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SPOTLIGHT

Modeling human diseases with induced pluripotent stem cells: from 2D to 3D and beyond

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ABSTRACT

The advent of human induced pluripotent stem cells (iPSCs) presents unprecedented opportunities to model human diseases. Differentiated cells derived from iPSCs in two-dimensional (2D) monolayers have proven to be a relatively simple tool for exploring disease pathogenesis and underlying mechanisms. In this Spotlight article, we discuss the progress and limitations of the current 2D iPSC disease-modeling platform, as well as recent advancements in the development of human iPSC models that mimic *in vivo* tissues and organs at the three-dimensional (3D) level. Recent bioengineering approaches have begun to combine different 3D organoid types into a single '4D multi-organ system'. We summarize the advantages of this approach and speculate on the future role of 4D multi-organ systems in human disease modeling.

KEY WORDS: Induced pluripotent stem cells, Disease modeling, Organoid, Organ-on-chip

Introduction

Understanding the mechanisms underlying human disease pathologies is important for the development of therapeutic treatments, but this is a challenging process due to the lack of biologically relevant disease models. Rodents have been widely used to simulate human diseases for decades, as they are a mammalian model that lends itself to experimental assessment and genetic engineering. However, primates and rodents diverged ∼75 million years ago, and fundamental interspecies differences make it impossible for rodent models to accurately mirror or fully recapitulate human clinical pathophysiology (Sayed et al., 2016). These differences make it difficult to extend findings on efficacy and toxicity testing of potential drugs from rodents to human, which has contributed to the failures of many clinical trials. In recent years, human induced pluripotent stem cells (iPSCs) have emerged as an attractive platform for overcoming these conventional limitations of animal models for disease modeling and drug discovery (Shi et al., 2017; Takahashi et al., 2007). iPSCs not only have the capacity for self-renewal and differentiation, but can also be directly generated from the patients' skin fibroblasts, blood cells and other somatic cell sources. Therefore, patient-specific iPSCs could provide unlimited disease-relevant cells in a personalized manner, serving as an extremely valuable resource for previously inaccessible cell types, including cardiomyocytes (Sayed and Wu, 2017) and neurons (Shi et al., 2017). However, concerns around the genome instability and epigenetic memory associated with the reprogramming process and iPSC maintenance remain, which pose challenges to the integrity of iPSC derivatives and the modeling of diseases that are epigenetically influenced by environmental factors (Tapia and Schöler, 2016).

Differentiated iPSCs in 2D monolayers are conventionally used to uncover disease phenotypes, but they lack the tissue- and organ-level structures and functions central to many disease etiologies. Recent efforts have been channeled to develop 3D iPSC models that can more accurately recapitulate tissue- and organ-level disease pathophysiology (Takebe et al., 2017). In this Spotlight article, we summarize the progress and potential challenges of modeling various human diseases using patient-specific iPSCs, focusing on the comparison of 2D and 3D systems, as well as integrated 3D systems that are also known as 4D multi-organ systems or 'body-on-chip'.

The progress and limitations of modeling human diseases in 2D

Currently, most iPSC disease modeling studies use the conventional 2D monolayer culture platform. Because iPSCs can be differentiated into any type of disease-relevant cells and can faithfully recreate the genetic background of patients, 2D iPSC models have been widely used to study monogenic diseases as well as more complex polygenic diseases of various organs (Table 1; Matsa et al., 2016). For example, studies using iPSC-derived dopaminergic neurons from patients with monogenic and sporadic Parkinson's disease (PD) have successfully illustrated key features of PD pathophysiology, including impaired mitochondrial function, increased oxidative stress and accumulation of α-synuclein protein (Torrent et al., 2015). Similarly, iPSC-derived hepatocytes from patients with inherited metabolic disorders, such as α1-antitrypsin deficiency, familial hypercholesterolemia and glycogen storage disease type 1a, recapitulate the pathological phenotypes of the disease with aggregation of misfolded α1-antitrypsin in the endoplasmic reticulum, deficient LDL receptor-mediated cholesterol uptake, and elevated lipid and glycogen accumulation (Rashid et al., 2010). Moreover, the introduction of CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 (CRISPR-associated protein 9) technology has now greatly facilitated the generation of isogenic iPSC lines (either by correction or insertion of mutations) that differ only at the genome-edited loci from the parental lines (Seeger et al., 2017). These precise isogenic controls have allowed researchers to correlate the genetic mutations with the disease phenotypes without any other confounding influences from the genetic background. Genome-edited isogenic iPSC lines have been used in various 2D disease modeling studies, such as dilated cardiomyopathy (Sun et al., 2012), familial Alzheimer's disease (Yagi et al., 2011) and cystic fibrosis in lung (Firth et al., 2015).

Despite the progress in 2D disease modeling, there are limitations to this technology (Table 1). Perhaps the most obvious of these is the

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Table 1. The comparison between 2D and 3D iPSC disease modeling systems

		3D systems		
	Conventional 2D system	Engineered tissue	Organoid	Organ-on-chip
Production method	Differentiated and grown on rigid flat surfaces as a monolayer	Fabricated with a scaffold and casting mold to mimic the ECM	Embedded in Matrigel and undergo self-organization in response to differentiation cues	Seeded in biofabricated and microengineered channels and chambers with perfusion
Production timing	Fast	Slow	Slow	Fast
Maturation status	Immature	Improved maturation	Improved maturation	Improved maturation
Cell morphology	Unnatural; they flatten out on a rigid culture surface	Cell type-specific size and shape similar to *in vivo*	Cell type-specific size and shape similar to *in vivo*	Depends on fabrication method
Cell types	Usually monotype; difficult to co-culture multiple cell types	Multi-type	Highly diverse cell types similar to *in vivo*	Multi-type
ECM	Limited composition and contacts with cells	*In vivo*-like composition and cell contacts	*In vivo*-like composition and cell contacts	Based on design and fabrication
Tissue architecture	Absent	Simple	Complex architecture reminiscent of organ development	Complexity based on design
Signal factor and nutrients diffusion	Short diffusion distance (directly exchanged through cell membrane); cells usually receive a supraphysiological dose	May diffuse down a concentration gradient and across cell layers; affected by ECM material properties	Inefficient transport to the interior cells, leading to cell death and a lack of maturity	Precisely controlled spatial and temporal diffusive gradients
Vascularization/ perfusion	Absent	Absent	Absent	Present
Fidelity	Low	Medium	High	Medium
High-throughput feasibility	Present	May be developed by bioprinting technology	Absent	Present
Controllability	High	Low	Very low	Very high
Variability and reproducibility	Low variability and high reproducibility	High variability and low reproducibility	High variability and low reproducibility	High variability and relatively low reproducibility
Genome-editing capability	Easy	Hard	Hard	Easy
Tissue/organ scalability	Absent	May develop to macroscale	May develop to macroscale	Microscale
Characterization and analysis	Limited to cellular and molecular analysis; easy cell retrieval	Tissue function analysis available; hard to retrieve cells; hard to analyze inner cell phenotypes. Improvements possible with fluorescent reporter and single cell 'omics' technology.	Tissue function analysis available; hard to retrieve cells; hard to analyze interior cell phenotypes. Improvements possible with fluorescent reporter and single cell 'omics' technology).	Tissue/organ simple unit function analysis; easy to retrieve cells; real-time multiplex monitoring and analysis with biosensors
Technical accessibility	Easy	Hard	Relatively easy	Very hard

ECM, extracellular matrix.

loss of the complex, 3D and heterotypic environment in which the cells normally reside *in vivo*. Parenchymal cells in living organs actually reside in a highly complex 3D environment supported by an organized extracellular matrix (ECM) and other cell types (Gattazzo et al., 2014). In the human heart, for example, cardiomyocytes represent only around 30% of the total cells, with the remaining 70% consisting of non-myocytes, such as vascular smooth muscle cells, endothelial cells, fibroblasts and leukocytes, in addition to the various structural ECM components (Pinto et al., 2016). Without the dynamic, reciprocal, biochemical and biophysical support from the ECM and surrounding cells, 2D iPSC models lack essential information regarding cell-cell communications, cell matrix mechanics and the unique *in vivo* niche environment in which parenchymal cells and tissue reside (Brafman, 2013; Gattazzo et al., 2014). Hence, fundamental questions related to the actual complex 3D status remain unanswered, specifically those related to non-cell autonomous pathogenesis and structurally related disease phenotypes (Passier et al., 2016).

A lack of 3D environmental cues could also explain why 2D iPSC derivatives are generally immature, resembling fetal cells more than adult cells in many cases (Wu and Hochedlinger, 2011). For example, the transcriptional pattern and metabolic profiles of embryonic stem cell- (ESC) or iPSC-derived hepatocytes have been shown to mimic fetal rather than adult hepatocytes (Baxter et al., 2015). For this reason, one could argue that 2D iPSC-derivatives are more suitable for modeling early-age onset diseases than adult-onset diseases. Nevertheless, multiple groups have successfully modeled adult-onset diseases such as PD and arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C) using various approaches to 'induce ageing' in culture (Kim et al., 2013; Vera and Studer, 2015). These approaches include prolonged culturing *in vitro*, the use of cellular oxidative stressors, induction of adult-like metabolism and overexpression of the ageing protein progerin (Vera and Studer, 2015). However, the artificially induced 'ageing' and the underlying immaturity of these cells may lead to misinterpretation of disease phenotypes and mechanisms. Overall, 2D monolayer platform has limitations when applied to disease

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modeling, and there is a compelling need for introducing an extra dimension to the existing modeling systems.

Going 3D: bioengineering and self-organization add a new dimension

With the emergence of new biomaterials as well as improved bioengineering methods, it is now possible to seed different types of cells as 'building blocks' into porous 3D scaffolds to form engineered tissue constructs (Fig. 1). Many different scaffolds made of materials that mimic the native ECM, including hydrogels (e.g. collagen, fibrin and Matrigel) and decellularized tissue extracts, have been investigated for this purpose (Zhu and Marchant, 2011). iPSC derivatives have been widely used to generate engineered intestinal, lung, hepatic and myocardial tissues, often by seeding the scaffolds with multiple different cell types in an attempt to recapitulate their native *in vivo* environment (Edgar et al., 2016). For example, engineered heart tissues (EHTs) have been fabricated with hydrogels by combing casting molds and supporting cells: iPSC-derived endothelial cells, smooth muscle cells and fibroblasts (Zimmermann and Cesnjevar, 2009). These iPSC-EHTs resemble their native physiological microenvironment and recapitulate coordinated contractile and electrophysiological interactions among the ECM and heterogeneous cell types that

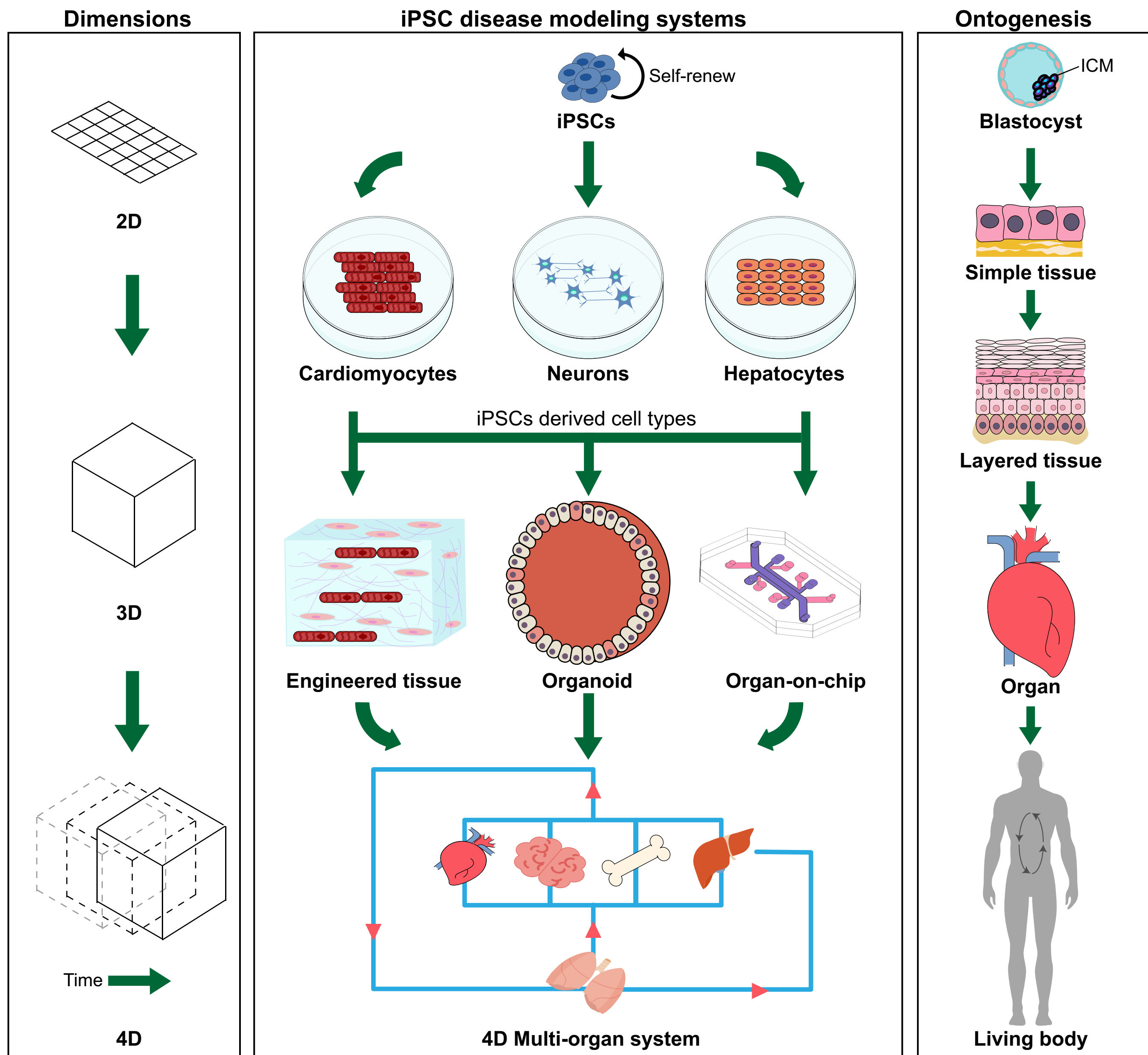

Fig. 1. Schematic overview of current iPSC disease modeling in 2D and 3D systems. iPSCs exhibit the capability to self-renew and differentiate into multiple cells (e.g. cardiomyocytes, neurons and hepatocytes), similar to ESCs that are derived from the inner cell mass (ICM) of the early embryo. Patient-specific iPSC-derived cells have been widely used to study various human diseases using a 2D monolayer platform, but this approach cannot recapitulate complex tissue architecture and organ functions seen *in vivo*. Various 3D systems have been developed to model human diseases under conditions that mimic more closely the bona fide physiological environment, including engineered tissues, organoids and organs-on-chip. In the future, converging these 3D systems and linking multi-organs together with engineered vasculature will enable modeling of the temporal dynamic processes in the living body and disease pathogenesis, adding a fourth dimension.

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make up the myocardium (Tzatzalos et al., 2016). EHTs from iPSC-derived cells have been used to successfully model cardiac diseases such as dilated cardiomyopathy and heart failure, helping to identify cardiac phenotypes crucial to pathogenesis (Hinson et al., 2015; Tiburcy et al., 2017).

Microfluidic organ-on-chip approaches offer a precise means to control tissue composition and architecture in an *in vitro* 3D microdevice that further incorporates vascular perfusion and micro-biofabrication (Takebe et al., 2017). This approach gives researchers finer control over multiple physiological phenomena such as tissue-tissue interactions and physicochemical niche cues, as well as physical forces that occur in living organs, such as breathing movement, shear stress, peristalsis and tension (Bhatia and Ingber, 2014). In 2010, a human lung-on-chip was created using classical soft lithography and microfluidic devices to reconstitute the functional alveolar-capillary interface of the lung (Huh et al., 2010). Since this discovery, organ-on-chip systems have been applied in iPSC disease modeling for an increasingly wide range of different diseases, including Barth syndrome-associated cardiomyopathy, drug-induced kidney glomerular injury, blood-brain barrier function and skin wound healing (Low and Tagle, 2017).

Organoids are 3D cell masses that recapitulate some level of tissue or organ architecture and function (Dutta et al., 2017). Unlike the carefully controlled environments afforded by bioengineered scaffolds or organs-on-chips, organoids are mostly self-organizing. Fundamental work by Eiraku et al. generated organoids of polarized cortical brain tissues and optic cups by exposing mouse ESCs to defined lineage-specification factors and embedding them in a hydrogel, often Matrigel (Eiraku et al., 2008, 2011). Since then, a range of iPSC-derived organoids resembling the brain, liver, gut, lung, kidney and heart have been developed (Dutta et al., 2017). Beginning with iPSCs or ESCs, specific differentiation cues guide the cells to self-organize and proceed along a developmental trajectory not unlike that which occurs *in vivo*. The result is an organoid that exhibits a relatively sophisticated 3D architecture and contains many of the cell types found in the *in vivo* organs they represent. Not surprisingly, many groups have used organoids as a platform to model human diseases, particularly those affecting developmental processes. One recent example is the successful engineering of human iPSC-derived intestinal tissues to model Hirschsprung's disease (Workman et al., 2017). This example is particularly noteworthy because, in order to accurately model the impaired intestinal-enteric nervous system (ENS) development of this disease, the authors introduced a second population of hiPSC-derived neural crest cells in order to form the ENS within the intestinal organoid. Another exciting application of organoids is the investigation of the interplay between host and infectious pathogens, such as Zika virus, *Helicobacter pylori* and Norovirus. By using brain organoids, researchers have been able to recapitulate important clinical malformations of Zika virus infection-induced microcephaly (Qian et al., 2017).

Whether in self-organizing organoids or the more controlled bioengineered scaffolds or organ-on-chip models, the addition of physiological parameters – spatial architecture, microenvironment, fluid flow, paracrine factors and mechanical regimens – in 3D systems tends to encourage iPSC-derivative cells to exhibit more mature properties and better organ-specific functions (Table 1). iPSC-generated EHTs demonstrated advanced maturation in many aspects, including ultrastructure, conduction velocity, electrophysiology and response to stimuli (Tiburcy et al., 2017). Similarly, brain organoids from iPSCs exhibit relatively mature features resembling those of the embryonic human brain, including the formation functional cortical circuits, glia-neuron interaction and myelination (Quadrato et al., 2016; Sloan et al., 2017). As novel 3D strategies emerge, increasing efforts are being made to comprehensively study various diseases using these 3D iPSC models (Table 2).

Despite their enormous potential, all current 3D technologies must overcome several hurdles in order to effectively model human diseases (Table 1). For example, organoids generated from iPSCs are heavily dependent on self-organization, but this results in considerable cellular heterogeneity and organoid-to-organoid variation, even within the same batch (Takebe et al., 2017). In addition, whether organoids will ever be able to recapitulate sophisticated organ architecture in a robust and reliable way remains to be seen. Furthermore, most established iPSC-derived organoids and organ-on-chips are only at a micrometer to millimeter scale, although engineered tissues may reach the centimeter scale similar to the actual human organs (Takebe et al., 2017). Another problem

Table 2. 3D iPSCs disease modeling studies

Tissue	Type of 3D strategy	Disease and reference
Blood vessel	Engineered tissue	Hutchinson-Gilford progeria syndrome (Atchison et al., 2017), aortic stenosis (Dash et al., 2016)
Liver	Organoid	Polycystic liver disease (Sampaziotis et al., 2015), drug-induced lethal liver failure (Takebe et al., 2013)
Lung	Organoid	Idiopathic pulmonary fibrosis (Firth et al., 2015; Wilkinson et al., 2017)
Brain	Engineered tissue	Glioblastoma invasion (Nayernia et al., 2013)
	Organoid	Miller-Dieker syndrome (Iefremova et al., 2017), Zika virus infection (Qian et al., 2016), microcephaly (Lancaster et al., 2013)
	Organ-on-chip	Blood-brain barrier (van der Helm et al., 2016)
Kidney	Organoid	Polycystic kidney disease (Freedman et al., 2015), nephrogenesis (Takasato et al., 2016)
	Organ-on-chip	Adriamycin-induced albuminuria and podocyte injury (Musah et al., 2017)
Pancreas	Organoid	Pancreatic facets of cystic fibrosis (Hohwieler et al., 2017)
Stomach	Organoid	*H. pylori* infection (McCracken et al., 2014)
Heart	Engineered tissue	Dilated cardiomyopathy (Hinson et al., 2015; Tiburcy et al., 2017), heart failure (Tiburcy et al., 2017), hypertrophic cardiomyopathy (Cashman et al., 2016)
	Organ-on-chip	Barth syndrome-associated cardiomyopathy (Wang et al., 2014)
Skeletal muscle	Engineered tissue	Muscular dystrophy (Smith et al., 2016)
Intestine	Organoid	Hirschsprung's disease (Workman et al., 2017), congenital gut defects (Spence et al., 2011), salmonellae infection (Forbester et al., 2015)
Skin	Organ-on-chip	Wound healing (Abaci et al., 2016)
Retina	Organoid	Retinal degeneration (Assawachananont et al., 2014; Völkner et al., 2016)

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is that iPSC-generated engineered tissues and organoids tend to lack supporting tissue, such as the vasculature or nervous system, which some recent studies have begun to address (Takebe et al., 2017; Workman et al., 2017; Zhang et al., 2016). Without these supporting tissues, important nutrients and response signals become inaccessible to cells that are embedded inside, limiting the life span and functionality of the tissue. Moreover, with increased 3D complexity, the cost-intensive and sophisticated manufacturing steps of all three technologies introduce high inter- and intra-operator variability, leading to inconsistent tissue construct quality and research outcomes compared with conventional 2D systems (Huh et al., 2011). Although less adaptable, the 2D iPSC modeling strategy has high reproducibility and greater potential for control and so may be acceptable for some applications such as for fetal/early onset diseases in which maturation is not relevant and where certain cell intrinsic defects (e.g. endoplasmic reticulum trafficking, mitochondrial respiration and cytoskeletal structure) do not depend on the 3D environment.

3D plus: adding a fourth dimension

Ontogenesis and disease progression are intrinsically dynamic processes in which both cellular and tissue-level biological activities are altered spatially as well as temporally (Yin et al., 2016). Current 3D systems that mainly model spatial events in a single organ fail to address fundamental questions associated with temporal events in multiple organs. These include the progression of organ development and ageing, the dynamics of tissue healing and regeneration, the exchange of metabolites between organs, and the sequential pathogenesis of inflammation, infection and multi-organ failure (Bhatia and Ingber, 2014). To further advance existing 3D models and to accurately model disease, it will be necessary to address this temporal dimension.

Organ-on-chip technology can integrate various patient-specific iPSC-derived 3D constructs into a dynamic system (4D multi-organ system, also known as ‘body-on-chip’) via a circulating flow that mimics the systematic interactions among different tissues and organs in the body (Fig. 1; Bhatia and Ingber, 2014). 4D multi-organ systems are still in the early developmental stages and are mostly used for studying the adsorption, distribution, metabolism, elimination and toxicity (ADMET) of drugs (Bhatia and Ingber, 2014). Recently, however, a proof-of-principle system comprising intestine, skin, liver and kidney chips was developed and maintained for more than 4 weeks (Maschmeyer et al., 2015). 4D multi-organ systems can also be incorporated with in-line sensors and fluorescent reporters for the real-time quantification and analysis of dynamic cellular and whole organ responses over the course of the study. In the future, 4D multi-organ systems are expected to be further improved by integrating with different 3D platforms to create engineered organoid-on-chips. These could be maintained in a physiological environment produced by integrated engineered organoids for extended periods of time to enable the temporal investigation of dynamic disease pathogenesis.

Closing thoughts

The advent of iPSC technology has allowed us to model various aspects of disease progression; however, better tools and technologies are needed to integrate these aspects and to understand disease at the whole-organ and whole-body levels. A wide range of obstacles, including variability, reproducibility and the complex nature of many diseases must be overcome to construct multi-organ systems that can closely mimic human diseases. Adding a fourth dimension to capture temporal aspects of disease progression is also crucial. By increasing the understanding of organogenesis and body development, bioengineering approaches that allow better control over organogenesis, tissue architecture, cellular composition and the extracellular environment will be fundamental in moving human iPSC-based disease modeling forward. With the creation of robust, reproducible and functionally relevant model systems, we can explore complex disease etiologies in a meaningful way to create precise therapeutic strategies in the coming era of precision medicine.

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