



# Biomimetic tissues on a chip for drug discovery

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Developing biologically relevant models of human tissues and organs is an important enabling step for disease modeling and drug discovery. Recent advances in tissue engineering, biomaterials and microfluidics have led to the development of microscale functional units of such models also referred to as 'organs on a chip'. In this review, we provide an overview of key enabling technologies and highlight the wealth of recent work regarding on-chip tissue models. In addition, we discuss the current challenges and future directions of organ-on-chip development.

## Introduction

Scientists in academia and industry rely heavily on *in vivo* animal models and *in vitro* cell culture platforms to investigate biological processes and develop therapeutic strategies. Although these approaches have been informative, and should continue to be so for years to come, they have significant shortcomings [1]. *In vivo* models can produce integrated multi-organ responses which are impossible to achieve using conventional *in vitro* models. However, within such a multi-organ system, isolating the salient tissues or cell groups related to a particular physiological or pathophysiological response is difficult and often requires the use of knock-outs or transgenic animals, leading to further complications and shortcomings. In addition to the ethics surrounding *in vivo* model usage, serious concerns exist over their biological relevance to humans [2]. The ability to extrapolate animal model data to human conditions is limited. Current *in vitro* platforms are useful for studying the molecular basis of physiological and pathological responses. In particular, recent advances in molecular biology enable the interrogation of different signaling pathways to identify the proteins, genes, receptors and ligands involved in physiological and pathological responses. However, *in vitro* platforms

often do not simulate the complex cell–cell and cell–matrix interactions crucial for regulating cell behavior *in vivo* [3]. The collective limitations associated with current *in vivo* and *in vitro* models are exemplified by the significant number of new drug candidates that fail to make it to market owing to low efficiency or severe side effects. These shortcomings together with regulatory restrictions limiting the use of animal models [4] have generated substantial interest in developing human-based tissue-like constructs for disease modeling and drug and chemical testing.

Recent developments in stem cell research, regenerative medicine, biomaterials, tissue engineering and microfluidics could be integrated into new three-dimensional (3D) *in vitro* models closely mimicking human organs and tissues. A prerequisite for industrial use of such models is scalability. Organ(s)-on-chip devices could provide not only the biological relevance but also the requisite high throughput applications. Herein, we provide an overview of the key microengineering technologies enabling the development of organ-on-chip devices. We then review recent work on exemplar biomimetic micro-organs, their physiological relevance and underlying technologies. We also offer our perspective on exciting future directions and the challenges that remain for creating miniaturized, functional and responsive integrated models of human tissues.

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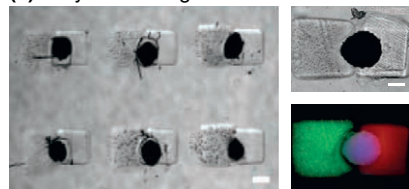
## Enabling technologies

Microfabrication, microfluidics and microelectronics are enabling microengineering technologies used in the development of physiologically relevant *in vitro* tissue models (Fig. 1). Microfabrication approaches such as photolithography, soft lithography, micro-contact printing and micromolding are enabling more-complex tissue cultures to be patterned on-chip. Extensive recent reviews outline a host of techniques that hold great promise for synthesizing tailored cellular microenvironments with high spatiotemporal

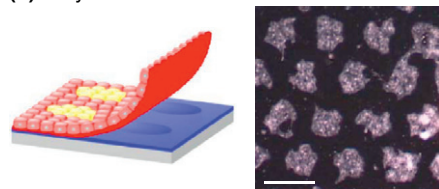
control over the mechanical and biochemical cues to regulate cellular behavior [5–11]. A recent example is the work of Qi *et al.* who used micromolding and photolithography to encapsulate individual embryoid bodies (EBs) within hybrid microgels to regulate stem cell differentiation (Fig. 1a) [12]. Various approaches have been used to engineer patterned co-cultures [13,14], a requirement for developing the complex tissues that comprise organ models. Micrometer-resolution cell positioning within co-cultures has been achieved by the directed assembly on a

### Co-cultures

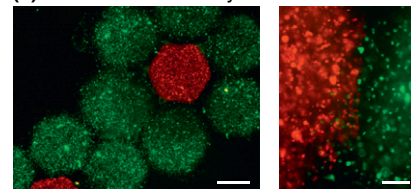
#### (a) Hybrid microgels



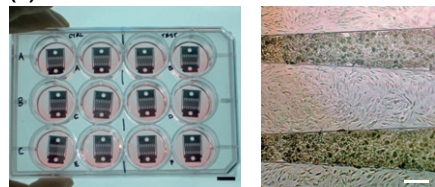
#### (b) Dynamic substrates



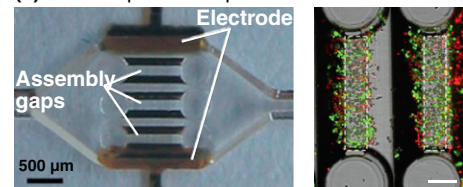
#### (c) Directed assembly



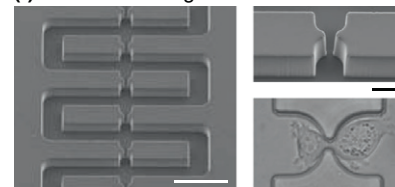
#### (d) Micromechanical substrates



#### (e) On-chip dielectrophoresis

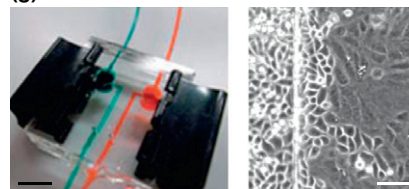


#### (f) Valves for single cell co-culture

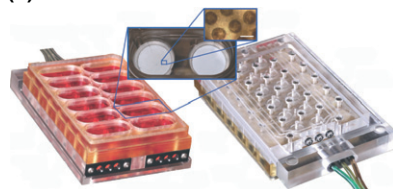


### Microfluidics and microdevices

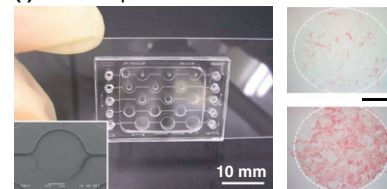
#### (g) Flow over co-cultured substrate



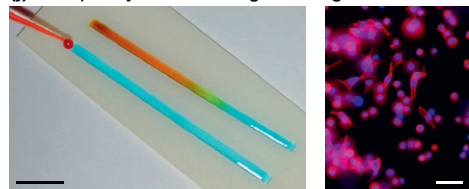
#### (h) Perfusion bioreactors



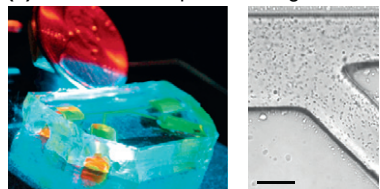
#### (i) On-chip mechanical stimulation



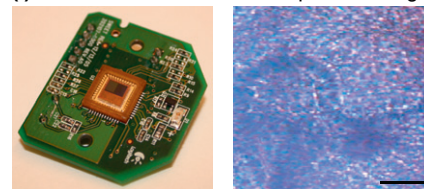
#### (j) Capillary flow drives gradient generation



#### (k) Microfluidic sperm sorting



#### (l) Lens-free real-time on-chip biosensing



Drug Discovery Today

FIGURE 1

Enabling technologies for on-chip tissue models. (a) Patterned microenvironment regulates differentiation of embryoid bodies. Scale bars 300  $\mu\text{m}$ . Source: Qi *et al.* [12], copyright (2010) John Wiley & Sons. (b) Dynamic substrate enables co-culture harvesting. Scale bar 1 mm. Source: Tsuda *et al.* [20], copyright 2006 with permission from Elsevier. (c) Directed assembly of cell-laden hydrogels for generating hierarchical tissue constructs. Scale bars 500  $\mu\text{m}$  (left), 100  $\mu\text{m}$  (right). Source: Zamanian *et al.* [15], copyright (2010) John Wiley & Sons. (d) Micromechanical substrates enable micrometer-resolution cell positioning and co-culture. Scale bars 1 cm (left), 250  $\mu\text{m}$  (right). Source: Hui and Bhatia [16], copyright (2007) National Academy of Sciences, USA. (e) Microfluidic cell culture chambers with integrated electrodes to assemble liver sinusoids by dielectrophoresis. Scale bars 500  $\mu\text{m}$  (left), 200  $\mu\text{m}$  (right). Source: Schütte *et al.* [17], copyright 2011 Springer Science and Business Media, LLC. (f) A microfluidic channel with an array of valves for single cell co-culture. Scale bars 100  $\mu\text{m}$  (left), 20  $\mu\text{m}$  (right). Source: Frimat *et al.* [19], reproduced with permission from The Royal Society of Chemistry. (g) Flow of growth factor directs the migration of one cell type into the other within a co-culture. Scale bars 5 mm (left), 100  $\mu\text{m}$  (right). Source: Kaji *et al.* [40], reproduced with permission from The Royal Society of Chemistry. (h) Multiwell plate with an array of perfusion bioreactors for 3D liver tissue culture. Source: Domansky *et al.* [38], reproduced with permission from The Royal Society of Chemistry. (i) Pneumatic microfluidic chip for the differentiation of stem cells under mechanical stimulation. Scale bars 10 mm (left), 1 mm (right). Source: Sim *et al.* [39], reproduced with permission from The Royal Society of Chemistry. (j) Capillary-flow-driven gradient generation within a fluid stripe for synthesizing gradient biomaterials to regulate cellular behavior such as spreading. Scale bars 1 cm (left), 100  $\mu\text{m}$  (right). Sources: Hancock *et al.* [114] (left), copyright (2011) John Wiley & Sons; and Hancock *et al.* [44] (right), copyright 2011 with permission from Elsevier. (k) A microfluidic device for separating motile and non-motile sperm. Scale bar 200  $\mu\text{m}$ . Sources: Cho *et al.* [42] (left), copyright 2003 American Chemistry Society; and Wu *et al.* [43] (right), copyright 2006 Springer Science and Business Media, LLC. (l) CMOS chip from webcam used to detect cardiomyocyte beating within a cell-based biosensor. Source: Kim *et al.* [48], reproduced with permission from The Royal Society of Chemistry.

free-surface (Fig. 1c) [15], micromechanical substrates (Fig. 1d) [16], on-chip dielectrophoresis (Fig. 1e) [17], and direct-write printing [18]. Single-cell co-cultures have been achieved using an array of valves along a microfluidic channel (Fig. 1f) [19]. A common issue associated with patterned tissue culture is removing and transferring the engineered tissue from the substrate used for patterning. This issue was addressed by Tsuda *et al.*, who produced their co-cultures on patterned thermoresponsive substrates (Fig. 1b) [20]. By reducing the temperature, the substrate released the co-culture. A second type of tissue transfer approach was pursued by Kobayashi *et al.* [21] and Nagamine *et al.* [22], who exploited the preference of cells to bind to certain gels rather than the substrate for patterning. Also, Tekin *et al.* [23,24] have used temperature-dependent shape-changing microwells to generate tissue structures of one or multiple cell types either in the form of cell aggregates or cell-laden hydrogels and used the shape-changing behavior of this system to retrieve the resulting tissue constructs. Additional examples of such microfabrication techniques applied to specific tissue models are outlined in subsequent sections.

Microfluidics is the study of systems that manipulate or process small (i.e. nanoliter and below) amounts of fluids within geometries measuring tens to hundreds of microns [25,26]. Extensive reviews exist on the widespread use of microfluidic devices in biomedical engineering, including drug discovery [2,27,28], stem

cell biology [29,30], cancer biology and experimental oncology [31], cell culture and processing [1,2,28,32,33], and probing cellular behaviors such as angiogenesis, migration and cell–cell interaction [34–36], even at the single cell level [37]. Microfluidic systems enable enhanced dynamic control over the cellular micro-environment within on-chip tissue models, such as providing nutrients and dissolved gasses (Fig. 1h) [38] and applying mechanical stimulation to cultured tissues (Fig. 1i) [39]. In a recent example, microchannels were immobilized onto co-cultured substrates to flow growth factors over the cells to promote directed migration (Fig. 1g) [40]. Microfluidic devices have also been developed to mimic *in vivo* processes, such as identifying smells [41] and sorting motile and non-motile sperm (Fig. 1k) [42,43]. Microfluidics, combined with microfabrication, enables the synthesis of more complex tissue constructs and biomaterials [5]. In a recent example, capillary flows within fluid stripes were used to generate prepolymer solution gradients that were crosslinked to form gradient biomaterials (Fig. 1j) [44].

Incorporating stimulation and sensing technologies on-chip and interfacing these with cultured cells is another important step for the real-time manipulation and detection of cellular behavior. Electrogenic cells such as heart and brain cells can be cultured directly atop complementary semiconductor–metal–oxide (CMOS) chips *in vitro* for pinpoint bidirectional connectivity between cells and the chip [45]. On-chip microelectrodes have

**TABLE 1**  
**Engineered on-chip tissue models in academia and industry**

<i>Tissue model</i>	<i>Type/comments</i>	<i>Academic research</i>	<i>Industrial research<sup>b</sup></i>
<b>Blood vessels</b>	Review	[34]	
	Live analysis	[96]	Quorum Technologies (Guelph, Canada)
	Capillary growth	[21,89,90,108,109]	
	Co-culture with endothelial cells	[40,91,92,95,110]	
<b>Brain</b>		[47]	
<b>Breast</b>	Review	[53]	
<b>Cornea</b>	Review	[53]	
<b>Gastrointestinal</b>		[64–66,104]	
<b>Heart</b>	Review	[53]	
	Example	[46]	
<b>Liver</b>	Review	[53]	
	Examples	[17,38,72,74,103,111]	CellASIC (Hayward, CA, USA); Hepregen (Medford, MA, USA); Hurel (New Brunswick, NJ, USA); RegeneMed (San Diego, CA, USA)
<b>Lung</b>		[49,80–82]	
<b>Multi-tissue</b>		[103–105]	
<b>Muscle</b>	Review	[53]	
	Myotube formation	[22,97,98,100,101,112,113]	Myomics (providence, RI)
	3D myotube	[97]	
	Stimulus-response	[22,100,101,112]	
<b>Skin</b>			Hurel (New Brunswick, NJ, USA); MatTek Corp. (Ashland, MA, USA); Phenion (Henkel AG & Co., Düsseldorf, Germany)
<b>Spleen</b>	In development <sup>a</sup>	[107]	
<b>Tumor</b>		[31,53,84,85,103]	

<sup>a</sup> A spleen model is currently under development at the Ingber Lab at Harvard University [107].

<sup>b</sup> Devices in development in industry; some listed in Ref. [107].



been used to stimulate myotubes of skeletal muscle [22] and interconnected cardiac cells [46]. Two-way microelectrode contacts have been used to excite and detect the signals from interconnected neurons non-invasively [47]. A second detection method was developed by Kim *et al.*, who attached a webcam chip to a cell culture chamber to detect cardiomyocyte beating in real-time (Fig. 1) [48].

Moving forward, the challenge is to develop more sophisticated microsystems that incorporate multiple tissues and vascular channels with active interfaces between them for transporting fluids, nutrients, immune cells and other regulatory factors [49]. An additional challenge is to apply spatiotemporally controlled mechanical forces to such microsystems to simulate the physiological environment, such as breathing in the lung, shear within blood vessels, peristalsis in the gut and tension in the skin [49,50].

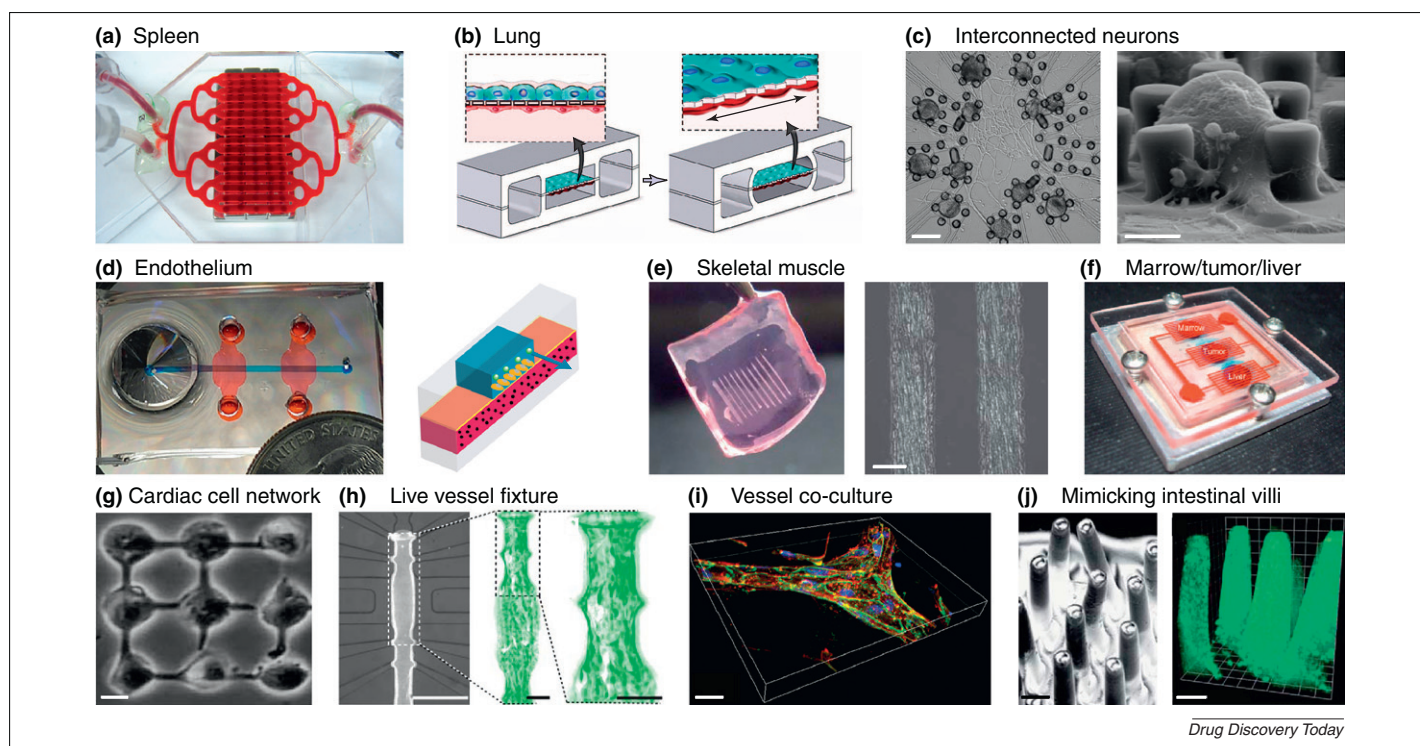
### Tissue models on a chip

A host of tissue models have been developed in academia and industry to mimic the sub-systems of organs or biological processes (Table 1 and Fig. 2). In the following, we provide an

overview of on-chip tissue models, their design, fabrication and physiological properties relevant to drug discovery. In particular, we highlight on-chip tissue models of the intestine, liver, lung and muscle, as well as models of tumors and blood vessels. We also outline recent attempts to combine the tissues of several organs on a single chip. Comprehensive reviews exist for bone, breast, cardiac, corneal, liver and tumor tissue models [51–53] as well as neural networks [54,55]; we expand on this literature by focusing more on liver and tumor tissue models. Other on-chip models that could be used for future studies on drug discovery and toxicity include those for sensory organs such as the nose [41,56,57] and biological processes such as sperm motility [42,43,58] and wound healing [59,60].

#### Intestine on a chip

Orally administered drugs are mainly absorbed in the small intestine, where they must diffuse across a mucous layer covering an epithelial cell layer lining the intestinal wall [61]. Therefore, early in most development processes, drugs and chemicals are first screened against intestinal cells and tissues to assess their



**FIGURE 2**

Microscale on-chip tissue models and culture platforms. **(a)** Spleen on a chip. Source: Baker [107], reprinted with permission from Macmillan Publishers, Nature, copyright 2011. **(b)** Lung on a chip with membrane housing epithelium/endothelium separating air flow from fluid flow chambers. Vacuum channels enable membrane stretching to simulate breathing. Source: Huh *et al.* [49], reprinted with permission from AAAS. **(c)** Neurons (dark patches) connected by neurites on a chip. Neurons held in place by posts were connected to two-way contacts in the substrate. Scale bars 100  $\mu\text{m}$  (left) and 20  $\mu\text{m}$  (right). Source: Zeck and Fromherz [47], copyright (2001) National Academy of Sciences, USA. **(d)** Microfluidic endothelium between chemokine and flow channel. Source: Song *et al.* [95], open access. **(e)** Fibrin gel with myotube line patterns for use with skeletal muscle-cell-based bioassay system. Scale bar 200  $\mu\text{m}$ . Source: Nagamine *et al.* [22], reproduced with permission from The Royal Society of Chemistry. **(f)** Multiple cell types representing liver, bone marrow and tumor cells cultured on-chip and connected via flow channels. Sources: Sung *et al.* [103] and Baker [107], reprinted with permission from Macmillan Publishers, Nature, copyright 2011. **(g)** Nine-cell cardiomyocyte network cultured on a chip. Scale bar 10  $\mu\text{m}$ . Source: Kaneko *et al.* [46], reproduced with permission from The Royal Society of Chemistry. **(h)** Microfluidic device for on-chip myography with simple resistance vessel fixation. Long-term culture and automated data acquisition of up to ten dose-response sequences. Scale bars 500  $\mu\text{m}$  (left) and 100  $\mu\text{m}$  (middle, right). Source: Günther *et al.* [96], reproduced with permission from The Royal Society of Chemistry. **(i)** Branched collagen gel tubes with encapsulated HUVECs labeled for b-catenin (green), actin (red) and nuclei (blue). Scale bar 10  $\mu\text{m}$ . Source: Raghavan *et al.* [90], the publisher for this copyrighted material is Mary Ann Liebert. **(j)** Micromolded polydimethylsiloxane (PDMS) (left) and collagen scaffold (right) mimicking human intestinal villi. Scale bars 100  $\mu\text{m}$ . Source: Sung *et al.* [66], reproduced with permission from The Royal Society of Chemistry.

absorption, distribution, metabolism, elimination and toxicity (ADMET) [62]. The small intestine epithelium is composed of two main cell types: enterocytes and goblet cells. Enterocytes constitute ~90% of the cell population and are covered with surface-enhancing microvilli. In most areas of the intestine, goblet cells constitute the remaining 10% of the cell population and secrete mucus which coats the epithelial layer and is important for innate immune responses and homeostasis. Coordinated intestinal movements (i.e. peristalsis) have also been shown to have an important role in intestinal biology. Every material absorbed through the intestine into the blood stream must first diffuse across the mucus layer, the enterocytes, the lamina propria and the capillaries formed by endothelial cells. Crossing the epithelial layer, however, has been shown to be the rate-limiting step [63].

Small intestine models destined for absorption and/or metabolism studies should include these key cellular (i.e. enterocytes, goblet and vascular endothelial cells), structural (i.e. villi and mucus) and dynamic (i.e. peristalsis) features. Some of these elements were addressed by Kimura *et al.* [64], who engineered an intestinal model with a membrane and vascular flow simulating the epithelial barrier and the epithelial–endothelial interface, respectively. Additional important model features include the mucus layer and the digestion process. These were partially addressed by Mahler *et al.*, who developed a microscale cell culture analog of the gastrointestinal (GI) tract incorporating digestion, a mucus layer and physiologically realistic cell populations [65]. To mimic drug transport across the intestinal wall better, additional structural features such as microscopic villi should be incorporated to enhance the intestinal epithelium surface area and to direct mucus. Such structural features have been fabricated in polydimethylsiloxane (PDMS) and hydrogels seeded with epithelial cells (Fig. 2j) [66], and could be incorporated into future functional tissue models.

Because healthy primary human cells are difficult to obtain, the majority of existing intestinal models were developed using immortalized cell lines. Although such cell lines can serve as informative and convenient surrogates for primary cells, their use limits the extrapolation of results to humans. Existing intestinal models also lack a host of ‘non-GI’ cells, including mast cells which play a key part in the barrier integrity and physiology of the intestinal epithelium [67].

#### Liver on a chip

Hepatotoxicity is a major side effect of many chemicals and drugs that are administered over prolonged periods of time. Almost half of all drug withdrawals occur as a result of acute liver toxicity [68]. Therefore, efficient, reliable, accurate and inexpensive tools for liver toxicity testing are required. Recent reviews provide an overview of microfluidic devices incorporating liver tissue or cells for metabolism, drug discovery and toxicity studies [53,69]. Existing tools for testing liver toxicity, involving hepatocyte cell lines, two-dimensional (2D) *in vitro* cultures and animal models, all suffer from major limitations. Cell lines and 2D cultures lack key metabolic activities found in human liver and major interspecies differences limit the predictive value of animal models [70,71]. The key challenge for on-chip liver tissue models is to maintain the metabolic activity and phenotype of the poorly viable cryopreserved human primary hepatocytes. Bioreactors have been

developed to meet this challenge, such as the perfused multiwell plate device of Domansky *et al.* [38], which supported the growth and functional integrity of hepatocytes in 3D culture for up to a week (Fig. 1h), and the multiwell micropatterned co-culture system of Khetani and Bhatia [72] which could maintain phenotypic functions for several weeks. Both groups co-cultured primary hepatocytes with other key liver cells such as sinusoidal endothelial cells containing stellate and Kupffer cells [38] or fibroblasts [72] to improve and maintain primary hepatocyte function considerably. Another desirable feature of liver tissue models is to simulate the morphology of the liver’s functional units (i.e. lobules) to provide the homogenous and heterogeneous cell–cell interactions and cellular matrix support to maintain hepatocyte functionality [72,73]. Toh *et al.* [74] incorporated this feature into their on-chip liver model that exhibited a reasonable simulation of the liver morphology and functionality for up to 72 hours. In a second example, Schütte *et al.* used a perfusion system and integrated electrodes to assemble liver sinusoids by dielectrophoresis (Fig. 1e) [17]. A second class of on-chip liver models involves intact tissues extracted from humans or animals and integrated on-chip for the *in vitro* assessment of metabolism and toxicity [69]. Recently, van Midwoud *et al.* [75] integrated liver and intestinal slices into different compartments of a microfluidic device with sequential perfusion between the compartments to study interorgan interactions. In addition to reflecting *in vivo* conditions adequately for investigating liver drug metabolism and toxicity, on-chip models incorporating primary hepatocytes or intact tissue slices must be able to maintain key metabolic activities and normal liver integrity, particularly with regard to repeat dose experiments [69]. This feature was demonstrated by Khetani and Bhatia [72], who performed nine-day repeat dose experiments with their co-culture system to test the chronic toxicity of troglitazone. Finally, an exciting new area is the ability to use *in vitro* cultures for disease modeling and to monitor disease progression and the impact of treatment in real-time. An example for such an approach is the recent work of Jones *et al.* [76], who used a fluorescent cell-based reporter system to monitor in real-time the infection of hepatitis C on hematoma cell lines and primary human hepatocytes.

#### Lung on a chip

The lung epithelium acts like a physical barrier between the host and the environment and responds to a variety of environmental insults such as pathogens and air pollution. The lung’s defense mechanisms include secreting antimicrobial peptides (e.g. defensins) and pro- and anti-inflammatory mediators, and recruiting innate and adaptive immune cells to the site of infection or damage. The efficiency of such protective responses as well as the homeostasis of the airways is underpinned by a variety of lung specialized epithelial cells (e.g. ciliated and mucus producing) and their crosstalk with other cell types such as antigen presenting cells, macrophages and myofibroblasts [77]. The human lung also provides one of the largest vascularized interfaces between the host and the environment, and is therefore considered a convenient port of delivery for local and systemic drugs. Drug delivery studies tend to focus on drug permeability across the lung epithelial barrier. Within this context, most existing lung tissue models are limited to single cell culture representations of the alveolar epithelium comprising epithelial cell lines [78,79], limiting their

physiological relevance to the human lung. Certain enhancements have been made to single cell culture models, such as microfluidics and flexible membranes to apply fluid and solid mechanical stresses to single cell alveolar models to simulate the effects of mechanical ventilation and physiologic or pathologic liquid plug flows [80–82].

More relevant lung tissue models destined for disease modeling and drug testing require additional cell types such as airway smooth muscle and immune cells, as well as key structural, functional and mechanical features of the human respiratory epithelium. Towards this goal, Huh *et al.* (Fig. 2b) [49] co-cultured alveolar epithelial, endothelial and certain immune cells on an artificial membrane and used adjacent vacuum channels to mimic the cyclic stretching during breathing. The inclusion of immune cells recapitulated certain aspects of human lung responses to infection and inflammation, enabling the assessment of the inflammatory response triggered by inhaled pathogens. Despite its many merits, this model used immortalized cell lines rather than human primary cells and did not include several other key structural or immune cells. It is not known whether the anatomical shape of the alveolar space or upper respiratory tract would have any bearing on the dynamics of cell-cell interaction. Future attempts, in addition to inclusion of primary cells, could focus on micropatterning scaffolds to improve the anatomical relevance and function of such tissue models.

#### Tumor on a chip

Developing anticancer therapies that target rapidly dividing cancer cells but leave normal healthy cells untouched is a grand challenge for cancer research. A recent review contains a comprehensive list of 3D tumor tissue models including multicellular spheroid, hollow fiber and multicellular layer models [53]. Perfusion delivers potential therapeutic agents to such 3D tumor cell cultures, which emulates the heterogeneous blood supply delivered to tumors *in vivo* [53]. In addition to these approaches, a popular strategy for developing anticancer drugs is HTS in which large combinatorial arrays of drug cocktails are produced and exposed to cells, which are then analyzed to assess the drug effects [31]. Performing HTS within microfluidic devices enables smaller volumes of reagents to be used than in typical multiwell plate experiments. One key engineering challenge is to generate an array of drug concentrations on-chip. To meet this challenge, Jang *et al.* developed a microfluidic combinatorial system that produced 64 or 100 combinations of two chemical solutions at different concentrations and stored them in isolated chambers [83]. The device inputs could be chosen such that the compositions of the stored mixtures spanned any triangle in the 2D space of chemical concentrations. A second engineering challenge is to expose different combinations or concentrations of drugs to cells cultured on-chip. Two groups recently accomplished this step by exposing human lung cancer cells to different concentrations of the anticancer drug verapamil (VP-16) [84,85]. In one study, the percentage of apoptotic cells in the verapamil-pretreated group was approximately double that of the control group, in agreement with previous flow cytometry analysis [85]. Further HTS advances could be made by using 3D cultures which mimic *in vivo* conditions better than

cell monolayers. For example, Fischbach *et al.* [86] cultured cancer cells in 3D alginate hydrogels and found that cell signaling was altered.

A second line of cancer research is to test anticancer therapies on-chip to optimize system parameters for different types of cancer cells, while requiring minute amounts of reagents and cells. For example, Jedrych *et al.* [87] created a microfluidics system for *in vitro* photodynamic therapy (PDT) [87]. In PDT, light induces a photosensitizer delivered to the carcinoma cells, which in turn reacts with oxygen to produce a chemical toxic to the tumor cells. A key step in developing PDT is to optimize the system parameters such as the photosensitizer concentration for different types of cancer cells. Jedrych *et al.* [87] demonstrated this step by testing different concentrations of photosensitizer on A549 cancer cells.

#### Vessels on a chip

Blood vessels are involved in most medical conditions and are thus integral components to organs-on-chip devices [88]. A key engineering challenge is to grow blood vessels and capillaries on-chip. Dike *et al.* addressed this issue by using a surface patterned with 10  $\mu\text{m}$  wide cell-adhesive lines surrounded by cell-repellent areas [89]. Endothelial cells cultured on the cell-adhesive lines differentiated to form capillary tube-like structures containing central lumens. A different approach was carried out by Kobayashi *et al.* [21], who patterned endothelial cells along parallel hydrophilic lines and then transferred the cells to a hydrogel scaffold. Once on the scaffold, the cells changed their morphology to form capillary-like tubular structures. The capillary structures were tested by flowing fluorescent solution through them, and also by transplanting them into mice where blood cells were subsequently found in the lumen. To control the endothelial capillary tube formation (tubulogenesis) further, Raghavan *et al.* cultured endothelial cells within microscale channels filled with collagen hydrogel (Fig. 2i) [90]. The cells were organized into tubes containing lumens with controlled branching and orientation. Future work must be done to generate capillary structures *in vitro* with similar mechanical properties to those *in vivo*.

A second major engineering challenge is to reproduce the microenvironment surrounding blood vessel walls, composed predominantly of vascular endothelial cells. A key component of this challenge is to understand how endothelial cells lining the vessel walls interact with other cell types. To address this issue, Chung *et al.* [91] and Kaji *et al.* [40,92] developed endothelium models to study the paracrine communication between endothelial and other cell types. Chung *et al.* found that MTLn3 cancer cells attracted endothelial cells to induce capillary formation, whereas smooth muscle cells suppressed endothelial activity [91]. Kaji *et al.* used complimentary substrates to co-culture endothelial and HeLa (cervical cancer) cells and found, similarly, that paracrine factors secreted from one cell type and directed via the culture medium to the other cell type affected the migration behavior of both types [40,92]. Research has also been devoted toward understanding how the cyclic stretch due to vessel deformation regulates cell function, and how disruptions in these forces produce diseased states such as hypertension or arteriosclerosis [93]. The effect of mechanical stimulation on endothelial cells *in vitro* has been previously reviewed [94]. An additional challenge is to apply chemical and mechanical stimuli simultaneously to cells



to engineer more biologically relevant models. To address this issue, Song *et al.* developed a membrane-based microfluidic device that models the adhesion of breast cancer cells on an endothelium (formed on the porous membrane) during intravascular metastasis (Fig. 2d) [95]. They showed that either the addition of the chemokine CXCL12 from the basal side or the elevation of fluidic shear stress could enhance the metastatic cell adhesion. Many additional processes remain to be studied, including the chemical and cellular exchange across vessel walls, regulation of the permeability of surrounding tissues, coagulation of blood, transmigration of leukocytes and the regulation of vessel diameter.

Microfluidic platforms for probing the structure and function of actual blood vessels under physiological conditions also exist. Recently, Günther *et al.* developed a microfluidic platform enabling on-chip fixation, long-term culture, controlled delivery of drugs and automated acquisition of consecutive dose–response curves of intact mouse mesenteric artery segments (Fig. 2h) [96]. The phenylephrine dose–response relationships produced by the platform were in good agreement with those obtained by conventional pressure myography.

#### Muscle on a chip

Skeletal muscle is one of the major insulin-target tissues responsible for the maintenance of whole body glucose homeostasis. Defects in the glucose uptake in skeletal muscle contribute to insulin resistance in type 2 diabetes. To investigate insulin- and contraction-induced glucose metabolism, on-chip skeletal muscle models require *in vivo*-like structure, electrode stimulation and co-culture with primary human skeletal cells with motor neurons. *In vivo*-like structural features include myotube alignment and assembly into sarcomeres. On-chip myotube alignment has been guided by substrate pattern [97,98] and stiffness [99,100] and by electrical stimulation [101]. An important aspect of a muscle tissue model is the ability to control muscle contraction. Such control has been accomplished by Tourovskaia *et al.* [102] by stimulating myotubes with a laminar stream of agrin, a chemical secreted by neurons *in vivo* at the location where nerves contact the muscle. A different approach was developed by Kaji *et al.* [100], who modulated the substrate stiffness with an atelocollagen and electrically stimulated the formed myotubes to demonstrate a positive correlation between the contractility of the myotubes and the glucose uptake. Control of individual myotubes was achieved by Nagamine *et al.* [22], who integrated a microelectrode array with aligned myotubes on a fibrin gel sheet (Fig. 2e). Future improvements to on-chip muscle tissue models include monitoring the glucose uptake and other metabolism events in real-time and incorporating neuromuscular junctions by co-culturing myotubes with neurons and stimulating them to induce muscle contraction.

#### Multiple organs on a chip

Most existing on-chip tissue models represent a single organ, preventing investigations on a drug's systemic effect. Microscale cell culture analog systems, also known as 'animal-on-a-chip', 'human-on-a-chip' or 'body-on-a-chip' systems, attempt to improve the prediction of the effects of drugs and toxicity on the whole body. In a typical multiorgan device, multiple cell types representing different organs are cultured in separate chambers

on a single chip. The chambers are connected by channels based on their sequence *in vivo* to assess systematically the effects of drug action and metabolism in different organs. A number of multi-organ devices has been developed with cell lines representing various organs or tissues, including: liver, tumor and bone marrow based on a mathematical pharmacokinetics–pharmacodynamics model and used to test the toxicity of the anticancer drug 5-fluorouracil (Fig. 2f) [103]; intestine, liver and breast cancer cells to evaluate the intestinal absorption, hepatic metabolism and the antitarget cell bioactivity of drugs [104]; liver, lung, kidney and adipose tissue [105]. Despite continued efforts and strong motivations to replace animal testing, these multi-organ systems are still in their infancy. The function of these systems is dictated by the cell types employed, and physiologically relevant cellular responses must be distinguished from artifacts due to *in vitro* cell culture. Also, barrier tissue analogs must be incorporated into existing multiorgan systems because these tissues can significantly reduce the bioavailability of drugs taken up through inhalation or absorbed through the skin. Finally, it is essential to benchmark the results of the on-chip systems with *in vivo* data under identical conditions and drug exposure. A more detailed discussion can be found in the literature by Esch *et al.* [106].

#### Concluding remarks

With recent advances in tissue engineering, biomaterials and microfluidics, developing functional organs on a chip is becoming a more realistic goal for various diagnostics and drug screening applications. However, barriers still exist before integrated *in vitro* models can be produced with the necessary capabilities to report and predict chemical metabolism and target interaction over long durations. One of the major challenges to recapitulating the true functional properties of organs is to build systems based on primary human cells rather than cancerous cell lines. An additional challenge is to create *in vivo*-like environments with enhanced architectural and cellular complexities. That said, once such complexities are in place, they will pose additional challenges for detecting cellular responses to external stimuli, monitoring the quality of the microenvironment (e.g. metabolites, pH and O<sub>2</sub> saturation) and supporting the diverse cellular requirements. Tools to address these issues will require additional advances in biosensing and engineering. In addition, mathematical modeling will guide the design of the next generation of organ-on-chip devices, and nanosensing will enable the real-time and *in situ* monitoring of the microenvironment and cellular and/or metabolic responses.

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