Review

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From 3D cell culture to organs-on-chips

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3D cell-culture models have recently garnered great attention because they often promote levels of cell differentiation and tissue organization not possible in conventional 2D culture systems. We review new advances in 3D culture that leverage microfabrication technologies from the microchip industry and microfluidics approaches to create cell-culture microenvironments that both support tissue differentiation and recapitulate the tissue–tissue interfaces, spatiotemporal chemical gradients, and mechanical microenvironments of living organs. These 'organs-on-chips' permit the study of human physiology in an organ-specific context, enable development of novel *in vitro* disease models, and could potentially serve as replacements for animals used in drug development and toxin testing.

3D cell culture

To understand fully how tissues form and function, as well as their pathophysiology, it is crucial to study how cells and tissues behave as parts of whole living organs that are composed of multiple, tightly opposed tissue types that are highly dynamic and variable in terms of their 3D structure, mechanical properties and biochemical microenvironment. Unfortunately, most studies on cell and tissue regulation have relied on analysis of cells grown in 2D cell-culture models that fail to reconstitute the *in vivo* cellular microenvironment; as a result, these cultures commonly do not maintain their differentiated functions.

Efforts to address these limitations led to the development of 3D cell-culture models in which cells are grown within extracellular matrix (ECM) gels (Box 1). This approach enhances expression of differentiated functions and improves tissue organization [1]. Nevertheless, even the most impressive 3D culture models fail to reconstitute features of living organs that are crucial for their function, including tissue–tissue interfaces (e.g. between epithelium and vascular endothelium), spatiotemporal gradients of chemicals and oxygen, and the mechanically active microenvironment that are central to the function of virtually all living organs. Gel-based culture systems also pose major technical challenges in terms of probing effects of physiological diffusion gradients (e.g. ion transport in kidney) or sampling cellular products that are often secreted in a polarized manner (e.g. biliary flow in the liver) because these tissues form in the center of the gel. As a result of these shortcomings of the existing 2D and 3D culture systems, systems-level analysis of cell and disease processes remains largely dependent on time-consuming and costly animal studies. However, as the pharmaceutical industry has come to discover, results obtained in animal experiments often fail to predict human responses.

In this article we focus on the next wave of 3D cell-culture models that better mimic the microstructure, dynamic mechanical properties and biochemical functionalities of whole living organs. These 'organs-on-chips' integrate microfluidics technologies with living cells cultured within 3D devices created with microfabrication techniques from the microchip industry, to study human physiology in an organ-specific context, and to develop specialized *in vitro* disease models. First we review how these whole organ mimics emerged from the convergence of cell biology with microengineering, and various new types of 3D culture models that have resulted along the way.

Microengineering meets cell biology

Microfabrication techniques, such as photolithography (Figure 1a), replica molding, and microcontact printing (Figure 1b), are well-suited to create structures with defined shapes and positions on the micrometer scale that can be used to position cells and tissues, control cell shape and function, and create highly structured 3D culture microenvironments [2–4]. Microfluidics – the science of manipulating small amounts (10^{-9} to 10^{-18} L) of fluids in microfabricated hollow channels (Figure 1) – is another core microsystems technology that has been used to generate and precisely tune dynamic fluid flows and spatiotemporal gradients, as well as deliver nutrients and other chemical cues to cells in a controlled manner [5].

One of the first major contributions made by applying microengineering approaches, such as soft-lithography-based microcontact printing (Figure 1b), to cell culture was the demonstration that ECM molecules presented in a 2D configuration can induce similar levels of tissue-specific differentiation to 3D ECM gel cultures, if presented on small, planar, microfabricated adhesive islands that restrict cell spreading so as to mimic the rounded or retracted shapes that cells exhibit when cultured on flexible 3D ECM gels. Under 2D conditions that partially restrict cell spreading, primary rat hepatocytes secrete higher levels of albumin and fibrinogen [6], capillary cells form hollow tubular networks [7], and vascular smooth muscle cells exhibit optimal response to vasoconstrictors [8]. The same approach, which permitted control of cell-shape distortion

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Box 1. Definition of 3D cell culture

Microengineered '3D cell culture' described in this review is broadly defined as the culture of living cells within microfabricated devices having 3D structures that mimic tissue- and organ-specific microarchitecture. Cell cultures in 3D matrix gels are referred to as '3D ECM gel cultures', '3D gel cultures' or 'conventional 3D cultures'.

independently of ECM coating densities or mechanical compliance under optimal growth factor conditions, was used to show that promoting capillary endothelial cell spreading stimulates growth, whereas fully preventing cell extension induces apoptosis within adherent endothelial cells [9]. Micropatterning techniques also have been used to culture cardiomyocytes on 2D elastomeric thin films micropatterned with ECM proteins to generate functional 2D heart tissues that generate stresses comparable to those measured *in vivo* [10]. Moreover, when the polymer is cut free from its adhesions to the rigid planar substrate, the heart tissue spontaneously contracts rhythmically and pulls the tissue into a 3D form, which is governed by the microcontact printed pattern of ECM [11].

Taken together, these findings emphasize that the mechanical environment is crucial for cell growth and tissue differentiation, suggesting that much of the power of 3D culture systems resides in the flexibility of the ECM gels that permits cells to change shape and form cell–cell connections that are prohibited on rigid conventional culture substrates. The observations also emphasize the importance of designing cell-culture systems that mimic the physical microenvironment of living organs as well as providing natural chemical cues.

Use of microfluidic cell-culture systems also has altered the way in which we study and manipulate living cells in both 2D and 3D systems. Owing to their small size, fluid flow in microfluidic systems is entirely laminar (no turbulence), and virtually no mixing occurs between neighboring streams that flow beside each other within the same hollow channel (Figure 1c). This novel property has been leveraged to generate abrupt step gradients on the micrometer scale to deliver gradients of chemicals across the diameter of a single cell [12], and to sustain chemical gradients with complex shapes over many hours to days to study cell motility in response to chemotactic stimuli [13,14]. It also has been extended to study more complex cell behaviors, such as stem cell differentiation [15–17], axon guidance [18], subcellular propagation of biochemical signaling [19], and embryonic development [20,21] in 2D culture, and to develop novel disease models (e.g. of Parkinson's disease [22]).

Microfluidic networks have been embedded directly within cell-laden hydrogels to enable efficient convective transport of nutrients and other soluble cues throughout the 3D scaffolds. Viable 3D tissue constructs have been produced consisting of calcium alginate hydrogels seeded with primary chondrocytes [23], hepatocytes encapsulated in agarose [24], mammary epithelial cells cultured in

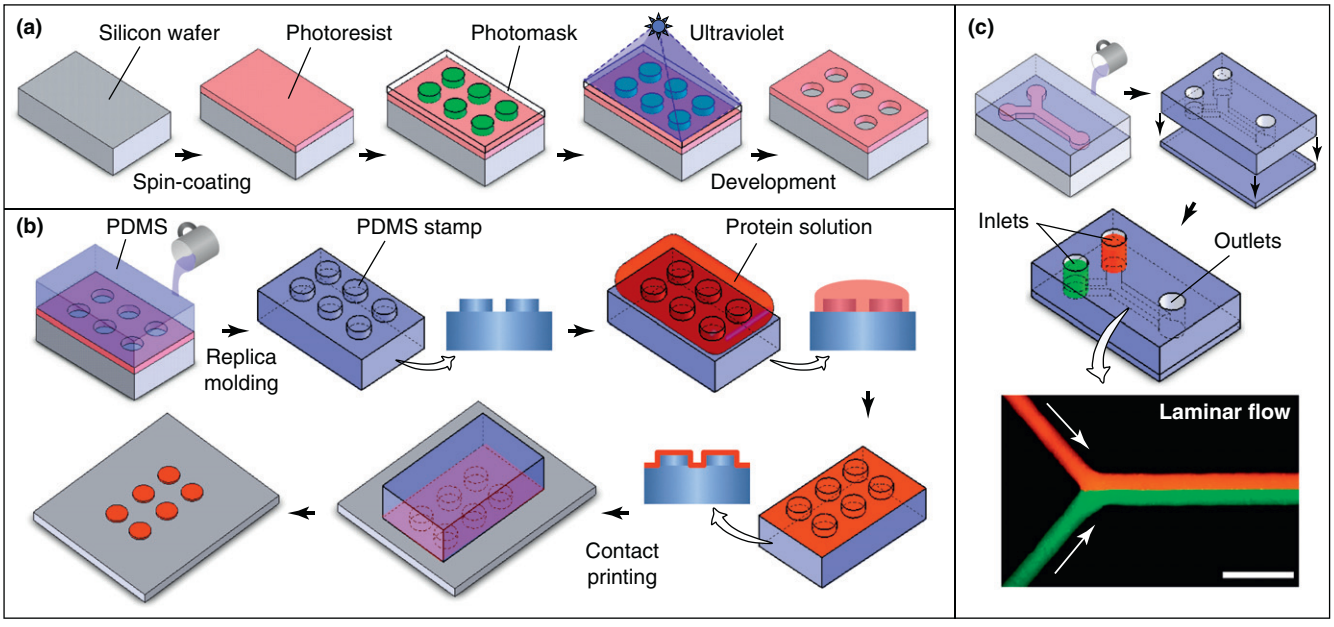

Figure 1. Microengineering technologies used to construct 3D culture systems and organs-on-chips. (a) Photolithography is a core microfabrication technique used to transfer microscale patterns to photosensitive materials by selective exposure to optical radiation. A silicon wafer is spin coated with a thin uniform film of a photosensitive material (photoresist), which is then aligned and brought in close contact with a photomask that typically consists of a transparent glass plate covered with a pattern defined by opaque chrome layers; the microscale pattern desired by the user is generated with computer-assisted design software. This is followed by exposure of the photoresist to high-intensity UV light through the photomask which protects some regions of the photoresist from UV and exposes others based on the design of the pattern. UV-exposed areas become soluble in a developer solution and dissolve away during the following step, termed development, thus leaving the desired microscale pattern etched into the photoresist. **(b)** Soft lithography involves fabrication of elastomeric stamps using a replica-molding technique in which liquid prepolymer of PDMS is cast against the bas-relief pattern of photoresist produced by photolithography in panel **(a)** to generate a PDMS substrate that replicates the 3D topography of the original master. In microcontact printing, the PDMS stamp is inked with protein solution, dried and brought in conformal contact with a surface for a period ranging from 30 s to several minutes. Upon removal of the stamp, a pattern is generated on the surface that is defined by the raised bas-relief structure of the stamp, and hence precisely recreates the microscale pattern of the original master. **(c)** Microfluidic devices are typically created by bonding a PDMS substrate containing microchannel features created by replica molding with a blank PDMS slab. In these microdevices, two fluids (red and green) introduced into independent inlets meet at a Y-junction and enter a straight microchannel in which they flow in adjacent, laminar streams without mixing (arrows indicate flow direction; scale bar, 500 μm). Reproduced from [84] with permission.

gelatin microdevices [25], and endothelial and smooth muscle cells embedded in 3D tubular poly(ethylene glycol) hydrogels [26]. More recently, a paper-based microfluidics technique in which fluid flow is guided in thin sheets of paper by printing chemicals that make the desired surrounding channels hydrophobic was used to create novel multilayered ECM gels for 3D culture [27]. The placement of the gels in paper sheets makes them easy to handle individually. But they can also be closely apposed to form layered cultures millimeters in thickness in which the gels interconnect in predefined spatial patterns, and then can be pulled apart at the end of the experiment to analyze cell responses within individual layers. This method permits study of defined chemical gradients *in vitro* and has been used to analyze the response of tumor cells and microvascular endothelium to oxygen gradients [28].

Microengineering cells into tissues on biochips

Recently, more complex microfluidic devices have been created to develop controlled microenvironments for manipulation and long-term differentiation of various types of cultured cells. For example, a microfluidic device containing a cross-flow bioreactor composed of two parallel arrays of silicon and stainless steel microchannels separated by a thin permeable membrane was developed to study the high metabolic demands of cultured primary rat hepatocytes [29,30]. Efficient delivery of oxygen and maintenance of physiological levels of fluid shear stress in the microreactor enabled the liver cells to reorganize into 3D tissue-like structures that remained viable for up to three weeks of culture.

Production of these early microsystems relied heavily on silicon microfabrication and micromachining techniques that are complex, costly and only accessible to specialized engineers. To overcome this, researchers developed microfluidic systems made of the silicone rubber, poly(dimethylsiloxane) (PDMS) [4], that are less expensive and easier to fabricate, and this opened entirely new avenues of exploration in cell biology. PDMS has several unique properties that make it a perfect choice for the fabrication of microdevices for the culture of cells and tissues. First, PDMS has a high gas permeability that ensures sufficient oxygen supply to cells in microchannels, eliminating the need for separate oxygenators that are commonly required in silicon, glass and plastic devices. This is a particular advantage for culture and maintenance of differentiated function of primary cells with high metabolic demands, such as liver epithelial cells, which are crucial for toxicology studies [31]. Similar PDMS microfluidic systems enabled the formation of viable and functional human tissues comprised of kidney epithelial cells [32], epidermal keratinocytes [33], osteoblasts [34,35] and chondrocytes [36] for drug screening and mechanotransduction studies.

Another advantage of PDMS is its optical transparency. This property was leveraged, for example, to carry out real-time microfluorimetric measurements of nitric oxide production and measure changes in pulmonary vascular resistance in a microfluidic model [37]. Optical monitoring of cell morphology and motility in PDMS microdevices also has proved to be useful for the analysis of tissue repair and reorganization [38] and of embryonic development [20,39]. In addition, the high flexibility of PDMS makes it possible to microfabricate tissue models with precise automated control of fluid flow. A self-contained microcirculating cell-culture system was created using a commercial Braille display composed of a computer-controlled array of piezoelectric pins that actuate microfluidic pumps and valves through localized elastic deformation of the PDMS channels [40,41]. This microdevice reproduces physiological fluid shear and pulsatile flow patterns for the analysis of human vascular endothelial cell responses to hemodynamic stresses. It also has been used in a handheld microfluidic device that enabled long-term (over two weeks) recirculation of cell-culture medium in ambient atmosphere to form muscle and bone tissues [42].

These intrinsic properties of PDMS become more powerful when combined with microfabrication techniques such as replica molding and embossing (Figure 1) to generate patterns and structures that provide more physiologically relevant cell-culture conditions. Replica-molding techniques were used, for example, to construct PDMS microfluidic organ scaffolds with branching vascular networks lined by living endothelium that recapitulate natural microvascular hemodynamics [43]. Related approaches have been used to microfabricate small 3D ECM gel building blocks that self-assemble into larger 3D organ-like structures with complex architecture, which could prove interesting as novel *in vitro* models [44]. Because microfabrication techniques are being utilized, electrical, chemical, mechanical and optical probes for direct visualization and quantitative analysis of cellular biochemistry, gene expression, structure and mechanical responses also can be integrated into virtually any microfabricated cell-culture device [45,46].

From 3D culture to organs-on-chips

Recreating tissue–tissue interfaces to mimic organ microarchitecture

Development of these microengineering approaches has opened entirely new possibilities to create *in vitro* models that reconstitute more complex 3D organ-level structures and to integrate crucial dynamic mechanical cues as well as chemical signals. To study polarized functions of various epithelial cells (e.g. intestine [47,48], lung [49], kidney [50,51], cornea [52]), for example, two PDMS cell-culture chambers were stacked and separated by a permeable synthetic membrane or ECM (Figure 2a). A similar approach has been used to integrate liver epithelium with microfabricated vascular networks [53], and fibroblasts were included as well in another liver model [54]. In this manner, microfabrication was leveraged to reconstitute a tissue–tissue interface that is a crucial element of whole liver organ structure, which was not previously possible in conventional 3D ECM gel cultures.

Replica-molding techniques also have been used to replicate complex surface relief patterns to produce biomimetic structures that mimic organ-specific microarchitecture. Microfabricated structures were built within microfluidic channels that resemble the endothelium that separates hepatocytes from the liver sinusoid in whole liver, with the goal of more closely approximating the mass transport properties of the hepatic microcirculation

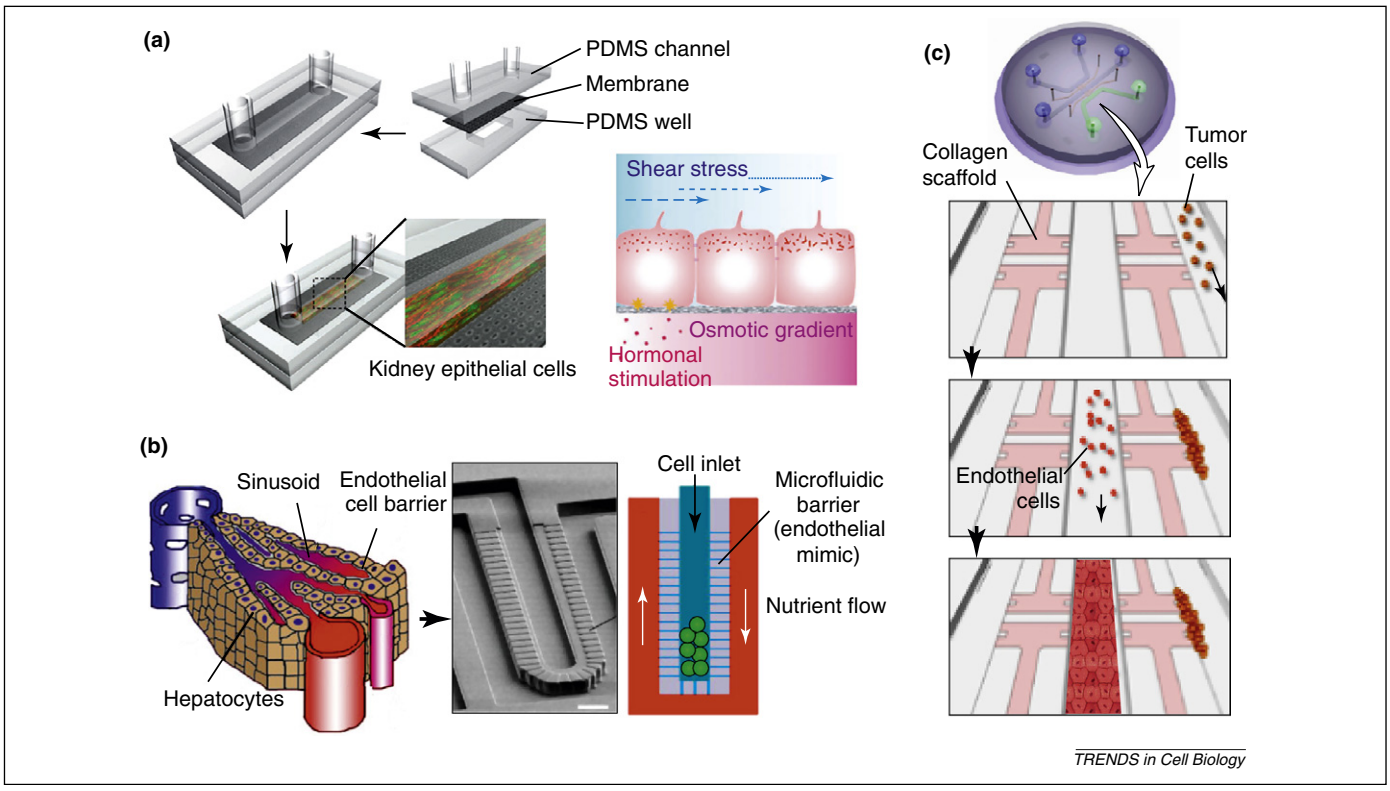

Figure 2. Microengineered organs-on-chips. **(a)** A microfluidic kidney epithelium model composed of a multilayered microdevice that incorporates stacked layers of PDMS microchannels and a PDMS well separated by a porous polyester membrane. The 3D architecture of this microsystem provides physiologically relevant culture environments for polarized kidney epithelial cells, and enables precise control of fluid flows, selective exposure of the apical and basal sides of the cells to fluid shear, hormones, and chemical gradients, and collection of samples from both sides of the polarized tissue. **(b)** A microengineered liver-on-a-chip reconstitutes hepatic microarchitecture. The functional unit of this microsystem consists of a central liver-cell culture chamber and a surrounding nutrient flow channel separated by microfabricated barrier structures patterned with a set of narrow (2 μm in width) microchannels that mimic the highly permeable endothelial barrier between hepatocytes and the liver sinusoid. This biomimetic device closely approximates transport of nutrients and waste products in the liver sinusoid and provides more favorable environments for the maintenance of primary liver cells in a differentiated state (scale bar, 50 μm). **(c)** Heterotypic interactions between tumor cells and endothelial cells are studied in a microfluidic device that permits co-culture of these cells in two separate microchannels connected via scaffold channels filled with 3D collagen gels (pink channels in the diagram). This microfluidic system is used to model the tumor microenvironment and gain better understanding of important disease processes such as angiogenesis and cancer cell invasion during cancer progression. Reproduced from [50,55,60,61] with permission.

(Figure 2b) [55]. This bioinspired liver-on-a-chip enabled primary human hepatocytes to maintain liver-specific metabolic activity for one week in culture without exogenous ECM coatings, and it induced formation of hepatic cord-like structures with functional bile canaliculi [56]. An array of PDMS microchambers interconnected by narrow (1 μm wide) channels was similarly used to enable growth and *in vivo*-like reorganization of osteocytes in a 3D environment that replicates the lacuna–canalicular network of bone [57]. Other examples include a compartmentalized microfluidic system that enables co-culture of neurons and oligodendrocytes to study neuron–glia communication during development of the central nervous system [58] and a microdevice incorporating ECM gels microinjected between two parallel microchannels to investigate the vascularization of liver tissues in 3D culture microenvironments [59].

Similar microsystems approaches have been used to develop disease models. For example, a microfluidic co-culture platform was developed that allows for communication between different tissue types through 3D ECM gels to examine capillary-cell invasion and sprouting in response to malignant breast and brain tumors (Figure 2c) [60,61] or behavior of breast cancer cells during the transition of ductal carcinoma *in situ* to invasive carcinoma [62]. A device incorporating two stacked PDMS microchannel layers separated by a thin porous membrane was used to form heterotypic multicellular spheroids in a 3D microfluidic culture system [63,64] that recapitulates the tumor microenvironment of metastatic prostate cancer cells [65]. A similar strategy permitted study of intravascular adhesion of circulating metastatic breast cancer cells in response to endothelial activation under physiological flow conditions [66]. Using this model, basal stimulation of the endothelium with the cytokine, CXCL12, was shown to significantly increase adhesion of circulating breast cancer cells.

Recapitulating organ-specific biochemical and mechanical microenvironments

Importantly, microengineering also can be used to mimic the complex biochemical microenvironment of living organs. A microfluidic perfusion bioreactor was constructed to impose oxygen gradients on hepatocytes co-cultured with fibroblasts to mimic physiological variations in hepatocyte form and function along the liver sinusoids, known as liver zonation, which is thought to depend on regional variations in oxygenation [67]. This microdevice effectively reconstituted the regional heterogeneity of liver microarchitecture as well as drug-induced zonal hepatotoxicity seen *in vivo*. A 3D microfluidic device was used to study chemotactic migration of malignant cancer cells

under conditions in which cancer cells were precisely positioned relative to 'source' and 'sink' cells, which produce and scavenge chemokines causing production of shallower and more physiological chemokine gradients [68].

More recently, mechanically active microfluidic culture devices have been created that reproduce the dynamic physical forces that are crucial for organ function, as well as for disease development. For example, primary human small airway epithelial cells were cultured in a microfluidic channel and integrated with a computer-controlled, two-phase (air–liquid) microfluidic switching system to mimic the propagation and rupture of liquid plugs that occur during the reopening of collapsed airways that causes mechanical airway injury in obstructive pulmonary disease [69]. This study revealed severe injurious cellular responses to plug propagation and rupture – and, impressively, these injury events could be detected acoustically in the device as crackling sounds nearly identical to the respiratory 'crackles' that physicians listen for through their stethoscopes to detect fluid in the lung.

In a related study, a mechanically actuatable, multilayered, microfluidic device was developed to expose alveolar epithelial tissues to a combination of cyclic wall-stretch and repeated propagation of a moving air–liquid interface that reproduces the complex pathological mechanical forces experienced by alveolar epithelial cells during mechanical ventilation [70]. Quantitative analysis of mechanical cell injury in this 'alveolus-on-a-chip' revealed that a combination of solid and fluid mechanical stresses causes significantly more severe cell injury than either alone, supporting clinical observations that alveolar stretch alone is not sufficient to induce ventilator-induced lung injury. Thus, these relatively simple microengineered tissue models offer an *in vitro* method to mimic some important facets of complex disease processes that depend crucially on 3D organ structure; again, this result could not be obtained using conventional 3D gel cultures.

Organomimetic microdevices with integrated functionality

The most recent advance in this area has been the development of integrated organ-on-chip microsystems that reproduce key structural, functional, biochemical, and mechanical features of living organs in a single device (Table 1). For example, we recently microengineered a device that recapitulates the 3D microarchitecture, tissue–tissue interfaces and breathing movements of the alveolus, as well as major physiological functions of the whole human lung [71]. This human lung-on-a-chip device consists of two PDMS microfluidic channel layers separated by a thin (10 μm), flexible, ECM-coated PDMS membrane with microengineered pores (10 μm in diameter) that mimics the alveolar–capillary interface of the living lung (Figure 3). Human lung alveolar epithelial cells are cultured at an air–liquid interface on one side of the porous membrane, while human lung capillary endothelial cells are grown on the opposite side under dynamic flow of culture medium that mimics the hemodynamics of the lung microvasculature. In addition, this microsystem reproduces breathing movements and the associated cyclic strain experienced by cells at the alveolar–capillary interface, by applying cyclic suction to neighboring hollow microchannels that deforms the central porous membrane and attached cell layers.

Most importantly, this lung-on-a-chip microdevice replicates the complex organ-level responses of living human lung to physiological inflammatory stimuli, such as bacteria or cytokines (e.g. TNF-α) introduced into the air space. The underlying endothelial cells rapidly become activated in response to these cues, and this induces adhesion of primary human neutrophils flowing in the capillary channel, as well as their transmigration across the capillary–alveolar interface and into the alveolar space where the neutrophils engulf the bacteria. Because of the optical clarity of the PDMS, the entire human inflammatory

Table 1. Summary of microengineered organ models

Organ	Incorporated cell types	Demonstrated organ-specific features	Refs
Liver	Hepatocytes Vascular endothelial cells Fibroblasts	Serum protein synthesis	[53,54]
		Bile canaliculi	[56,59]
		Liver sinusoid	[55]
		Liver zonation	[67]
Lung	Airway epithelial cells Alveolar epithelial cells Pulmonary microvascular endothelial cells	Airway closure and reopening	[69,70]
		Small airway protein (CC10) synthesis	[69]
		Alveolar–capillary interface	[71]
		Surfactant production	[49,71]
		Lung inflammation	[71]
		Extrapulmonary absorption	[71]
Kidney	Renal tubular epithelial cells	Molecular transport	[50,51]
Gut	Intestinal epithelial cells	Intestinal absorption	[47,48]
Bone	Osteoblasts Osteocytes	Lacuna–canalicular network	[57]
Breast	Mammary epithelial cells Mammary fibroblasts Vascular endothelial cells	Malignant tumor invasion	[62]
		Cancer metastasis	[66,68]
Eye	Corneal epithelial cells Vascular endothelial cells	Epithelial barrier function	[52]
Brain	Neurons Astrocytes Oligodendrocytes	Axon–glia interaction	[58]
		Tumor angiogenesis	[60]

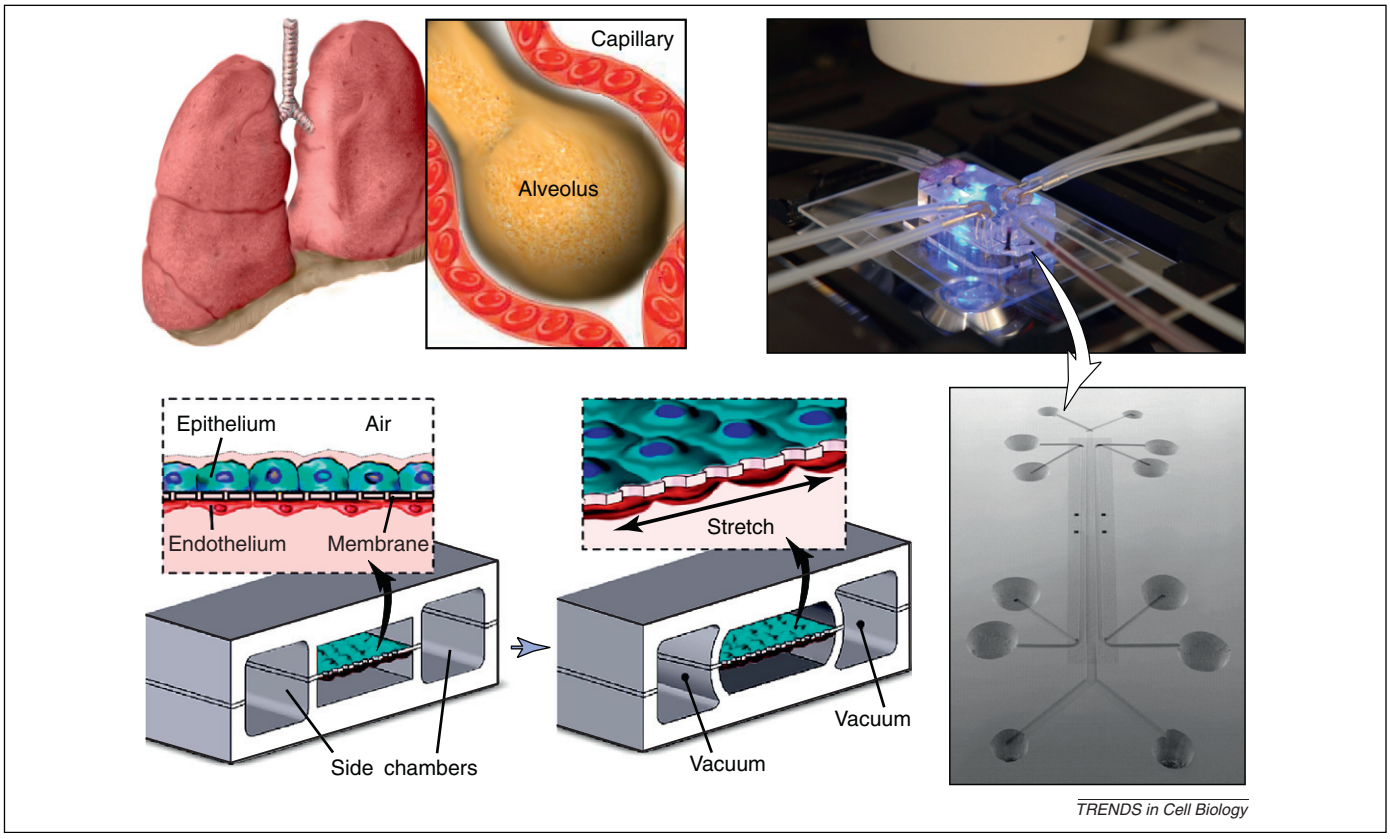

Figure 3. A human breathing lung-on-a-chip. A microengineered model of the alveolar–capillary interface within a clear flexible microfluidic chip approximately the size of a computer memory stick (top right visualized on the microscope under fluorescence illumination; bottom right shows scanning electron micrographic view) that reconstitutes the cellular, biochemical and mechanical functions of the living human lung. The crucial tissue–tissue interface of the alveolus (top left) is replicated in this bioinspired microdevice by co-culturing human alveolar epithelial cells and pulmonary capillary endothelial cells on the opposite sides of a thin, flexible, porous, ECM-coated PDMS membrane (bottom left). To accomplish mechanical actuation that mimics physiological breathing movements, air pressure in two hollow side chambers microfabricated within the device is decreased and increased in a cyclic manner by using a small vacuum pump; this causes the membrane and attached human cell layers to cyclically stretch and relax under physiological mechanical strain. The lung epithelial cells are cultured at an air–liquid interface, and culture medium is pumped through the lower microchannel containing the capillary cell layer to mimic blood flow through lung microvasculature. This system effectively mimics the entire human inflammatory response when pathogens or inflammatory cytokines are placed in the air channel and human neutrophils are introduced into the capillary channel; the device also can be used to study absorption and toxic effects of airborne particles, chemicals or drugs. Reproduced from [71] with permission.

response can be visualized in real-time within this microdevice (for video see http://wyss.harvard.edu/viewpage/240/lungonachip).

The lung-on-a-chip also was used for nanotoxicology studies in which various types of nanoparticles were introduced into the air channel. These studies revealed that silica nanoparticles (12 nm) that mimic airborne particulates are transported through both the epithelial and endothelial cell layers using a transcytosis mechanism, and that they induce cell toxicity and inflammation as measured directly within the microchannel in real-time using microfluorimetry. A surprising result was that physiological breathing movements significantly increase the transport of these nanoparticles from the air space into the vascular channel, and this prediction was confirmed in an animal model. This is a prime example of a new and potentially important physiological mechanism that would never have been detected in a conventional 2D or 3D cell-culture model.

'Human-on-a-chip'

Despite the considerable advances in the creation of microengineered tissue and organ models, much remains to be accomplished in the development of complex 3D models that reconstitute whole-organ metabolism and physiology because virtually all organs are functionally integrated in the human body. In fact, investigators are already developing integrated 'human-on-a-chip' models that consist of interconnected compartments, each containing a cell type representing a different organ, linked through a microfluidic circulatory system [72]. In one study, a microfabricated bioreactor was created that contained 2D cultures of liver and lung cells in different microchambers interconnected by microfluidic channels under conditions that approximated physiological liquid-to-cell ratios, hydrodynamic shear stresses and liquid residence times in living whole-organ systems to develop physiologically relevant pharmacokinetic models [73]. Toxicity studies using this system demonstrated, for example, that naphthalene metabolites produced in the liver compartment lead to glutathione depletion in the lung epithelial cell compartment [74]. This microfluidic system was later modified to incorporate 2D and 3D cultures of adipocytes, cancer cells and bone marrow stem cells to study drug accumulation, distribution, metabolism and toxicity [75–77]. Similar approaches were used to develop microfluidic models of multiorgan interactions useful for studying intestinal absorption, hepatic metabolism and the activity of breast

cancer drugs [78]. Various laboratories are currently working to link more complex, mechanically active, organ-on-chip models via microfluidics to meet similar goals, while providing more complete physiological biomimicry.

Potential applications and future prospects

These studies have revealed that although 3D cell cultures represent a great improvement over planar 2D models, and altering the ECM mechanical compliance can further control cell differentiation and fate switching in 3D systems, the reality is that we can do even better to achieve our goal of recapitulating organ-level functionality by combining microengineering with cell biology. The key to meeting this challenge has been to recognize the importance of reconstituting the appropriate tissue microarchitecture, complex biochemical milieu and dynamic mechanical microenvironment (e.g. cyclic mechanical strain, fluid shear). Fortunately, microfabrication strategies and microfluidics are well-suited to meet these challenges because they provide precise dynamic control of structure, mechanics and chemical delivery at the cellular size scale.

Microengineered 3D cell-culture models, and particularly more sophisticated organ-on-chip microdevices, have many potential applications. The pharmaceutical industry is under intense pressure economically, ethically and scientifically to find ways to accelerate the drug-development process and to develop drugs that are safer and more effective in humans at a lower cost. Traditional animal-testing approaches are costly, and they often fail to predict human toxicity or efficacy; in fact, many now question the ethics of sacrificing animals if they cannot reliably predict clinical outcomes. The chemical and cosmetics industries are facing similar challenges, and they too are seeking alternative *in vitro* methods that are more predictive of clinical outcomes [79].

Microengineered cell-culture systems that mimic complex organ physiology have the potential to be used for the development of human-relevant disease models that are more predictive of drug efficacy and toxicity in patients, while also providing greater insight into drug mechanism of action. More predictive *in vitro* assays incorporating cultured human cells also will protect public health by identifying environmental toxins and providing a better understanding of their mechanisms of action, as well as improving our ability to predict risks for specific compounds. In addition, the organ-on-chip microdevices could be integrated to study the interplay of different organs in determining pharmacokinetic properties of compounds at a higher level of functionality than was possible in the past.

This ability to integrate functional organ mimetics, such as gut-, liver-, lung- and skin-on-chips within a 'human-on-a-chip' (Figure 4), could provide improved methods to explore different routes of drug delivery (oral, aerosol and transdermal), as well as their effects on the efficacy or toxicity of different drug formulations. Other examples include linking the lung-on-a-chip with a heart-on-a-chip to

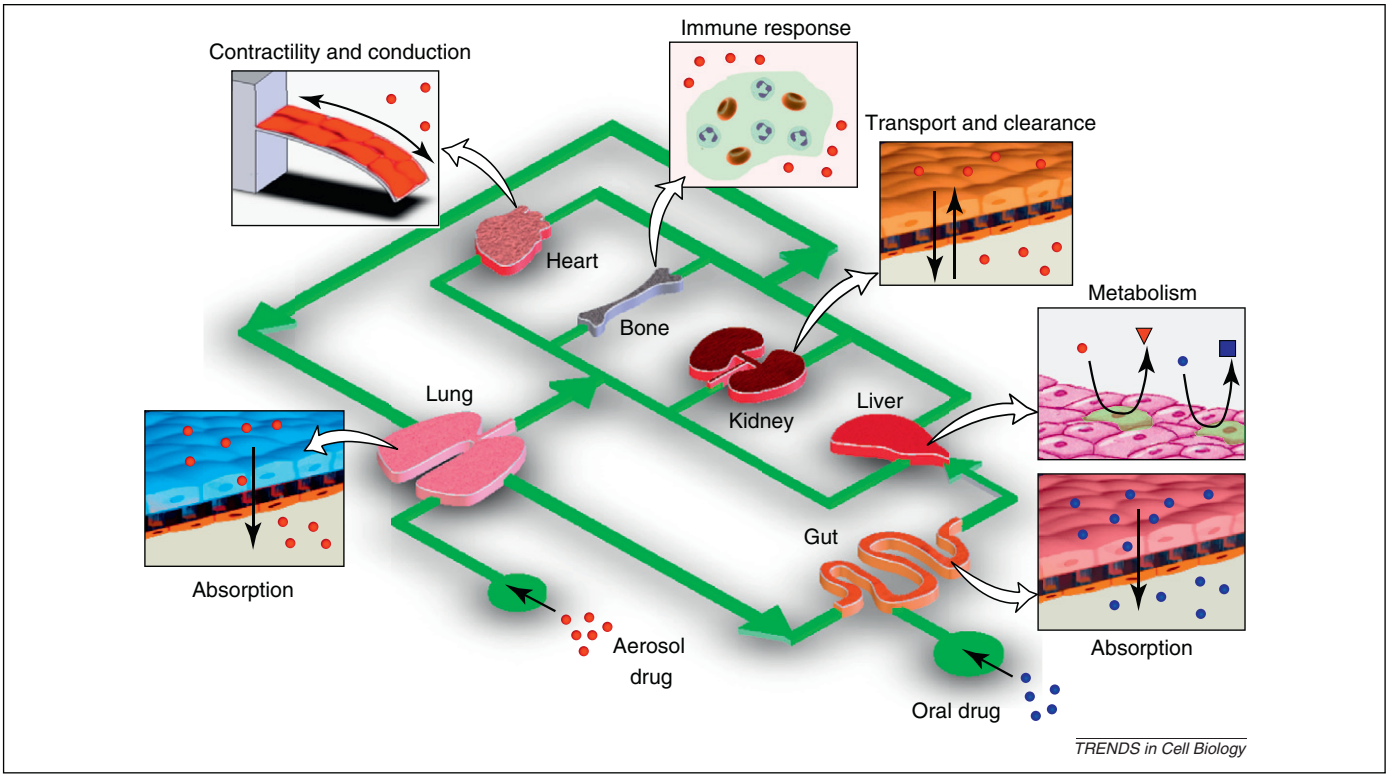

Figure 4. The human-on-a-chip concept. Biomimetic microsystems representing different organs can be integrated into a single microdevice and linked by a microfluidic circulatory system in a physiologically relevant manner to model a complex, dynamic process of drug absorption, distribution, metabolism and excretion, and to more reliably evaluate drug efficacy and toxicity. As shown in this example, an integrated system of microengineered organ mimics (lung, heart, gut, liver, kidney and bone) can be used to study the absorption of inhaled aerosol drugs (red) from the lung to microcirculation, as well as to measure their cardiotoxicity (e.g. changes in heart contractility or conduction), transport and clearance in the kidney, metabolism in the liver, and immune-cell contributions to these responses. Drug substances (blue) also can be introduced into the gut compartment to investigate interplay between orally administered drugs and molecular transporters and metabolizing enzymes expressed in the various organs.

provide better insight into potential cardiotoxicity of aerosolized drugs, inhaled nanoparticles or other air pollutants, or adding a bone marrow-on-a-chip to other organ mimetics to provide an immune-response element to the integrated human-on-a-chip microsystem. In the future, it even might be possible to inhabit these devices with cells obtained from different human 'responder' or 'non-responder' genetic populations (e.g. HLA subgroups or people who express particular molecular transporters or metabolic enzymes) such that drugs can be developed for specific human subpopulations and, eventually, virtual human clinical trials could be carried out using these microengineered models.

One of the key requirements for these systems to be implemented as new screening paradigms is to further characterize the response of these microchips to pharmacological modulation, and to validate their abilities to predict human responses with well-characterized drugs. Another key future challenge will be identifying the optimal source of human cells that best mimic *in vivo* responses. Recent organ-on-a-chip studies suggest that established human cell lines can be used to model complex lung functions (e.g. inflammatory response) *in vitro*, and that these cells exhibit more highly differentiated phenotypes when presented with physiologically relevant microenvironmental cues than when maintained under conventional 2D or 3D culture conditions [71]. Alternatively, inducible pluripotent stem (iPS) cells or embryonic stem (ES) cells may be the solution when primary cells are not a practical option. However, iPS and ES cells are still at very early stages, and much work is required to drive the cells from a neonate-like state to the more mature adult phenotype required for use in organ-on-chip microsystems. An additional challenge lies in integrating these microengineered organ mimics with sensors that can detect and measure optical, chemical, electrical and mechanical signals from cells to analyze their structure and function. Also, the development of automated control of flow and pressure within microfluidic devices using microengineered pumps and valves [41,80–83] will probably be required for more reliable and facile operation, and will constitute an important step towards multiplexing these microsystems for high-throughput analysis and screening.

Concluding remarks

Although bioengineered 3D microsystems and organ-on-chip technologies are relatively new and still require further validation and characterization, their potential to predict clinical responses in humans could have profound effects on drug discovery and environmental toxicology testing. The scale-up of these complex technologies, together with systems integration of the engineering (e.g. fluidics handling, pumps) into easy to use, scalable, reproducible and user-friendly systems will be the key to their future success. Equally crucial will be for the field to move from lab-based prototyping to commercial manufacturing of these chips in materials suitable for drug-discovery applications. This is crucial because, despite the many desirable properties of PDMS most commonly used in microfluidic systems, PDMS has poor chemical resistance to certain solvents and it can absorb small hydrophobic molecules (e.g. many drugs, fluorescent dyes), thus compromising rigorous chemical testing of potential therapeutic agents. In addition, it will be important to ensure that appropriate biomarkers and assays are developed for use with these microsystems, and to validate the extrapolation of *in vitro* results to the human situation. Nevertheless, in the near term these microengineered culture devices provide uniquely powerful alternatives to existing 2D and 3D culture models for cell biologists and others interested in analyzing cell physiology and visualizing complex cell behaviors in a more physiologically relevant context.

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