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Microfluidics in Nanomedicine

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Keywords

Microfluidics
The science and technology that involves the manipulation of nanoscale amounts of fluids in microscale fluidic channels for applications that include chemical synthesis, and biological analysis and engineering.

Nanotechnology
The manipulation of matter on atomic and molecular scales.

Nanomedicine
The medical application of nanotechnology for the advanced diagnosis, treatment and prevention of a number of diseases.

Biomimetic microsystem
A microscale device that mimics biological systems and is used to probe complex human problems.

Clinical translation
Clinical translation involves the application of discoveries made in the laboratory to diagnostic tools, medicines, procedures, policies and education, in order to improve the health of individuals and the community.

Nanomedicine is the medical application of nanotechnology for the treatment and prevention of major ailments, including cancer and cardiovascular diseases. Despite the progress and potential of nanomedicines, many such materials fail to reach clinical trials due to critical challenges that include poor reproducibility in high-volume production that have led to failure in animal studies and clinical trials. Recent approaches using microfluidic technology have provided emerging platforms with great potential to accelerate the clinical translation of nanomedicine. Microfluidic technologies for nanomedicine development are reviewed in this chapter, together with a detailed discussion of microfluidic assembly, characterization and evaluation of nanomedicine, and a description of current challenges and future prospects.

1 Introduction

Nanomedicine is the medical application of nanotechnology that uses engineered nanomaterials for the robust delivery of therapeutic and diagnostic agents in the advanced treatment of many diseases, including cancer [1–3], atherosclerosis [4–6], diabetes [7–9], pulmonary diseases [10, 11] and disorders of the central nervous system [12, 13]. One key advantage of nanomedicine is the ability to deliver poorly water-soluble drugs [14–16] or plasma-sensitive nucleic acids (e.g., small interfering (si)RNA [17, 18]) into

the circulation with enhanced stability. Nanomedicine is also capable of providing contrast agents for different imaging modalities and the targeting of specific sites for the delivery of drugs and/or genes [19–23]. Engineered nanomaterials, developed as particulates that are widely referred to as nanoparticles (NPs), have been formulated using a variety of materials that includes lipids, polymers, inorganic nanocrystals, carbon nanotubes, proteins, and DNA origami [24–36]. The ultimate goal of nanomedicine is to achieve a robust, targeted delivery of complex assemblies that contain sufficient amounts of multiple therapeutic and diagnostic agents for highly localized drug release, but with no adverse side effects [37, 38], and a reliable detection of any site-specific therapeutic response [39, 40].

1.1 Nanomedicine Development

Typical nanomedicine development processes for the clinical translation include benchtop syntheses, characterizations, *in-vitro* evaluations, *in-vivo* evaluations with animal models, and scaled-up production in readiness for clinical trials. Although, previously, several NPs have been reported as superior platforms, many are still far from their first stages of patient clinical trials due to several critical challenges [41, 42]. Such challenges mainly result from batch-to-batch variations of NPs produced in the benchtop synthesis process, and from insignificant outcomes in the *in-vitro* evaluation process under physiologically irrelevant conditions. These limitations ultimately lead to highly variable results in the *in-vivo* evaluation, or to failure in clinical trials. In order to address these challenges, the following methodologies need to be established in the nanomedicine development process:

- Nanomedicine needs to be continuously produced in a high-throughput fashion. The large-scale, continuous production of nanomedicines will allow a robust supply of highly reproducible materials for the *in-vitro* and *in-vivo* evaluation stages and clinical trials, ultimately increasing the success rate in clinical trials.
- Nanomedicines synthesized using large-scale, continuous production methods also need to be characterized in a high-throughput manner. Rapid characterization will create an efficient production cycle for an optimized nanomedicine via feedback loops between the synthesis and characterization stages.
- The *in-vitro* evaluation of nanomedicine must be conducted in more physiologically relevant environments. Highly repeatable results obtained from these biomimetic conditions will allow the obviation of a number of simple screening experiments in animal studies, not only saving costly animal models but also accelerating the clinical translation.

1.2 Microfluidics Technology

Microfluidics technology provides highly compatible platforms to create a new nanomedicine development pipelines that include the required methodologies introduced above. Basically, microfluidics presents a number of useful capabilities to manipulate very small quantities of samples, and to detect substances with a high resolution for a wide range of applications, including chemical syntheses [43, 44] and biological analysis [45, 46]. More importantly, the adaptability of microfluidics allows its integration

with many other technologies, such as micro/nanofabrication, electronics, and feedback control systems [47–52]. Recently, microfluidic platforms integrated with control systems and advanced microfabrication technologies have been used to address the critical challenges in nanomedicine [53–57]. For example, the continuous synthesis of NPs in microfluidics has demonstrated a versatility to produce a variety of NPs with different sizes, shapes, and surface compositions [58, 59]. Several advances have recently been made in the label-free detection, characterization and identification of single NPs [60]. The confluence of microfluidics and biomimetic design has enabled the creation of physiologically relevant microenvironments for the evaluation of drug candidates [61–63]. The key microfluidic technologies in nanomedicine, including microfluidic assembly, and the characterization and evaluation of nanomedicines, are discussed in the following sections (see Fig. 1), and their current challenges and future research directions are highlighted.

2 Microfluidic Assembly of Nanomedicines

The bulk synthesis of NPs typically has strong dependencies on nonstandard multistep processes which are time-consuming, difficult to scale up, and depend heavily on specific synthetic

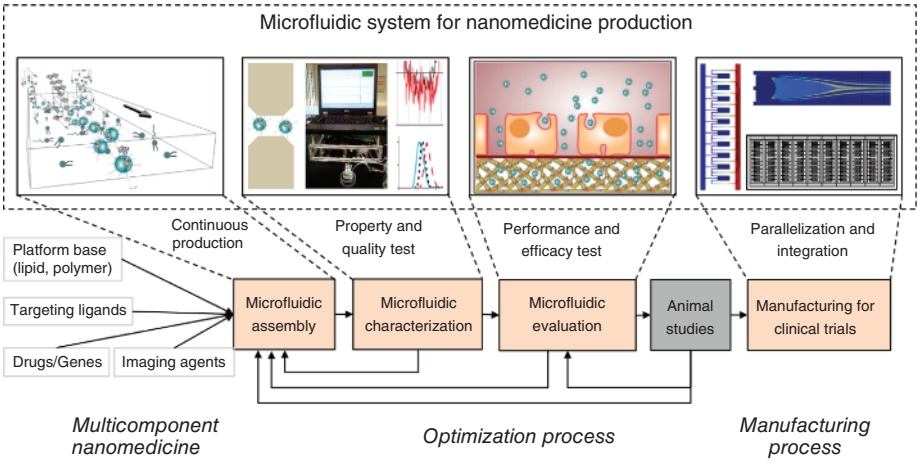


Fig. 1 A new nanomedicine development pipeline using microfluidic systems. First, a designed nanomedicine with multiple precursor components is continuously assembled through controlled strong mixing patterns in the Microfluidic Assembly stage, and the properties of the nanomedicine produced are identified at the Microfluidic Characterization stage. Only if those properties meet the nanomedicine design criteria will the performance and efficacy of a selected nanomedicine be evaluated in *in-vitro* biomimetic microsystems that recapitulate the structure and function of human organs in the Microfluidic Evaluation stage. If the targeting, therapeutic, and imaging efficacies are satisfactory in the *in-vitro* model system, the nanomedicine will then be validated with animal models. All nanomedicine candidates that are unsuccessful at the above stage will be reformulated in the Microfluidic Assembly stage and go through the iterative processes. If successful in animal models, the selected nanomedicine will then be manufactured through parallelized microfluidic platforms. The pressure and flow patterns in the integrated microfluidic system are regulated by high-precision control systems.

conditions in the laboratory. This reliance of NPs on such nonstandard multistep processes inevitably causes high batch-to-batch variations in their physico-chemical properties [64–69]. Batch size is also subject to custom protocols that vary among laboratories, leading to difficulties in screening and identifying optimal NP physico-chemical characteristics for enhanced drug delivery. Furthermore, the introduction and combination of multiple materials for creating multicomponent NPs compromises the expected functionality of the individual elements. This is largely because of an inability to precisely control the continuous assembly process in various conventional bulk syntheses that involve the macroscopic mixing of precursor solutions [58, 70]. As the micrometer- and nanometer-scale interactions of precursors will direct the characteristics of NPs, it is essential that their composition is fine-tuned in order to attain the anticipated functionalities of multicomponent NP assemblies. In general, the central challenge for the synthesis of multicomponent NPs is to establish large-scale and continuous manufacturing methodologies with high reproducibility.

Amphiphilic blocks self-assemble spontaneously into NPs through size-dependent formation mechanisms and on timescales governed by diffusion [71]. The physico-chemical properties of NPs are, at least in part, determined by the timescales at which the multiple solutions mix in the system [72], as well as the thermodynamic characteristics of the block polymers [73]. Thus, a mixing timescale that is longer than the characteristic time for chemical chain formation will result in an uncontrolled aggregation due to incomplete solvent change. Conversely, a complete solvent change through shorter mixing times in rapid precipitations can result in stable assembly kinetics that lead to the production of homogeneous NPs [74]. One critical difference between conventional bulk synthesis and microfluidic assembly is the mixing time, which occurs on the order of seconds in bulk synthesis and contrasts with those in the millisecond and microsecond range in microfluidic assembly [75]. This shorter mixing time results in more homogeneous NPs by reducing the aggregation of precursors, leading to high reproducibility, which in turn prevents the subsequent thermal and mechanical agitation needed in conventional bulk synthesis for NP homogenization. Therefore, a precise control of microfluidic flow patterns with tunable characteristic mixing times will offer a better understanding of the effect of the mixing time on NP reproducibility and homogeneity.

Microfluidic technologies have demonstrated a better control over effective mixing of the precursor solutions for assembling a range of NP types (Fig. 2a) when compared to conventional bulk methods, due to the larger contact surface areas given per unit volume of fluid in microfluidics [58, 70]. For example, typical laminar flows in microfluidics enabled the controlled syntheses of several NPs (Fig. 2b), including liposomes [76–78] and polymeric NPs [75, 79, 80], with a narrower size distribution compared to those of conventional bulk synthesis. Under laminar flow conditions at a low Reynolds number (~1),[1)] mixing occurs only through diffusion across the interface between two miscible fluids moving next to each other in viscous flows. Unfortunately, NP synthesis by diffusive mixing does not allow for the development

[1)] The Reynold's number is a dimensionless number that provides a ratio of inertial to viscous forces to quantify the relative importance of these two types of force for given flow conditions.

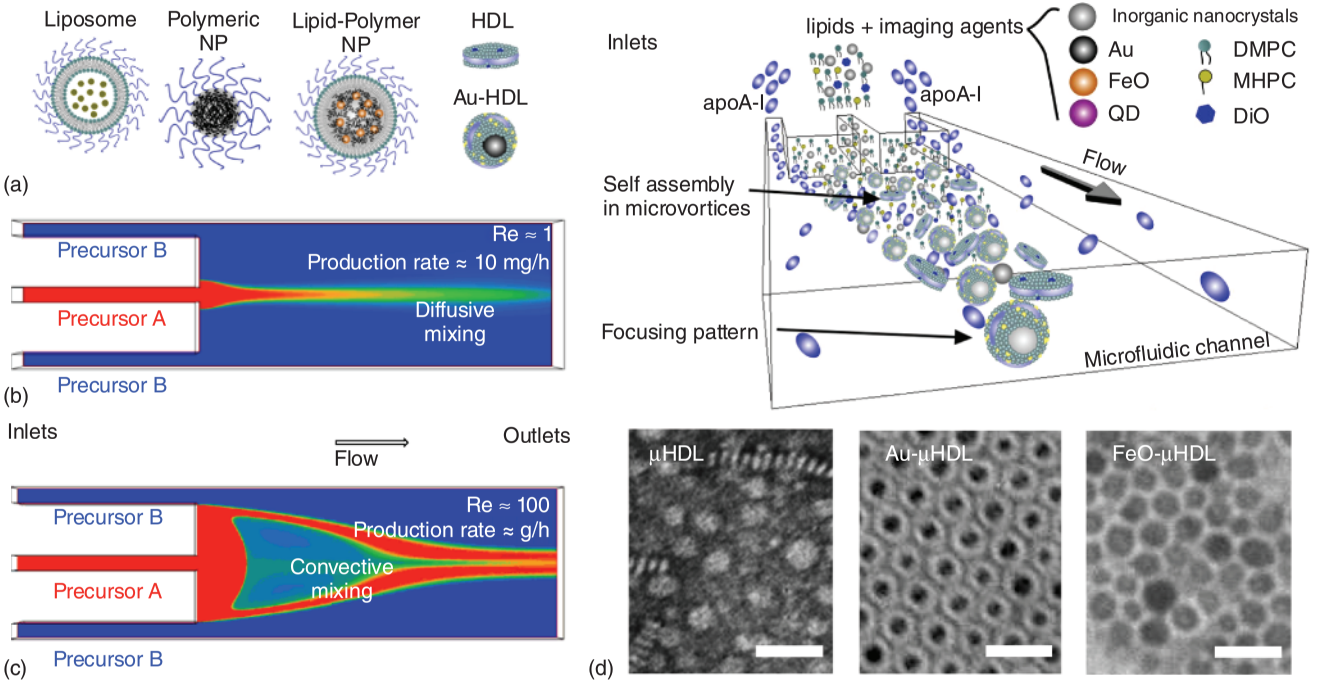


Fig. 2 (a) Representative nanomedicine types (liposome, polymeric NP, lipid-polymer NP, and high-density lipoprotein (HDL)) that have been assembled by microfluidic technology; (b) A microfluidic channel that creates diffusive mixing across laminar flow interfaces at Re ~ 1; (c) A microfluidic channel that creates convective mixing between two precursors at Re ~ 100; (d) Microfluidic reconstitution of HDL (referred to as ■ μHDL) with inorganic nanocrystals, such as gold (Au) and iron oxide (FeO), using controlled microvortices. The transmission electron microscopy images show μHDL, Au-μHDL, and FeO-μHDL (scale bars = 20 nm). Reproduced with permission from Ref [100].

of materials such as lipid–polymer hybrid NPs [81], which require a strong mixing of solutions in the aqueous and organic phases. Lipid–polymer hybrid NPs have shown a higher drug loading within the polymer core and a slower drug release due to the lipid shell when compared to pure polymeric NPs [82, 83]. Furthermore, the diffusive mixing required is difficult to scale up and ultimately leads to a limited controllability of the precursor mixing time, thereby restricting NP homogeneity and leading to a low-throughput production of NPs.

One approach to facilitate precursor mixing (i.e., shortening the mixing times) is to use convective mixing, thereby increasing the interfacial surface area between fluids and reducing the diffusion length scales. Whereas, conventional microfluidic systems exploit easy-to-control flow patterns, which are strictly laminar at low Re values (~1), an increase in Re ($10 < Re < 30$) generates complex flow patterns under a variety of geometric conditions of the microfluidic channel, such as local microvortices and flow separation due to an increase in inertial forces [84–86]. In order to implement convective mixing in microfluidic devices, microfluidic platforms have been designed for the rapid mixing of fluids using relatively higher inertial forces in localized regions with moderate Re values ($10 < Re < 100$) [87–93]. Furthermore, microvortices have demonstrated the ability to rapidly manipulate, sort, and excite particles in microfluidics [94–97]. Recently, a new generation of three-dimensional (3D) focusing patterns in a simple, single-layer microfluidic channel has allowed the development of a pattern-controllable microvortex platform (Fig. 2c). This device has been used for the highly reproducible synthesis of lipid–polymer hybrid NPs with multiple drugs and imaging agents [98, 99], and multifunctional high-density lipoprotein-derived NPs [100] with high productivity (up to $1\,g\,h^{-1}$) (Fig. 2d).

3 Microfluidic Characterization of Nanomedicines

The most important properties of NPs to be characterized before probing their interaction with biological systems are size, shape, surface chemistry/charge, and stability. The development of novel NP characterization tools will impact heavily on nanomedicine, as the lack of characterization standards and quality control tools for NPs has inhibited their clinical adoption to date. One practical obstacle to the clinical-scale commercialization of NPs is an inability to certify the stability of formulations, as even small property variations will have significant effects on *in-vivo* distribution, causing unpredictable therapy outcomes. Recently, several studies have been conducted on NP quality evaluation in microfluidics. For example, a rapid liposome quality assessment in microfluidics allows for quantitative results on liposome formulation composition and stability using dielectric spectroscopy and multivariate data analysis methods [101]. Instantaneous immobilization by ultrarapid cooling in microfluidics reveals the formation of nonequilibrium liposomes in detail [102]. Yet, in spite of recent advances in the label-free characterization of single NPs [60], it remains difficult to effectively integrate a high-throughput microfluidic technology capable of detecting NPs (or their motion) with currently available characterization equipment that includes dynamic light scattering (DLS) [103], transmission electron microscopy

(TEM) [104], atomic force microscopy (AFM) [105], Auger electron spectroscopy (AES) [106], nuclear magnetic resonance (NMR) [107], and flow cytometry [108].

Meanwhile, recent advances in nanofabrication and microfluidics have allowed for the development of high-throughput devices capable of characterizing NP properties, including size and surface charge. The most common electrical technique is to probe impedance changes in nanowire-embedded microfluidic and nanofluidic

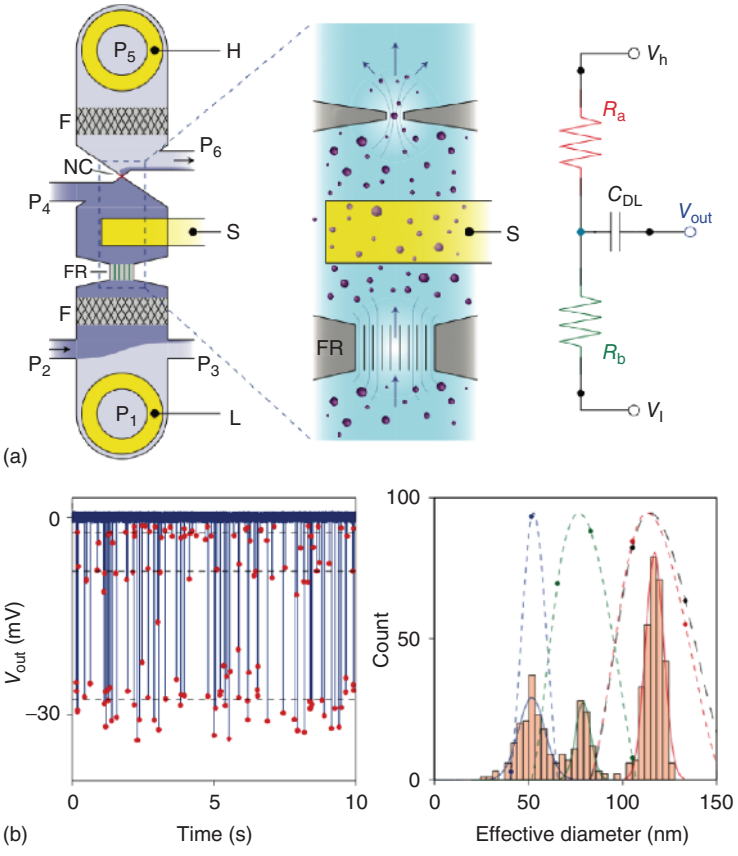


Fig. 3 (a) Overall device layout (left) depicting the electrical and fluidic components: external voltage bias electrodes (H, L) and sensing electrode (S); embedded filters (F); fluid resistor (FR); nanoconstriction (NC); pressure-regulated fluidic ports (P_1~P_6). The nanoparticle (NP) suspension enters at P2 and exits at P6. A detailed image of the dashed box area in (a) (middle) shows the key sensing parts. While NPs flow in the direction of the arrows, changes in the electrical potential in the NC are detected by the electrode S. Electrical circuit expression of the device (right): a constant bias voltage V_h (V_l); Resistors R_a and R_b represent the resistance of the nanoconstriction and the fluidic resistor, respectively; (b) An example analysis of a NP mixture with polydispersity. Left: Output voltage over time for a mixture of NPs of different diameters. Events marked with red circles cluster around three values of V (horizontal dashed black lines). Right: Histogram of effective diameters (40 s measurements). Reproduced with permission from Ref. [117].

channels, and to detect any perturbation in the local electrical properties of the nanowires in response to disturbance by NP solutions. For example, nanowire field-effect transistors allowed for the real-time sensitive detection of label-free molecules [109–111]. A combination of these technologies with nanoscale mechanical systems offers real-time, high-precision, single-molecule/NP/cell detectors, such as advanced mass spectrometry [112] and microfluidic and nanofluidic channel resonators [113–115].

With recent advances in the fundamental physical chemistry of nanoscale pore sensors, several pore-based sensors have been developed in the nanoscale range, offering a rapid and specific, yet simple, biosensing strategy with an improved measurement sensitivity over a wide particle size range [116]. An example of this is a high-throughput microfluidic analyzer that has been developed to detect and characterize unlabeled NPs in a multicomponent mixture at a rate of 500 000 particles per second (Fig. 3) [117]. In this case, a real-time single-nucleotide detection of a model G487A mutation (which is responsible for glucose-6-phosphate dehydrogenase deficiency) was achieved by leveraging the *in-situ* reaction-monitoring capability of the nanopore platform [118]. Tunable pore sensors, which can elastically adjust the size of their pore, have been used to count and detect the size and concentration of smaller NPs compared to other techniques such as flow cytometry [119]. This platform also allows for a simultaneous extraction of the size and zeta-potential of NPs from their charge density, under electrophoretic forces [120]. NP translocation was also detected using a pressure-reversal technique through a cone-shaped nanopore membrane [121]. Today, these approaches, all of which employ size-tunable pore sensors, are starting to provide a better understanding of the fundamental behavior of NPs, as well as a high-throughput characterization of their properties.

4 Microfluidic Evaluation of Nanomedicines

Nanomedicines needs to be nontoxic, biodegradable, sufficiently stable to be delivered to targeted sites, and to have a superior therapeutic advantage over the free drug [122, 123]. Conventionally, nanomedicine evaluation has been made in static cell culture plates, but unfortunately this neglects the important effects of flowing conditions and subsequent transport phenomena on the microenvironment. In contrast to static conditions, flowing conditions assist in the homogeneous distribution of NPs with no gravitational sedimentation, which is similar to the physiological conditions encountered *in vivo*. For example, microfluidic approaches have been used to measure the cytotoxicity of quantum dots (QDs) in a flowing condition [124, 125] and to examine the stability of multicomponent NPs across a laminar flow interface [126]. Compared to tests conducted in conventional static plates, these approaches have provided a more accurate approximation of nanomedicine performance *in vivo*. Microfluidic approaches were also used to evaluate the selective binding of NPs to cells while varying the fluid shear stress, the targeting ligand concentration, receptor expression on target cells, and NP size [127, 128]. The targeted delivery of a nanomedicine represents a powerful technology for the development of safer and more effective therapeutics compared to systemic delivery by nontargeted formulations [83]. Indeed, such approaches show

that the targeting performance of an NP can be examined under more physiologically relevant conditions, which is preferential to examining a wide array of cell–particle interactions prior to *in-vivo* experiments. The accurate detection of biomarkers also holds significant promise for "personalized" cancer diagnostics, with more physiologically relevant 3D platforms having been developed for identifying and validating ubiquitous biomarkers [129].

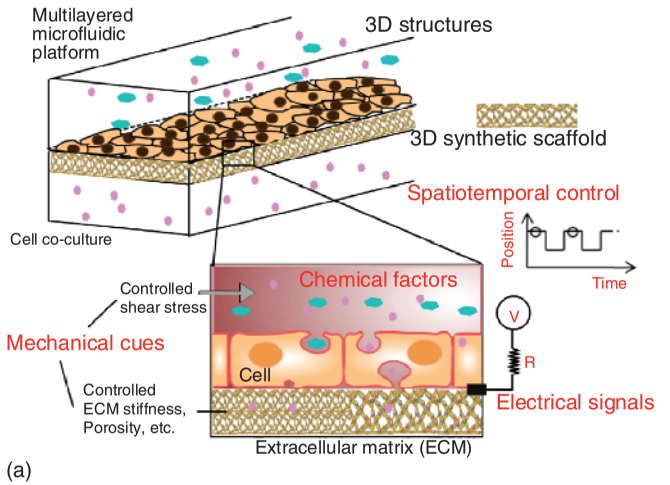
4.1 Mimicking Physiological Environments

Today, an increasing number of engineered NPs requires a reliable high-throughput screening methodology with more physiologically accurate conditions. Whereas, microfluidic approaches have demonstrated the potential to closely approximate physiological environments, current preclinical studies on drug candidates mostly rely on costly and highly variable animal models, mainly because existing cell culture models fail to recapitulate the organ-level pathophysiology of humans. This lack of accurate predictive models highlights the need for better approaches to mimic the structure and function of cells, tissues and organs, as well as the dynamically changing environments *in vivo*. Recently, the evolution of microfluidics has witnessed the integration of *in-vitro* cellular approaches onto chips, which allow real-time, *in-vitro* microscopic observations to be made as well as an evaluation of cell function [130]. But, in order to probe the targeting, therapeutic and diagnostic efficacy of NPs in spatially and temporally regulated environments, it is important first to examine how the NPs interact with cells, tissues and organisms under more physiologically realistic conditions [131, 132]. Consequently, the microfluidic approaches for replicating organ-level structure and function will be discussed at this point (Fig. 4a) [133], and current applications and potentials for the *in-vitro* evaluation of nanomedicines highlighted.

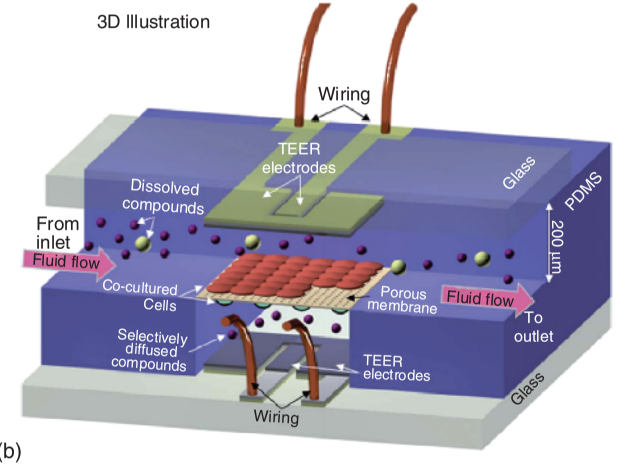
4.2 Endothelial Cell Systems

The vascular endothelium is a crucial target for therapeutic intervention in pathological processes that include inflammation, atherosclerosis, and thrombosis. Endothelial cells exist under dynamically changing mechanical stresses that are generated by blood flow patterns.

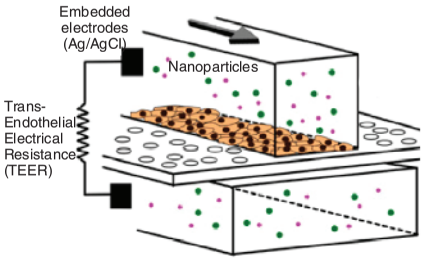
Fig. 4 (a) Schematic of microengineered biomimetic systems with spatiotemporal control over physiological effectors, including mechanical cues, chemical factors, electrical signals, multilayered platform with 3D scaffold; (b) Schematic depiction of a biomimetic model that mimics the function of the blood–brain barrier. b.END3 brain endothelial cells and C8-D1A astrocyte cells are cultured on either side of a porous membrane between two microfluidic flow chambers; (c) Schematic of a microfluidic model that mimics the permeable endothelium in artery-surrounding microvessels. Permeability is detected using microelectrodes embedded in the chip. The fluorescent image shows the disrupted endothelial connections. Adherens junctions are shown in green, and nuclei in blue (scale bar = 20 μm). Schematic and TEM image of nanoparticles used for NP translocation studies in the chip (scale bar = 100 nm); (d) Schematic of a lung-on-a-chip device showing IL-2-induced pulmonary edema (scale bar in contrast image = 200 μm). The graph shows barrier permeability in response to IL-2, with and without cyclic strain. Error bars indicate SEM. The fluorescent images show that immunostaining of epithelial occulidin (green) and vascular endothelial cadherin (VE-cadherin; red) with 10% strain with and without IL-2 (scale bars = 30 μm). Reproduced with permission from Refs [133, 143, 149, 152].

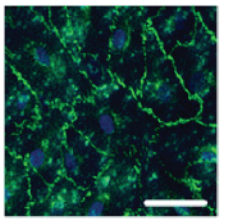

Multilayered microfluidic platform
3D structures
3D synthetic scaffold
Spatiotemporal control
Position
Time
Cell co-culture
Controlled shear stress
Chemical factors
Mechanical cues
Cell
V
R
Controlled ECM stiffness, Porosity, etc.
Electrical signals
Extracellular matrix (ECM)

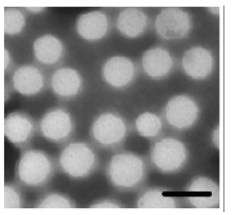
(a)

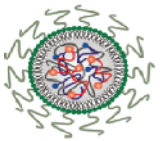

3D Illustration
Wiring
TEER electrodes
Glass
PDMS
Dissolved compounds
From inlet
Fluid flow
200 μm
Co-cultured Cells
Porous membrane
Fluid flow
To outlet
Selectively diffused compounds
TEER electrodes
Glass
Wiring

(b)


Embedded electrodes (Ag/AgCl)
Nanoparticles
Trans-Endothelial Electrical Resistance (TEER)

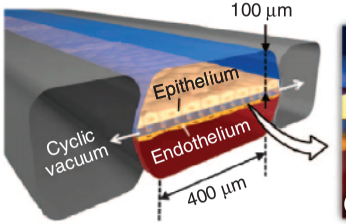


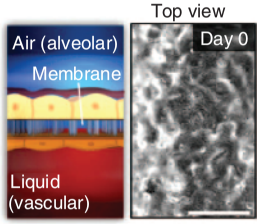


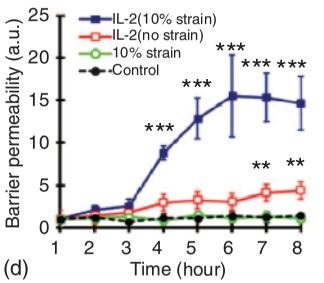


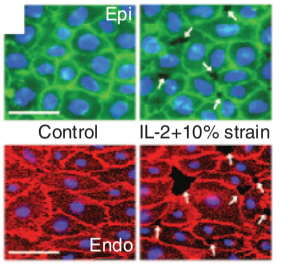

Au-PLGA
PLGA
Cy5.5-PLGA
DSPC
DSPC-PEG2000

(c)


100 μm
Epithelium
Cyclic vacuum
Endothelium
400 μm


Top view
Air (alveolar)
Membrane
Liquid (vascular)
Day 0


IL-2(10% strain)
IL-2(no strain)
10% strain
Control
Barrier permeability (a.u.)
Time (hour)


Epi
Control
IL-2+10% strain
Endo

(d)

Yet, endothelial cell monolayers cultured in conventional multiwell plates fail to reproduce the complex architecture of a vascular network *in vivo* and thus fail to capture the relationship between shear stress experienced by the cells and the local concentration of the drug used. Recent developments in microengineered vascular systems have shown the potential for evaluating nanomedicines under physiologically realistic conditions. For example, replicating the structure and function of blood vessels *in vitro* can be helpful for investigating NP behavior and interaction in and around the targeted sites [134–138]. An accurate reconstitution of the geometric configuration of natural blood vessels is also important, as the interactive effects between blood flow and drug concentration were not captured by a rectangular channel coated with endothelial cells. Rather, a branching network with tubular channels was constructed in order to reproduce these effects [139]. In addition, microvessels supported by the extracellular matrix (ECM), when patterned in a tubular structure, establish the endothelial monolayer, maintain permeability, and are not prone to delamination (which was relatively common in the rectangular channels). The rectangular channels were also very susceptible to delamination that disrupted local permeability, due mainly to the poor connections between the endothelial cells and ECM at the sharp corners [140, 141].

Several additional methods to reproduce microvessels accommodating multiple cells (endothelial cells, pericytes, and astrocytes) have allowed the development of microfluidic models of the blood–brain barrier (BBB) (Fig. 4b) [142–148], as well as for endothelial dysfunction and permeability control in atherosclerosis (Fig. 4c) [149–151].

4.3 "Organ-On-A-Chip"

In combining microfabrication techniques with tissue engineering, the "lung-on-a-chip" device offered a novel *in-vitro* approach to drug screening by mimicking the mechanical and biochemical activities of the human lung (Fig. 4d) [152]. For example, a recent study using this device revealed that mechanical strains associated with physiological breathing movements play an essential role in the development of the increased vascular leakage which leads to pulmonary edema, and that circulating immune cells are not necessary for this disease to develop. The same studies also led to the identification of potential new therapeutics, including angiopoietin-1 (Ang-1) and a new transient receptor potential vanilloid 4 (TRPV4) ion channel inhibitor (GSK2193874), which might prevent the severe toxicity associated with interleukin (IL)-2 therapy. An *in-vitro* model of the intestine has also been developed, together with its crucial microbial symbionts [153, 154]. Whereas, previous *in-vitro* models of intestinal function depend on the use of epithelial cell lines (e.g., Caco-2 cells) which create polarized epithelial monolayers but fail to mimic human intestinal functions for drug development. The recent development of a "gut-on-a-chip" device recreated the gut microenvironment with low shear stress ($0.02\,\mathrm{dyne\,cm^{-2}}$) with cyclic strain (10%; 0.15 Hz) that mimicked physiological peristaltic motions. This precise regulation allowed for an increased exposure of the intestinal surface area and a robust 3D intestinal villi morphogenesis, which mimicked the enhanced cytochrome isoform-based drug-metabolizing activity and the absorptive efficiency of the human intestine.

4.4 Renal Toxicity and Hepatotoxicity

A further use of biomimetic microfluidic platforms for nanomedicine is to evaluate renal toxicity and hepatotoxicity in preclinical studies. Renal excretion represents a clearance pathway for the removal of molecules from vascular compartments, during which time the circulating NPs enter the glomerular capillary and undergo a size-dependent filtration. Those NPs smaller than the pore size of glomerular filtration (~5 nm) can be filtered to enter the proximal tubule, where the brush border of the epithelial cells is negatively charged. As a consequence, positively charged NPs are readily resorbed from the luminal space compared to the negatively charged NPs. The recent development of a microfluidic device lined by human kidney epithelial cells that can be exposed to a shear rate demonstrated a significant increase in albumin transport, glucose reabsorption and brush border alkaline phosphatase activity, all of which are crucial functions of the human kidney proximal tubule [155]. This approach also confirmed that cisplatin toxicity and Pgp efflux transporter activity detected on-chip more closely mimicked the *in-vivo* responses than those obtained with cells maintained on conventional culture plates.

It should be noted that any NPs which are not cleared via the kidney are excreted via the hepatobiliary system. The hepatocytes, which are referred to as potential sites for toxicity, play an important role in liver clearance through endocytosis and the enzymatic breakdown of NPs. As NPs between 10 and 20 nm in size are efficiently eliminated via the liver, any NPs designed in this size range must be modified in order to avoid their prolonged retention in the liver as they undergo excretion. Recently, microfluidic devices have been developed that reconstitute the function of the hepatocytes; for example, a 3D hepatocyte chip has been fabricated for *in-vitro* drug toxicity examinations aimed at predicting drug hepatotoxicity *in-vivo* [156]. This device allowed for the controlled delivery of multiple drug doses to functional primary hepatocytes, while an incorporated concentration gradient generator created *in vitro* dose-dependent drug responses in order to predict *in-vivo* hepatotoxicity.

4.5 Live Tissue Explants

The introduction of *ex-vivo* live tissue explants into microfluidics may provide additional physiological conditions to be investigated. For example, embryonic tissues excised from live frog embryos were used to examine dynamic responses to time-varying chemical stimuli (Fig. 5a) [157]. Carcinoma tumor biopsies were also introduced into a reproducible glass microfluidics system to study the tumor environment, thus offering a preclinical model for the creation of "personalized" treatment regimens [158]. The culturing of brain tissue slices on transistor arrays fabricated on silicon chips may also become a novel platform for neurophysiological and pharmacological studies. Typical microfluidics and semiconductor technologies can be integrated to produce high-resolution, planar transistor arrays for mimicking neuronal structures in long-term studies of topographic mapping [159], and also for mapping evoked extracellular field potentials in organotypic brain slices of rat hippocampus [160].

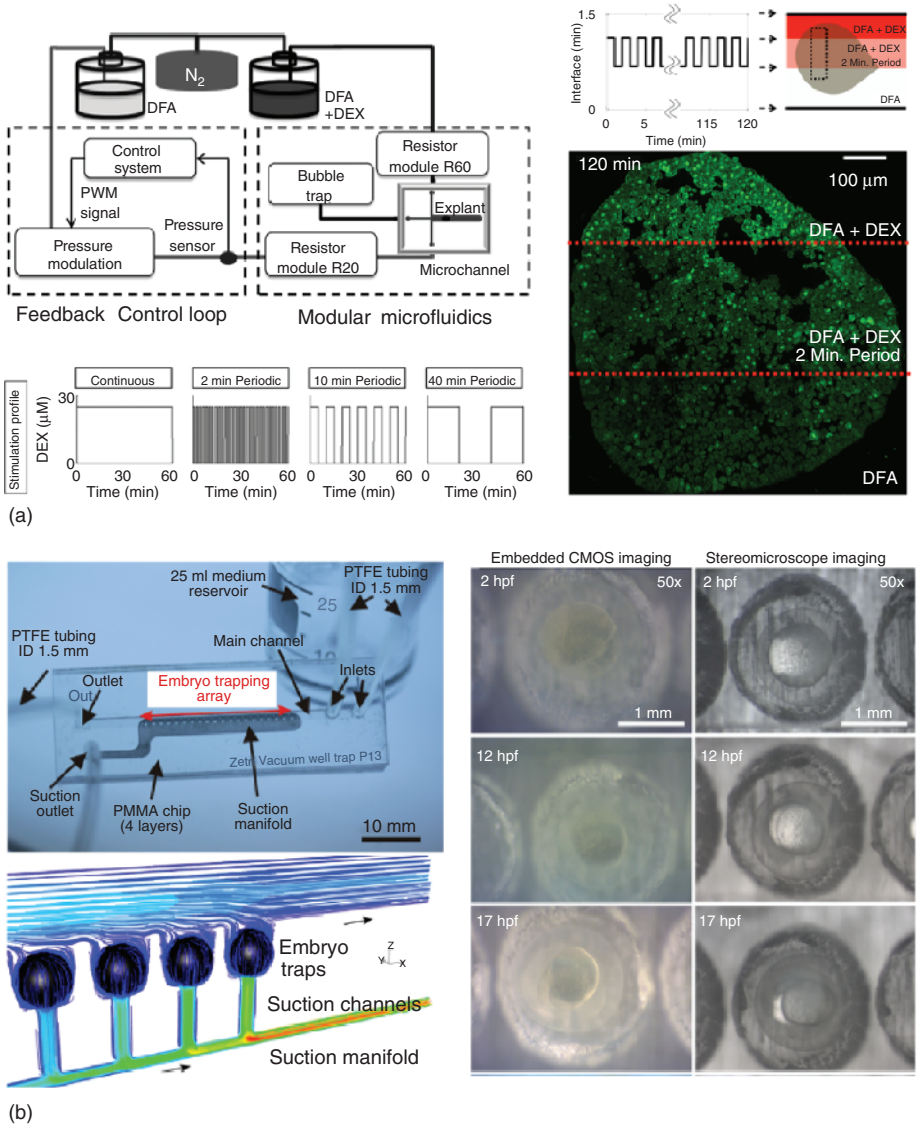


Fig. 5 (a) Schematic of feedback control system that allows for long-term culture of an embryonic tissue excised from a live frog embryo within a microfluidic channel to examine dynamic responses to spatially and temporally varying chemical stimuli. The fluorescent image show the distinct localized responses of an embryonic tissue to dynamic stimuli in a single tissue explant for 2 h, showing that stimulation with localized bursts versus continuous stimulation can result in highly distinct responses. This platform can be used to investigate the cell intercommunication in response to localized drug stimulation for toxicity tests; (b) Schematic of microfluidics-based chip integrating embedded electronic interfaces, and CFD simulation for the flow stream line prediction. Embryos cultured in the device that allowed for immobilization, culture, and treatment of developing zebrafish embryos for toxicity tests. Reproduced with permission from Refs [157, 167].

4.6 Intact Organisms

Small multicellular organisms such as nematodes, fruit flies, clawed frogs and zebrafish, allow for toxicological screening in the normal physiological environments of intact organisms, providing substantial advantages over cell lines and extracted tissues [161–163]. While the fully automated analysis of these model systems in a high-throughput manner remains challenging, the application of microfluidics to these model organisms has demonstrated the ability to handle multicellular organisms in an efficient manner and to precisely manipulate the local conditions to allow for the assessment and imaging of these small organisms [163–166]. For example, manipulating small organisms, such as the worm *Caenorhabditis elegans* , allowed the observation of neuronal responses in order to correlate the activity of sensory neurons with the worm's behavior *in vivo* [161]. Moreover, the integration of embedded electronic interfaces with microfluidic chip-based technologies allowed for the automatic immobilization, culture, and treatment of developing zebrafish embryos during fish embryo toxicity (FET) biotests (Fig. 5b) [167].

To capture the interactions between multiple organs on microfluidic chips would potentially enable a more accurate model of how organs function and interact with one another for potential drug development applications [168, 169]. For example, the combination of a mathematical model and a multiorgan approach provided a novel platform with improved predictability for testing the toxicity of an anticancer drug, 5-fluorouracil, in a pharmacokinetics-based manner [170]. These approaches can help to achieve a better insight into the mechanisms of action of drug candidates, perhaps leading to patient-specific therapies in the future [171]. While multiorgan chips have the potential to simulate human body functions for patient-specific point-of-care devices and therapies, the inherent complexity of each organ itself hinders the development of reliable "human-on-a-chip" model systems. For example, the practical challenges include an optimization of organ size, the control of fluid volumes, the maintenance of coupled organ systems, and the development of a universal blood substitute [172]. The key question for building multiorgan systems is how to simplify the organ complexity without losing physiological accuracy.

5 Challenges and Opportunities

A new nanomedicine development pipeline using microfluidics technologies includes microfluidic assembly, characterization, evaluation, and the manufacture of nanomedicines. These technologies will allow the robust supply of highly reproducible nanomedicines to the entire development process and thereby increase the success rates in clinical trials. In addition to the stages discussed above, microfluidics technology for nanomedicine manufacture is key to the successful translation of a nanomedicine from the laboratory to the clinic. A long-term vision for the manufacture of nanomedicines is to create reliable, continuous and scalable assembly methodologies for a variety of multifunctional NPs with high reproducibility, yield, and homogeneity [173]. The development of these assembly methods requires microfluidic approaches to allow for an efficient and strong mixing of

precursors, modular methods for incorporating multicomponents (e.g., therapeutic compounds, imaging agents, targeting ligands, etc.) into multifunctional NPs, and automated control systems for the large-scale integration and parallelization of microfluidic modules [117, 174]. The ability to integrate microfluidics with dynamics, control, and more complex microfabrication techniques opens the door for high-throughput, automated manufacturing.

A current challenge of nanomedicine manufacture using microfluidics is to optimize and maximize microfluidic devices with tunable mixing flow patterns that are applicable to the synthesis of a wide range of nanomedicine types, without losing the physico-chemical properties of the designed nanomedicine [175]. The key technologies required for the development of these nanomedicine manufacturing techniques are computational fluid dynamics to allow simulation of mixing flow patterns in microfluidic devices, highly reliable microfabrication techniques capable of integrating microscale pumps, valves and detecting sensors [176], and high-precision control systems that regulate parallelization and automation capability [177–180].

While the quantities of NPs synthesized by microfluidic devices are often in the microgram to milligram range, the parallelization of microfluidic channels has the potential to scale-up the synthesis by several orders of magnitude to a clinical scale of grams to kilograms. With parallel and stackable microfluidic systems, gram to kilogram scales of NPs could be prepared with the same properties as those prepared at the bench scale, as long as a precise control of either flow rates or pressure in the microfluidic platform is achieved. Pressure control is far better than flow control for controlling the flow rate into a microfluidic network because the flow rate is proportional to the inlet pressure, which can be easily measured for high bandwidth feedback control [181]. To maintain a precise control over fluid pressure with the potential to scale-up production, it is necessary to isolate the pressure-regulating mechanism from the fluid reservoir and the microfluidic device, so that larger reservoir volumes and diverse microfluidic devices can be used independently and integrated as needed. Three important features should be considered for robust and reliable parallelization:

- Fouled modules must be easily replaced or disconnected from other systems.
- Unexpected disturbances due to air bubble formation in microfluidic devices need to be compensated, as this increases the hydraulic resistance between neighboring devices in the parallelized network, leading to an imprecise regulation of the entire system.
- Bridging or networking channels that connect microfluidic modules need to be well designed with minimal secondary flows, which may affect the main bulk flow streams, leading to chip-to-chip variations.

While the microfluidic assembly of nanomedicine has demonstrated much progress using several platforms and various mixing patterns, practical development has been significantly constrained due to a lack of tools capable of detecting, characterizing, and analyzing NPs in a high-throughput manner. Although single NP detectors and characterization tools using microfluidics and nanofabrication technologies have been demonstrated, these are still limited to specific solutions and NP types and need to be generalized

in order to function with multicomponent NPs. In addition, there is a need for a technique allowing the easy preparation of highly concentrated NP samples to be developed, including the purification of toxic solvents via either separation or filtration. Furthermore, while many approaches have shown the promise of combining optical systems with microfluidics [60], the precise and reliable control of light delivery to targeted areas in microfluidic platforms remains an active topic of research and engineering. A combination of microfluidics and optics – termed optofluidics – has demonstrated a synergistic effect for new capabilities in several applications including lens, colloidal suspensions, and flow cytometry [182–185]. In addition, optofluidics technology could be employed to incorporate the microfluidic characterization capabilities into NP production platforms in a high-throughput fashion.

Reliability of biomimetic microsystems that mimic the structure and function of human organs for nanomedicine evaluation is crucial. Current challenges include the reliability of long-term cultivations of multiple cell types [186], as well as real-time monitoring of nanomedicines, cellular response to nanomedicines, and critical chemical cues (e.g., reactive oxygen stress) in 3D microenvironments [187–189]. In addition, the development of synthetic biomaterials remains a critical topic of research for physiological accuracy and niches for specific cells, tissues and organs. Furthermore, the development of *in-vitro* model systems that can accurately replicate the structure and function of *in-vivo* systems necessitates a precise 3D control of dynamically changing properties, such as mechanical properties of the ECM, at a scale comparable to human cells, tissues and organs [132, 172, 190].

6 Concluding Remarks

Microfluidics in nanomedicine has demonstrated the ability to overcome critical issues with conventional approaches used for nanomedicine development. When combined with advanced nanofabrication, synthetic biomaterials and high-precision control systems, microfluidic technologies constitute a novel platform capable of replacing the entire nanomedicine production process in a scalable manner. Although microfluidics as applied to nanomedicine is still in its infancy, it will surely continue to expand to provide innovative systems at industrially relevant scales in the near future.

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