentrations need to be carefully optimized. Alternatively, spray-drying can also be employed to dry the therm-tolerant nanoparticle formulations, which is particularly beneficial for the products not requiring sterility [211]. The concentration of as-

prepared nanoparticle formulations is usually in a relative low level. Besides stability enhancement, drying can also be used to increase the concentration of nanoparticle suspensions, because the dried products can be reconstituted at a desired concentration. Overall, the development of dry formulations of nanocarriers is important to ensure a more stable drug product and to avoid the use of the 'cold chain' during distribution.

Currently, the translation of nanoparticles to industry and clinical is very slow, while it is anticipated that microfluidics will bring a revenue and solve many of the current challenging problems in nanomedicine production in the future [26]. Microfluidic technology is one of the most promising technology to support and speed-up the development and clinical translation of nanomedicines. In addition to the advantages in fabricating monodisperse and batch-to-batch repeatable nanoparticles at industrial-scale, microfluidics also contribute significantly to the fast, cost-effective, and *in vivo* mimetic screening of nanoparticles. The fluidic/shear stress mimetic, 3D cell culture, small organisms-on-a-chip, organ-on-a-chip, and human-on-a-chip platforms can mimetic the *in vivo* situation much better than the convectional cell culture in Petri dishes. Moreover, the microfluidic channels can be designed transparently (*e.g.*, glass capillaries), facilitating the real-time imaging.

Because of the ability to high-throughput produce formulations, thousands of nanoparticles can be engineered fast and easily by microfluidic approach for targeting drug delivery. Among these nanoparticles, most of them have only been tested *in vitro*, which is probably due to the low rate and high cost of *in vivo* tests. The future of microfluidic screening is to design easy to use, cost effective, less instrument demanding, and industrial standard platforms. Advanced microfluidic platforms that can characterize multiple properties of nanoparticles in one system are demanded. The microfluidic platforms that can precisely mimetic biological systems, resulting in high correlation between the *in vitro* and *in vivo* studies, are also highly desirable. Recently, an innovation method has been developed to simultaneously evaluate the biodistribution of a variety of chemically distinct nanoparticles, such as carrying specific nucleic acid barcodes [212]. After the administration of a library of nanoparticles in

a single mouse, the quantification of particle biodistribution was achieved by deep sequencing the barcodes. By monitoring the biodistribution of 30 nanoparticles to eight tissues, the chemical properties promoted the targeting drug delivery to certain tissues and were successfully identified. This nano-barcoding system enables the *in vivo* evaluation of thousands of nanoparticles in just one single animal, which can dramatically accelerate the discovery and understanding of targeted nanocarriers [212].

Overall, microfluidics has great potential to speed-up the development of nanoparticle formulations and their clinical translation. For this highly interdisciplinary research, the microfluidic-based nanoparticle screening technology is only in the proof-of-concept stage. More efforts should be channeled to develop a general acceptance method for nanoparticle screening. The close collaborations among the researchers from different fields, such as biology, medicine, materials, microfluidics and biomedical engineering, will definitely accelerate the progress of the microfluidic-based nanoparticle screening technology. Considering the favorable characteristics offered by microfluidics, it is envisaged that the microfluidic-assisted preparation and screening of nanoparticulate drug delivery systems has a great potential in accelerating the clinical translation of nanomedicines.

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