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NANO/MICROSCALE TECHNOLOGIES FOR DRUG DELIVERY

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Nano- and microscale technologies have made a marked impact on the development of drug delivery systems. The loading efficiency and particle size of nano/micro particles can be better controlled with these new technologies than conventional methods. Moreover, drug delivery systems are moving from simple particles to smart particles and devices with programmable functions. These technologies are also contributing to *in vitro* and *in vivo* drug testing, which are important to evaluate drug delivery systems. For *in vitro* tests, lab-on-a-chip models are potentially useful as alternatives to animal models. For *in vivo* test, nano/micro-biosensors are developed for testing chemicals and biologics with high sensitivity and selectivity. Here, we review the recent development of nanoscale and microscale technologies in drug delivery including drug delivery systems, *in vitro* and *in vivo* tests.

Keywords: Nanotechnology; microtechnology; drug delivery; *in vitro* test; *in vivo* test.

Abbreviations

3D: three-dimensional; ADMET: absorption, distribution, metabolism, elimination, and toxicity; CNT: carbon nanotubes; CYP: cytochrome P450; DNA: deoxyribonucleic acid; ECM: extracellular matrix; LbL: Layer-by-layer; MEMS: microelectromechanical system; PCR: polymerase chain reaction; PDMS: poly(dimethylsiloxane); PK: pharmacokinetic; PD: pharmacodynamic; RNA: ribonucleic acid; μCCA: micro cell culture analog.

1. Introduction

Proper formulation design helps to deliver active pharmaceutical ingredients through biological membranes to the right target with increased efficacy and safety.

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The pharmacokinetic (PK) and pharmacodynamic (PD) profiles of the formulation need to be assessed in the process of designing new drug delivery systems.[1] The assessment of PK, PD, and safety profiles of new drug delivery systems is usually achieved by *in vitro* tests (e.g., cell-based assays) and *in vivo* tests based on animal experiments.

Nanoscale (1–100 nm) and microscale (0.1–100 μm) technologies are emerging as powerful tools for drug delivery process.[2] Nano/micro-fabrication techniques, such as soft lithography and electrospinning, have been well developed and widely applied in many research fields including drug delivery in the past decade.[3,4] Due to the low costs and simple procedures, both nano- and micro-fabrication have offered possibilities for the study of chemical/biological processes at cellular/molecular levels and for the design of drug carriers capable of interacting with biological systems at these levels.[5,6]

The rapid progress of the novel nano/micro-scale technologies also provided useful tools to evaluate new drug delivery systems by means of *in vitro* and *in vivo* tests. There is a growing need to improve cell-based *in vitro* test models, potentially to replace animal models.[7,8] Moreover, in some European industries (e.g., cosmetics and chemicals), animal testing is being phased out entirely, thereby forcing companies to adopt new *in vitro* screening methods to predict drug toxicity in human effectively.[9] Thus, the need for concordance between *in vitro* assays and *in vivo* responses is becoming greater and more pressing, particularly in high throughput that would enable prioritization of compounds for further development involving animal testing of pharmaceutical candidates. Nano/micro-scale technologies are providing opportunities to meet this need. The technologies can create local cellular microenvironment to closely mimic the physiological and pathological environments.[10] Microscale technologies have been utilized to fabricate complex microscale structures. Multiple cell types can be cultured inside them to create novel microscale systems mimicking multi-organ interactions. Such devices allow us

	Drug delivery systems	*In vitro* test	*In vivo* test
Nanoscale	Photolithography, Layer-by-layer deposition, Etching and Nanofluidics	Nanogrooves Nanofibers	Nano-biosensors Nanofluidics
Microscale	Silicon and polymeric microparticles Microfluidics to prepare particles Microneedles, Microchips and Micropumps	Cells-on-a-chip Tissue-on-a-chip Body-on-a-chip	Micro-biosensors Microfluidics

Fig. 1. Nano/microscale technologies used for drug delivery.

to observe the systematic, whole-body response to drugs rather than the response of single cell lineages.[11] Nano/microscale technologies have also been used for *in vivo* tests. Some nano/microscale biosensors were developed to detect biomolecules as well as small molecules *in vivo*.[12] Microdevices based on microfluidics were developed to facilitate *in vivo* tests from blood sampling to sample preparation and analysis.

In this paper, we review the recent development of nano/microscale technologies for fabricating drug delivery systems and methods to evaluate these systems (Fig. 1). First, we review nanofabrication techniques for drug delivery systems and emerging nanotechnologies for *in vitro* and *in vivo* tests. We then discuss microscale technologies for drug delivery systems and lab-on-a-chip techniques.

2. Nanotechnology

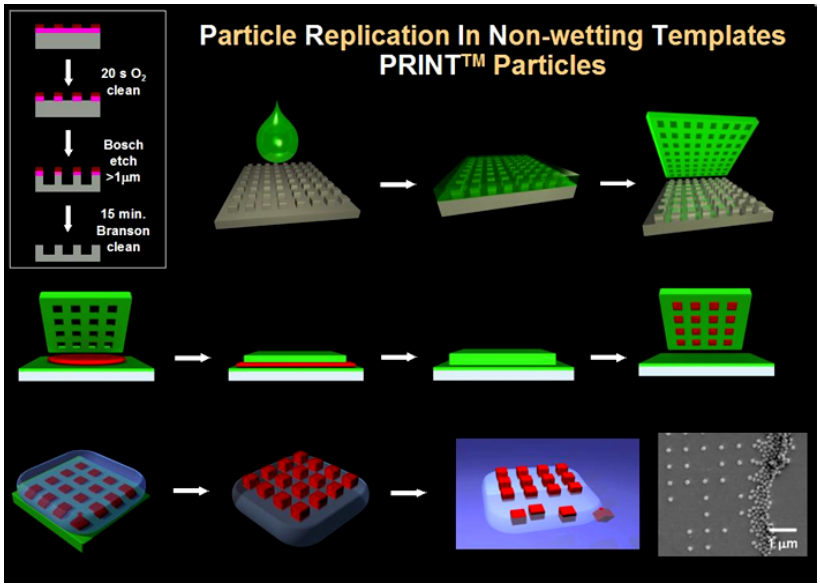
2.1. *Nanotechnology for drug delivery systems*

Since liposomes were first prepared in 1960s, extensive research has been devoted to the development of nanoscale drug delivery systems, such as liposomes, micelles, dendrimers, polymer particles, and colloidal precipitates.[13] However, conventional techniques for the production of these nanoparticles, such as solvent evaporation/extraction, require multi-step procedures and often result in a wide distribution of particle sizes.[13] Recently, increasing attention has been devoted to the use of nanofabrication technologies, such as lithography, etching, and layer-by-layer (LbL) assembly, to fabricate nanocarriers for drug delivery. These technologies offer the possibility for highly reproducible mass-fabrication of systems with complex geometries and functionalities.

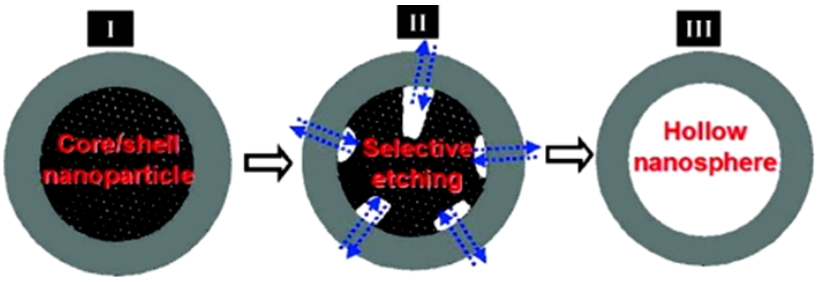
2.1.1. *Photolithography*

Photolithography is one of the most widely used nanofabrication techniques. Photolithography allows the precise control of particle size (20 nm to 100 μm) and shape (spheres, cylinders, discs, and so on) by using a predesigned template.[14] Particle size and shape play a significant role in the *in vivo* performance of delivery vehicles.[15,16] Particle size and size distribution determine their *in vivo* distribution, biological fate, toxicity, and targeting ability of these delivery systems, while particle shape alters drug release, affects particle transport characteristics, and influences cell–particle interactions.[15,17,18]

Rolland *et al.* reported particle replication in nonwetting templates as a very useful technique for the fabrication of monodisperse particles with simultaneous control over structure (i.e., shape, size, composition) and function (i.e., cargo, surface structure).[19] The idea stemmed from "top-down" fabrication of particles using lithography. It enabled the top-down fabrication to be below 100 nm with controlled size, shape, and composition (Fig. 2(a)).[14,19,20] Recently, the particles have



(a)



(b)

Fig. 2. Nanofabrication. (a) Fabrication of the silicon master template (box, upper left); wetting of the silicon master with liquid fluoropolymer, followed by curing (top row); perfluoropolyether elastomeric mold produced with nanoscale features from the master (upper right); confining organic liquid to cavities by applying pressure between mold and a perfluoropolyether surface (middle row); removal of organic particles from mold with adhesive layer (bottom left); and dissolution of adhesive layer producing free particles (bottom right).[14] (b) Illustration of the selective etching strategy for zinc oxide hollow nanoparticles using a sacrificial template.[28]
Note: (a) Reprinted from Gratton SE *et al.*, Nanofabricated particles for engineered drug therapies: A preliminary biodistribution study of PRINT nanoparticles, *J Control Release* **121**:10–18, Copyright 2007, with permission from Elsevier. (b) Reprinted with permission from Zeng H *et al.*, ZnO-based hollow nanoparticles by selective etching: elimination and reconstruction of metal-semiconductor interface, improvement of blue emission and photocatalysis, *ACS Nano* **2**:1661–1670, 2008. Copyright 2008 American Chemical Society.

been investigated for drug delivery. Petros *et al.* reported a Trojan horse particle composition based on a disulfide cross-linker that released doxorubicin in response to environmental changes.[21] Cell viability revealed that the particles were more efficient in killing HeLa cells than free doxorubicin.

Glandchai *et al.* conducted a novel application of step and flash imprint lithography that can be used to fabricate stimuli-responsive nanoparticles of precise sizes, shapes, and compositions.[22] The method utilized the topography of a quartz template to mold ultraviolet crosslinkable macromers into patterns on a silicon wafer.[23] Nanoscale features of varying sizes (50–400 nm) and shapes (square, pentagonal, and triangular) can be obtained. They demonstrated that biologics can be successfully incorporated within these nanocarriers during the imprinting process by pre-mixing them with the macromer solutions.

Apart from photolithography, an approach has been reported to produce nanoparticles without templates. The approach is similar to photolithography but without the use of photomasks. A block copolymer that is soluble in water can self-assemble into core-shell structural nanoparticles upon addition of salt.[24] After ultraviolet-crosslinking to cure the nanoparticles to form nano-sized gel particles, dialysis was used to remove the salt and the nanogel particles were generated with an inner space to load drugs. These nanogels offer the potential to simultaneously address several different design criteria, such as particle size, shape, composition, and surface functionality.

2.1.2. *Etching*

Etching is a process to create topographical features on a surface by selective removal of material through physical or chemical means.[25] Etching has been widely utilized in nano drug carriers. In recent years, porous silicon has been investigated for applications in microelectronics, biological sensors, and biomedical devices.[26] Porous silicon composites showed great promise in improving the mechanical stability and controlling release rates of a delivery system. Li *et al.* prepared a caffeine-impregnated poly(L-lactide) film, cast from a thermally oxidized porous silicon template.[27] After removal of the template, replication of the optical spectrum was observed in the polymer that can be used to monitor the release of drug from the biocompatible polymer.

Hollow micro- and nanoscale structures have attracted much interest because of their specific structures and properties differing from their solid counterparts, such as high surface-volume ratio, low density, excellent loading capacity, and high permeability.[28,29] An *et al.* presented a novel strategy called "selective etching" and synthesized various hollow nanoparticles retaining the size and shape uniformity of the original nanocrystals from manganese oxide and iron oxide.[30] Because of the unique hollow structure and the spin relaxation enhancement, these nanoparticles may be used as drug delivery carriers or magnetic resonance imaging contrast agents.[30] Zeng *et al.* reported a simple weak acid selective etching strategy to fabricate zinc oxide-based hollow nanoparticles at low temperature (Fig. 2(b)).[28] Zinc/zinc oxide core/shell nanoparticles were used as a sacrificial template. When the template was immersed in a weak acid solution, the H^+ ions in the solution entered the core parts, leading to preferential etching of the core parts in addition

to weak etching of the shell layer, because metals and oxides have different chemical potentials in their reactions with acid. A weak acid was used to adjust the relative etching rates of active metal in core parts and oxide in shell parts, so that the core parts can be preferentially and exhaustively consumed, and thus resulted in the formation of hollow nanoparticles. Chen *et al.* developed a novel "structural difference-based selective etching" strategy to prepare highly dispersed hollow mesoporous nanostructures with controllable particle/pore sizes, which was achieved by making use of the structural differences of the fabricated nanostructures.[31] These nanostructures showed high loading capacity (1.2 g/g) for anticancer drug doxorubicin, while the synthesized hollow mesoporous silica spheres with large pores showed ultrafast immobilization of protein-based biomolecules (hemoglobin). These hollow spheres loaded with doxorubicin exhibited significantly greater cytotoxicity than free doxorubicin, while the hollow spheres themselves had no cytotoxicity even at very high concentration. The hollow mesoporous silica spheres are potentially useful for cancer therapy.

2.1.3. *LbL deposition*

LbL assembly, one of the self-assembly methods, utilizes the electrostatic attraction of molecules containing complementary charge onto a solid substrate to form thin films.[32] Currently, a variety of components including polymers, proteins, and polysaccharides have been used for preparing LbL films. Because of its versatility, LbL assembly has been applied in biomimetics, biosensors, functionalized membranes, and drug delivery.[33]

Wood *et al.* assembled nanoscale electroactive thin films by using a nontoxic, electroactive material, i.e., Prussian blue.[33] The LbL thin films can be remotely controlled to release precise quantities of chemical agents in the presence of a small applied voltage (1.25 V), because Prussian blue (negatively charged) can be switched to a neutral form at this potential and thus the nanocomposite thin film was dissolved to release the drug. Furthermore, they demonstrated that Prussian blue particles exhibited no observable toxicity on a panel of mammalian cell lines. These characteristics made the electroactive thin films potentially useful for controlled release.

Pierstorff and Ho demonstrated the triblock copolymer-mediated deposition and the release of multiple therapeutics from a single thin film.[34] They constructed a dual-therapeutic eluting polymer-hybrid film that released functional anti-inflammatory (dexamethasone) and an anti-cancer drug (doxorubicin) simultaneously.

In addition, Tomita *et al.* developed a new strategy to fabricate LbL thin films composed of carboxyl-terminated poly(amidoamine) dendrimer and poly(methacrylic acid) at acidic pH through both electrostatic attraction and hydrogen bonding.[35] These LbL films were decomposed in strong acidic and neutral pHs.

The dendrimer-containing LbL films are potentially useful as a nano-device for developing pH-sensitive drug delivery systems.

2.1.4. *Nanofluidics*

Kobayashi *et al.* succeeded in generating simple colloidal droplets in parallel in high throughput by using systems incorporating submicron-channel arrays. The droplet size distributions were 1.4–3.5 μm and coefficient of variation was approximately 9%.[36] Recently, another nanofluidic technology improved the size distribution.[37] Based on the capillary focusing effect, Malloggi *et al.* generated colloidal droplets with sizes between 0.9 and 3 μm and coefficient of variation of 1%.[37] The system included a nanofluidic section that comprised a cross junction and a terrace. With the use of dedicated nanofluidic devices, it was possible to generate simple and multiple droplets, particles, Janus droplets (i.e., droplets with two separate compartments), and capsules whose size could be controlled by the nanochannel depth. These droplets are potential drug carriers.

2.2. *Nanotechnology for* in vitro *test*

For *in vitro* drug test, cell-based methods are useful. The ability to control cell culture substrate microstructure may improve *in vitro* methods. Compared with microtechnology that has been widely used in fabricating *in vitro* test models, nanotechnology has just begun to show its potential by providing nanostructures, such as nanogrooves and nanofibers, as guidance for cell growth and reconstruction of extracellular matrix (ECM). These nanoscale features may offer new *in vitro* tissue culturing systems for drug testing.

2.2.1. *Nanogrooves*

Nanostructured materials can mimic the nanometer topography of the native tissues and recent findings showed that cellular responses can be directed by nanotopography.[38] Loesberg *et al.* evaluated the morphological behavior of fibroblasts cultured on nanogrooves (groove depth: 5–350 nm, width: 20–1,000 nm) formed by electron beam lithography.[39] It was found that the cells adjusted their shape according to nanotopographical features down to a cut-off value of 100 nm width and a depth of 70 nm. Given sufficient culturing time, fibroblasts would even align themselves on groove depths as shallow as 35 nm, provided that ample ridge-surface (150 nm wide ridges) was available to the cells. Lamers *et al.* created nanogrooved substrates with a large area by using laser interference lithography and subsequent reactive ion etching and found that nanogrooves had a profound influence on osteoblast behavior.[40] Osteoblasts were responsive to nanopatterns down to 75 nm in width and 33 nm in depth. Osteoblast-driven calcium phosphate mineralization and osteoblast-specific gene expression were also regulated by nanogrooves. These

nanogrooves offer precise control of cell microenvironment. They are potentially useful to construct drug testing chips by lodging cells at desired positions.

2.2.2. *Nanofibers*

Nanometer-sized fibers and pores are useful to create a 3D environment for cell culture.[41] Nondegradable polyurethane-based nanofibers can be integrated into microfluidic systems for mimicking vascularized tissues embedded in ECM nanofibers.[42] A microwell-based platform to produce consistent and functional cell clusters was reported by Gallego-Perez *et al.*[43] The well bottom was made entirely of nano/microscale polymer fibers, which was achieved using electrospinning, a versatile technique that can produce nonwoven mats of fibers with essentially any chemistry and diameters ranging from 15 nm to 10 mm. The porous well bottom provided an open pathway for medium inflow/outflow that facilitated suitable nutrient/waste exchange for improved cell viability and functionality. Nanofibers can be easily integrated into standard well plates to mimic *in vivo* conditions to create better drug testing models.

2.3. *Nanotechnology for* in vivo *test*

Nanotechnology for *in vivo* test includes nano-biosensors and nanofluidic devices. Biosensors can be used to detect specific cellular biochemical outputs mediated by isolated enzymes, organelles, cells, tissues, or immunosystems. A whole array of micro/nano-biosensors have been developed for measuring various compounds, such as glucose, ethanol, cholesterol, uric acid, lactate, and hydrogen ions.[44] Some of these biosensors can be implanted *in vivo* for real-time monitoring, while others can be used to handle and analyze small-quantity samples.

2.3.1. *Nano-biosensors*

Recently, various nanoscale biosensors have been developed for biological detection *in vivo*, including biosensors based on carbon nanotubes (CNTs), magnetic nanoparticles, and gold nanoparticles.

CNTs exhibit many unique intrinsic physical and chemical properties and have been explored for biological and biomedical applications in the past few years.[45] Single-walled CNTs are highly absorbing materials with strong optical absorption in the near-infrared range.[45,46] Two glucose sensors were reported by Barone and Strano.[47] The first one assembled glucose oxidase as functional protein onto the surface of CNT using a dialysis method.[48] Once glucose oxidase was assembled, potassium ferricyanide can be irreversibly adsorbed on the surface of CNT and quench CNT fluorescence. In the presence of β-D-glucose, glucose oxidase catalyzed the reaction of β-D-glucose to D-glucono-1,5-lactone with a co-product, i.e., hydrogen peroxide, which can partially reduce the functionality of potassium ferricyanide and thus partially recover the intensity of CNT fluorescence. The partially recovered

fluorescence intensity was proportional to glucose concentration.[48] The other sensor was fabricated by coating nanotubes with dextran, which is a glucose analog.[49] The coating of dextran barely affected the intensity of CNT auto-fluorescence. However, the addition of concanavalin A reduced the CNT fluorescence intensity, because concanavalin A can bind dextran. In the presence of glucose, concanavalin A would be freed from the CNT because concavalin A preferred to bind glucose. Hence, the CNT auto-fluorescence intensity was restored to a certain level, proportional to the concentration of glucose.[49] Besides, CNT-based sensors were also developed to detect proteins and deoxyribonucleic acids (DNA).[50,51]

Magnetic particulate biosensors have unique advantages over other detection techniques, such as broad applicability to different types of targets (DNA, proteins, metabolites, and cells), minimal sample preparation requirement and the ability to perform measurements in turbid media.[52] Magnetic resonance-based sensors for calcium were fabricated by conjugating an organic compound to the surface of a magnetic nanoparticle.[53] When calcium ions were added, they induced the nanoparticles to cluster. This work suggests many chemicals of nonbiological origin, such as those employed for ion-selective electrodes, may be adapted to obtain magnetic resonance-based sensors for certain ions.

Distinctive optical properties of gold nanoparticles have established them as a useful color indicating agent for optical sensing. Various biosensing devices based on gold nanoparticles were developed to detect important bioanalytes such as ascorbic acid, uric acid, glucose, and neurotransmitters such as dopamine and epinephrine.[54] For example, gold nanoparticles integrated in a nanotube array were used as enzyme-free glucose sensors and showed good stability and reproducibility.[55]

2.3.2. *Nanofluidics*

Nanofluidics has been applied in biomolecular separation at nanoscale due to many different mechanisms, including steric, hydrodynamic, entropic, electrical, and ratcheting. The most common target molecule for separation at the nanoscale is DNA.[56] Fabrication technologies for nanostructures inside microchannels can improve DNA separations. It was found that these nanostructures generated unique separation modes, which was otherwise impossible by using random-sized pores of conventional gels or polymers.[57] Baba *et al.* demonstrated that nanopillar array structures and nano-plane gap structures fabricated using electron beam lithography can separate relatively small DNA molecules (∼10 kbp) based on molecular sieving effect and size exclusion chromatography.[58] Fu *et al.* reported a novel anisotropic nanofluidic sieving structure that was etched into a silicon wafer.[59] The predesigned structural anisotropy caused biomolecules of different sizes or charges to follow distinct trajectories, leading to efficient separation. High-resolution continuous-flow separation of a wide range of DNA fragments (between 0.05 and 23 kbp) and proteins (between 11 and 400 kDa) was achieved in just a few minutes by using this approach. Zeng *et al.* created 3D nanofluidic sieves within microfluidic devices by

using a facile microfluidic colloidal self-assembly strategy.[60] The system achieved fast separation of biomolecules with a wide size distribution, proteins (20−200 kDa) and double strain DNA (0.05−50 kbp).

Nanofluidics is also used to concentrate biomolecule analytes. A device to extract and concentrate biomolecule analytes was developed by integrating microtubules (one type of cytoskeletal proteins) with nanofluidic devices.[61] The microtubules were functionalized to provide binding sites for specific target biomolecules. This device made it possible to selectively extract target molecules such as streptavidin and albumin and then concentrate them up to five orders of magnitude higher from a complex mixture of analytes ranging from 1 nM to 10 fM.

3. Microtechnology

3.1. *Microtechnology for drug delivery systems*

3.1.1. *Microparticles*

Microparticulate drug delivery systems have been in use for over two decades for controlled and targeted drug delivery.[62,63] The majority of these particles are prepared using conventional methods, such as emulsification, which mostly yields particles with heterogeneous shape and size distribution as well as sub-optimal drug loading efficiency.[64] In contrast, microfabrication offers precise control over microarchitecture and size distribution.[64]

Several novel microfabricated oral drug delivery systems have been developed.[65,66] Ainslie *et al.* fabricated particles of flat, thin, or disc shapes to maximize the contact in the intestinal lumen and minimize the areas in contact with flowing gastric and intestinal fluids.[67] Ahmed *et al.* fabricated silicon microparticles using lithographic and reactive ion etching techniques.[65] Chemical modifications using silane chemistry and carbodiimide coupling reagents were employed to bind proteins to the releasing side of microparticles.[65] Other than silicon, polymeric bioadhesive microparticles have also been fabricated for delivery of peptides, proteins, DNA, and other molecules.[68,69] Specific ligand molecules may be attached to the polymeric surfaces to increase the strength of bioadhesion or to increase the specificity for certain area of the gut.[68] Despite drawing much attention from drug delivery scientists, monolayered microparticles have inherent problems such as burst release, inefficient to provide zero-order release rates and the lack of controlled release phenomena. To overcome these problems, double- and triple-layered polymeric microparticles have been fabricated with the aim of controlling release rates, providing pulsatile release and loading multiple drugs.[63,70] Lee *et al.* reported a novel, one-step fabrication method for triple-layered microparticles from poly(DL-lactide-co-glycolide), poly(L-lactide), and poly(ethylene co-vinyl acetate).[63] Ibuprofen and metoclopramide hydrochloride were loaded into these microparticles. Drug distribution in the microparticle layers depended on the affinity between the drug and the polymer. Hence, ibuprofen being hydrophobic localized in the long ethylene

chains of poly(ethylene co-vinyl acetate) whereas metoclopramide hydrochloride localized in the hydrophilic poly(dl-lactide-co-glycolide). Manipulation of layer thickness could be achieved by varying the polymer ratios and this approach can be employed to adjust drug loading efficiencies of the multilayered polymeric microparticles.

3.1.2. *Microfluidics*

Microfluidics features the techniques of constraining the fluids on a micrometer or sub-micrometer scale. For drug delivery applications, various microfluidic devices showed advantages for the fabrication of microparticles and nanoparticles. They have been usually used in preparation of monodisperse polymer microparticles. Particles with different morphologies varying from solid particles[71] to liquid filled and hollow microcapsules[72] were fabricated.

Lo *et al.* fabricated niosomes using microfluidic hydrodynamic focusing technique 40% narrower size distribution than those fabricated by conventional method.[73] Thiele *et al.* used a similar technique with a poly(dimethylsiloxane) (PDMS)-based microfluidic device to fabricate monodisperse block copolymer vesicles, the size range of which could be tuned between 40 nm and 2 μm.[74] Xu *et al.* demonstrated the reduced burst release and controlled release profile of monodisperse particles fabricated by using a microfluidic flow-focusing device as compared to conventional polydisperse particles.[75] Utilizing the adaptability of the technique for rapid mixing, Valencia *et al.* formulated monodisperse lipid polymer and lipid quantum dots in a single step process.[76] Breslauser *et al.* fabricated monodisperse silk microspheres from reconstituted silkworm cocoon using a glass capillary-based microfluidic system.[77] The microspheres thus produced had a homogenous size distribution and different diameters could be achieved by modulating the system flow rates.

Confinement and well-defined mixing made it possible for microfluidic systems to produce liposomes of nano/micrometer dimensions. Tan *et al.* devised a novel shear focusing-based method of droplet generation for liposomal fabrication and encapsulated cancer cells, yeast cells, microbeads, and proteins.[78] Jahn *et al.* developed two different methods using two microfluidic channels fabricated on a silicon wafer to produce liposomes using a hydrodynamic fabrication process.[79,80] Pradhan *et al.* demonstrated a novel microfluidic injection device-based fabrication of liposomes, wherein the diameter of liposomes was a function of the needle diameter.[81] Microfluidics thus offers the precise control of the fabrication environment for development of various micro- and nano-scale drug delivery systems.

3.1.3. *Microdevices*

The advent of microelectromechanical systems (MEMS) and miniaturization techniques has provided an ideal platform for creation of biomedical microdevices to be used as drug delivery systems. Such novel microscale devices are designed to

be minimally invasive and offer control over release rates.[82] The following section reviews the microfabricated devices for drug delivery.

3.1.3.1. Microneedles

Over the past four decades, drug delivery *via* skin has been useful to circumvent the harsh conditions inside gastrointestinal tract and the first pass effect of liver.[83] The fabrication of micron scale needles that are a few hundred micrometers long to penetrate skin but do not stimulate the nerve endings offers a suitable alternative to hypodermic injection. In addition, it provides a convenient alternative for the drugs labile to enzymatic degradation in the gut.

Microneedles from silicon were initially developed using a deep reactive ion-etching technique described by Henry *et al.*[84] The needles formed had extremely sharp tips were approximately 150 μm long (Fig. 3(a)) and had sufficient strength to penetrate the top layer of skin, i.e., stratum corneum, without breaking and were successful in achieving a 1,000-fold increase in calcein permeability.[84] Paik *et al.* designed in-plane single-crystal silicon microneedles, integrated with a PDMS microfluidic chip, which was used to deliver rhodamine B and black ink in *in vitro* and *ex vivo* models.[85] Wilke *et al.* devised a wet etching technique using potassium hydroxide to fabricate silicon microneedles.[86] The technique although complex, offered cost and processing benefits over the dry etching process. Similar technique was used by Li *et al.* to fabricate short microneedles measuring 70–80 μm in length.[87] Shikida *et al.* devised a novel anisotropic wet etching and dicing-based process, which did not use any photolithography or the plasma-based equipment.[88] These novel methods were suitable for fabricating solid microneedles. To make an adaptation of the process for fabrication of hollow microneedles, Shikida *et al.* proposed an enhancement of the previous processes with the use of metal plating to minimize the number of photolithography steps to fabricate hollow microneedles.[89]

Because of the high material and processing cost of silicon, researchers looked for cheaper alternatives and came out with metals such as stainless steel,[90,91] palladium,[92] titanium,[93] and nickel[94] for fabricating microneedles. Martanto *et al.* fabricated stainless steel microneedles from 75-μm-thick sheets using infrared laser.[91] Gill and Prausnitz successfully demonstrated the use of steel microneedles (Fig. 3(b)) for the transdermal delivery of various compounds such as vitamin B and calcein.[90] They also devised a method to seclude or "pocket" microparticles within the holes of the microneedles to deliver particles of small dimensions such as barium sulfate without wiping off on the skin.[95]

Although silicon and metals offer robust microneedles and significant research has been carried out on them, they are potentially harmful, if the microneedles break off in the skin or are reused.[96] Polymeric microneedles have since drawn the attention of many researchers, as they obviate the problems inherent with their predecessors. The needles are biocompatible and biodegradable and have been shown to be strong enough to penetrate the skin.[96,97] Microneedles of this class have been

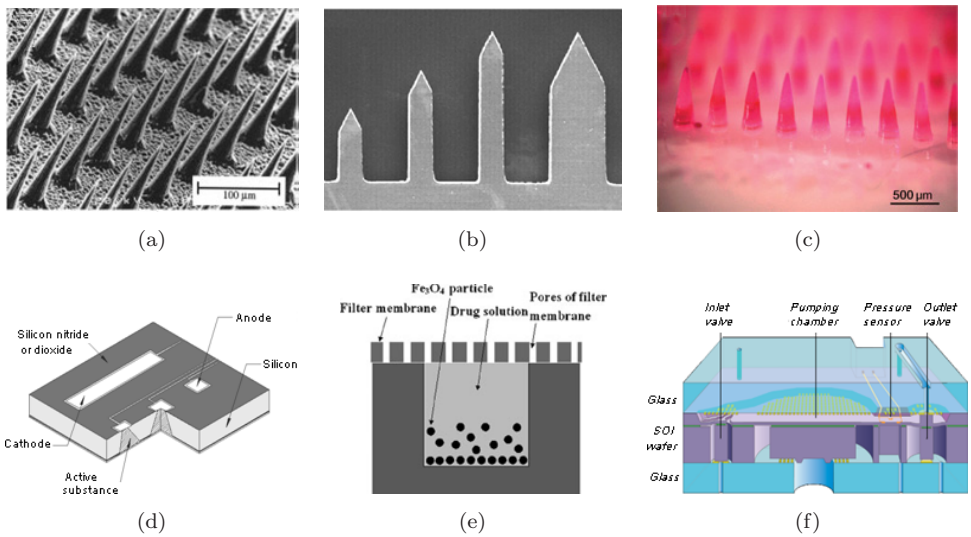


Fig. 3. Different microdevices. (a) Silicon microneedles.[84] (b) Steel microneedles.[90] (c) Polymeric microneedles encapsulating sulforhodamine.[96] (d) A prototype microchip for controlled release of drugs.[104] (e) Polymeric microchip with magnetically triggered drug release.[107] (f) Cross section of MEMS pump chip.[111]

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fabricated from different polymeric materials[96–98] and sugars.[99–101] Park *et al.* fabricated microneedles from poly(lactide-co-glycolide) incorporating calcein and Texas Red-labeled albumin using a micromolding technique.[98] Lee *et al.* fabricated microneedles from carboxymethylcellulose, albumin, and amylopectin. The model drugs sulforhodamine B, albumin, and lysozyme were either incorporated in the needles or in the backing for bolus or sustained release respectively.[97] Sullivan *et al.* designed a novel room temperature molding technique especially for biomolecules.[96] The process involved *in situ* polymerization of monomer vinyl pyrrolidone to encapsulate albumin (Fig. 3(c)). Ito *et al.* used another simple process to fabricate microneedles from dextrin.[102] Kolli *et al.* used maltose microneedles to study the permeation characteristics of nicardipine hydrochloride across hairless rat skin and observed an increase as compared to passive diffusion.[103]

3.1.3.2. Microchips

Implantable microsystems have been developed using the technologies of microfabrication for placing under the skin to reduce the risk of infection and frequent need of drug administration by injection. Santini *et al.* reported the fabrication of a first of its kind solid state silicon microchip (Fig. 3(d)), which could provide controlled release of chemicals.[104] Microchips containing microreservoirs were fabricated using silicon wafers by lithography, chemical vapor deposition, electron beam evaporation, and reactive ion etching. The filled reservoirs were protected by thin anode membranes made of gold, which dissolved in presence of chloride ions and application of voltage, thus releasing the drug.[104] These microchips offered more flexibility for incorporating the chemicals in solid, liquid, and gel forms as compared to microfluidic devices, which were limited to only liquids and could be used for multiple drugs.[105] They also demonstrated that the device was capable of achieving controlled release amenable to both constant and pulsatile release patterns as release from each reservoir could be controlled individually.[105] Metallic membranes, which can be electrothermally activated to release the drugs, have also been fabricated.[106]

The silicon microchips, however, present two undesirable disadvantages. First, silicon as a material is not biodegradable and needs a second operation to be removed from the body. Second, the electrochemical and electrothermal dissolution phenomena are limited to burst release.[107] To overcome the disadvantages, polymeric microchips have been developed from biodegradable polymer poly(L-lactic acid). The reservoirs were covered by poly(DL-lactic-co-glycolic acid) membranes of varying molecular masses to control the release of chemicals. These biodegradable chips showed a pulsatile release pattern for heparin, human growth hormone, and radio-labeled dextran.[4] Cai *et al.* hypothesized that pulsatile release could be achieved by using magnetic particles of ferrous-ferric oxide in polymeric microchips.[107] They fabricated microchips (Fig. 3(e)) using poly(DL-lactic acid) as the substrate and porous polycarbonate as the sealing membrane, both of which had long degradation times to allow for drugs to be completely released before the membrane completely degraded. The magnetic particles were designed to guard the pore openings in the membrane depending upon the direction of application of magnetic field. Such a device offers great potential in incorporating various drugs with a pulsatile release pattern.

3.1.3.3. Micropumps

Micropumps may be broadly classified as mechanical and nonmechanical. Mechanical pumps need a physical actuating mechanism, which have some undesirable properties such as requirement of high driving voltage and insufficient biocompatibility.[108] In contrast, nonmechanical pumps do not have any moving parts or valves and thus the fabrication and design are relatively simpler. These pumps offer the advantages of high pressure head, adjustable flow rates, and simple

design.[108] Lu *et al.* recently designed a microchip electroosmotic flow pump on a planar soda lime glass substrate.[109] The pump demonstrated a maximum pressure head of more than 1,000 psi and a maximum flow rate of 282 nl/min. A simple approach of fabrication was used to pack nanoparticles into the microchannels, which aided in achieving high flow rates. Such a micropump can be potentially used for delivering liquids at desired pressure and flow rates. Maillefer *et al.* developed a disposable high-performance silicon micropump with silicon-on-insulator wafer and dry reactive ion etching techniques.[110] The micropump chip was integrated with an external insulin pump. The microdevice maintained a stroke volume of 160 nl and could achieve flow rates of up to 2 ml/h. Schneeberger *et al.* at Debiotech incorporated the first of its kind pressure sensor in the insulin pumps (Fig. 3(f)) to achieve desired flow rates and precise delivery.[111] A novel compact disposable transdermal microdevice integrating a micro gear pump and a microneedle array was fabricated by Matteucci *et al.* The device was able to deliver at a rate of 0.1–1.2 ml/min.[112]

Microfabrication techniques are thus advantageous as compared to conventional methods and offer potentially useful drug delivery systems for the next generation therapeutics. Using these methods, a wider range of drugs can be delivered to the body through various routes. Microneedles developed from different materials have been shown to physically enhance the permeation of various hydrophilic, protein, and other biological drugs, which was not possible using the passive permeation enhancement methods. Polymeric microchips offer the flexibility to achieve the desired drug release profile with the additional advantages of their biocompatibility. Micropumps have been designed to achieve desired flow rates to control drug release precisely.

3.2. *Microtechnology for* in vitro *test*

Microscale technology is playing an important role in constructing various *in vitro* models. Integration of microfabrication and cell culture techniques has resulted in "cells-on-a-chip" technology, which is showing promise for drug testing, as compared to conventional cell-based methods.[113] Furthermore, "organ-on-a-chip" that mimics multi-tissue interactions and "body-on-a-chip" that mimics multi-organ interactions were also reported.[8,114,115]

3.2.1. *Cells-on-a-chip*

One advantage of a microfluidic cell culture system is to form concentration gradient. Several types of devices have been developed for the formation of concentration gradient.[116] A microfluidic device named Gradient Maker has been used for toxicity testing.[117,118] The device was fabricated with soft lithography, casting PDMS to obtain channels connected to a chamber where the gradient was formed. Cells were seeded in the culture chamber of the device and the drug was introduced into one of the two inlets of the device, while medium was pumped through the second inlet

to produce a steady-state concentration gradient in the culture zone. The device was demonstrated to generate large concentration gradients in just few millimeters. By performing cell viability assay, a quantitative dose–response relationship was achieved. This type of study illustrates the potential of utilizing microfluidic devices to implement high-throughput drug screening.

Early determination of absorption, distribution, metabolism, elimination, and toxicity (ADMET) profiles of drug candidates is crucial in the drug discovery process, because deficiencies in ADMET characteristics are the leading cause of attrition during drug development.[119] The traditional cell-based testing cannot provide accurate information about ADMET of potential drugs because its setting (e.g., static condition and no interaction between cells) is not a close resemblance of the physiological conditions. During the past few years, there have been several attempts to develop *in vitro* microscale systems for assessing metabolism-dependent toxicity. A metabolizing enzyme toxicology assay chip (MetaChip) was developed for rapid and inexpensive assessment of ADMET profiles.[120] The chip was fabricated by arraying sol–gel spots with cytochrome P450 (CYP) enzyme onto a glass slide and seeded with a monolayer of cancer cells to study enzyme-dependent cytotoxicity.[120] The system was further improved by superimposing the chip with an array of hydrogel-encapsulated cells (called DataChip by authors).[121] Using this system (Fig. 4(a)), three CYP isozymes against nine compounds were tested. The half maximal inhibitory concentration values obtained from this system were comparable to those obtained from a conventional 96-well plate assay. The *in vitro* toxicity results also correlated well with *in vivo* rat data.

3.2.2. *Tissue-on-a-chip*

Compared with single-layer cell culture, 3D cell aggregates resemble native tissues and may provide a better model for *in vitro* testing. Microfabricated systems offer the opportunity to grow cells under conditions that maintain normal 3D environmental cues, for instance, gradients of cytokines, secreted proteins from neighboring cells, and interactions with the ECM.[122] Kim *et al.* have reported such systems to immobilize cells in a 3D hydrogel and to confine the 3D culture constructs to the middle of microchannels by the mechanisms of hydrodynamic focusing and microvalving.[123,124] 3D cell culture is also useful in characterizing anticancer treatments. 3D microfabricated electrospun collagen membranes were constructed to culture prostate cancer cells, which were demonstrated to be a better approximation of the tumor microenvironment.[125] 3D chitosan-alginate scaffolds were reported to provide a 3D microenvironment for glioma cells representative of the *in vivo* tumor.[126] In another example, an alginate-based microfluidic system was developed for tumor spheroid formation.[127] These approaches may be useful for the development of anticancer therapeutics.

Liver has a central role in drug metabolism and toxicity. Efforts have been made toward microscale liver tissue on a chip to mimic the liver function. A microfluidic

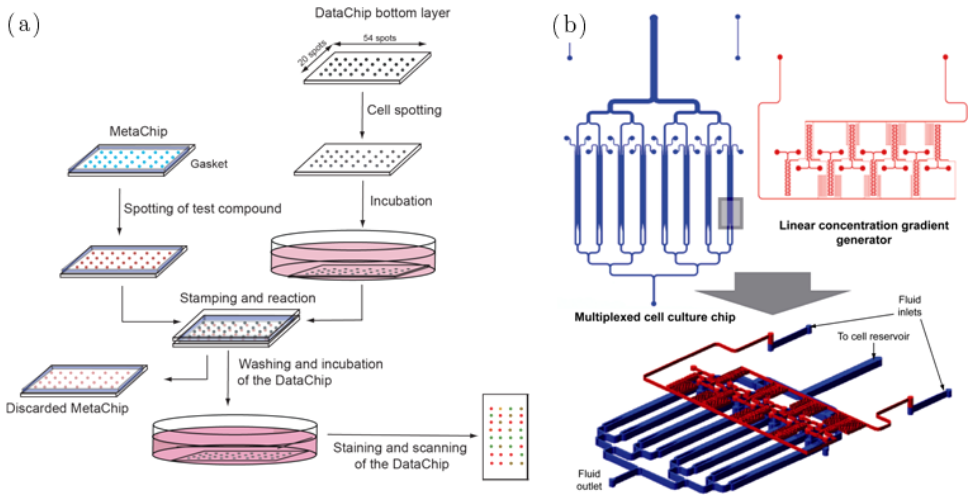

Fig. 4. Microtechnology for *in vitro* test. (a) Schematic of the DataChip platform for direct testing of compound toxicity or coupling with the MetaChip for evaluating toxicity of CYP-generated metabolites.[121] (b) Microfluidic design and assembly of a linear concentration gradient generator and multiplexed cell culture chip to construct the 3D hepatocyte chip.[128]
Note: (a) Reprinted from Lee MY *et al.*, 3D cellular microarray for high-throughput toxicology assays, *Proc Natl Acad Sci USA* **105**:59–63, 2008. Copyright (2007) National Academy of Sciences, USA. (b) Reprinted from Toh YC *et al.*, A microfluidic 3D hepatocyte chip for drug toxicity testing, *Lab Chip* **9**:2026–2035, 2009. Reproduced by permission of *The Royal Society of Chemistry*.

3D hepatocyte chip (Fig. 5(b)) for *in vitro* drug toxicity testing was developed based on multiplexed microfluidic channels where a 3D microenvironment was engineered in each channel to maintain the hepatocytes' synthetic and metabolic functions.[128] The multiplexed channels allowed for simultaneous administration of multiple drug doses to functional primary hepatocytes. Ho *et al.* developed a heterogeneous patterning of hepatic and endothelial cells on a chip *via* the enhanced dielectrophoresis trap design.[129] The design was used to construct the radial pearl-chain patterns of liver cells. The microfluidic chip facilitated the construction of liver tissue *in vitro*.

Renal tubular cells are important to study, as many drugs are secreted into urine *via* these cells. Jang *et al.* developed a simple multi-layer microfluidic device by integrating a PDMS microfluidic channel and a porous membrane substrate to culture and analyze the renal tubular cells.[130] Primary rat inner medullary collecting duct cells were cultured inside the channel. Enhanced cell polarization, cytoskeletal reorganization, and molecular transport by hormonal stimulations were observed.

Vascularization plays an important role in *in vitro* tissue engineering. To mimic the bifurcations, tortuosities, and cross-sectional changes found in microvascular networks *in vivo*, Rosano *et al.* developed a synthetic microvascular network on a PDMS chip that can serve as an *in vitro* model.[131] Microvascular networks from a cremaster muscle were mapped using a modified geographical information system,

and then used to manufacture the synthetic networks on a PDMS chip. A confluent layer of bovine aortic endothelial cells was successfully cultured in the anatomically realistic microvascular network derived from *in vivo*. This microfluidic system may provide insight into characterization of drug delivery particles in microvascular networks.

Instead of constructing replicate tissue micro-architectures, tissue-on-a-chip can also be fabricated by directly integrating *ex vivo* tissue into a microfluidic device.[132] Liver tissue has been kept functional and viable for over 70 h in this pseudo *in vivo* condition. Furthermore, a microfluidic biochip was developed for the incubation of precision-cut liver slices under flow conditions, based on a PDMS device containing 25-μl microchambers for integration of the slices.[133] The microdevice was coupled to a perfusion system, which enabled constant delivery of nutrients and continuous removal of waste products. Liver slices were viable for at least 24 h in the microdevice. The metabolism of 7-ethoxycoumarin was tested by this microdevice and the metabolic rate was found comparable to that obtained from experiments using well plates. Carraro *et al.* developed a microfrabricated bilayer device with a discrete parenchymal chamber based upon computational modeling of hepatic vascular flow, and showed that this perfusion device design can support a highly metabolic cell type during extended culture.[134] Recently, they reported a process using mechanical dissociation of liver slices into hepatic organoids with preserved intrinsic tissue architecture from swine liver.[135] Then, the organoids were cultured in an oxygen-supplemented bilayer perfusion device that can better preserve organoid viability, morphology, serum protein synthesis, and urea production, compared with standard and oxygen-supplemented static cultures. This device showed potential for *in vitro* disease modeling and xenobiotic testing.

In addition to fabricating biochips on a tissue level, microscale technology was also developed to create mechanically active "organ-on-a-chip" microdevices that reconstitute tissue–tissue interfaces critical to organ function. Recently, Huh *et al.* developed a living and breathing lung-on-a-chip (Fig. 5) that can mimic the boundary between the lung's air sacs and its capillaries.[114] This microdevice reproduced complex integrated organ-level responses to bacteria and inflammatory cytokines introduced into the alveolar space. In nanotoxicology studies, this model revealed that cyclic mechanical strain accentuated toxic and inflammatory responses of the lung to silica nanoparticles. Mechanical strain also enhanced epithelial and endothelial uptake of nanoparticulates and stimulated their transport into the underlying microvascular channel. Similar effects of physiological breathing on nanoparticle absorption were observed in mouse lungs. The lung-on-a-chip can predict how human lungs absorb airborne nanoparticles and mimic the inflammatory response triggered by pathogens. In another study, Ould-Dris *et al.* reported a technique to integrate polyethersulfone membranes inside a PDMS microchip to mimic the glomerular filtration function of kidney.[136] This technique has the potential as an *in vitro* model of a miniaturized artificial kidney.

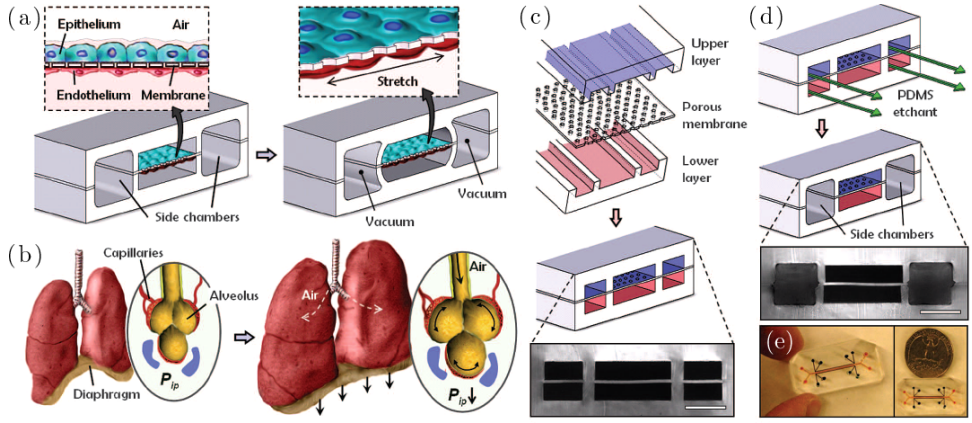

Fig. 5. Biologically inspired design of a human breathing lung-on-a-chip microdevice. (a) The microfabricated lung mimic device uses compartmentalized PDMS microchannels to form an alveolar-capillary barrier on a thin, porous, and flexible PDMS membrane coated with ECM. The device recreates physiological breathing movements by applying vacuum to the side chambers and causing mechanical stretching of the PDMS membrane forming the alveolar-capillary barrier. (b) During inhalation in the living lung, contraction of the diaphragm causes a reduction in intrapleural pressure (P_{ip}), leading to distension of the alveoli and physical stretching of the alveolar-capillary interface. (c) Three PDMS layers are aligned and irreversibly bonded to form two sets of three parallel microchannels separated by a 10-μm-thick PDMS membrane containing an array of through-holes with an effective diameter of 10 μm. Scale bar, 200 μm. (d) After permanent bonding, PDMS etchant is flowed through the side channels. Selective etching of the membrane layers in these channels produces two large side chambers to which vacuum is applied to cause mechanical stretching. Scale bar, 200 μm. (e) Images of an actual lung-on-a-chip microfluidic device viewed from above.[114]
Note: Reprinted from Huh D *et al.*, Reconstituting organ-level lung functions on a chip, *Science* **328**:1662–1668, 2010. Reprinted with permission from AAAS.

3.2.3. *Body-on-a-chip*

Microscale technology is used to integrate multiple miniaturized organ model systems into a single device to recapitulate interactions between different organs and enable more realistic *in vitro* assays of the whole body's response to drugs, namely, body-on-a-chip.

Currently, two types of microfluidic cell culture systems were developed as *in vitro* models to mimic body's dynamic response to various drugs and chemicals. The first one uses a closed design to culture mammalian cells in interconnected microchambers to represent key organs linked through a circulatory system. A micro cell culture analog (μCCA) (also known as a "body-on-a-chip" or an "animal-on-a-chip") used such a kind design by Shuler as a physical realization of a physiologically based PK model.[11] A four-chamber μCCA ("lung"-"liver"-"fat"-"other tissue") was fabricated to study bioaccumulation, distribution, and toxicity of selected compounds.[115] μCCA was also used to evaluate combination therapy for multidrug resistant cancer and colon cancer.[137,138] In another experiment, the μCCA system

was coupled with a micro model of the gastrointestinal tract to examine the response to oral exposure of drugs, chemicals, or nanoparticles.[139] These coupled gastrointestinal tract/body modules have been used to mimic human response to acetaminophen plus ethanol and have shown that nanoparticles can interfere with normal physiological responses such as iron uptake and nutrition. Furthermore, a μCCA was developed to fit with a PK–PD model to quantitatively analyze the effect of the drug.[140] Three cell lines representing the liver, tumor, and marrow were cultured in the chip to test the toxicity of an anticancer drug, 5-fluorouracil. It provides a novel platform with improved predictability for testing drug toxicity and can help researchers gain a better insight into the drug's mechanism of action. Besides, Nakayama *et al.* developed a disposable-type three-compartment micro-cell culture using PDMS-based microfabrication and small magnetic stirrer-based internal pumping system (for disposability and space-saving).[141] The device enabled cells derived from different tissues/organs to be maintained in different culture protocols for toxicokinetic studies.[141]

The other type of microfluidic cell culture systems is to use an open design, so that different "organs" are fluidically linked by liquid and yet relatively isolated from each other. In an effort toward realizing the ambition of human-on-chip, Zhang *et al.* developed a multi-channel 3D microfluidic cell culture system with compartmentalized microenvironments to culture multiple cell types.[8] Different types of cells were cultivated, including liver, lung, kidney, and adipose tissue inside the system. It was shown that controlled release of substitute growth factors (for example, transforming growth factor β1) inside such a compartment was possible by means of gelatin microspheres mixed with cells. Potentially, such a multi-channel system can be used as an alternative to animal models in drug screening.[8]

3.3. *Microtechnology for* in vivo *test*

Microtechnology has been used to facilitate *in vivo* test for sampling, processing, and analysis.[5] Moreover, lab-on-a-chip system can be used for *in vivo* drug concentration monitoring.

Obtaining blood samples from animals is challenging, especially from small animals such as mice and rats. Blood loss must be minimized, so that the physiological state of the animal is not disturbed. To obtain small blood samples, automated systems, based on a microfluidic platform that can be connected to the animal blood vessels through a catheter, have been developed.[142] Furthermore, a microsystem for automated blood sampling from laboratory mice was developed for PK studies with the advantage of eliminating the need for a tethered catheter with large dead volume.[143] It was designed to be worn as a backpack on a mouse, and used a microneedle, reservoir, and an actuator to instantaneously prick the animal for a time-point sample. The device also showed the potential to be integrated into microanalytical systems.

Microscale technology is also useful in sample processing, such as extraction and purification. An electrokinetically driven microfluidic chip was developed to realize beads-based solid-phase extraction of amino acids from a mixture sample, yielding an average extraction efficiency of 55% with coefficient of variation of 10.6% under the typical experimental conditions.[144] Another microfluidic device was developed to carry out integrated volume reduction and purification of nucleic acids from dilute, large volume biological samples.[145] The dual-phase device seamlessly integrated two orthogonal solid-phase extraction processes, a silica solid phase using chaotrope-driven binding and an ion exchange phase using totally aqueous chemistry (chitosan phase), providing the unique capability of removing polymerase chain reaction (PCR) inhibitors used in silica-based extractions (guanidine and isopropanol). The device reliably yielded a volume reduction for DNA and RNA (ribonucleic acid) purification on the order of 50- and 14-fold, respectively, both compatible with downstream PCR analysis.

To analyze biochemical samples including DNA, RNA, proteins, peptides, amino acids, neurotransmitters, and a variety of metabolites, microdevices have been developed.[146] These devices may provide new approaches for PK and PD studies. Nandi *et al.* reported a PDMS microchip-based analysis system that integrated *in vivo* microdialysis sampling with reagent delivery and mixing followed by injection and analysis of the derivatized sample by laser-induced fluorescence detection.[147] The microdialysis-microchip electrophoresis system showed the potential to be employed in the future for simultaneous monitoring changes in blood–brain barrier permeability and levels of amino acid neurotransmitters in the rat stroke model. Digital microfluidics involves the manipulation of microliter to picoliter droplets on hydrophobic surfaces under voltage control, whereby many lab-on-a-chip functions can be performed on a common and programmable platform.[148] Mousa *et al.* developed a digital microfluidic method and applied it to the extraction and quantification of estrogen in 1-μl samples of breast tissue homogenate.[149] Another digital microfluidic platform was developed to prepare and purify protein samples for measurement by matrix-assisted laser desorption/ionization mass spectrometry.[150] The complete integrated sequence of protein processing steps were performed on this platform, including disulfide reduction, alkylation, and enzymatic digestion, followed by cocrystallization and analysis of the sample *in situ*.

To monitor various compounds *in vivo*, many biosensors based on MEMS technology were developed, for example, glucose,[151] dopamine,[152] nitric oxide,[153] D-serine,[154] and glutamate.[155] An implantable enzyme-based carbon fiber microbiosensor was developed for *in vivo* monitoring of dopamine.[152] The biosensor was fabricated using tyrosinase immobilized in a biocompatible matrix consisting of a biopolymer, chitosan, and ceria-based metal oxides, deposited onto the surface of a carbon fiber microelectrode with a diameter of about 100 μm. The system provided continuous, real time monitoring of electrically stimulated dopamine release in the brain of an anesthetized rat. Levels of dopamine up to 1.69 μM

were measured. In addition, various sensors were fabricated to measure compressive stress,[156] intravascular shear stress,[157] blood pressure,[158] blood flow,[159] cutaneous temperature,[160] tissue stiffness, and contact force.[161] *in vivo*. These monitoring and measurement will be potentially useful for PK and PD studies.

4. Conclusion

Nano/microscale technologies offer many new opportunities to develop effective drug delivery systems At nanoscale, conventional nanoparticulate drug delivery systems can be greatly improved by using photolithography, etching, and nanofluidics. It has been demonstrated that nanoparticles can carry chemicals or biologics and release them in response to environmental changes. Uniquely, thin films containing several therapeutic agents can be assembled by LbL deposition. The films can release precise quantities of the therapeutic agents, in response to either electrical or pH signals. At microscale, particles with different morphologies varying from solid particles to hollow microcapsules can be fabricated On the other hand, microneedles and micropumps provide new means for drug delivery, especially for the delivery of biologics. These microdevices are not possible with conventional methods.

For *in vitro* study, nano-structures such as nanogrooves and nanofibers are useful to control cell microenvironments. Moreover, microchips to mimic tissue, organ, and body functions have been developed from conventional cell culture models. These lab-on-a-chip devices provide alternative methods, which are potentially useful to replace animal models. In the light of rapid development of tissue engineering, improved correlation between chip testing and clinical trials can be expected. However, it should be pointed out that these chips were not designed to reconstitute tissues or organs for regenerative medicine, but to provide cell-based platforms for drug testing.

For *in vivo* study, biosensors at both nanoscale and microscale have been developed aiming to monitor various biological molecules (e.g., glucose and dopamine) and body responses (e.g., cutaneous temperature and tissue stiffness). The sensors can be useful for PK and PD studies. In addition, nano- and microfluidic devices have been developed to separate and analyze biological samples. These devices will be especially useful to process small-quantity samples because of their high efficiencies and low sample consumptions.

Despite all these progresses, efforts are still required to adapt and improve these techniques for drug delivery applications. Many of these nanoscale and microscale techniques are based on silicon and other materials from the semi-conductor industry. These materials may cause biocompatibility problems for clinical applications. To this end, biomaterials with which microstructures can be created need to be developed to fabricate various drug delivery systems. Next, these lab-based techniques need to be scaled up for applications in pharmaceutical industries. Most of the techniques, however, require clean room and other sophisticated facilities, which

are more expensive than conventional instruments, thus posing as obstacles. Lastly, extensive studies are needed to prove the novel drug delivery systems created by using these techniques to be clinically safe and effective.

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