Advanced Drug Delivery Reviews 128 (2018) 29–53

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Microfluidics in nanoparticle drug delivery; From synthesis to pre-clinical screening☆

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ARTICLE INFO

Article history:
Received 11 December 2017
Received in revised form 15 March 2018
Accepted 2 April 2018
Available online 5 April 2018

Keywords:
Microfluidics
Drug delivery
Nanotechnology
Nanomedicine
Organ-on-a-chip
3D culture

ABSTRACT

Microfluidic technologies employ nano and microscale fabrication techniques to develop highly controllable and reproducible fluidic microenvironments. Utilizing microfluidics, lead compounds can be produced with the controlled physicochemical properties, characterized in a high-throughput fashion, and evaluated in *in vitro* biomimetic models of human organs; organ-on-a-chip. As a step forward from conventional *in vitro* culture methods, microfluidics shows promise in effective preclinical testing of nanoparticle-based drug delivery. This review presents a curated selection of state-of-the-art microfluidic platforms focusing on the fabrication, characterization, and assessment of nanoparticles for drug delivery applications. We also discuss the current challenges and future prospects of nanoparticle drug delivery development using microfluidics.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Microfluidic Devices for Drug Delivery Systems".

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https://doi.org/10.1016/j.addr.2018.04.001
0169-409X/

1. Introduction

Advances in drug delivery technologies can improve pharmacological factors such as efficacy and bioavailability [1], leading to the discovery and development of more effective drugs for better patient prognoses and quality of life. It is not surprising that the US market for drug delivery system has grown dramatically since 2000 [2,3]. Conventional drug delivery development is an arduous, multi-step process requiring extensive efforts for mass production, chemical characterization, feasibility and toxicity testing (*in vitro*), and preclinical animal and clinical human trials. At present, there are several potentially promising nanoparticle drug delivery platforms in development, only a few have been successful in the clinical trial phase [4]. Nanoparticle drug delivery faces challenges resulting from fabrication quality controls, product batch-to-batch variation, and the inability to gain physiologically relevant test results in conventional *in vitro* prescreening platforms [5]. Recent developments in microfluidics have enabled a new generation of nanoparticle synthesis and delivery techniques, and shown the potential of the improved predictive power of preclinical nanoparticle testing through biomimetic microfluidic platforms (Fig. 1).

Microfluidics has expanded beyond merely micro to nanoliter fluid handling, now incorporating a multidisciplinary approach to encompass a wide range of applications [6–8]. The small scale of microfluidics provides a means to implement highly controllable, reproducible, and scalable fabrication methods for nanoparticle production compared to conventional methods. Organ-on-chip microfluidic technology offers highly relevant organ specific testing platforms capable of biologically relevant experimental time scales while using a fraction of sample and media volumes compared to conventional *in vitro* culture systems [9–11]. The application of powerful microfluidic techniques to nanoparticle development processes may address critical challenges in the clinical translation of nanoparticle drug carriers (Fig. 2).

2. Microfluidics in drug delivery: fabrication

Conventional nanoparticle fabrication methods are largely associated with unstandardized, multi-step processes such as nanoprecipitation and emulsification-based solvent evaporation methods. Accounting for >50% of all nanoparticle fabrication methods, nanoprecipitation forms nanoparticles from a colloidal suspension between two aqueous solvent

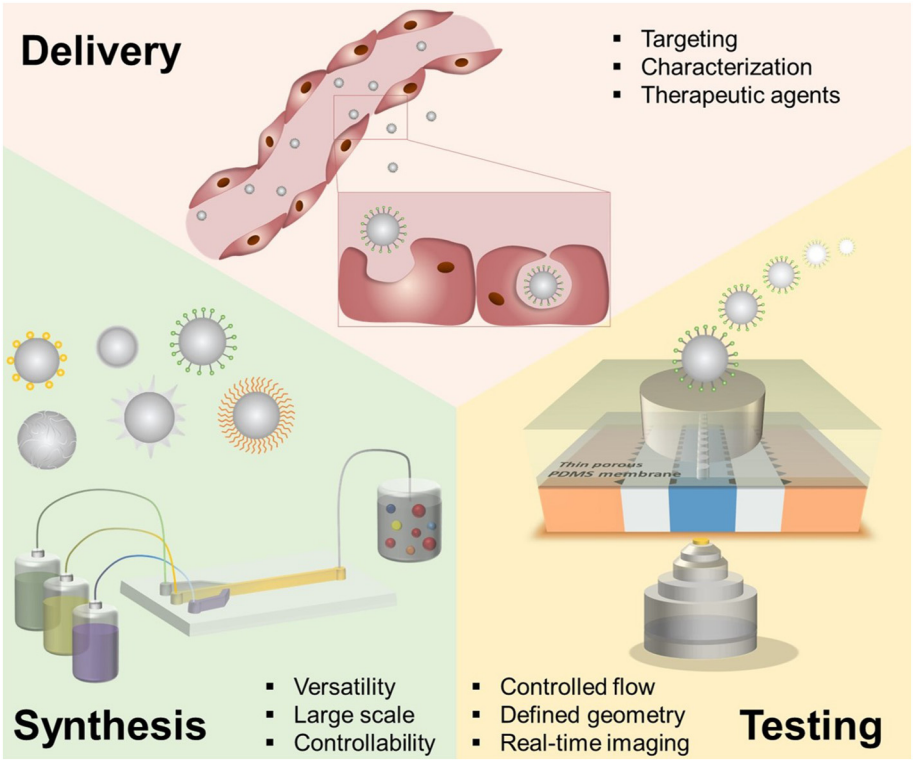

Fig. 1. Illustration of Microfluidics in particle synthesis, delivery and testing. Recent development in Microfluidics enabled a new generation of nanoparticle synthesis, delivery and preclinical testing.

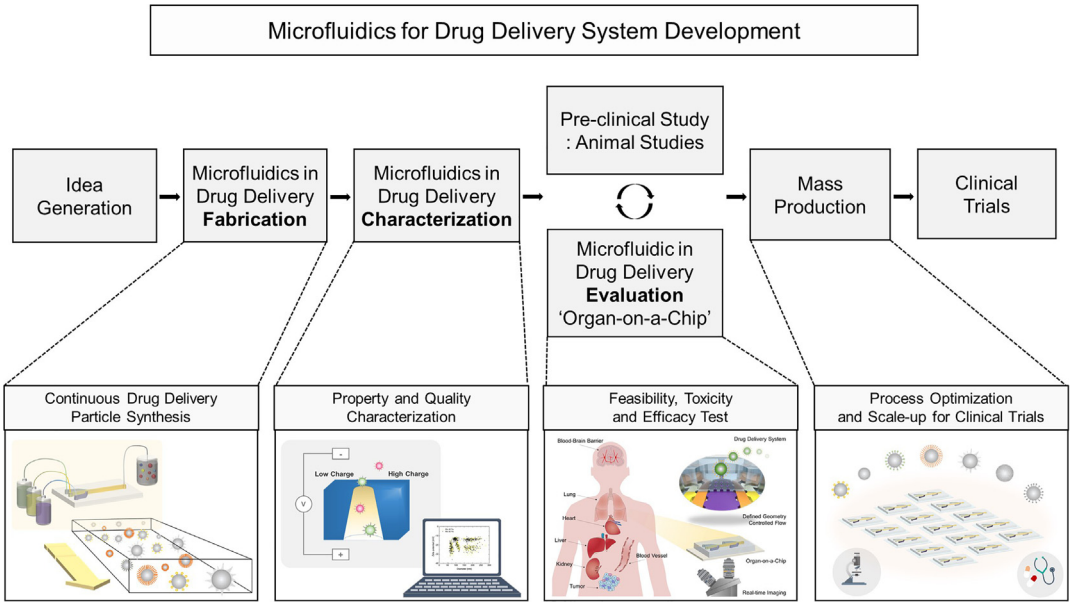

Fig. 2. Pipeline of microfluidics for drug delivery. Microfluidic synthesis at micro and nanoscales allows continuous fabrication of nanoparticles. Particle property and quality can be characterized using microfluidics in a high-throughput manner. Only if those properties meet the drug delivery design criteria will be evaluated in *in vitro* biomimetic microsystems: organ-on-a-chip. If the targeting, therapeutic and imaging efficacies are satisfactory in organ-on-a-chip model and animal models, the drug delivery particle candidates will be prepared for optimization and move on to scale-up production for the clinical test. Modified from [5].

phases though the addition of lipophilic or polymeric drugs dissolved in one solvent type as droplets into an agitated body of a different solvent [12,13]. The characteristics of particles synthesized by this method can be controlled by agitation speed and polymer drip rate. Another common method, emulsification-based fabrication forms nanoparticles by agitating and diluting emulsions formed at the interface between two solvent aqueous layers. In this practice, particle size can be controlled by turbulence; smaller particles are formed by reducing the size of larger particles. Both conventional nanoparticle synthesis methods are easy to lead to polydisperse distributions and batch to batch variations, and thus an additional steps are required to homogenize the synthesized particles.

Effective drug delivery carriers enhance the therapeutic effect of drugs and reduce the toxic effect of drugs while improving the absorption of poorly soluble and unstable drugs [14]. Especially, reliability and controllability of a drug release profile are an important factor of a successful drug delivery system, which depends on the physicochemical properties including the size, shape and composition. For instance, nanoparticles with diameter <10 nm are filtered out through pores in the glomerular membrane of the kidney [15]. On the other hand, larger particles are easily recognized by the immune system [16]. Recent applications of microfluidic technologies to drug delivery system development have demonstrated highly optimized drug carriers. Basically, microfluidic provides a number of useful capabilities to manipulate very small quantities of samples and allow versatile production of nanoparticles with tunable sizes, shapes, and surface compositions to promote the efficacy of drug transport, release profile, and elimination during treatment [5]. Furthermore, microfluidics when parallelized can provide reliable and reproducible scale-up production in a precisely controlled manner. In this section, we highlight recent advances in (2.1) the fabrication, (2.2) the precise control, (2.3) the scale up production of drug delivery particles using microfluidic platforms, and (2.4) outlook and challenges.

2.1. Drug delivery particle fabrication using microfluidics

Microfluidic technologies provide new opportunity in drug delivery. In conventional synthesis methods, mass transport in fluids is governed by the inertial and viscous effect both, which are associated with nonlinearities that give rise to numerous instabilities, like turbulence [17]. However, in microfluidics the inertial effect becomes negligible [18]. This characteristic enables microfluidics to synthesize nanoparticles in a highly controlled and reproducible way, which was difficult to achieve in the conventional macroscale synthesis methods. In this section, we introduce several microfluidic methods that can be used to produce drug delivery nanoparticles, including (2.1.1) flow focusing, (2.1.2)

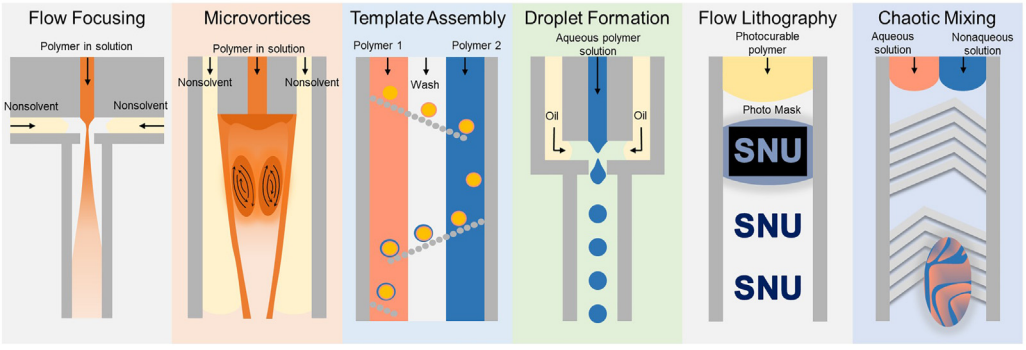

Fig. 3. Microfluidics in drug delivery: fabrication. Various microfluidic based drug delivery particle fabrication methods have offered capabilities to produce particle in a controllable and reproducible manner.

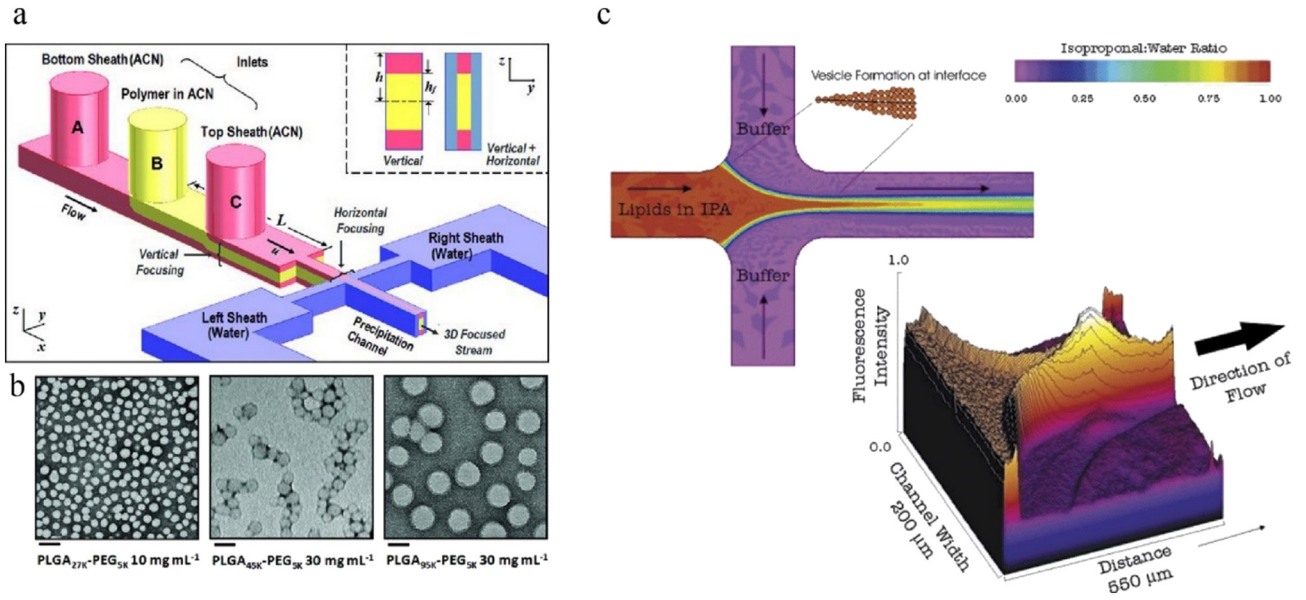

Fig. 4. Microfluidic methods to fabricate micro and nanoparticle using flow focusing. (a,b) Synthesis of size tunable polymeric nanoparticles enabled by 3D hydrodynamic flow focusing. (a) Schematic of a device for 3D hydrodynamic focusing consisting of three sequential inlets for vertical focusing and a separate inlet for side sheath flow. (b) TEM images show PLGA-PEG nanoparticles. Scale bar: 100 nm, 100 nm and 200 nm respectively. Adapted with permission from [22]. (c) Schematic of liposome formation process in the T-shape microfluidic channel with 3D colour contour map. Adapted permission from [29].

microvortices, (2.1.3) chaotic flow (2.1.4) droplet-based approaches, and (2.1.5) the other methods (Fig. 3).

2.1.1. Flow focusing method

Hydrodynamic focusing is a powerful tool for microfluidics when requiring mixing and diffusion controlled chemical reactions. Hydrodynamic focusing develops when fluids with different velocities are introduced side by side. The most common way to perform hydrodynamic focusing is to use 3 inlet microfluidics, where the core flow containing the samples of interest is sheathed by side fluids. The ability of microfluidics to rapidly mix reagents provides homogeneous reaction environment and adds reagents in a precise manner during the reaction process [19]. Rapid and tunable microfluidic mixing allowed for the synthesis of drug-encapsulated biodegradable polymeric poly(lactic-*co*-glycolic acid)-polyethylene glycol (PLGA-PEG) nanoparticles with a defined size, lower polydispersity, and higher drug loading with a slower release [20]. Tunable size coming from nanoprecipitation through slower mixing resulted in a higher portion of PEG incorporation in the nanoparticles during the self-assembly in addition to increased size [21]. In addition, 3D hydrodynamic flow focusing has been suggested by a monolithic single layer with three sequential inlets for a vertical focusing pattern followed by a conventional horizontal focusing flow (Fig. 4a, b) [22]. This 3D flow focusing structure enabled the isolation of the precipitation polymer from the channel walls, which was one of the challenges for 2D flow focusing. This 3D flow focusing successfully produced nanoparticles over a long period without the channel fouling even with the use of high molecular weight (45 kDa) PLGA precursors and allowed those to target cancer cells when being premixed with multiple drugs [23].

In addition to microfluidic synthesis of polymeric nanoparticles, liposomes with a controlled size have been produced using similar flow focusing microfluidics [24–26], where a central flow stream of a lipid-containing ethanol solution is sheathed by two side streams of an aqueous solution. The lipid stream is focused into a narrow sheet, in which the nanoparticle size can be tuned by modulating the volumetric flow rate ratio between the central and side streams [27]. According to a theory proposed by Lasic et al. the lipids dissolved in an organic solvent transform into the intermediate bilayer phospholipid fragment (BPF) structure [28]. Continuous diffusion between the water and ethanol solutions reduces solubility conditions of lipids and triggers instability at the boundary layer of BPFs, inducing bending and closing of the lipids and leading to the formation of lipid vesicles [25,28]. Jahn et al. firstly reported the production of liposomes using this technology. T-shaped microfluidics were employed to produce liposomes in the range of 100 to 300 nm [29]. Notably, as the ratio of the flow rate increased, a lower vesicle size was produced. As the shear stress exerted on the liposomes increased, the vesicle size decreased (Fig. 4c). The same group later suggested multiple inlet channel microfluidics to control the liposome size by adjusting the ratio of the precursor flow rates. It was found that the liposome formation depends more heavily on the focused ethanol stream width and its diffusive mixing with the aqueous stream in the side channel, rather than on the shear force at the solvent-buffer interface. The study demonstrated that the vesicle size and its distribution are tunable from 50 to 150 nm [26]. A recent study demonstrated the importance of lipid formulation, lipid concentrations, the presence of residual solvents, and payload characteristics in the production of liposomes, and also proposed a microfluidic design for scaled up liposome fabrication [30].

Encapsulation materials should not only exhibit target specificity as a drug delivery vehicle, but also pose little or no adverse off target effects. For instance, hydrophobically modified chitosan nanoparticles were used to encapsulate a hydrophobic anticancer agent, paclitaxel, at 95% + efficiency using a T-shaped PDMS microfluidic device [31].

In general, hydrodynamic flow focusing systems are easy to fabricate and operate, and are capable of generating particles with uniform size distribution. Particles generated through flow focusing tend to be <1 μm, too small for long term payload release applications. Throughput scale is another key limitation of flow focusing techniques, as individual microfluidic chips are rate limited to microscale outputs without the arrangement of a high throughput array of devices to maximize mass transfer. In addition, PDMS based flow focusing platforms are unsuitable for high pressures and organic solvents (e.g., acetonitrile), limiting particle fabrication to organic varieties [32].

2.1.2. Microvortices method

Microfluidics performing at higher Reynolds numbers (>100) can be designed for the formation of drug delivery nanoparticles through controlled microvortices. Using simple changes in inlet pressures, Kim et al.

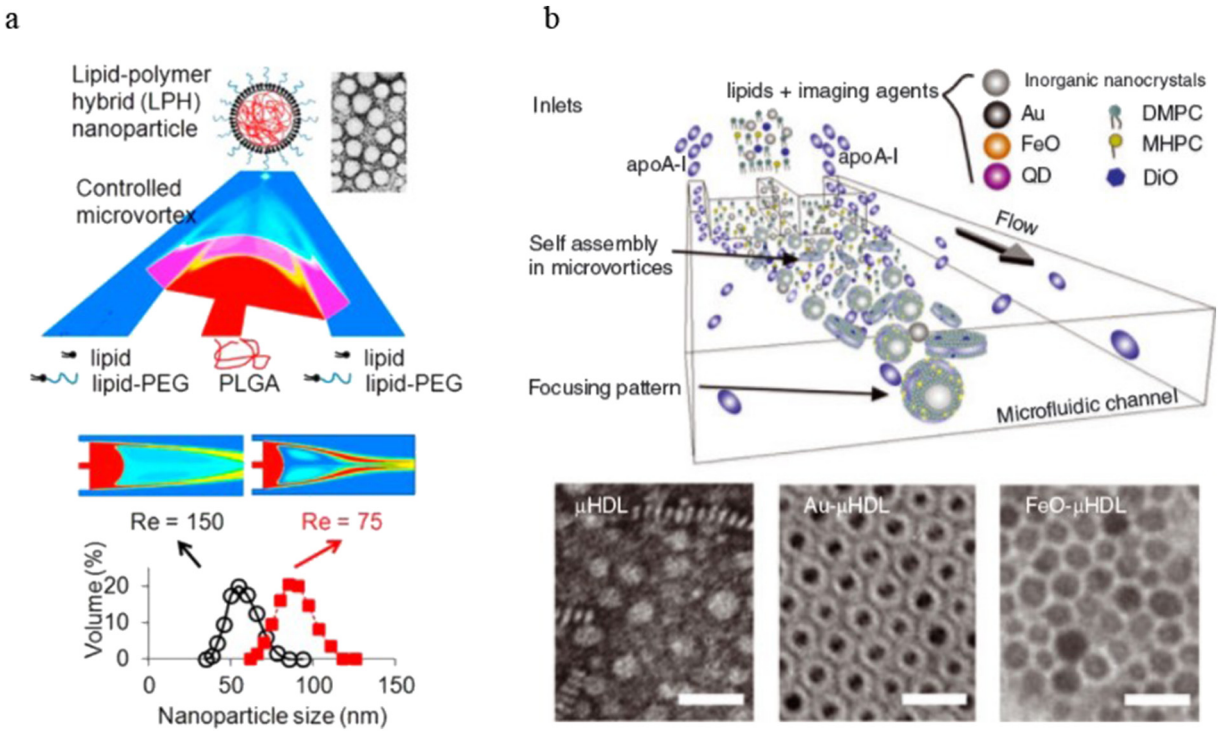

Fig. 5. Microfluidic methods to fabricate micro and nanoparticles using microvortices. (a) Schematic images show that mass production and size control of lipid-polymer hybrid nanoparticles are achieved through microvortices. Microvortex-based nanoparticle synthesis shows 1000 folds higher productivity than conventional diffusive synthesis methods. Adapted with permission from [34]. (b) Microfluidic reconstitution of HDL-mimetic nanomaterials using microvortices. A schematic depiction of a microfluidic platform that enables single step and mass production of HDL mimetic nanomaterials with various imaging and therapeutic agents. Microvortex pattern visualization and computational fluid dynamics simulation showing the microvortices at *Re*~150. Scale bar: 500 μm. Adapted with permission from [36].

achieved dynamic control of 3D chemical profiles with a single 2D microfluidics, in which microvortices play an crucial role in creating tunable 3D patterns in microfluidics [33]. Inspired by this 3D microvortices pattern, lipid-polymer hybrid nanoparticles were produced by symmetric microvortices at the intersection of the three inlets. These microvortices enabled up to 1000 times higher productivity of lipid-polymer nanoparticles than diffusive mixing-based nanoparticle synthesis (Fig. 5a) [34]. Kim et al. investigated the nanoparticle size distribution by varying the Reynolds numbers, and found that an increase in the Reynolds number reduces the produced particle size, resulting in the size decrease from 93 to 55 nm with varied Reynolds numbers from 30 to 150 nm [34,35]. This approach was also applied to the synthesis of biologically active high-density lipoprotein (HDL) particles. HDL-mimetic nanoparticles were reconstituted using a single-step, self-assembly method in a single layer 3-inlet device. A central flow stream of a phospholipids-containing ethanol solution is sheathed by two side streams of an apolipoprotein A-I (apoA-I)-containing aqueous solution. This microfluidic approach allowed for the identification of an optimal composition ratio (lipid:apoA-I) and demonstrated that HDL-mimetic nanoparticles have similar bioactivity properties to native discoidal HDL. Furthermore, multiple imaging agents and drugs were able to incorporate within these nanomaterials (Fig. 5b) [36]. This platform was also extended for the synthesis of PLGA-HDL hybrid particles [37]. In this application, phospholipids and PLGA were solubilized in a mixture of acetonitrile/ethanol and then introduced in the central channel and sheathed by the apoA-I solution. Upon mixture of the different solutions, instantaneous self-assembly results in the formation of a hydrophobic PLGA core surrounded with phospholipid and apoA-I. Interestingly, the spherical structure of the biomimetic PLGA-HDL nanoparticles resembles that of mature spherical HDL and its biological capabilities were similar to those found in native HDL.

Microvortex techniques were developed to overcome the inefficiencies of slow diffusive mixing (e.g., lower productivity due to the wasteful generation of undesirable polymer aggregates). As microvortices operate under relatively high Reynolds numbers (>100), and pressures, air bubbles can pose a serious problem. Computational simulations can be utilized in conjunction with experimental testing to optimize designs and mitigate potential inefficiencies.

2.1.3. Chaotic flow method

Although laminar flow through conventional microchannels preclude the use of turbulence under normal circumstances, flow disruptive patterns such as herringbone mixers can be used to passively mix fluids within a channel. Chaotic advection is a technique for enhancing mixing efficiency by utilizing geometric patterns to induce transversal flow components that stretch and fold volumes of fluid over the cross section of a microchannel. The staggered herringbone mixer, noted for its effectiveness and fabrication simplicity, utilizes an array of "herringbone grooves" on one or more surfaces of a microchannel to induce turbulent mixing within a continuous flow [38]. Fluid is redistributed over the entire channel cross section, significantly reducing Taylor dispersion [38] and resulting in a nearly even residence time distribution [39,40]. A recent study utilized herringbone mixers to fabricate lipid nanoparticles [41], with a further study establishing the effects of lipid concentrations and mixing performance on particle sizes [42]. It was determined that low lipid concentrations agitated by herringbone mixing arrays could successfully produce small size lipid nanoparticles (30 nm) within a narrow size distribution [42]. Staggered herringbone mixers were also used to synthesize doxorubicin loaded lipid nanoparticles [43] and siRNA carrying lipid nanoparticles [44].

The herringbone mixer exponentially increases the surface area between two fluids with distance traveled, resulting in faster diffusional mixing when compared with hydrodynamic flow focusing approaches at equivalent flow rate ratios. While mixing channels patterned with herringbone arrays on one face may exhibit less chaotic mixing on the face far from the patterned side, the addition of additional herringbone array sides can increase mixing performance and particle synthesis quality.

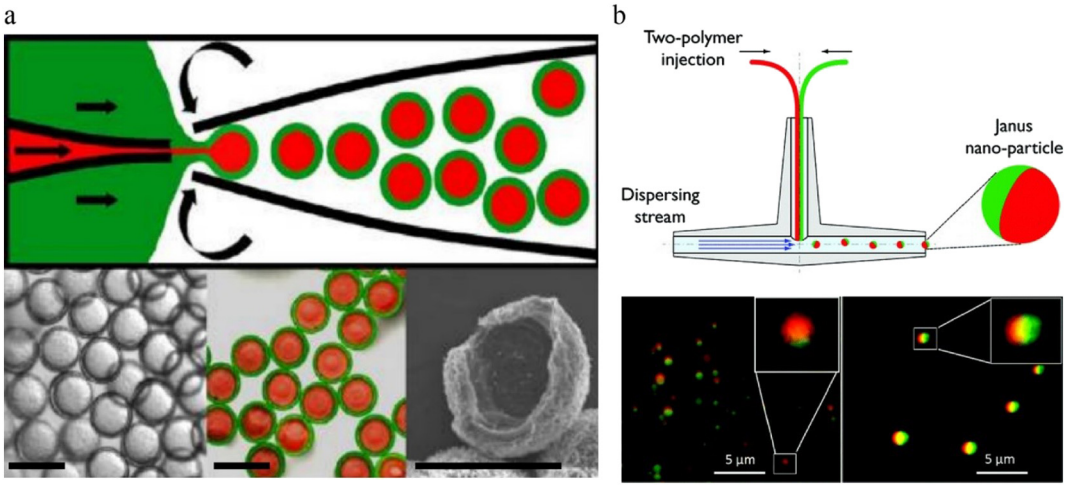

Fig. 6. Microfluidic methods to fabricate micro and nanoparticles using droplets. (a) Generation of double emulsion droplets for the formation of a core-shell structure using a capillary device. This microfluidic device allows the precisely controlled injection of different fluids. The two inner (red and green) fluids are hydrodynamically sheathed by the outer white fluid. The scale bars: 100 μm. Adapted with permission from [37] (b) Schematic of synthesizing Janus nanoparticles using a droplet method induced by a dispersing stream. Sample inlets are inserted into the dispersing channel via a T connector. Below images show that droplet based Janus particles loaded with Nile red and hydrophilic dye FITC-dextran. Scale bar: 5 μm. Adapted with permission from [39].

2.1.4. Droplets method

Droplet-based microfluidics is a popular carrier synthesis method capable of generating highly reproducible and homogenous drug-loaded particles. This method controls discrete volumes of fluid in an immiscible phase (water/oil and liquid/gas) with laminar flow regimes. The capability of microfluidics to generate discrete droplets is leveraged to produce particles [13]. This droplet generation is usually aided by the well-controlled interfaces and flow rates in a microfluidic system. Furthermore, a wide range of organic and inorganic materials have been investigated for microparticle formation. To form controllable monodisperse emulsions, microfluidic capillary devices and PDMS devices are usually used [45–47]. Shestopalov et al. described a droplet-based microfluidic method for performing multi-step synthesis of nanoparticles on millisecond time scale [48]. Two aqueous reagent streams were brought together in a short segment channel where they were allowed to generate a laminar flow alongside each other. They further demonstrated the utility of this droplet-based microfluidic method to perform a multi-step synthesis of CdS/CdSe core–shell particles. In addition, core-shell drug delivery system fabricated in a one-step, solvent-free process using microfluidics was introduced (Fig. 6a) [49]. A hydrophilic drug (doxorubicin hydrochloride) is encapsulated in an aqueous core by a lipid shell of a hydrophobic drug (paclitaxel). The use of microfluidic synthesis techniques enabled high encapsulation efficiency and precise control of particle size, composition, and release profiles, compared to conventional synthesis methods.

Generally, the size of a drug delivery particle strongly affects the drug release rate; smaller PLGA particles release drugs faster than larger ones due to the greater surface-area-to-volume ratio [47,50]. Furthermore, Hui et al. suggested one-step fabrication of polymeric Janus nanoparticles using droplet-based microfluidics [51]. The fluidic nanoprecipitation system contains dual inlets, one for each half of the particle, that connect into the precipitation stream. They demonstrated biocompatible Janus

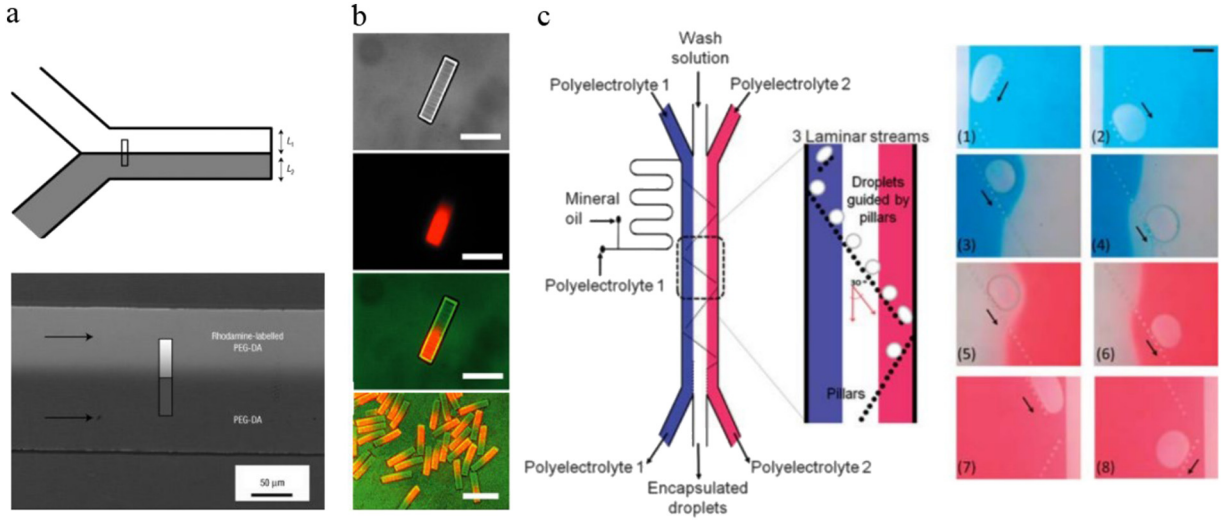

Fig. 7. Continuous-flow lithography and template assembly methods using microfluidics. (a) Schematic showing the synthesis of Janus particles using continuous flow lithography. Two streams containing PEG-DA (grey) and PEG-DA with rhodamine-labeled (white) are co-flowed through a channel. (b) DIC and fluorescence microscopy images of particles show that homogenous synthesis. The scale bars: 100 μm. Adapted with permission from [44] (c) Overview of a device for continuous generation of polyelectrolyte microcapsules by a layer-by-layer deposition. Blue and red dyes were used to represent two different polymer solutions, whereas plain water was used to represent the washing solution. The scale bar: 200 μm. Adapted with permission from [48].

nanoparticles that encapsulate a hydrophobic drug (paclitaxel) and hydrophilic drug (doxorubicin hydrochloride) on one side and the other (Fig. 6b). Similarly, a microfluidic device implementing side-by-side capillaries also allowed for rapid synthesis and incorporation of two molecules with different physicochemical properties in Janus particles for co-delivery [52]. Among the molecular milieu of the cell, the lipid bilayer performs as an intricate synthetic target. Matosevic et al. reported a microfluidic assembly line that produces uniform cellular compartments from droplet, lipid, and oil/water (O/W) interface [53]. With this approach, the size of the droplet precursor determined the vesicle size and encapsulation of small molecules.

Overall, droplet-based microfluidics is one of the most robust fabrication methods for multifunctional drug carriers with size tunability and drug release profile. The main challenges facing droplet-based synthesis concern the inability to produce nanoscale drug carriers, the overall complexity of droplet based fabrication protocols, and low production yields [54].

2.1.5. Other methods

Precisely shaped polymeric particles and structures are widely used for designing drug delivery system [55,56]. Flow lithography is a photolithographic technique where a pattern is directly projected into a photocurable polymer to produce particles [57]. The shape of the particles in the x-y plane is regulated by the shape of the transparency mask, whereas the z-plane projection is decided by the height of the channel used and also the thickness of the oxygen inhibition layer [57]. Exploiting the diffusion-limited mixing observed in a laminar flow, bifunctional Janus particles were synthesized by polymerizing rectangular particles across the surface of co-flowing of rhodamine-labeled oligomer streams (Fig. 7a, b) [57]. Output resolution can be improved by use of stop-flow lithography, in which pre-polymer flow is halted during exposure steps to prevent flow induced shape distortions [58]. Adoption of SFL techniques have enabled reliable production processes for cell encapsulating particles, although cell viability concerns may arise from the necessity of UV exposure and the use of cytotoxic prepolymers [59]. This method has been used to generate encoded particles for biomolecule analysis. Based on continuous flow lithography combining particle synthesis and the encoding into a single process to generate multifunctional particles, it could demonstrate with high specificity the same multiplexed detection using individual multiprobe particles [60].

Flow lithography methods can fabricate particles of various shapes. Due to a simplified channel design, flow lithography based platforms are easier to operate than droplet based systems, but are limited to non-photosensitive drug molecules, incapable of synthesizing sub-micron particles, and are unsuitable for high throughput production.

Multilayered polymer particles can be precisely engineered by a layer-by-layer technique using a multitude of templates and materials. These multilayered particles are emerging as a useful platform for various biomedical applications [55]. A microfluidic technique that utilize micropillars in a flow channel to continuously generate layer-by-layer microcapsules has been demonstrated [61]. Particles moved within three parallel laminar polymer streams and deposition of three bi-layers were achieved in <3 min with a thickness of ~3 nm per layer (Fig. 7c). Moreover, these nanoengineered delivery systems can encapsulate a wide variety of novel therapeutics with a well-defined time and place [62].

2.2. Microfluidics precisely controls drug delivery particle

The size and uniformity of drug delivery particles greatly affect their biodistributions *in vivo*. For example, particles may become susceptible to aggregation and trapping within specific organs and tissues based on size due to a variety of mechanisms which may include immune response, physical barriers, and other phenomena. Naturally, aggregation and trapping will limit the availability of payload therapeutics to accessible regions [63]. The most crucial benefit of using microfluidics to fabricate drug delivery particles is the ability to precisely control particle size. Compared to conventional synthesis methods, microfluidics can generate typically narrower particle size distributions [21]. Flow focusing and microvortices in microfluidics lead to discrete and uniform size particle synthesis at the flow interface. The size of the produced

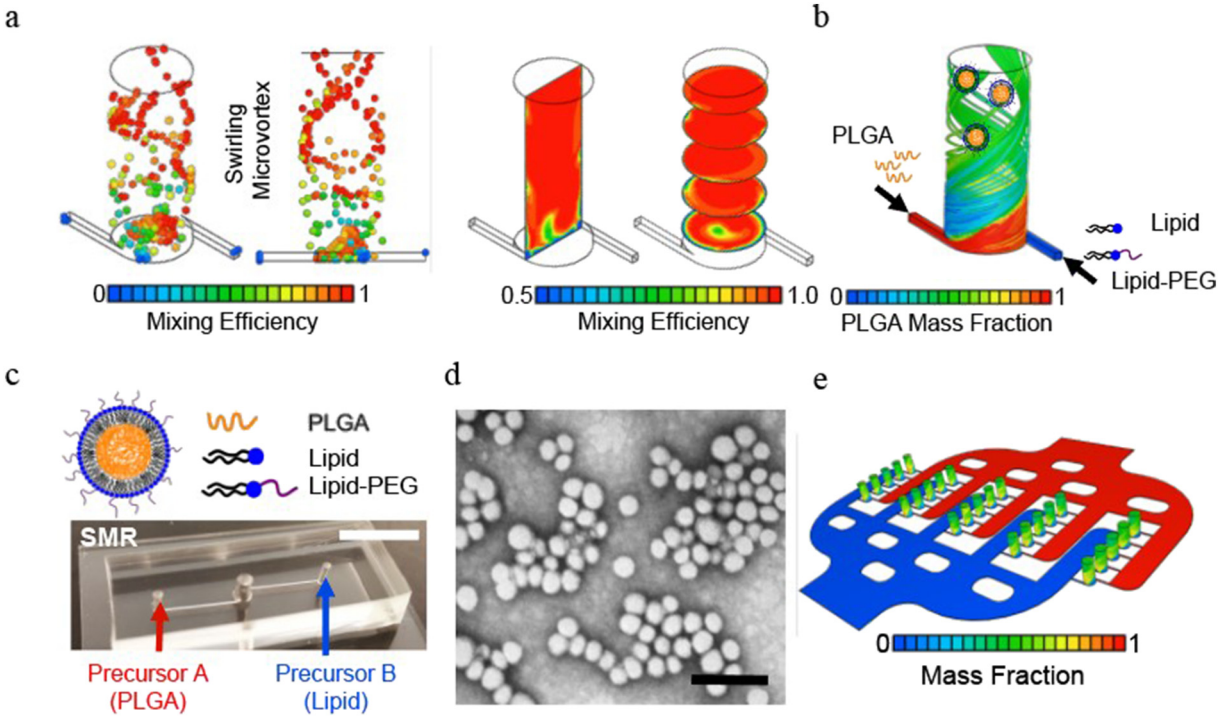

Fig. 8. Microfluidics provides scale-up production. (a) Engineering of a swirling microvortex reactor (SMR). Mixing efficiency was maximized at 2 mm diameter. (b) Schematic showing the synthesis of lipid-polymer nanoparticles through swirling microvortex. (c) Photo of the SMR. The scale bar: 5 mm. (d) TEM image of lipid-polymer nanoparticles. The scale bar: 100 nm. (e) Development and optimization of microvortex array. CFD simulation predicting a mass fraction distribution of precursor solutions. Adapted with permission from [56].

particles can be easily controlled by changing the flow rate [23–25]. Microfluidics can provide a basis for the rapid and robust mixing of different fluids where the three stages of carrier fabrication (nucleation, growth through aggregation and stabilization) are well-controlled, resulting in the production of drug delivery particles with high reproducibility and size uniformity [13]. In addition to particle size, the shape of drug delivery particles has gained more attention because of its effect on cellular internalization and intracellular trafficking [64]. Using flow lithography, the fabrication of non-spherical shaped particles could be made continuously in a precise manner by simple UV exposure [58,59]. This feature demonstrates that microfluidics-based drug delivery fabrication can be leveraged for mass production of drug delivery particles with various shapes to meet the biological needs. Furthermore, the precise control of particle shell thickness enables the fine tuning of the drug release profile of drug delivery particles for various drug release applications [61,65].

2.3. Microfluidics provides scale-up production

The conventional bulk synthesis of drug delivery particles relies on non-standard multistep procedures which are time-consuming, difficult to scale up, and heavily depend on specific synthesis conditions [5,66]. A key challenge in the transition of drug delivery particles from bench to the clinical heavily depends on the development of reliable methods of scalable production. While microfluidics relies on small volumes and dimensions of microchannels to exert precise controls on continuous assembly, some processes can be sized up to milliliter scales. Millifluidics can interface with existing purification and monitoring techniques in order to enable the high-throughput functionalization of gold nanoparticles and real-time monitoring for quality control [67]. On the other hand, Kim et al. reported symmetric microvortices microfluidic method with fast flow rate (i.e., high Reynolds number ~150) that showed 1000 times higher productivity than previous microfluidic approaches. Furthermore, as compared to conventional approaches, their methodology resulted in an improved reproducibility and homogeneity of the nanoparticle batches [34]. Recently, Toth et al. introduced robust and reliable manufacturing of lipid-polymer nanoparticles by integrating the parallelized swirling microvortex reactors platform with high precision feedback control system that can address unpredictable disturbances during the synthesis procedures. The SMRs consist of two inlets for precursor solutions and one outlet for nanoparticles and parallelized microvortex network for connecting all 25 swirling microvortex reactors. Interestingly, feedback pressure control system regulated the inlet pressure of parallelized microvortex array whereas mitigating external disturbances and reducing precursor flow fluctuation (Fig. 8) [68].

Most microfluidics based combinatorial synthesis and screening platforms discussed in this publication have not yet addressed issues concerning scalability for industrial production, and are mostly limited to micro and milligram ranges per unit. Theoretically, fabrication platforms can be arrayed in parallel to deliver the gram to kilogram scale yields required for industrial scale implementation, although much work is needed for developing platforms capable of exerting precise pressure controls and withstanding higher pressures required for higher mass transfers [5]. In the design of the parallelization, microfluidic modules should be well designed to avoid secondary flows, which can lead problems in the main bulk flow streams, causing chip-to-chip variations.

2.4. Outlook and challenges: microfluidics in drug delivery: fabrication

Since the debut of liposomal drug carriers in the 1970s [69], enormous efforts have been made to develop nanoparticle synthesis platforms for diagnostic and therapeutic applications. Classical nanoparticle synthesis methods heavily rely on bulk mixing and tend to suffer poor reproducibility from batch to batch and fail to optimize nanomaterial properties. In this perspective, microfluidic reactors have shown the capability to produce particle in a controllable and reproducible manner suggesting possible solutions to the aforementioned problems.

Over the past several years, examples showing the use of microfluidics for the synthesis of nanoparticle with different sizes, shapes, and compositions have emerged. At the moment, microfluidics enabled combinatorial synthesis of large number of distinct nanoparticles. Production rate can be enhanced through parallelization of multiple devices. Although microfluidics enables rapid mixing of reagents, control of temperature, and precise spatiotemporal manipulation of reactions, there is ample room for further development in microfluidic processes for nanoparticle synthesis (e.g., drug loading efficiency and stealth layer coverage). The potential of microfluidics to create complex and multifunctional nanoparticles for nanomedicine remains largely unexplored.

In terms of fabrication, many challenges remain. Conventional fabrication methods have the advantage comparatively easy scalability. Microfluidic-based approaches, in contrast, offer precise control over particle size and characteristics at the cost of yield volumes. Current attempts at incorporating high throughput production within a microfluidic platform (e.g., the industrial-scale) face challenges concerning reproducibility and output quality. In order to maintain long-term stability and robustness during the drug delivery system fabrication, one possible solution may be the incorporation of feedback pressure control loop systems to stabilize operating pressures.

3. Microfluidics in drug delivery: characterization

The most important properties of nanoparticles to be characterized before probing their interaction with biological systems are size, shape, surface chemistry/charge, drug loading and stability. Indeed, the development of novel drug delivery particle characterization tools heavily impact the odds of a successful clinical translation. One actual barrier to the clinical-scale of nanoparticles is an inability to validate the stability of the drug delivery system *in vivo*. Therefore, particle characterization is an important step for clinical translation. In this section, we introduce several microfluidic methods capable of characterizing drug delivery nanoparticles, including (3.1) size and morphology, (3.2) charge, (3.3) drug loading and release profiles, and (3.4) outlook and challenges (Fig. 9).

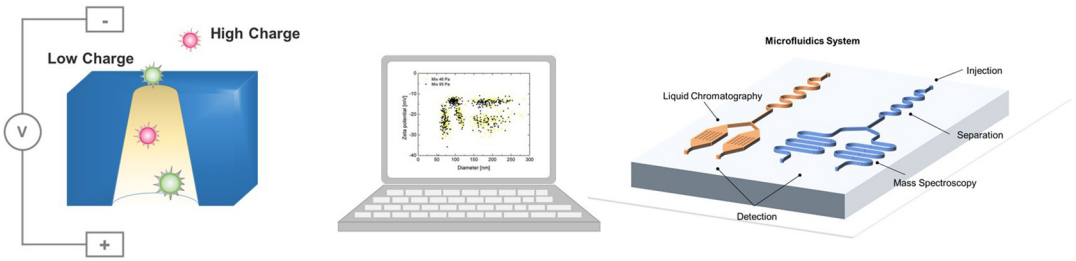

Fig. 9. Microfluidics in drug delivery: characterization. Microfluidic platforms have conducted on nanoparticle quantity and quality characterization especially size, charge and amount of reagent.

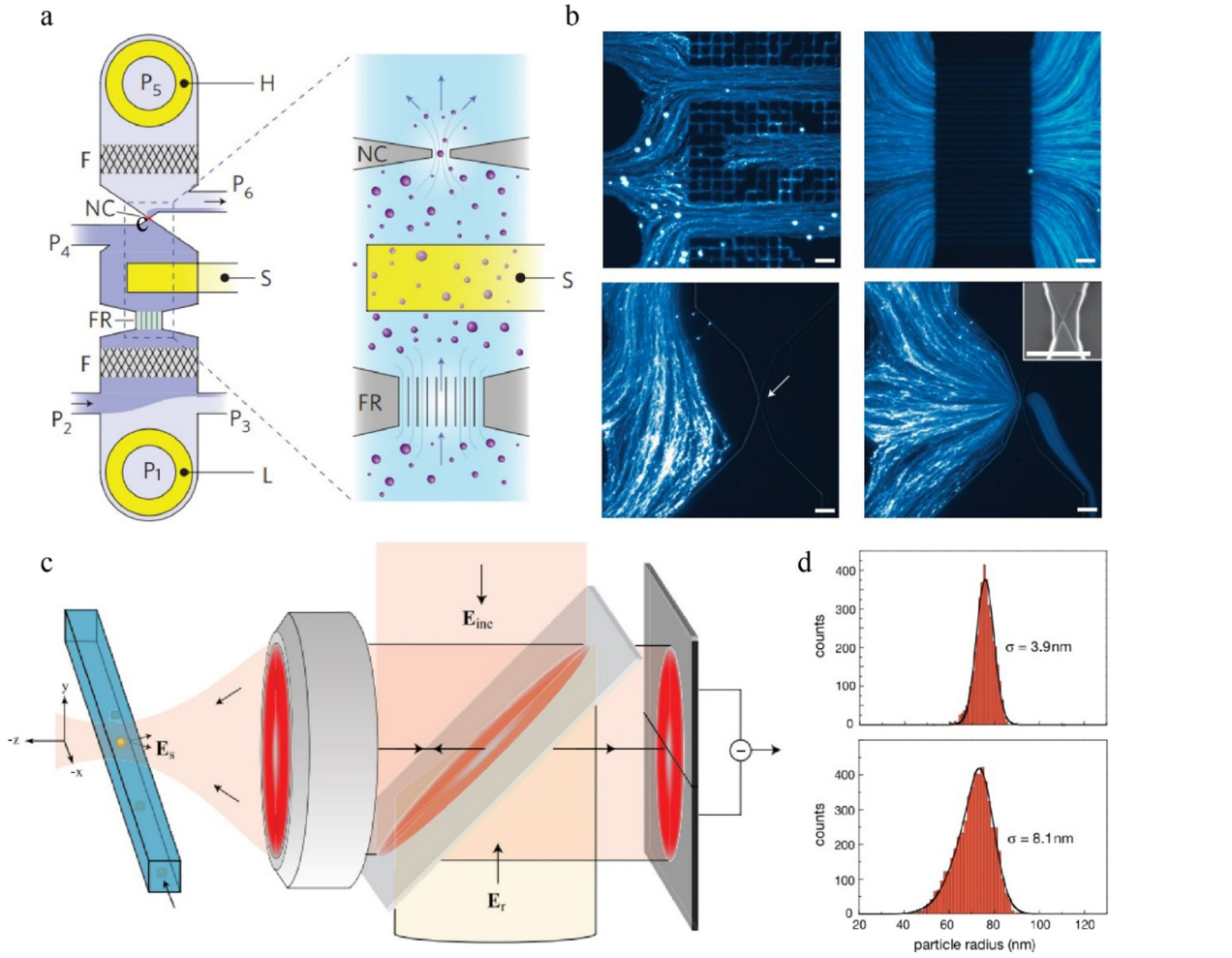

Fig. 10. Microfluidics provides drug delivery particle characterization. (a,b) A high-throughput label-free nanoparticle analyzer. (a) Chip layout showing relative placement of the electrical and fluidic components of the device consisting of external voltage bias electrode (H,L), sensing electrode (S), embedded nanometer-scale filter (F), fluid resistor (FR) and nanoconstriction (NC). (b) False-colour fluorescence micrographs and scanning electron micrograph showing controlled particle flow within microfluidic components. Scale bars: 10 μm. Adapted with permission from [63]. (c,d) Nano-optofluidic detection of single nanoparticle. (c) Heterodyne interferometric detection of light scattered by a nanoparticle or a virus (yellow) as it traverse a laser focus. The schematic employs an excitation laser that is reflected off a beamsplitter and focused through an objective into a nanofluidic channel. (d) Experimental particle size distributions measured with the heterodyne approach. Adapted with permission from [68].

3.1. Size and morphology characterization

Particle size is the most basic aspect of drug delivery nanoparticles, and is a major determinant of bio-distribution and retention in target tissues [70]. Dynamic light scattering (DLS) measurements are commonly used for particle size determination. According to Stokes-Einstein equation, DLS can measure size of particles in suspension [71]. Microfluidic devices can serve as a platform for the real time *in situ* monitoring of nanoparticle formation, enabling the possibility of investigating the fundamental reaction processes of nanoparticle synthesis. The investigation of mechanisms behind nucleation and growth is critical for optimizing nanoscale particle production. In one platform, the synthesis of cysteine-capped quantum dot nanocrystals between two interdiffusing reagent streams was examined in a continuous flow microfluidic device using spatially resolved photoluminescence imaging and spectroscopy [72]. Furthermore, small angle X-ray scattering (SAXS) were used to demonstrate the kinetics and mechanisms of nanoparticle nucleation and growth during synthesis in a microfluidic channel. Polte et al. represented a continuous-flow SAXS setup for time-resolved studies of nanoparticle formation mechanisms. The method allowed "in house" SAXS at a time resolution of about 100 ms without requiring a synchrotron radiation facility and the setup is applicable in general to a wide range of chemical liquid phase synthesis of nanoparticles.

Despite recent development in the label-free characterization of particle [73], particle detection still remain a challenge for integrating high-throughput microfluidic technologies. Instead, characterization of particles based on the size and morphology can be determined using such advanced microscopic techniques as atomic force microscopy (AFM) [74], scanning electron microscopy (SEM) [75], transmission electron microscopy (TEM) [76] and DLS.

3.2. Charge characterization

Recent advanced in nano/microfluidic enabled the development of high-throughput devices capable of characterizing particles such as effective diameter and surface charge [77]. The fundamental principle of the electrical detection is based on investigating conductance or capacitance changes of particle samples [78,79]. Fraikin et al. suggested a microfluidic analyzer that detects individual nanoparticles and characterized complex, unlabeled nanoparticle suspensions [77]. The analyzer has two components: microfluidic channel, which directs the pressure-driven flow of analyte through the electrical sensor and the senor itself, comprising two voltage-bias electrodes and a single readout electrode

which embedded in the microchannel (Fig. 10a, b). This analyzer has been developed to detect and characterize unlabeled nanoparticles in a multicomponent mixture at 500,000 particles per second. In addition, nanopore readout platform could detect single-nucleotide polymorphism by in-situ reaction monitoring [80]. It is known that the characteristic spectral dependence of the surface plasmon resonance (SPR) behavior allows metallic nanoparticles to be distinguished among other nanoparticles and also gives additional information such as local environment and shape anisotropy [81]. An Interferometric techniques also have been demonstrated for distinguishing single dielectric nanoparticles. Inspired by this techniques, Mitra el al. reported real-time detection of sub-100 nm polystyrene particles, viruses and larger proteins flowing through a microfluidic channel based on their polarizability (Fig. 10c, d) [82]. In addition, eliminating the phase sensitivity in interferomertic particle detection, they could improve the accuracy of particle characterization and identification. Stretch actuated variable pore size membranes enabled simultaneous determinations of size, concentration and zeta-potential of nanoparticles from charge density under electrophoretic force [83].

These approaches, all of which adapt size-tunable pore sensors, are providing a better understanding of the fundamental characteristics of nanoparticle and high-throughput characterization of their properties. However, these approaches are based on theoretical models. Therefore, applying the best suitable and plausible theoretical models is the key challenge.

3.3. Drug loading and release characterization

Drug release behavior is a crucial factor for nanoparticle application, and is directly related to drug stability and therapeutic effects. In general, the drug release rate depends on (1) drug solubility, (2) desorption of the surface-bound or adsorbed drug, (3) drug diffusion out of the nanomaterial matrix, (4) nanomaterial matrix erosion or degradation, and (5) the combination of erosion and diffusion process [84]. In this way, it is very essential to determine extent of the drug release and to acquire such information most release method require that the drug and its delivery vehicle be separated [85]. Drug loading capacity of the particles is commonly defined as the amount of drug bound per mass of carrier. Various conventional technique such as UV spectroscopy [86] or high performance liquid chromatography (HPLC) [87], gel filtration [88] are used to determine this parameter.

Microfluidic-based liquid chromatography (LC) has drawn much attention due to its enhanced sensitivity, reduced sample consumption and ability to multiplex measurements. In general, microfluidic-based LC platforms consist of pump/gradient generation, injection ports, columns, and detectors (Fig. 9) [89]. A microfluidic HPLC chip was fabricated by laminating polyimide films with laser-ablated channels and port [90]. HPLC chip integrated an enrichment column, a separation column and a nanoelectrospray tip in a single device and enabled to reduce the delay and dead volumes between components, as well as reduced the post-separation volume. Gao et al. developed an integrated microfluidic device for high-throughput drug screening with mass spectrometry (MS) detection. This microfluidic device incorporated a concentration gradient generator, cell culture chamber and solid phase extraction columns in a PDMS chip. By using combination systems, the process of drug absorption and evaluation of cytotoxicity could be simultaneously accomplished [91]. Integrated devices can offer a means of high-throughput drug analysis with a low amount of reagent consumption. Furthermore, there have introduced several microfludic-based nanospray emitters [92,93] and microfluidic-based LC-MS analysis [94,95].

Although some of these microfluidic systems for drug loading and releasing characterization are being actively developed, there is a long way to go. Many different detectors have been integrated to MS/LC chips, and most induce flow through electrophoretic forces. While MS/LC chips are highly sensitive, the bulkiness of auxiliary components such as the power supply or pump, as well as the detector itself, can pose a challenge for on-chip miniaturization and integration. Further information can be found in a number of review articles cited here [89,96,97].

3.4. Outlook and challenges: microfluidics in drug delivery: characterization

Due to the importance of characterizing nanoparticle properties, characterization platforms are crucial to nanoparticle development

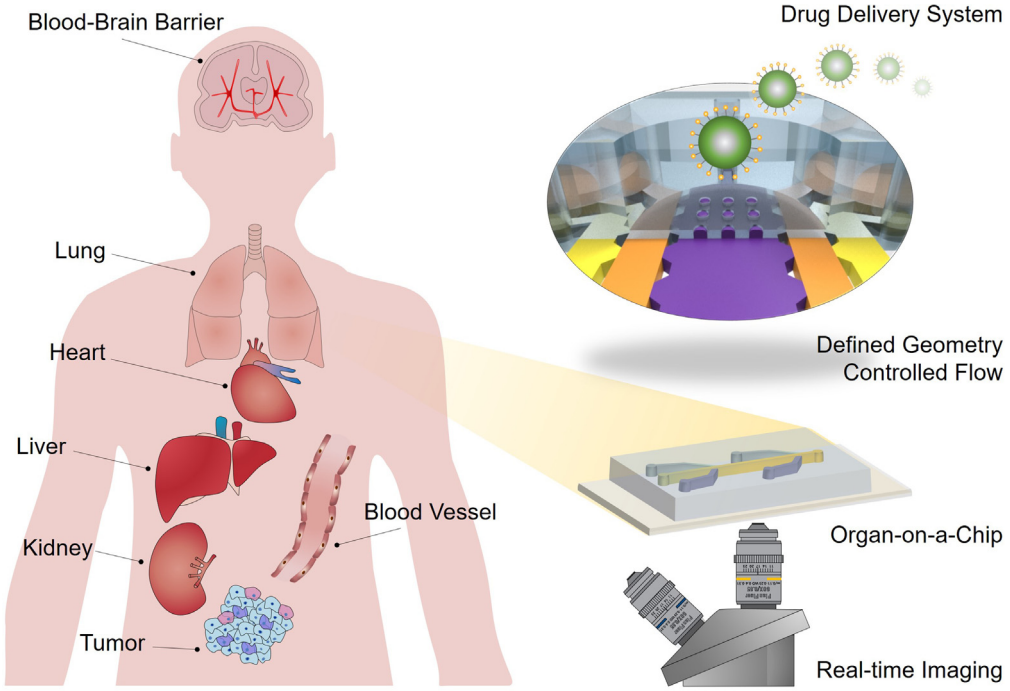

Fig. 11. Microfludics in drug delivery: evaluation. Recent development of microfluidics has witnessed the integration of *in vitro* cellular approaches onto chips, which allow real-time imaging and *in vitro* microscopic observation as well as an evaluation of cell function and behavior.

prior to biological testing. DLS is the most common method for nanoparticle analysis. In general, this technique is the best used for submicron particles and can be used to measure particle with size less than a nanometer. DLS can also be used as a probe of complex fluids such as concentrated solutions. From the Stokes Einstein relation, diffusion coefficients measured by dynamic light scattering can be used to determine particle size [98]. In terms of drug delivery system using microfluidics, although several microfluidic models have introduced to characterize nanoparticle, these microfluidic platforms do not predominately used like DLS measurement instruments. Indeed, current lithographic techniques can limit the manipulation of fluid. In addition, common procedures (e.g., preparation of chromatographic columns) are not well suitable at the microscopic level and implementing sample injection of subnanoliter volumes with sensitive detection.

In the context of nano-size drug delivery system characterization, "nanofluidics" would be desirable for drug delivery system characterization. Nanofluidics is the study and application of fluidics in and around geometries with nanoscale characteristic dimensions. In the past decade, nanofluidic transport phenomena and effects have been intensively studied, and a few striking applications (e.g., nanopore-based DNA sequencing [99] and resistive-pulse sensing [100]) have been demonstrated. However, nanofluidics is an immature technology, especially in comparison to microfluidics [101]. Due to the inaccessibility of nanoscale closed space systems, most standard tools widely used in microfluidics are unsuitable for nanofluidic applications [101,102]. In terms of material perspective, polymer-based materials are attractive, because of their flexible processing and low cost. However, dimensional instability and roof-collapse of channel structures, because of the low stiffness of PDMS. Therefore, material selections for nanofluidics are the top priority. With the future development of nanofluidics, we expect dramatic advance in drug delivery system characterization.

4. Microfluidics in drug delivery: evaluation

Drug delivery systems need to be nontoxic, biodegradable, sufficiently stable to be delivered to targeted sites, and to have a greater therapeutic advantage over the naked drug [103]. Conventionally, drug delivery system evaluation has been made in static tissue culture plates, but unfortunately this neglects the important effects of 3D, flowing conditions and other mechanical or biochemical environmental conditions. Today, an increasing number of microfluidic approaches have been demonstrated the potential to closely mimic physiological microenvironements. Current pre-clinical studies on drug candidates heavily rely on costly and highly variable animal models, mainly because existing cell culture systems fail to recapitulate organ-level pathology of humans. This lack of predictive models emphasize the need for better approaches to mimic the structure and functions of cells, tissues and organs (Fig. 11). Recently, the development of microfluidics has witnessed the integration of *in vitro* cellular approaches onto chips, which allow real-time imaging and *in vitro* microscopic observation as well as an evaluation of cell function and behavior. Consequently, in this section, we introduce microfluidics in drug delivery focusing on evaluation. In detail, this section includes (4.1) organ-on-a-chip, (4.2) preparation of organ-on-a-chip, (4.3) drug delivery evaluation using microfluidics, and (4.4) outlook and challenges.

4.1. Organ-on-a-chip

Conventional two-dimensional monolayer cell culture system usually do not accurately recapitulate the structure, function, physiology of living tissues, as well as highly complex and dynamic three-dimensional *in vivo* [104]. With the introduction of "organ-on-a-chip", the microfluidic technologies can recapitulate the multicellular architectures, tissue-tissue interfaces, physicochemical microenvironments and vascular perfusion of the body [105]. Organ-on-a-chip technology produces organ functionality not possible with conventional 2D or 3D culture systems with high-resolution, real-time imaging and *in vitro* analysis including biochemical, genetic and metabolic activities of living cells [105]. So far, several individual organs on chips have been developed, including lung-on-a-chip [9], blood vessel-on-a-chip [106,107], blood-brain barrier-on-a-chip [108], tumor-on-a-chip [109], liver-on-a-chip [110] and heart-on-a-chip [111]. The simplest system is a single, perfused microfluidic chamber including one cultured cell (e.g. hepatocyte), whereas in more complex configurations two or more fluidic channels can be connected by a porous membrane, lined on the other sides by different cell types (e.g., lung alveolar-capillary or blood brain barrier or tumor-tumor vasculature) [105]. In the context of drug delivery and development, it should be especially valuable for the study of molecular mechanisms of action, toxicity and efficacy testing and biomarker identification. For more detail, we suggest recent review articles on organ-on-a-chip system [112,113].

4.2. Preparation of organ-on-a-chip

A majority of the microfluidic platforms covered in this publication are based on devices fabricated from soft lithographically micropatterned PDMS bonded to a glass substrate. PDMS is a commonly used substrate for microfluidic platform fabrication due to a combination of material biocompatibility, ease of handling, and optical clarity. PDMS soft lithography has been a standard in microfluidic device fabrication since its popularization by the Whitesides group in 1998, and is capable of producing a functional microfluidic device from a mere design within a period of days [114].

Soft lithographic microfabrication processes, which may substantially vary depending on the final product, can be conservatively described as the following processes: mask design and fabrication, mold fabrication, PDMS molding and demolding, punching, and bonding. Most mold patterning is done through photolithography using a UV curable novolac polyepoxide resin negative photoresist known as SU-8, on a substrate of silicon wafer [115,116]. As such, micropatterned mold designs are drafted in CAD and printed on transparent films to be utilized as negative photomasks in the photolithographic process. After patterning the silicon wafer with the desired designs, uncrosslinked PDMS is poured into the mold, cured, and demolded. The demolded micropatterned PDMS substrates are then punched to create input and output ports for any media reservoirs, hydrogel seeding ports, actuation chambers, and other features which may be included in a given design [117,118]. Patterned and punched PDMS substrates can then be plasma bonded to glass, or bonded to other PDMS substrates to form multilayer designs. Due plasma induced hydrophilicity in PDMS, many microfluidic chip designs requiring substrate hydrophobicity require bonded PDMS devices to be post baked in an oven for hydrophobicity recovery [114].

Many microfluidic devices discussed in this publication construct two and three dimensional microscale tissues by patterning fluid suspensions of cells in extracellular matrices (ECM). Cell suspensions are patterned into 3D cellular hydrogels in microchannels, or as 2D cell sheets on membrane or smooth substrate surfaces. Cell species origin varies by study, ranging from human donor cells to rodent cells. Among human cell based studies, many discussed platforms utilize immortalized cell lines with organ specific origins such as Caco-2 (human epithelial colorectal adenocarcinoma) for gut platforms, HepG2 (human hepatocellular carcinoma) for liver platforms, and A549 (adenocarcinomic human alveolar basal epithelial cells) for lung platforms [119]. Primary cells directly donated from patients and other human tissue sources, such as HUVEC (human umbilical vein endothelial cells) and hLF (human lung fibroblasts) can also be used [120]. Cell species and cell line/primary cell origin selection depends on a variety of factors ranging from logistical concerns to resource availability. Animal derived cells tend to be least financially burdensome of the three, followed by immortalized human cell lines. Given the vast genetic and physiological differences between humans and non-humans, non-human tissue derived data are often used to supplement preliminary studies prior to human cell

Table 1
Microfluidic models for drug delivery evaluation.

Microfluidic model	Cells	Culture method	Matrix	Nanoparticle	Drug loading	Application (Brief summary of the study)	Reference
Blood vessel	–	–	–	Avidin and biotin conjugated microsphere/Microsphere in multiple sizes/Liposome or metal particles	–	Synthetic microvascular network(SMN) to study particle adhesion depending on vessel anatomical characteristics or particle shape and size	[137]
	Red blood cells	Human blood perfusion	–	Microsphere(2 μm)	–	Studying margination propensity related with hemodynamics and hemorheology	[136,139,140]
	Bovine aortic endothelial cells(BAEC)	2D seeding on SMN channel	–	Antibody conjugated microsphere (anti-ICAM-1 or IgG1)	–	Synthetic microvascular network to study characterization of particles and dynamic flow condition in microvascular network	[141]
	Red blood cells	Human blood perfusion	–	Anti-ICAM-1 coated micro/nano particles	–	Characterization of nanoparticle delivery depending on vessel geometry, shear rate, blood cells, particle size and particle antibody density	[142]
	Hy926(Human endothelial cell line), Human Platelet	EC: 2D seeding on microfluidic channel/Platelet: Flow through channel	–	Fluorescent mesoporous silica(FMS) nanoparticle	–	Studying effect of nanoparticle on platelet adhesion and aggregation	[143]
	Human endothelial cell	2D seeding on microfluidic channel	–	Mesoporous silica nanoparticle (MSN)	–	Evaluation of effect of shear stress on endothelial cytotoxicity	[144]
	HUVEC	2D seeding on microfluidic channel	–	Gold nanoparticle	–	Studying influence of gold nanoparticle size and shear stress on endothelial viability	[145]
	HUVEC	2D seeding on microfluidic channel	–	Gold nanoparticle	–	Studying the effect of gold nanoparticle toxicity depending on flow condition towards endothelial cells	[146]
	HUVEC	2D seeding on microfluidic channel	–	Liposomes coated with APN and VCAM-1 targeting peptides	–	Development of system to quantify nanoparticle accumulation on HUVEC layer with effect of flow condition	[147]
	Bovine aortic endothelial cells	2D seeding on microfluidic channel	–	Fluorescent nanoparticle coated with tissue plasminogen activator(tPA)	–	Development of shear-activated nanotherapeutics having maximized drug delivery efficacy and its verification on microfluidic system	[148]
	–	–	–	Size-tunable polyion complex vesicle (PICsome)	–	Studying permeation of nanoparticles in microfluidic system having straight micropores	[149]
	HUVEC	2D seeding on microporous microchannel	–	Lipid-polymer nanoparticles	–	*In vitro* microfluidic atherosclerosis model to study endothelial translocation of nanoparticle	[150]
	HUVEC Lung fibroblast co-culture	3D gel pattering	Fibrin	High-density lipoprotein(HDL) mimetic nanoparticle	–	Vascular network –HDL mimetic nanoparticle interaction; biphasic effect of HDL mimetic nanoparticles	[151]
	bEnd.3 (mouse brain endothelial cells line)	2D seeding on microporous microchannel	–	gH625(membranotropic peptide) conjugated polystyrene nanoparticle	–	Studying effect of flow condition and peptide coating on transportation of nanoparticle through brain endothelium	[152]
Tumor	Human breast cancer cell (LCC6/Her2)	Droplet formation (Encapsulation)	Alginate	CaCO3 nanoparticle	Doxorubicin	The dose-dependent cytotoxic effect of doxorubicin is measured, and observed decreasing viability and proliferation with increasing doxorubicin concentration by new microfluidic platform	[169]
	Human breast cancer cell	Spheroid formation (MTS)	Gelatin, matrigel	–	Doxorubicin	The culture of uniformly sized multicellular tumor	[170]

	(MCF-7)					spheroid (MTS) in a hydrogel scaffold and further applications in a microfluidic channel to evaluate therapeutic efficiency of doxorubicin	
	Human breast cancer cell (MDA-MB-435)	Spheroid formation (MTS)	Matrigel	Gold nanoparticle (PEG coated, Transferrin coated typed)	–	Developing a tumor-on-a-chip microfluidic platform to study the transport of synthetic carriers through a three-dimensional tissue environment and characterizing nanoparticles within a tumor tissue.	[171]
	Human breast cancer cell (MCF-7), Human microvascular endothelial cells (MVECs)	Pseudo 3D (Monolayer of Endothelial cells and Tumor in 3D matrix)	Type I collagen, Matrigel	Fluorescent nanoparticles (100,200,500 nm)	–	Establishing a tumor-microenvironment-on-chip to recapitulate the key features of complex transport of drugs and nanoparticles within the tumor microenvironment for more effective targeted delivery strategy.	[172]
	Human breast cancer cells (MCF-7, MDA-MB-231, and SUM-159PT)	3D gel pattering	Type I collagen	Dox-HANP nanoparticle (Dox-loaded 250 nm hyaluronic acid nanoparticle)	Doxorubicin	Developing an integrated experimental and theoretical analysis of cellular drug transport of cancer cells in the microfluidic platform. Studying the effect of nanoparticle-mediated drug delivery, the transport and action of doxorubicin encapsulated nanoparticles are examined	[174]
	Human breast cancer associated endothelial cells (HBTAEC), Human breast cancer cells (MDA-MB-231 and MCF-7)	Pseudo 3D (Monolayer of Endothelial cells and Tumor in 3D matrix)	Fibronectin/gelatin (Endothelial cell), Matrigel (Tumor)	Liposomal drug carriers (Nanoscale carrier)	–	An *in vitro* tumor microenvironment has been established that approximates *in vivo* tumor barrier properties to reproduce enhanced permeability and retention (EPR) similar to permeability values reported in vivo.	[175]
Lung	Human alveolar epithelial cells and microvascular endothelial cells	Pseudo 3D (cell sheet on porous membrane)	–	–	–	An alveoli model using a pneumatically actuated mechanotransduction method with an apical and basal polarized layered cell sheet configuration.	[9]
	A549	2D dish culture	–	Cerium oxide nanoparticle	–	Oxidative stress and cytotoxicity assay of CeO2 nanoparticles on A549 lung cancer cells.	[181]
	Human bronchoalveolar carcinoma	2D dish culture	–	Silica 15 nm and 46 nm nanoparticle	–	A study on the effect of silica nanoparticle size on toxicity in human bronchoalveolar carcinoma.	[182]
	Primary lung alveolar, small airway epithelial cells, and human non-small-cell lung cancer	Pseudo 3D (cell sheet on porous membrane)	–	–	Erlotinib and rociletinib	Creating *in vitro* human orthotopic models of non-small-cell lung cancer that recapitulate organ microenvironment-specific cancer growth, tumor dormancy, and responses to tyrosine kinase inhibitor therapy.	[192]
Liver	Caco-2 TH29-MTX HepG2/C3A	2D culture (cell sheets)	–	50 nm carboxylated polystyrene nanoparticle	–	Using the GI tract-liver-other tissue system allowed observation of compounding effects and detection of liver tissue injury	[195]
	HepG2 and C3A cells	Spheroid formation	GelMA hydrogel	–	Acetominophen	Development of a liver-on-a-chip platform for long-term culture of three-dimensional human HepG2/C3A spheroid for drug toxicity assessment and the bioreactor allowed for *in situ* monitoring of the culture environment.	[196]
Kidney	Human proximal tubular	Pseudo 3D (cell sheet on porous membrane)	Type IV collagen	–	Cisplatin	Using primary human kidney proximal tubular epithelial cells, investigated cisplatin toxicity and Pgp efflux transporter activity.	[198]

experimentation [121]. Immortalized human cells are standardized lines of cultured cancer cells which tend to exhibit a general enhanced culture viability, proliferative ability, and near infinite passaging ability, thus affording easier handling and logistics over primary human cells [121]. Compared to healthy primary cells of the same origin, cancer cell lines possess many phenotypic differences which can translate to differences in metabolic and physiological activity [122]. Primary cells are directly isolated from healthy human donor tissues and closely resemble *in vivo* human tissues at the cost of price and handling difficulty. As with most other healthy somatic cells, primary cells are subject to senescence, and cannot be passaged beyond the Hayflick limit [123]. Irrespective of cell origins, the majority of the organ platforms discussed in this publication utilize macrofluidic dish cultures to proliferate their cells to the desired confluency prior to trypsinization and seeding within their respective microfluidic organ devices.

The various microfluidic organ platforms discussed in this publication employ a combination of ECM, growth factors, mechanotransduction, and other mechanical or biochemical environmental conditions to organize cells into tissues as well as model pathological states. ECM hydrogels are used as scaffolds for cellular growth and arrangement, can take the form of natural matrices such as fibrin and collagen, and synthetic hydrogels such as Matrigel [124]. ECM hydrogels are often employed to pattern cell suspensions within fluid patterning microchannels. Growth factors are chemotactic biochemical signaling molecules which can be used to stimulate and direct growth, as well as polarize tissues in a basal/apical arrangement along a concentration gradient axis [125]. Growth factors are often administered in the form of conditioned media, or through factor producing stromal cell co-cultures. Mechanotransductive factors such as substrate and shear stress play a crucial role in tissue differentiation, as it is in the case of endothelial cell derived vascular networks, and are often induced through engineered features of a given microfluidic device [107,126,127]. Flow based factors such as shear stress can be induced through the use of hydrostatic pressure gradients and pumps, while substrate strains can be induced through the mechanical actuation of flexible bulk surfaces and membranes [107]. Other environmental conditions such as oxidative stress, hypoxia, and hypothermia can be induced through modification of incubation conditions.

Microfluidic platforms modify and combine various micropattern designs and cell culture conditions to emulate *in vivo* like organ microenvironments for cultivating human relevant tissue culture systems. Additional environmental control methods are developed with each new leap in understanding with regards to the complex biochemical and mechanical interactions of tissue differentiation and maintenance, as well as better understanding of pathological states.

4.3. Drug delivery evaluation using microfluidics

Over the last few decades, the rapidly developing field of nanomedicine significantly impacts on human disease therapy [11,128]. Nanomedicines need to be non-toxic and biodegradable, and possess a high drug loading capacity and sufficiently stable to be delivered to targeted sites [103,129]. Conventionally, nanomedicine evaluation has been made in static cell culture dishes, but this method neglects various physiological conditions such as flow and three dimensional culture. Recent developments in microfluidic technologies provide evaluation and prescreening methods to address issues of poor predictability that limit the pace of clinical translation of nanotherapeutics [130]. Easy manipulation of nano-to-micro liter volume of liquids has enabled these models to become a platform where dynamic crosstalk between cells can be achieved [131]. Furthermore, the system geometry and structure recapitulate physiological length scales, interstitial flows, and concentration gradients. Therefore, these highly biomimetic models overcome the drawbacks with conventional tissue culture models. Also, if human cells are incorporated into microfluidics, organ on a chip technologies can resolve the main drawback of animal models; species difference. Rodent models have correctly predict human toxicity only 43% of the cases comparing human and animal drug toxicities [132,133]. Because of the low predictive rate, the US Food and Drug Administration (FDA) requires drug testing should be done in at least two different species; however, the prediction of human toxicity remains only accurate in 71% of the cases [132]. By better mimicking the physiological conditions and more accurately predicting the effect of drug delivery, these sophisticated *in vitro* evaluation and screening platforms may bridge the gap between the outcomes of animal studies and human clinical trials [130]. In this section, we introduce specific organs on chip technologies and their interactions with drug delivery systems focusing on (4.3.1) blood vessel, (4.3.2) blood-brain barrier, (4.3.3) tumor, (4.3.4) lung, (4.3.5) liver, (4.3.6) kidney, and (4.3.7) heart (Table 1).

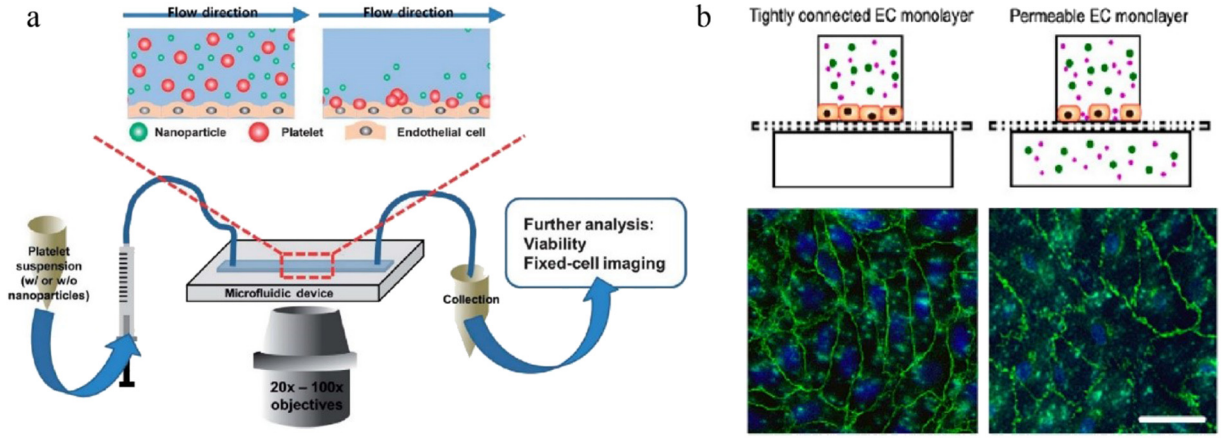

Fig. 12. Blood vessel-on-a-chip and drug delivery. (a) Schematic of microfluidic on-chip assay for platelet-endothelial adhesion and platelet aggregation. Endothelial cells were first injected into microfluidic device in cell suspension, and let cultured to become a confluent layer on microfluidic channel within two days. Once endothelium is constructed, human platelets isolated from fresh human blood is injected with or without mesoporous silica nanoparticle. Platelet suspension was collected from outlet for aggregation study and microchannel was imaged and analyzed for adhesion study. Adapted with permission from [143]. (b) By applying inflammatory cytokine, TNF-α, and shear stress through the microfluidic channel, endothelial monolayer become highly permeable (up right) which was reconstructed to be pathological state. Endothelial intracellular junction was disrupted and it was verified by immunostaining of VE-cadherin (green). (bottom, right) Due to the change of permeability translocation of nanoparticles has increased and this could be measured with intensity change of FITC-albumin suspended on bottom channel. Scale bar: 20 μm. Adapted with permission from [150].

4.3.1. Blood vessel-on-a-chip and drug delivery

Major usage of nanoparticles in the field of medicine is to deliver nano-drugs in targeted site in safe and accurate manner. Particles should be designed properly depending on complex factors such as its load or site of target, but their microcirculatory pathways also should be considered crucial, since most of drug delivery nanoparticles are injected intravenously and travel through blood vessels [134,135]. Especially, margination of nanoparticles has been issued to explain how spherical particles are localized or attached to blood vessel walls, since they exhibited uneven distributions inside branched microvasculature, resulting in decreased efficiency of targeted drug delivery [136,137]. Microfluidic *in vitro* models are expected to provide insight to hemodynamics in microcirculation of nanoparticles through mimicking dynamic flow conditions in physiologically relevant microenvironments [138]. Early studies of drug delivery systems using microfluidic platforms had focused on effects of unique anatomical features of vessels; therefore, an *in vitro* system called synthetic microvascular network (SMN) was widely adopted in research even without culturing vascular endothelial cells. This approach allowed for the analysis of the flow of particles in real time depending on their size and shape, and specified what geometry of a blood vessel facilitates the accumulation of particles [137,139–141]. Hemorheology, moreover, is another consideration in determining an efficiency rate of nanoparticle delivery [136]. Blood cells were introduced in a microfluidic assay in order to reveal how interaction between those cells and particles could affect the accumulation of delivery systems. Variation in particle size or the surface chemistry of nanoparticles directed the interaction between particles and red blood cells [136,142]. One of related studies have suggested that existence of red blood cells enhanced the binding of particles on a vessel wall and this was significant when particles got bigger with a higher density of antibody coating [142]. Furthermore, adhesion and aggregation of platelets in human blood had different tendency depending on concentration of mesoporous silica nanoparticle which are recently highlighted in drug delivery system researches (Fig. 12a) [143]. In virtue of microfluidics, many researchers were able to conduct simple and cost effective assays to find out the effect of shear stress on endothelium accumulation or cytotoxicity. Human endothelial cells, mostly HUVECs were plated inside microfluidic channel and mesoporous silica nanoparticles [144] or gold nanoparticles [145] in different size were applied with laminar flow in which HUVECs experience shear stress while exposed to nanoparticles. Conventional viability test was followed after to determine cytotoxic influence of shear stress while nanoparticle treatment on endothelium compared to static state which will provide more physiologically relevant information when these drug delivery system is actually used in clinics [146,147]. On the other hand, shear stress was artificially applied in shear-activated nanotherapeutics and was tested in microfluidic model of pathological blood vessel in case of thrombosis or embolism. Aggregates of nanoparticles were dispersed by high fluid shear stress to have activation in therapeutic effect which expected to decrease the dose and minimize side effects [148]. One of the important physiological features in microvascular system is their selective permeability and this could be controllably mimicked in *in vitro* microfluidic model [149]. *In vitro* assay for measuring vessel permeability is necessary for studying nanoparticle translocation across the endothelium. Common microfluidic design of having extremely thin porous membrane between two layers of microchannel enabled measuring how much nanoparticles has pass through endothelial layer from one channel to another and gave an insight that this depends on endothelial cells permeability [150]. From the study by Kim et al., compartmentalized microfluidics were used as an atherosclerotic endothelium model to study translocation of lipid polymer hybrid nanoparticle. This model was compared to an *in vivo* rabbit model and the similarities between microfluidic model and the *in vivo* model provides the potential as a model for probing the translocation of nanoparticle (Fig. 12b) [150]. Recently, Ahn et al. investigated the effect of HDL mimetic nanoparticle on the angiogenesis process [151]. Using microfluidic synthesis method that generates tunable microvortices [36], HDL mimetic nanoparticle was reconstituted by self-assembly of precursors. This study revealed critical effects of HDL mimetic nanoparticle on angiogenesis exhibiting a biphasic effect on angiogenic sprout growth while inhibiting TNF-α stimulated angiogenesis. This study may lay the groundwork for the integration of microfluidic technologies to examine cell-nanoparticle interactions critical to discovery and screening new drug delivery system. For a decade, development of *in vitro* vascular model has stepped a huge leap forward by taking advantage of microfluidic and microfabrication technology. Its application on characterizing nanoparticle drug delivery system, therefore, has also been highlighted recently. Since pharmacokinetics extremely depends on vessel morphology and physiology, microfluidic platform would be an ideal tool for testing unrevealed mechanisms which will contribute to development of high performance drug delivery system.

Previous studies focusing on addressing the effects of flow and nanoparticle size on the translocation of particles across an endothelium, were largely restricted to 2D endothelial culture systems. The mechanism of particle translocation in 3D microvascular system would be more accurate since the phenotype and function of 3D system differ from simple 2D endothelium. Moreover, beyond the efficiency of particle delivery through the endothelium, the final target across the endothelium should be examined. In vivo, the human vascular system is embedded in a complex matrix structure. Particle delivery through this 3D matrix should be simulated to find out maximized success rate of delivery to targeted cells.

4.3.2. Blood-brain barrier-on-a-chip and drug delivery

Blood-brain barrier (BBB) is a specialized vascular unit in central nervous system (CNS) naturally designed for protection against toxic substance within bloodstream and maintenance of brain homeostasis. On the other hand, it has been regarded as a major hindrance in developing therapies for CNS disorders so that it is the greatest challenge for pharmaceutics to design drug or its delivery system to reach sufficient penetration level across BBB [152–154]. Various kinds of nanoparticles ranging from metal or polymeric nanoparticles to lipid organic nanoparticles have been emerged since several decades and competed each other for optimized tool as CNS drug cargo, still developing novel drug delivery system optimized to pass BBB is on progress [153,155–157]. Meanwhile, the validation of developed CNS drug delivery nanoparticles were conducted in different types of models. *In vitro* BBB model outstood over *in vivo* models because they were more favorable when estimating drug pharmacokinetics and particle distribution [155]. Beyond simple *in vitro* system using transwell platform, BBB models using microfluidic system has introduced since 2012 [158]. Numerous models have developed in form of microfluidic BBB-on-a-chip which enabled not just reconstruction of three dimensional structure of BBB but also feasible measurement of transendothelial electrical resistance (TEER), as parameter of BBB permeability, and also helped to study cellular and molecular mechanism of barrier system [108,159–161]. Above all, major purpose of BBB-on-a-chip was to utilize in preclinical screening of drugs for CNS diseases. A number of studies have shown drug treatment assays in their own BBB-on-a-chip models which had promising results, a few cases were reported for drug delivery nanoparticles to be tested inside the chip. Transcytosis of shuttle-mediated nanoparticles through endothelial cells was tested in microfluidic system in existence of controllable flow by Falanga et al. [162]. Endothelial monolayer as BBB monolayer were constructed on porous membrane between two microfluidic channels and nanoparticle transcytosis effect was quantified by measuring particles from one channel to another. They have proved that delivery particles coated with specific peptide called gH625 and condition of having flow had enhancement of particle transcytosis. As this study shows, numerous novel BBB-on-a-chip platforms now being developed and coming in to the spotlight of pharmaceutics should optimized for testing CNS drug nanomedicines.

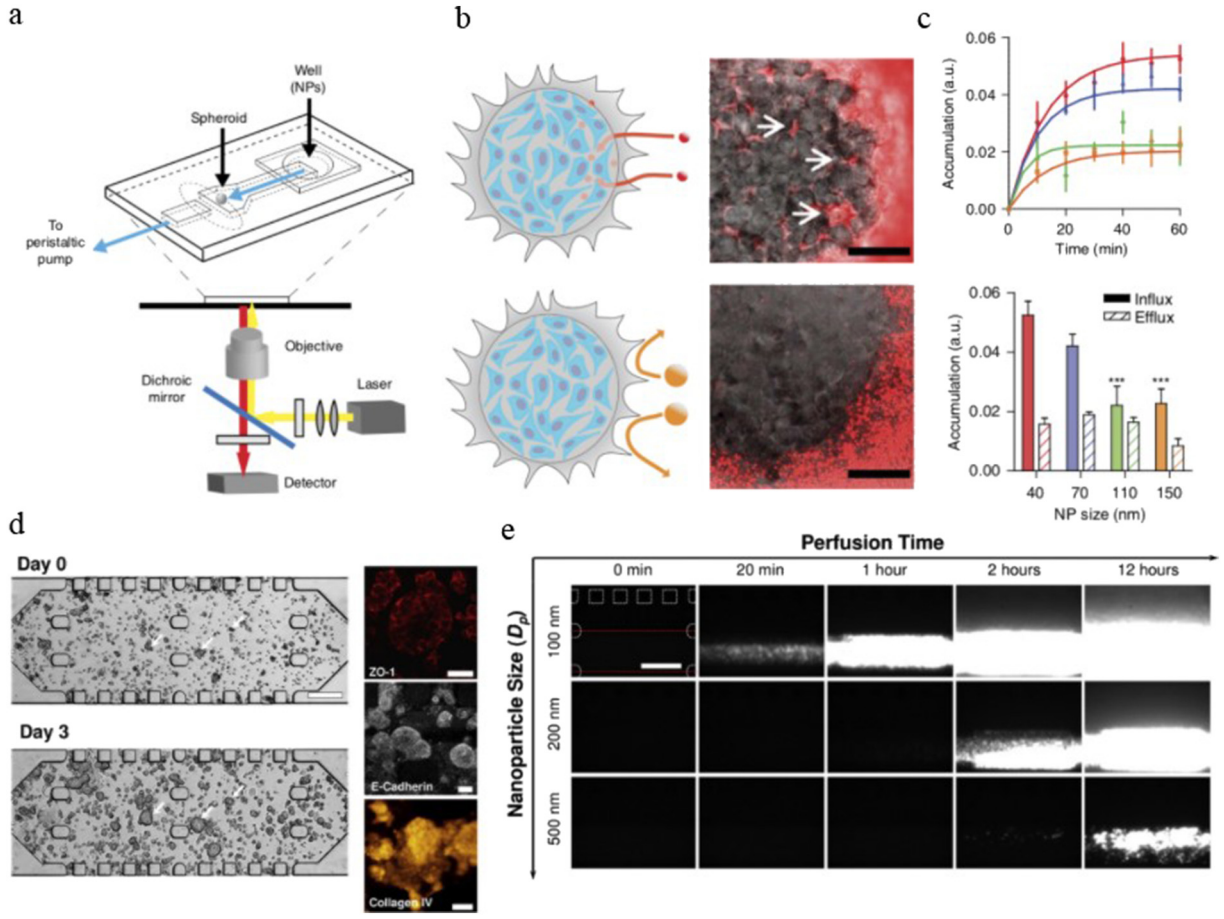

Fig. 13. Tumor-on-a-chip and drug delivery. (a–c) Tumor-on-a-chip provides an optical window into nanoparticle transport. (a) Tumor-on-a-Chip system is a two-layered microfluidic chip with space for spheroids and nanoparticles. The integration of the spheroid in the microfluidic chip generates a controllable flow condition for the tissue and provides an optical window for real-time, uninterrupted analysis using confocal microscopy imaging. (b) Tissue accumulation depends on size of nanoparticle. Injection of 40 nm fluorescent PEG-NPs into the microfluidic chip, the NPs reach the spheroid and accumulate in interstitial spaces. On the other hands, 110 nm fluorescent PEG-NPs are excluded from the spheroid. Scale bar: 100 μm. (c) profile of tissue accumulation of nanoparticle depending on size: 40 (red), 70 (blue), 110 (green) and 150 nm (orange). Adapted with permission from [171]. (d,e) Simulation of complex transport of nanoparticles around a tumor using Tumor-Microenvironment-on-Chip (d) Tumor cell growth with collagen matrix in the microfluidic chip. Scale bar: 300 μm. The cells proliferated and aggregated firmly between each cell and the expression of tight junction proteins is shown by fluorescence micrograph. Scale bar: 50 μm (e) The effects of nanoparticle size on transport efficiency. Each size of fluorescence nanoparticle is administered along the capillary channel. The temporal and spatial changes in fluorescence intensity were further analyzed to determine the nanoparticle concentration profile. Adapted with permission from [172].

The blood-brain barrier is a complex system consisting of multiple types of cells surrounding brain endothelial cells. These cells have diverse effects on particle delivery through BBB microvascular networks, and need to be accounted for in any study characterizing nanoparticle interactions with the BBB.

4.3.3. Tumor-on-a-chip and drug delivery

Drug delivery assays using Tumor-on-a-chip platforms focus on evaluating delivery efficacy and toxicity of cancer nanomedicine. Microfluidics offer significant advantages over conventional macroscale cell cultures by allowing precise control of physiological signals such as hydrostatic pressure, shear stress, oxygen and nutrient gradient, enabling the reproducibility of tumor microenvironments [109]. Microfluidic platforms can be used to screen both drugs and carrier nanoparticles by providing a physiologically accurate tumor microenvironment for testing [163]. Nanoparticle screening through microfluidic systems can be divided into two categories; (i) models constructed from pre-formed mono and coculture spheroids [164–166] and (ii) models mimicking tumor microenvironment through the incorporation of biological barriers within ECM filled microchannels [167,168]. Yu et al. developed a droplet-based microfluidic system for fabricating breast cancer tumor cell encapsulating alginate beads. After formation, the alginate beads were captured in a micro-sieve structure within a continuous perfusion system. This microfluidic system allows tumor cells to augment in a uniform position within the equal environment. The dose-dependent response of the tumor spheroid to doxorubicin showed a higher survival rate in multicellular spheroid cultures compared to conventional single cell cultures. Moreover, it can also monitor the drug response over time in the same spheroid. Not only cells can be entrapped inside tiny droplets but also reagents can be administered using very low volumes, even 1000 times smaller than the ones used in conventional assays [169].The purpose of this study was to develop an *in vitro* 3D tumor model as a tool to evaluate the therapeutic effects of anticancer drugs. Multicellular tumors of hydrogel scaffolds using micro-wells can be cultured and the therapeutic effect of doxorubicin can be evaluated. Microwells with geometric structures in which tumor cells can form a multicellular spheroid were constructed to include a hydrogel scaffold. By loading matrigel-embedded preformed spheroids in the central channel of the microfluidic device, the medium flow was continuously supplied to the side channels to reproduce blood flow conditions. In addition, Shin et al. observed the degree of doxorubicin-micelles accumulation in multicellular spheroids by fluorescence microscopy [170]. It is necessary to evaluate the degree of accumulation according to *in vivo* environmental conditions such as interstitial flow.

Platforms to model the distribution and transport of nanoparticles within three dimensional tissue microenvironments were developed

using controllable flow conditions [171]. The proposed microfluidic device allowed precise control of media flow conditions, and the spheroids were coated with a layer of laminin that served as an Au-nanoparticles transport barrier to mimic the conditions at the tumor region found *in vivo*. Under physiological flow conditions, tissue penetration and accumulation of fluorescent nanoparticles were monitored in real time. Nanoparticle size, surface functionalization and flow conditions in the microenvironment were found to affect Au-nanoparticles accumulation near the tumor microenvironment. The nanoparticles were functionalized with target groups accumulated in the periphery and could not penetrate deeply into the core. Tumor-bearing mice were used to determine whether the *in vitro* study results were similar *in vivo* (Fig. 13a–c). These studies provide important insights that will help design better nanoparticles for improved *in vivo* targeting. Kwak et al. developed an *in vitro* tumor model to reproduce the microenvironment for determining delivery around the tumor site [172]. The main purpose is to characterize the effects of pathophysiological conditions of tumors on the nanoparticle transport. This model is known as tumor-microenvironment-on-chip which is consisting of consists of a three-dimensional microchannel. Breast tumor cells (MCF-7) and endothelial cells are cultured within the extracellular matrix under perfusion of interstitial fluid. To explore nanoparticle delivery efficiency under tumor microenvironmental variation, studies were conducted including cut-off pore size, interstitial fluid pressure, and tumor tissue microstructure. The results suggest that tumor-microenvironment-on-chip can mimic complex transport around the tumor and provide detailed information on nanoparticle transport behavior. The design of nanoparticles for targeted delivery takes into account the dynamic interaction of nanoparticles in the tumor microenvironment (Fig. 13d–e) [172].

A previous study indicated a connection between tumor transport ability and local micro environmental conditions. Bagley et al. demonstrated the upregulation of transport capacity in an ovarian cancer model by introducing plasmonic nanoantennae as a heat-generating nanomaterial with a microfluidic device. This new device is capable of evaluating the diffusion ability of nanoparticles according to the level of temperature and studying endothelial responses that were difficult to observe *ex vivo* [173].

Investigating the characterization of cell-type-specific drug transport is necessary for quantitative characterization of drug response and resistance to cancer cell types. Three types of human breast cancer cell lines (MCF-7, MDA-MB-231 and SUM-159PT) were cultured on this tumor-microenvironment-on-chip platform and the drug absorption and response were observed. The results demonstrate that cell drug delivery can be quantitated cell-type-specifically by rate constants indicating the uptake of doxorubicin across cell membranes. The developed experimental and theoretical models enable quantitative analysis of cell drug delivery and drug resistance mechanisms. The proposed experimental platform provides a highly relevant cellular microenvironment to test drug response of various cancer types, since it provides controllable 3D extracellular environments under perfusion [174]. Tumor microenvironment has unique characteristics including leakage and discontinuity of tumor endothelial cells of vascular system, poor oxygenation, low pH and high interstitial pressure as well as communication between various cells at micro environmental level. Nanoparticles can be a great tool to make a breakthrough for this microenvironment. The different porosity and pore size of the tumor vascular endothelium can be targeted by many types of nanoparticle carriers. Therefore, mimicking the EPR effect *in vitro* is an important research topic indicating the microenvironment of the tumor [135].

Recently, the microfluidic platform consists of 3D solid tumors (MDA-MB-231 and MCF-7) cultured in a tumor compartment and an endothelium compartment characterized by a vascular network that forms a complete lumen under shear flow condition. Endothelium permeability for both small dye molecules and large liposomal drug delivery systems was quantified using fluorescence microscopy. Endothelial cell permeability was significantly increased in the presence of tumor cell conditioned media or tumor cells. The magnitude of this increased permeability was significantly higher in metastatic breast tumor cells compared to non-metastatic breast tumor cells. The biomimetic microfluidic tumor microenvironment platform mimics the tumor microenvironment, including the EPR effect. The platform has significant potential in applications such as cell-cell/cell-drug carrier interaction studies and rapid screening of cancer drug treatments [175].

Developing a microfluidic platform for rapid combination synthesis and optimization of nanoparticles is a key to overcome tumor. Valencia et al. introduced a number of nanoparticle precursors in which nanoparticle libraries of varying size, surface charge, target ligand density and drug load are generated in a reproducible manner. They evaluated the nanoparticle screening capability by avoiding macrophage absorption as well as by operating rapidly synthesize 45 formulations of different size and surface composition. A correlation between *in vivo* drug pharmacokinetic study results and *in vitro* behavior has been established. Targeted nanoparticles selected *in vivo* showed an increase in tumor accumulation in mice compared to untargeted nanoparticles. From this study, selected nanoparticle synthetic parameters showed longer blood half-lives and enabled microfluidic platforms (e.g., targeting ligands for cancer cells) to synthesize targeted nanoparticles with a variety of targeting ligand densities. The proposed microfluidic platform represents a tool that can potentially accelerate the discovery and clinical translation of nanoparticles [176].

The tumor microenvironment consists of interactions between various components (cancer, fibroblast, immune cell and endothelial cell). There is a lack of models for evaluating the function of nanoparticles in models that reflect this complex environment. In addition, the majority of the models in which the behavior of tumor is statically reflected. As tumors exhibit hyperactive growth and metastasis, a static model is not suitable for meaningful data. Although microfluidic tissue culture platforms enable real time imaging, very few of the studies discussed in this paper have fully exploited this capability. In addition, although many tumor vasculature models are discussed, the vascular models utilized often do not sufficiently reflect *in vivo* microenvironmental vascular conditions.

Results of studies on the penetration and accumulation of nanoparticles on a microfluidic platform including MTS (Multicellular Tumor Spheroid) have been reported. In addition to the spheroid model, the results of examining complex stimuli and responses within a 3D scaffold which use various hydrogels are needed. The EPR effect, solid tumor stress, and normalization of tumor vasculatures that have not been fully studied in previous animal models may be further studied using microfluidic platforms.

In summary, preexisting microfluidic drug screening platforms for patient specific tumor microenvironments used in conjunction with nanoparticle testing shows great promise, and may potentially lead to an optimized developmental pipeline for personalized nanoparticle drug delivery assays, and further lead to advances in highly patient specific nanoparticle therapies for cancer treatment [11].

4.3.4. Lung-on-a-chip and drug delivery

The lung is a vital respiratory organ which serves as a gas exchange interface between the circulatory system and the outside. In terms of gross anatomy, the lung possesses an elaborate hierarchical architecture of branching pathways wherein the large trachea into progressively smaller networks of bronchi and bronchioles, ultimately terminating in closed alveolar sacs. The alveolar sacs, enveloped by extensive networks of capillaries, serves as the primary functional interface unit between blood and inhaled gases, and serves to facilitate the exchange of oxygen, CO_2, and other volatile molecules [177]. Substances that are absorbed through the lungs can be directly distributed systemically through the circulatory system, a phenomenon which has been utilized as a major pathway of fast acting drug administration [177–179]. Direct accessibility to the circulatory system also provides a platform for the delivery of payloads that would otherwise be inactivated through first pass metabolic and digestion processes that oral administration entails

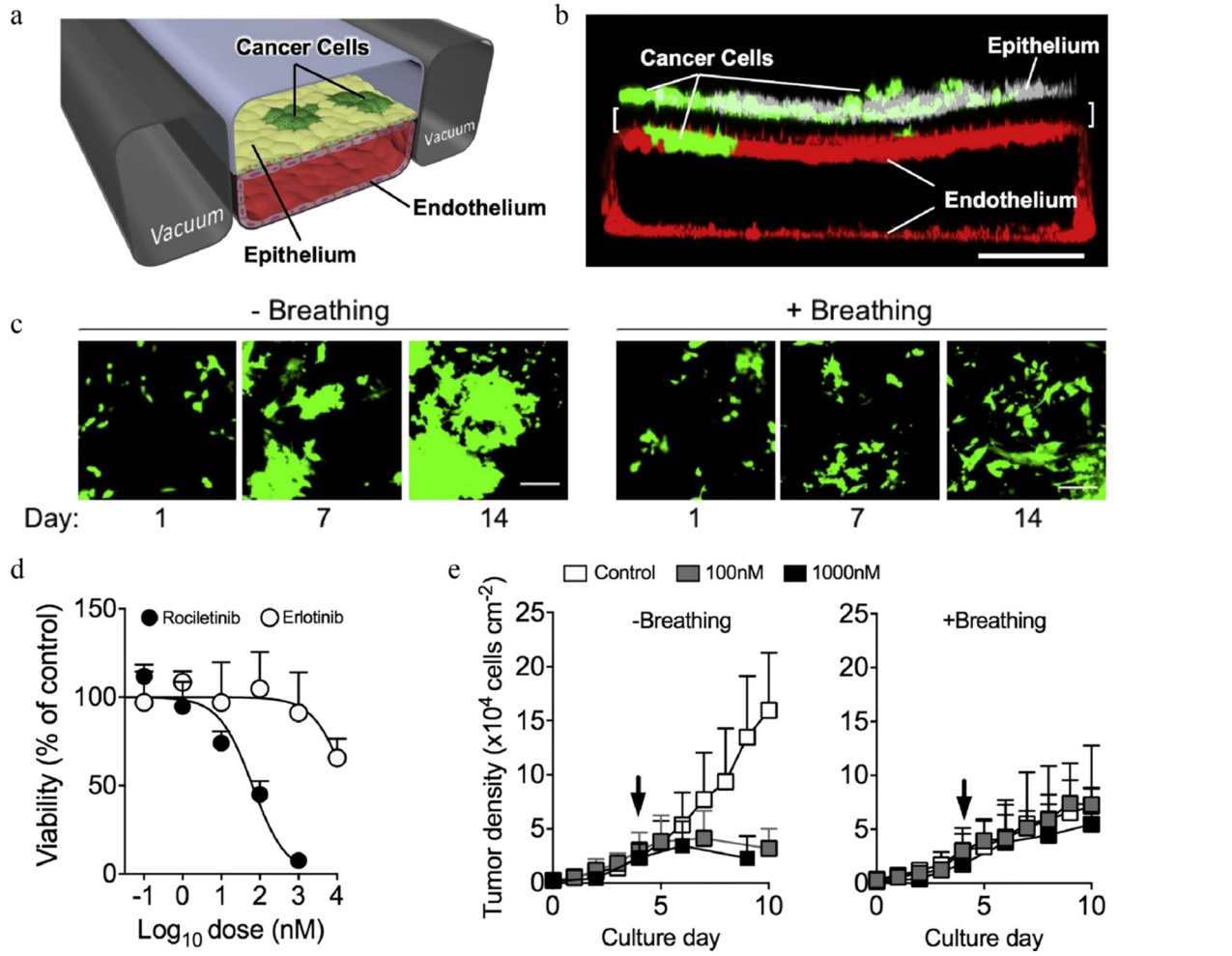

Fig. 14. Lung-on-a-chip and drug delivery. (a) Schematic images of a cross-section through the 2-channel lung-on-a-chip. (b) Human lung epithelial cells and NSCLS tumor cells cultured on the upper membrane with human lung microvascular endothelial cells. Confocal image shows GFP-labeled lung cancer co-cultured with primary lung alveolar epithelial cells immunostained with tight junction protein ZO-1 (white). Primary lung microvascular endothelial cells labeled with anti-VE-cadherin (red). Scale bar: 200 μm. (c) Fluorescence image shows that GFP-labeled NSCLS tumor cell clusters growing within the epithelium of the chip. (d,e) The evaluation of the growth of NSCLC tumor cells in microfluidic chips cultured in the presence of rociletinib and erlotinib. Adapted with permission from [192].

– allowing for the noninvasive delivery of protein payloads such as insulin and hGH at higher rates of bioavailability [177]. Pharmacological substances delivered through inhalation can take many forms, from simple small molecule gases like nitrous oxide, to vaporized liquids such as chloroform and nicotine, to aerosolized dry powders like insulin and fluticasone propionate [177]. With the rise of nanoparticle research, efforts to incorporate nanoparticle carriers for drugs, biologics, and other therapeutic payloads have emerged as a prominent study. A variety of nanoparticle types and materials including chitosan, cerium oxide, silica, PLGA, alginate, silver and CNTs have been investigated as potential drug and biologics carriers with mixed results [180–186]. In the cases of insulin encapsulated in chitosan nanoparticles, and elcatonin encased in chitosan treated PLGA nanoparticles, qualitative drops in blood glucose and blood calcium levels have been observed from the respective carriers in *in vivo* models [180,185]. Conventional inorganic, non-biodegradable nanoparticle carriers like silver, silica, and cerium oxide have shown evidence of cytotoxicity and adverse effects in both conventional 2D *in vitro* cultures and *in vivo* testing, however, limitations in conventional testing methods have generated considerable difficulty in obtaining usable data [181–184]. Due to the branching structure of the lung, as well as the long pathways that drugs must take to reach the alveoli, successful delivery is difficult to control and subject to poor yields, and validation of realistic successful delivery conditions is challenging *in vivo*, and impossible with conventional *in vitro* 2D cultures [177,179,187]. The difficulty of emulating both macroscale tissue structures as well as cellular level histological functions in conventional 2D *in vitro* platforms as well as the resource intensiveness of utilizing *in vivo* animal models generated considerable need for an *in vivo* like *in vitro* microfluidic platform capable of bridging the gap between the economical deployment of in vitro devices and the realistic and complicated micro and macro scale tissue environments and functions of *in vitro* testing [188–191]. The Wyss institute Lung-on-a-Chip, introduced in 2010, engineered a lung alveolar tissue model composed of primary tissue cultures and integrated mechanical stretching functionality to provide the basis for a degree of mechanotransducive and histological layering conditions. The platform was used to assay for silica nanoparticle toxicity [9]. Recently, lung-on-a-chip was used to recapitulate human lung cancer (non-small-cell lung cancer) growth and invasion patterns. This study revealed that local microenvironmental cues elicited by cells that comprise the epithelial and endothelial tissues of the lung, as well as by mechanical breathing motions, can significantly influence human lung cancer growth *in vitro*. More importantly, tumor cells become resistant to anti-cancer drug (rociletinib) without breathing motions (Fig. 14) [192].

Due to the highly hierarchical nature of lung physiology and the critical role of macroscale tissue organization on the mechanical function of the lung, lung on a chip platforms have technological and logistical challenges to overcome if they are to fully emulate macroscopic lung

function in a way that is physiologically relevant to aerosolized nanoparticle delivery methods. Technological challenges have largely limited lung on a chip platforms to emulating certain functional aspects of individual highly localized tissue groups such as the alveoli and larger air channels, and the viable informative window afforded by feasible culture times is thus far insufficient to gauge comprehensive post nanoparticle administration observation beyond the order of weeks. In all, further progress on the forefront of *in vitro* lung nanoparticle assays require a means to both assess the interactions of investigated particles on the macroscale functionalities of lung tissues, and would greatly benefit from a means to observe intermediate to chronic scale particle effects through longer term cultures.

4.3.5. Liver-on-a-chip and drug delivery

The liver is a vital organ with an immense number of distinct functions, including facilitating many systemic metabolic processes. In the context of pharmaceutical research, the liver is particularly important for several key reasons stemming from the high degree of metabolic activity. As a metabolic hub, the liver serves as a first pass metabolic screen for all drugs administered orally. Depending on liver interactions, orally administered drug dosages can be rendered almost completely inert from liver based first pass metabolism, before even making it into blood circulation. Pharmaceutical substances that reach blood circulation, either through non-oral alternative administration routes or through moving through the initial first pass, are constitutively screened and filtered by the liver as part of the circulatory system. With regards to nanoparticle therapeutics, liver bound Kupffer cells serve as a component of the mononuclear phagocyte system (MPS), which serves as a macrophage immune response that tends to sequester and accumulate between 30 and 99% of circulating nanoparticles, and can contribute to increased hepatotoxicity [193,194]. Due to the high degree of liver metabolic interactions with pharmacologically active substances, hepatotoxicity contributes heavily to the attrition rate of drug studies [187,195]. Unsurprisingly, hepatotoxicity assays are in high demand. Many distinct microfluidic hepatic tissue models exist, each trading varying levels of simplicity and ease of use in exchange for *in vivo* like control conditions for more biologically relevant data. For instance, a study incorporated a multi cell-line-derived tissue model of Caco-2/TH29-MTX intestinal co culture and HepG2/C3A liver co culture platform to gauge the penetration of nanoparticles through intestinal cell line co-culture tissues to liver cell line tissues, as well to observe the degree and effects of nanoparticle aggregation on cell tissue viability and cell-cell junction conditions [195] (Figure). Bhise et al. developed a bioprinted hepatic cell line spheroid bioreactor platform consisting of HepG2/C3A co-cultures to attempt a hepatotoxicity assay with three dimensional tissue structures for qualitative viability testing [196]. The use of cell lines like HepG2 and C3A rather than primary cells and ex vivo biopsied tissue cultures raise potential concerns with data relevancy, however use of primary and ex vivo tissue cultures come at the cost of a drastically increased degree of culture difficulty and a much shorter viable culture time [187]. Furthermore, ex vivo rat biopsied liver and intestinal tissue slice chip successfully incorporated and maintained *in vivo* metabolic processes, rates, and inter-tissue interactions for up to 8 h for the intestinal component, and 24 h for the liver subunit [197]. Other approaches to address the issues of nanoparticle hepatotoxicity involved bypassing liver nanoparticle uptake altogether through nanoparticle surface modification to adhere to red blood cells. Other efforts to reduce nanoparticle uptake by Kupffer cells have been attempted in *in vivo* models by modifying nanoparticle substrates and surface treatments, dosage profiles, and concurrent medications to varying degrees of success – indicating the potential for further study and optimization [193].

While current liver on a chip platforms are certainly an improvement over the 2D hepatocyte monocultures of the past in terms of *in vivo* like organization of polarized endothelial membranes, the 3D structures constructed by current platforms have yet to form functional multilayer hepatic tissues such as those seen in the hepatic lobule. Tissue organization is crucial to assessing the accessibility of nanoparticles to the liver as a whole, as a theoretical nanoparticle would need to enter the hepatic lobule through the circulatory vasculature, into the sinusoid with endothelial lining and Kupffer cells, prior to accessing hepatocytes. As the metabolic first pass activity of the liver, like the Kupffer cell mediated MPS, is also highly tissue dependent, further work is needed to model nanoparticle engulfment and delivery bioavailability with any degree of accuracy. In all, hepatic metabolic and viability screening for nanoparticle delivery would benefit greatly with the development of engineered *in vivo* like *in vitro* tissues, as well as from using primary cells.

4.3.6. Kidney-on-a-chip and drug delivery

A Large number of kidney-on-a-chip system consist of renal cells embedded on the interface of ECM or membranes located next to perfusable microchannels that can provide nutrients, waste clearance, and stimulate flow [198–201]. Traditionally, *in vitro* studies are conducted under static conditions on plastic tissue culture plates. However, renal proximal tubule epithelial cells *in vivo* are subjected to continuous luminal fluid shear stress [15]. By virtue of microfluidics, 3D microfluidic kidney models grown in extracellular matrix were more sensitive to drug-induced toxicity and better suited to monitor chronic toxicity compare to 2D counterparts [202]. Kidney-on-a-chip enables high-

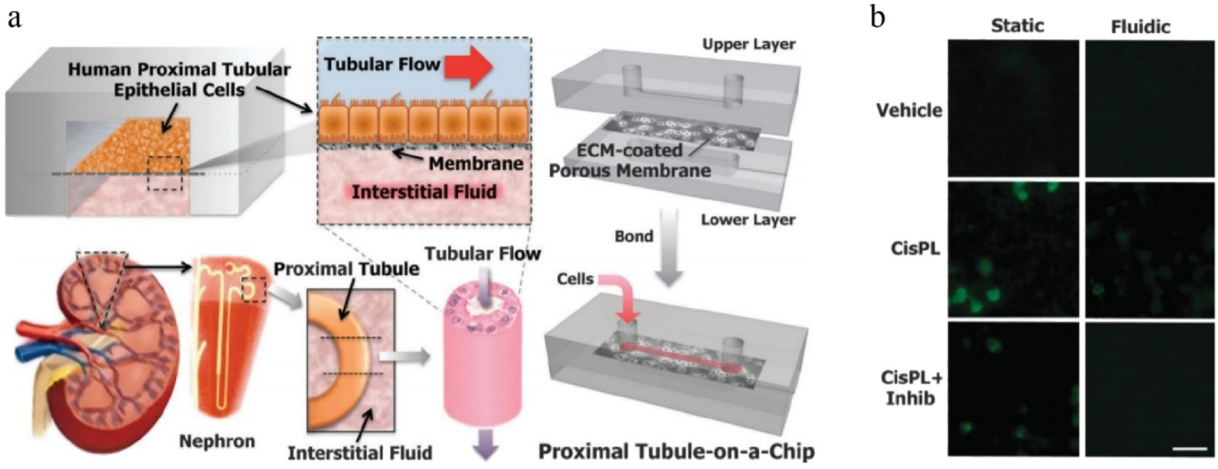

Fig. 15. Kidney-on-a-chip and drug delivery. (a) Design for the human kidney proximal tubule-on-a-chip. The microfluidic device consists of an apical channel separated from a bottom reservoir by ECM-coated porous membrane which primary proximal tubule epithelial cells are cultured in the presence of a fluid shear stress. (b) Cisplatin toxicity measured *in vitro*. Immunofluorescence views of Annexin V shows cell apoptosis. Scale bar: 50 μm. Adapted with permission from [198].

resolution and real-time molecular imaging of *in vitro* system, major benefit for gaining insights into drug delivery mechanism. In the early study of kidney-on-a-chip, Jang et al. suggested kidney proximal tubule on-a-chip which can precisely predict toxicities that can be produced by drug in humans. The kidney proximal tubule on-a-chip consists of an apical channel separated from a bottom reservoir by an ECM coated porous membrane upon which human proximal tubule epithelial cells are cultured in the presence of apical fluid shear stress [198]. After administered cisplatin via injection into the interstitial compartment of the device, the proximal tubular cells exhibited increases in cell injury both static and dynamic conditions. However, the cisplatin damaged proximal tubule cells cultured in the presence of flow recovered a significantly greater extent than cells in static condition (Fig. 15). Among the tissues of interest for systemic toxicity interactions, the kidneys are one of the crucial site for elimination of chemicals and drugs via their glomerular filtration. Drug delivery evaluating using kidney-on-a-chip may provide an important insight into exploring mechanistic interpretation of cellular mechanism for predicting kidney toxicity and renal drug clearance *in vitro*.

4.3.7. Heart-on-a-chip and drug delivery

The heart is associated with a high degree of susceptibility to drug toxicity, driving significant demand for *in vitro* cardiac models for drug testing [203]. Numerous cases of cardiac tissue engineering have developed by using novel biomaterials such as coiled fiber scaffolds embedded with gold nanoparticles [204], hydrogel sheets embedded with carbon-nanotubes [205] or tri-layered elastomeric scaffolds [206]. Lab-on-a-chip technology has contributed in developing models representing physiological features for high-throughput pharmacological studies. One of the early heart-on-a-chip models consists of cantilever structures called muscular thin films (MTF) for quantitative analysis of heart autonomous contractility. Alignment of cardiomyocytes and matrices used in the platform can be used to quantify cardiac function [111,207]. In addition, various models of heart-on-a-chip have applied human iPSC-based cardiac cells [208] or 3D bioprinting technology [209] in order to mimic the physiology and microenvironment of the cardiac system. All of aforementioned models are mainly targeted to have application on high-throughput or high-content screening of drugs and they have actually shown these applications with small molecule drugs that are already released in market. They have shown different cardio-physiological response to each drug stimulus, change in contraction rate or alignment, implying that the models are suited to test the performance of drug delivery system.

All of the aforementioned models target applications in high-throughput or high-content screening of drugs and have actually shown applications with small molecule drugs on market. Appropriate cardio-physiological responses to each drug stimulus was observed *in vitro*, demonstrating the suitability of organ chip models for drug performance observation.

4.4. Outlook and challenges: microfluidics in drug delivery: evaluation

The majority of smart nanoparticle delivery systems work well *in vitro* testing, only to fail in more sophisticated *in vivo* environments. Overall, the application of microfluidics to 3D cell culture organ-on-a-chip technologies shows great potential in mimicking more physiologically relevant *in vivo* microenvironments. Organ-on-a-chip represent an innovative step forward to use as a pre-clinical screening system. Organs-on-chips have been focused of a public–private collaboration between government initiatives such as the FDA, the federal Defense Advanced Research Project Agency (DARPA) and the National Institute of Health (NIH) since 2012. Millions of dollars have been invested in universities to advance this research.

Data released by FDAReview.org indicated that only 1 in 10 drugs that enter clinical trials ultimately gain FDA approval [210]. However, the current dish culture and animal models do not always give us the complete picture. As an emerging candidate, organ-on-a-chip can manipulate mechanical and chemical micro-physiological environments. Therefore, the use of organ-on-a-chip represent an innovative step forward to make high-throughput drug screening and characterization of nanoparticle with faster and inexpensive, while creating information that better associate to human pathophysiology comparing conventional dish culture and preclinical animal models.

Despite the great promise, creating an organ-on-a-chip systems is not a simple process, with a number of hurdles to overcome. Reproducing the architectural complexity of human tissues and organs in a miniaturized fashion and linking them in the right arrangement that the interconnect systems are the representative challenges. Further obstacles exist in the validation of organ-on-a-chip results, especially when real-time, repeated measurements are essential. Recently, full sensor integrated organ-on-a-chip system has been demonstrated [211]. Fully integrated organ-on-a-chip enabled to build optical, physical (pH, oxygen), and biochemical sensor onto microfluidics. These innovations can greatly improve the ability to monitor long term culture responses of organ-on-a-chip of disease, drug effects and evaluating drug delivery system.

Organ-on-chips can lay the ground for the future personalized nanomedicine, with the integration of patients-derived cells. The true meaning of personalized therapy can be achieved by microfluidics including both personalized organ-on-a-chip and personalized drug delivery system.

5. Challenges and future perspective

Novel drug delivery nanoparticle development is an interdisciplinary effort at the intersection of engineering, biology, chemistry, medicine, and material science. The use of microfluidics for the fabrication, characterization, and evaluation of nanoparticle drug delivery platforms can improve both the controllability and reproducibility, and the efficiency of preclinical studies [212]. These technologies will enable the robust supply of highly reproducible particles to the entire development process and therefore increase its chance of the successful clinical transitions.

An ideal drug delivery system is biocompatible, biodegradable, shelf-stable, and easy to produce, while possessing a high payload capacity and targeting efficacy. Microfluidic fabrication methods have the potential to circumvent many inefficiencies and bottlenecks in scalability and batch variability controls associated with traditional bulk synthesis. Therefore, microfluidic technology for drug delivery particle fabrication is key to the successful translation of a drug delivery particle from laboratory to the clinic.

Microfluidic drug delivery particle fabrication has vastly improved the tunability of mixing precursor reagents and simplified the modular inclusion of imaging agents, therapeutic compounds, and targeting ligands to form a multifunctional multicomponent platform [128]. One main challenge in microfluidic particle synthesis is the optimization of a modular platform that maintains certain physicochemical properties [5]. Since computational fluid dynamics allows simulation of mixing flow patterns or particle formation, it can be applied in a wide range of drug delivery fabrication. For example, as aforementioned, in the design of the parallelization (towards industrial-scale), microfluidic modules should be well designed to avoid secondary flows, which can lead problems in the main bulk flow streams. In context of scale-up production, computational fluid dynamics is a valuable tool for optimizing these demanding processes.

In addition, there is the most recurring issues in microfluidics - air bubbles. Because of the microscale dimensions of the channel, air bubbles can be very difficult to remove and be detrimental for the experiments. The origin of bubbles inside microfluidic devices can be start of the experiment, fluid loading, porous materials, leaking issue and dissolved gas. Indeed, unexpected disturbance due to air bubble formation in microfluidic device need to be resolved, as this increases not only

increases the hydraulic resistance within the channel but also leads to an inaccurate fabrication and characterization of drug delivery system.

Another key feature of microfluidics in drug delivery system applications is the ability to evaluate drug delivery particles using organ-on-a-chip technologies. Microfluidic platforms can incorporate 3D cell culturing and stem cell technologies to engineer *in vivo* like tissues to be used for drug testing [112,213,214]. Coculturing multiple cell types within engineered microenvironments can potentially reconstitute specific organ-like functional tissues capable of giving biologically relevant test data in real time. These models can address the key challenges of conventional platforms and enable the reconstitution of increasingly complex and realistic microenvironments.

Improvements in realistic models may be achieved through the incorporation of increasingly sophisticated microenvironmental control methods at the cost of additional complexity. Complexity poses a myriad of problems which must be addressed if the benefits of producing sophisticated and faithful reconstituted tissues are to outweigh the costs associated with complexity related platform operation difficulties and additional potential variables in results interpretation. Difficulties inherent to increases in sophistication can be observed in the relationship between conventional 2D cell culture assays and microfluidic 3D tissue culture platforms.

Microfluidic tissue culture platforms are a step forward from 2D cell culture in terms of *in vivo* like microenvironmental generation, and are capable of patterning multiple types of cells into heterogeneous, polarized tissues resembling physiologically relevant *in vivo* counterparts. Microfluidic tissue culture platforms accomplish microenvironmental engineering through sophisticated designs using micropatterns, ECM arrangements, targeted delivery of growth and chemotactic factor gradients, compartmentalized and targeted delivery of multiple media types, *in vivo* like cellular patterning, and many other features. As sophisticated platforms, microfluidic tissue culture devices are capable of delivering *in vivo* like, more physiologically relevant tissue and cellular responses compared to those derived from 2D macrofluidic cultures, at the cost of increased cost and difficulty associated with producing and operating microfluidic devices. Consisting of microchannels, chambers, and other micropatterned structures, microfluidic platforms require more resources, time, and specialized infrastructure to produce than simple 2D devices. In general, fabrication costs and difficulties directly increase with sophistication.

The relatively larger number of handling processes in tissue cultures in inducing microphysiological conditions over 2D cell cultures also introduces heightened technical and skill requirements to proficiently utilize tissue culture platforms. Where 2D microtiter and dish cultures are generally employed as simple assays to report simple quantitative or qualitative values such as individual cell viability through optimized and easy to interpret, multiplexable reporting methods, tissue culture platform experimental results can require analysis of much more complex tissue level physiological responses across a broad range range of possible multiscale morphological reactions. The combination of many engineered factors and environmental conditions compound into a drastic increase in degrees of freedom associated with both the input and output of microfluidic tissue culture platforms, compared to simple 2D cultures. The comparatively higher degrees of freedom in cultivating 3D tissues translates to increased challenges in developing and executing controlled protocols, as well as additional levels of complexity in experimental results; In practice, 3D tissue culturing methods and results quantification metrics are less standardized and defined, compared to 2D alternatives.

Ultimately, increases in sophistication will translate to similar exacerbations of fabrication, utilization, and interpretation challenges within microfluidics. Microfluidic platforms, as they are now, also face many challenges which must be overcome before large scale implementation on the industrial scale. Issues concerning mass producibility and high throughput screening compatibility are some key features addressed by initiatives to improve adoption.

Many of the microfluidic platforms discussed in this publication are based on soft lithographic PDMS designs that were developed and fabricated in-house or procured from limited fabrication runs from an associated institution. Initially developed as a means of rapid prototyping, soft lithography is ideal for microfabrication at the pilot scale, with low per-design initial investment for producing a limited run. Although soft lithography enables the production of a novel functional microfluidic design in PDMS within 24 h of drafting, the method utilizes fragile silicon wafer molds and heavily bottlenecked PDMS casting steps which limits the scalability of higher throughput fabrication. To address the larger scale production inefficiencies inherent to soft lithographic microfabrication, many commercial and academic ventures such as Emulate, MIMETAS, and Curiochips have looked to comparatively more upscalable manufacturing techniques such as injection molding and 3D printing for microfluidic device production. Incorporating large scale manufacturing practices into microfluidic chip production is a significant step towards ensuring that industrial scale demands can be supplied.

Development efforts to scale up production capabilities have also enabled a greater degree of quality control and form factor standardization for high throughput compatibility. Many conventionally produced PDMS microfluidic devices are punched manually or punched in a device-specific configuration, producing distinct form factors and input/output ports which may vary significantly even within devices of the same design. The implementation of standardized, mass production friendly templates produced in large, uniform batches enables the possibility of automated handling through existing microtiter plate HTS infrastructure.

While the forefront of academic microfluidic tissue platform research continues to specialize towards increasingly sophisticated designs for higher fidelity *in vivo* like reconstitution, development for industrial applications have focused on simplifying designs in favor of flexible platforms that are simultaneously easier to use and are capable of applying to a wider range of tissues. Although the strategy of simplification sacrifices aspects of realistic tissue generation, a balance between lesser but still meaningful levels of realism and greater accessibility for a wider user base may prove instrumental for industrial scale adoption. Although organ-on-a-chip technology is still in its infancy, it will surely continue to expand to provide physiologically/pathologically relevant microenvironments to evaluate multifunctional drug delivery particles in the near future.

6. Concluding remarks

In summary, microfluidics can produce drug delivery particles in a well-controlled, reproducible and high-throughput manner, as well as characterizing and validating particle performance in reconstituted 3D physiologically relevant microenvironmental conditions. Microfluidics have the potential to mimic the characteristics of human organs and may enable scientists to predict the safety and efficacy of therapeutic drug candidates prior to clinical phase studies. Furthermore, organ-on-a-chip systems can spatiotemporally manipulate 3D extracellular environments through the utilization of microfabrication, miniaturization and controlled engineering techniques. The integration of microfluidics in nanoparticle drug delivery testing will prove to be a key factor in the acceleration of pre-clinical phase trials, thereby further optimizing the time and resources required to bring drugs to market.

Acknowledgements

This study was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning NRF-2015R1A2A1A09005662, 2016R1A4A1010796 (N.L.J.), and also supported by the American Heart Association National Scientist Development Grant 15SDG25080314 (Y.

K.) and by the National Science Foundation CAREER award CMMI 1653006 (Y.K.), and the Institute of Engineering Research at Seoul National University provided research facilities for this work.

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