Microengineered systems with iPSC-derived cardiac and hepatic cells to evaluate drug adverse effects

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Abstract

Hepatic and cardiac drug adverse effects are among the leading causes of attrition in drug development programs, in part due to predictive failures of current animal or in vitro models. Hepatocytes and cardiomyocytes differentiated from human induced pluripotent stem cells (iPSCs) hold promise for predicting clinical drug effects, given their human-specific properties and their ability to harbor genetically determined characteristics that underlie inter-individual variations in drug response. Currently, the fetal-like properties and heterogeneity of hepatocytes and cardiomyocytes differentiated from iPSCs make them physiologically different from their counterparts isolated from primary tissues and limit their use for predicting clinical drug effects. To address this hurdle, there have been ongoing advances in differentiation and maturation protocols to improve the quality and use of iPSC-differentiated lineages. Among these are in vitro hepatic and cardiac cellular microsystems that can further enhance the physiology of cultured cells, can be used to better predict drug adverse effects, and investigate drug metabolism, pharmacokinetics, and pharmacodynamics to facilitate successful drug development. In this article, we discuss how cellular microsystems can establish microenvironments to better control differentiation protocols and increase the physiological relevance of iPSC-derived cardiomyocytes and hepatocytes. Development and standardization of technologies will enable evaluation of the potential value of cellular microsystems to improve the in vitro models used in drug development programs. Future steps in this field include controlled connections of organ systems to better recreate clinical metabolism and pharmacokinetics.

Keywords: Pharmacological screening, iPSCs, microenvironment, microfluidics, cellular systems, drugs

Impact statement

Cardiac and hepatic adverse drug effects are among the leading causes of attrition in preclinical and clinical drug development programs as well as marketing withdrawals. The insufficiency of animal testing models has led to considerable interest in the employment of cardiac and hepatic models using human-induced pluripotent stem cells (iPSCs) for drug toxicity testing. However, current batches of iPSC-derived cardiomyocytes and hepatocytes are variable and not matured as adult primary tissues, which limit their prediction of drug effects. This article discusses how the use of microfluidics can create microenvironments to better control differentiation protocols and increase the physiological relevance of iPSC-derived cardiomyocytes and hepatocytes. Development and standardization of technologies will enable evaluation of the potential value of cellular microsystems to improve the in vitro models used in drug development programs. Future steps in this field include controlled connections of organ systems to better recreate clinical metabolism and pharmacokinetics.

Introduction

The ability of cells differentiated from human induced pluripotent stem cells (iPSCs) to predict clinical drug effects is limited by the immaturity of their cellular function, variability between cells or differentiation batches, and the lack of physiologically relevant properties for several applications in modeling primary cells. In this article, we discuss the potential of microfluidic and microfabricated devices to recreate microenvironments in cellular systems...
that can address the hurdles of differentiating, maturing, and using iPSC-derived cardiomyocytes and hepatocytes. These lineages are of interest to the drug development field because cardiac and hepatic adverse effects are the leading causes for drug attrition. Relevant in vitro research with these two lineages seeks to understand drug mechanisms of action and improve the predictivity of drug effects at the preclinical stage. Reliable cellular models with strong biological relevance are critical in drug development. To this end, microengineered cellular systems have been developed utilizing a range of strategies often tailored to respective cell lineages to create biomimetic microenvironments that have resulted in improved maturity and function of iPSC-derived cell lineages. Key physiological elements of cellular microenvironments include the presence of multiple cell types and organ- or tissue-specific properties that stabilize and mature cell function. The fields of microfluidics, micrometrology, and microfabrication have enabled technologies that can control and define more physiological three-dimensional (3D) multicellular cultures as well as sense or manipulate their cellular function. In addition, engineered microfluidic connections of multi-organ systems, such as interconnected heart-liver systems, can further enhance in vitro models of drug response through direct flow of metabolites and soluble factors. Such approaches can be implemented to control and improve differentiations, cellular maturity, and overall physiological relevance.

Differentiation of iPSCs involves cellular populations with compositions that progress along stages of the differentiation processes, creating a naturally heterogeneous microenvironment with complex cellular interactions and, in 3D settings, these differentiating multicellular cultures have been demonstrated to develop spontaneously at the microscale. Although microengineered 3D cultures have been reported to improve differentiation quality, owing to difficulties in handling 3D cultures, the field currently favors monolayer differentiation approaches. This approach may evolve with the development of microfabricated devices designed to perform with higher robustness and reliability for handling multicellular 3D microenvironments and for phenotyping their function in higher throughput settings. Such devices offer an unprecedented opportunity to monitor, control, and study differentiation stages and to provide models for early-stage prediction of drug effects.

We review microengineered approaches to mature iPSC-derived hepatocytes and cardiomyocytes and examine how specific features of microfluidic cellular systems are used to mimic in vivo microenvironments for improved differentiation, maturation, and monitoring of iPSC-derived cells. For using these systems, we describe potential drug development applications for the employment of iPSC-derived cardiac and hepatic cellular models. The fundamentals of reprogramming and differentiating iPSCs of microfabrication and microfluidics, and of cardiac- or hepatic-specific function, and of in vitro drug evaluation assays have been reviewed in detail elsewhere. Concepts from these fields are referenced and summarized for discussing how microfabricated systems with iPSC-derived hepatic and cardiac cells can be developed and used for predicting drug adverse effects.

**Maturity of iPSC-differentiated cells improves with enhanced extracellular physiology**

The clinical predictivity of cellular models is highly dependent on the ability of cellular functions to mimic adult human physiology. However, the fetal-like profile of many iPSC-derived cardiac and hepatic cells has been a principal challenge for modeling human tissue. The definition of maturity differs by context and is dependent on mechanistic properties that establish specific cellular activities related to tissue function. Much current research seeks to address these challenges with promoting and defining aspects of maturity, both for iPSC-derived cardiomyocytes and hepatocytes. A variety of approaches using either or both biochemical and biophysical stimuli have been developed to enhance the maturity of iPSC-derived lineages, including extended time in culture after differentiation, activation of signaling pathways using chemical factors, 3D culture settings, electromechanical stimulation, and modulation of cell morphology. Cellular systems can integrate these features, often in combination, and be further microengineered to facilitate measurements of biological properties that define maturation. To this end, cellular systems can be microengineered to recreate tissue- or organ-specific conditions. Here we discuss approaches specific to applications with hepatocytes and cardiomyocytes.

**Hepatocytes**

Despite advances in hepatocyte differentiation methods, the fetal-like profile of iPSC-hepatocytes remains a challenge for translational research. Hepatocyte maturity directly relates to cellular metabolic and transport functions, notably in lipid storage, glycogen synthesis, formation of biliary canaliculi, expression of cytochrome (CYP) P450 enzymes, other drug-metabolizing proteins, transporters and biomarkers. To address this, 3D culture formats, extracellular microenvironments under fluid flow, and coculture with non-parenchymal cell types have been demonstrated to enhance the maturity of iPSC-hepatocytes. Accordingly, future work should investigate the biological mechanisms underlying the maturation of these cells.

Data from studies involving microengineered systems with primary hepatocytes generally suggest that more stable cultures are achieved with 3D settings, fluid flow, and co-culture with other cells, which prolonged and stabilized hepatic function for several weeks. Co-cultures with hepatocytes (as reviewed in depth by Cotovio and Fernandes) may involve derivation followed by mixing and loading of individual cell lineages (e.g. hepatic endoderm and endothelial cells) or differentiation to multiple cells types (e.g. hepatic endoderm and cholangiocytes) and/or Kupffer cells within the same culture (see Figure 1(c)). Therefore, a combination of structural support and activation of signaling pathways from biomimetic microenvironments may lead to the maturation of iPSC-hepatocytes with 3D co-cultures under flow.
Mechanistically, studies with 3D hepatocyte culture systems have demonstrated enhanced cell polarity, formation of bile canaliculi, and increased expression of cytochromes P450 (CYPs). These results in improved function of primary hepatocytes suggest that microfluidic systems can be developed to better understand the fundamentals of maturation and further optimize microenvironments for culturing iPSC-hepatocytes.

The next challenge to advance cellular hepatic systems is to engineer microfluidic capabilities using gradient combinations of different factors along arrays of miniaturized systems. These types of systems can be used to identify signaling factors and concentrations to optimize differentiation methods and cellular maturation, as well as to perform higher throughput functional assays (see Figure 1(d), (f), and (g)). In addition to maintaining multicellular cultures in 3D under flow, efforts have been undertaken to further emulate liver microarchitecture, either by attempting to separate bile from the cell culture medium or by inducing cells to organize into acinar-like microtissues. The potential to implement improvements such as these for understanding and controlling the maturation of iPSC-hepatocytes has not yet been comprehensively explored.

Cardiomyocytes

Electrophysiology, calcium handling, metabolism, contractility, and the organization and composition of subcellular structures and organelles are biological properties of cardiomyocyte function that affect the duration and magnitude of contraction for each heartbeat and therefore define cardiomyocyte maturity. iPSC-cardiomyocytes differ from primary cardiomyocytes for each one of these functional modes, and cellular systems have been microengineered to enhance the maturity of cellular function, as extensively reviewed by several authors. Briefly, the microenvironment can be engineered to stimulate cells mechanically and electrically, expose cells to chemical factors dissolved in the cell culture medium or attached to the surface of materials, and induce cells to assume a level of structural organization and morphology that is observed in primary tissue. In addition to recreating the organization of primary tissue, 3D engineered cellular constructs, termed engineered heart tissues, have the advantage of facilitating the co-culture of different cell types (see Figure 1(c)), which has been demonstrated to enhance the maturity of iPSC-cardiomyocytes. Cellular organization and composition of the human heart tissue are complex and difficult to fully replicate in vitro. In addition to cardiomyocytes, various cell types exist in the heart in high numbers relative to cardiomyocytes, such as endothelial cells, smooth muscle cells, and different types of stromal fibroblasts organized within an intricate and well-defined three-dimensional network of extracellular matrix proteins. Establishing in vitro co-culture systems generally involves a limited number of available cell types (e.g. iPSC-cardiomyocytes with fibroblasts or mesenchymal stem cells) at an experimentally defined ratio.
Soluble components with demonstrated enhanced enhancement of cellular maturity include factors secreted by other co-cultured cell types, metabolic conditioning with glucose-free media (including other more physiological carbon sources), and the addition of combinations of small molecules such as tri-iodo-L-thyronine and dexamethasone. Modulation of gene expression can also increase maturity, as seen in published applications for arrhythmogenicity and calcium handling studies, as well as overexpression of micro-ribonucleic acids. Strategies involving soluble factors do not primarily involve an engineered microenvironment, although micro-fluidic formulators and perfusion of small extracellular volumes can optimize the timing and concentration in the delivery of reagents to cells, either for modulating gene expression, hormonal treatment, or metabolic manipulation.

The mechanistic causes that drive cellular maturity of iPSC-cardiomyocytes are still poorly understood and may be difficult to dissect with platforms that combine several maturation factors. As reviewed by Schroer et al., microdevices can focus on a specific microenvironment property. Therefore, functional effects of cell shape, cell-cell adhesions, specific properties of extracellular mechanics, and electrical stimulation can be individually evaluated. For example, Lundy et al. discovered that the structural maturation of iPSC-cardiomyocytes, defined by the expression and organization of the contractile machinery and associated subcellular structures, was related to functional maturation. Consequent work used microengineered devices to further investigate these observations and confirmed the dependence of cardiomyocyte maturity on the subcellular organization of its contractile machinery. Overall, these studies showed that single cell maturity was enhanced with an aligned rectangular morphology enabling myofibril alignment and setting sarcomeres size within 1.9–2.2 μm. Similar relationships between these properties and functional maturity were further confirmed in multicellular systems. Other studies have focused on the importance of cell-cell adhesions for enhancing maturity. This dependence of maturity on contractile and associated structures is still poorly understood and exemplifies the additional work that is needed in the field to set cardiac maturity in vitro. Microengineered platforms for culturing iPSC-cardiomyocytes have been developed to simultaneously address two main goals: (i) enhance the maturity and physiological significance of their functional modes (electrophysiology, electrical conduction, calcium signaling, contractility, structural organization, metabolism, chamber specificity, etc.) and (ii) enable measurement of parameters that define these functional modes. While fabricating devices, implementing extracellular factors and proteins and combinations of cell types that can recreate microenvironments to enhance cellular maturity, features have been created within systems to assay cardiac function. Soft lithography, micromolding, 3D printing, and hydrogel formation are the most common approaches for fabricating such devices. Decellularized matrices as biomimetic cues to enhance cell maturity also hold great potential for recreating physiological settings in vitro. Incorporation of cells is dependent on the design of devices and can involve seeding them directly on micromolds followed by centrifugation to force cellular aggregation, loading them in culture media into microfluidic chambers under pressure, or mixing them homogeneously in a hydrogel to be molded or printed into a specific shape. To assay cellular properties for testing drug effects on different mechanisms of cardiac function, devices can be engineered or combined with instrumentation to quantify parameters that mechanistically define electrophysiology, contractility, calcium cycling, electrical conduction, structural organization, and bioenergetics.

Despite progress in the field, there remains a gap in functional modes between primary myocytes and iPSC-cardiomyocytes. Moving forward, more successful models with enhanced maturity should be evaluated in multiple sites and translated by independent laboratories for prediction of clinical drug effects. Of note, significant efforts have been made in the last decade to miniaturize engineered tissues with iPSC-cardiomyocytes and multiplex the amount of functional modes that can be evaluated.

Assaying functional maturity in microsystems

Microsystems can facilitate the analysis of cellular function in relation to maturity. With cardiomyocytes, functional analysis is often based on parameters that provide information about mechanisms that drive cardiac contractility, such as electrophysiology, calcium flow, force generation, and metrics of cellular mechanics and conduction. These methods have been extensively applied in two-dimensional (2D) cultures and reviewed in depth elsewhere, such as by Laurila et al. To transition to engineered cellular systems, comparable methods can be integrated into devices to perform analyses with image-based techniques, force sensors, or other stimulating or recording features. For example, engineered heart tissues have deformable microposts for measuring contraction force and rate. Similarly, 2D approaches for cardiac functional assays can be engineered into 3D systems, for example, electrical probes to pace cells or to record electrophysiology metrics, or optical windows for image-based assays. In addition to these methods, cardiac and hepatic assays can involve the detection of soluble factors excreted into the media, i.e., the secretome, which may include indicators of cellular maturity, biomarkers, or drug metabolites that can be collected with automated microfluidic approaches.

Image-based assays can especially benefit from the use of stable lines of iPSCs with fluorescently labeled proteins. In contrast to other established methods such as immunostaining, gene expression analysis, and flow cytometry, cell lines expressing fluorescently tagged proteins offer advantages by enabling non-destructive and minimally invasive assays for tracking the status of cellular function. Deep learning software can be particularly advantageous when analyzing subtle changes in cellular phenotypes from cell imaging data, either by monitoring cultures of iPSCs and early-stage differentiation, determining maturation, or
assaying drug responses using experimental cultures. Such methods have recently been validated to determine the quality of iPSC cultures, detect early differentiation changes, and for late-stage analysis such as the categorization of drug responses using iPSC-cardiomyocytes.

To generate imaging data with live cells, many engineered reporter iPSC-lines are being developed to aid in the determination of cellular structure and function, including fluorescent reporter lines for structural features and a GCaMP calcium sensor. In addition to screening function, image-based methods can be developed to provide information concerning the purity of differentiations.

Cell-to-cell and batch-to-batch variability is another recognized challenge in the standardization of iPSC differentiations, and it can confound experimental results concerning their reproducibility. To address this challenge, algorithms can be developed to screen for cellular variability from images acquired with microscopy to quantify variability and assess its experimental implications.

Towards contexts of use of iPSC-derived cardiac and hepatic systems for drug development

From a historical perspective in the evolution of the 3D cellular systems field, Simian and Bissel discussed translational steps that may lead to the broad adoption of these technologies. Despite considerable advances in the field of 3D cellular systems during the past decade, a stronger commitment by drug development stakeholders will be necessary to validate and translate these technologies. Since cellular systems lack several components of the self-controllable and often poorly understood multi-factorial complexity of in vivo models, these systems are not expected to replace the employment of animals in drug development in their current state. These systems must be developed for well-defined regulatory contexts of use, such as assessing the effects of drugs on specific mechanisms.

Toxicity

The liver and the heart are among the most sensitive organs to drug toxicity. Cardiac and hepatic toxicity-related side effects are a leading cause of attrition during drug development, post-marketing withdrawals, and black box warnings added to FDA-approved drug labels. Therefore, increasing the predictivity of in vitro tools for clinical drug toxicity is a high priority for regulatory stakeholders. Overall, the contexts of use in predicting toxicity for cellular systems need to be defined and validated to enable confidence in their use by the pharmaceutical industry.

Microengineered cellular systems can improve the predictive outcome of toxicity relative to traditional 2D cultures. For example, the presence of Kupffer cells in hepatic systems is essential to investigate inflammatory roles in drug toxicity, and traditional 2D platforms do not maintain such type of co-cultures to the same degree as 3D systems. A similar need has been identified to include macrophages or monocytes for predicting immune-related effects in cardiac systems. In addition, both 3D cardiac or hepatic systems can more robustly predict toxicity mechanisms that rely on prolonged drug exposures as their function systematically lasts longer and is more stable than cells in 2D platforms.

As contexts of use relate to biological mechanisms, Weaver et al. proposed a roadmap for implementing preclinical models of hepatotoxicity in which different mechanisms of drug-induced liver injury are comprehensively reviewed. Toxicity mechanisms in the liver can involve hepatocyte mitochondrial dysfunction, abnormal transporter activity, abnormal formation of lysosomes, formation of reactive oxygen species, damage of endoplasmic reticulum, and immune-induced injury or damage of non-parenchymal cells that support liver function. Similar publications also review the mechanistic causes of cardiotoxicity. Cardiotoxicity can lead to the dysfunction of cardiomyocyte physiological mechanisms via drug effects that directly target cardiomyocytes or indirectly affect the function of other cell types that regulate cardiomyocyte function.

Liver metabolism that converts parent drugs into metabolites also accounts for a potential source of cardiotoxic effects that can only be predicted in vitro with interconnected heart-liver cellular systems. Commonly screened cardiotoxicity mechanisms relate to electrophysiology, calcium cycling, mitochondrial function/bioenergetics, structural tissue and subcellular properties, contractility, and cell death.

In addition to assaying phenotypes and markers screening toxicity mechanisms, lists of compounds with known mechanistic drug effects are often used to validate drug development tools and serve as comprehensive controls. For example, the Comprehensive in vitro Proarrhythmia Assay initiative established a set of compounds with varied levels of proarrhythmic clinical risk to validate in vitro assays, and similar lists have been proposed to screen for mitochondrial toxicity, tyrosine-kinase inhibitor toxicity, and contractility. As iPSCs can be developed to model patient- or population-specific risk factors, they offer the potential to predict toxicity in cases where such factors play a role in drug mechanisms of action.

Pharmacokinetics and pharmacodynamics

Drug toxicity depends on the level of exposure and availability of a drug. Reichel and Lienau reviewed preclinical pharmacokinetic data enhance the success rate of drug discovery and referenced practical examples from the industry. A key concept in pharmacokinetic studies is drug clearance. Human-specific organ function related to drug clearance is systematically determined during drug development, but it is usually estimated from indirect measurements and it is rarely determined directly with clinical testing, which could be potentially done with engineered cellular systems.

The liver plays a major role in the pharmacokinetics of the majority of drugs. In contrast, myocardial metabolism is mostly for energetic cellular requirements, although the possibility of drug transformation and
clearance in the heart has been identified.\textsuperscript{149-151} At the mechanistic level, the expression and activity of enzymes and transporters tightly regulate drug clearance,\textsuperscript{152} and the use of iPSC-differentiated cells for such studies must ensure the stability of the expression and activity of enzymes and transporters.\textsuperscript{153} Related to the stability of cellular function, culturing hepatic cells in 3D platforms and under fluidic flow has been shown to stabilize transport and metabolism.\textsuperscript{41,52,154}

Initial studies focusing on differentiation and maturation of iPSC-hepatocytes in 3D systems yielded promising results for obtaining functional and gene expression profiles that are closer to the activity of primary tissues.\textsuperscript{155-157} However, in addition to better investigating the mechanistic implications of 3D microenvironments in iPSC-hepatocyte differentiation, the potential employment of these systems with differentiated cells for drug clearance studies still requires elucidation. Once validated for this context, engineered systems with iPSC-differentiated cells will provide the opportunity of replicating patient-specific properties that relate to the expression of enzymes and transporters.\textsuperscript{158}

Differences in drug metabolism and transport have been identified between populations,\textsuperscript{159-161} which has strong implications in inter-population variations in drug pharmacokinetics that may be modeled in engineered cellular systems containing cells differentiated from iPSCs that represent the genetic background of specific populations. In addition to enabling population-specific studies, iPSC-hepatocytes can also harbor disease-causing mutations or conditions that affect hepatic drug clearance.\textsuperscript{148,162-164} Altered metabolic induction can occur in association with genetic determinants, as it has been reported to occur with rifampicin induction when P-glycoprotein is downregulated, or with CYP1A1 inducibility upon mutations in the aromatic hydrocarbon receptor, which activates CYP enzymes along with other transcription factors.\textsuperscript{165-167}

However, induction of enzyme activity in iPSC-hepatocytes, such as CYP3A4, CYP1A2, and CYP2C9 activities, is low compared with primary hepatocytes. Engineered 3D systems have been reported to enhance the maturity of metabolic induction,\textsuperscript{32,168,169} and further research should develop this application with iPSC-hepatocytes.

To our knowledge, cardiac cellular systems containing iPSC-cardiomyocytes have not yet been tested for the evaluation of drug metabolism in cardiac tissue.\textsuperscript{149,151} However, another pharmacokinetic concept of major interest for using cardiac cellular systems is drug systemic concentration,\textsuperscript{170} which can be replicated \textit{in vitro} by setting physiological levels of drug exposure in cell culture medium with microfluidic formulators.\textsuperscript{83} Future work should be developed in drug clearance and varied drug exposure levels with both cardiac and liver systems to evaluate how clinical pharmacokinetic data can be predicted.

The discipline of pharmacodynamics studies drug mechanisms of action on living systems, including drug-induced reactions at the cellular level, drug binding to biological receptors, and effects on the biochemistry and physiology of cellular functions.\textsuperscript{171} As drug pharmacodynamics are intimately dependent on pharmacokinetics, which govern the time and magnitude of tissue exposure to a drug, pharmacokinetic and pharmacodynamic studies are often conducted simultaneously.\textsuperscript{172}

For engineered cellular systems, the use of microfluidic formulators that can expose cultures to well-tuned drug concentrations is a promising approach to replicate clinical drug pharmacokinetics \textit{in vitro}.\textsuperscript{83} Such high-resolution studies are necessary in drug development as there is often a considerable gap between the proposed mechanisms of action based on preclinical research and the clinical therapeutic targets of new drugs.\textsuperscript{113}

Bedside-to-bench approaches aim to use clinical data as a starting point for mechanistic investigations with \textit{in vitro} methods of drug effects, as reviewed by Brackman and Giacomini\textsuperscript{113} for the gene that encodes the breast cancer resistance protein. Similar reverse translational approaches could be applied to evaluate the predictivity of cellular systems based on known clinical effects of drugs. The main challenge for validating cellular systems, however, relates to replicating physiological mechanisms that correlate to potential drug effects (e.g. expression of receptors, involvement of multiple cell types) and discordance between results from these systems and clinical data can inform on their limitations for predicting drug effects.

Integrating iPSC-differentiated cells in more predictive clinical effect platforms would also be key for applications that relate to rare diseases and personalized and precision medicine. In the cardiac field, several mutations and combined genetic conditions are known to cause cardiomyopathies and other rare cardiac conditions.\textsuperscript{173} In addition, drugs effects such as doxorubicin toxicity\textsuperscript{174,175} can also be related to the genetic background of patients and related gene expression profiles, which further illustrates the opportunity of using patient-specific cells.

Similar opportunities for addressing patient-specific drug effects also exist for the use of iPSC-differentiated cells in liver cellular systems.\textsuperscript{176} In addition to recreating settings dependent on genetic backgrounds, cellular systems can be engineered to replicate microenvironments that enhance the physiological effect of drugs. For example, a variety of liver systems can recreate oxygen zonation by setting extracellular oxygen tension, which can be critical to detecting specific drug effects.\textsuperscript{57,177} Physical exercise and general conditioning of cardiovascular physiology can also play a strong role in drug cardiac effects\textsuperscript{178-182} and several cardiac microphysiological systems have the ability to stimulate cells electrically and mechanically to recreate physiological conditioning.\textsuperscript{72,127,183} Altogether, patient-specific conditions can be recreated with iPSC-derived lineages and the pathophysiology of cardiac or hepatic function can be emulated by engineering the extracellular microenvironment.

**Features of microfluidic cellular systems for cardiomyocyte and hepatocyte differentiation**

Despite the progress in the fields of microfluidics and microfabrication, use of 2D cultures remains vastly
predominant in differentiations, and testing of the potential for cellular microsystems, non-invasive functional monitoring, and programmed media changes is in early stages of initiation.

The differentiation methods for deriving cardiomyocytes or hepatocytes from iPSCs are currently well-standardized in the field for 2D culture. Derivation of cardiomyocytes most often relies on carefully timed modulation of WNT signaling to induce mesoderm and specify the cardiomyocyte progenitors along with ongoing modulation of insulin signaling.\textsuperscript{184} The pathways of hepatocyte differentiation are reproduced \textit{in vitro} using Activin A to induce formation of definitive endoderm, followed by the addition of BMP4 and FGF2 to specify hepatic progenitors.\textsuperscript{185,186} Figure 1(b) represents these two differentiation pathways. Most current differentiation protocols have been established using 2D or monolayer culture systems. In this review, we seek to emphasize the value of incorporating 3D systems at early or initial stages of differentiation, an approach that has led to development of tissue platforms with iPSC-CMs.\textsuperscript{187,188} Transiting differentiations from 2D systems to 3D devices involves challenges at many stages. Firstly, the process of seeding or loading iPSCs into 3D devices may require more rigorous dissociation or additional manipulation. Compromising cell quality through experimental handling is a known challenge for sensitive cells such as iPSCs,\textsuperscript{189} although alternative approaches to reduce stress during cell loading into devices have been developed, such as transient on-chip vacuums\textsuperscript{190} or inducing aspiration using degassed PDMS (polydimethylosiloxane).\textsuperscript{191} Some additional challenges during cell differentiation may include the need to adjust the timing of signaling factor addition since stage progression may be paced differently due to spatial-temporal differences in a 3D format,\textsuperscript{192} and addressing the retention of dead cells within a device that may accumulate as a natural process of ongoing differentiation.\textsuperscript{193}

Engineered cellular microsystems, such as 3D organoids or microphysiological systems, seek to recreate elements of the \textit{in vivo} microenvironment by facilitating cell–cell interactions and allowing self-organization of structural features.\textsuperscript{194} Ronaldson-Bouchard et al.\textsuperscript{84} observed that iPSC-cardiomyocytes co-cultured with stromal cells in 3D cultures and under electrical training yielded more mature modes of function when cells were seeded into these settings relatively early in differentiation (around Day 12). In a similar manner, microsystems used for deriving iPSC-hepatocytes have demonstrated promising results when integrating elements of 3D culture and microfluidic perfusion.\textsuperscript{19,195,196} Furthermore, most differentiation methods rely on soluble factors as differentiation cues. When culturing iPSCs for differentiation in microfluidic devices, as exemplified in Figure 1, there is potential to tune the microenvironment to either enhance or replace signaling factors based on cell–cell interactions and co-cultures of cell lineages.\textsuperscript{197} The extracellular matrix (ECM) is also a critical aspect of both \textit{in vivo} and \textit{in vitro} cell function and biology and can be experimentally defined both in 2D and 3D culture settings, as well as in devices that facilitate differentiation and maturation stages in 3D. The ECM is known to modulate cell signaling and differentiation cues, for example, ECM-integrin signaling has roles both in early-stage differentiation of iPSCs as well as through later stages of cardiomyocyte differentiation and maturation.\textsuperscript{198} ECM studies open the possibility of biomimetic approaches to stem cell differentiation methods, which could be controlled in microsystems designed to generate specific lineages.\textsuperscript{199}

Different designs have been applied for the development of microengineered liver and cardiac cellular systems, which include open and closed systems, static or dynamic flow, as well as systems that require different quantities of cells.\textsuperscript{19,57,200} Figure 1(a) represents a general closed microfluidic device, featuring an inlet for loading cells as well as microfluidic channels for media perfusion. Cells can be loaded into culture chambers or wells as a mono- or cocultures, generally by dissociating and mixing lineages, when applicable, before resuspending in culture medium at the appropriate density.\textsuperscript{19} Depending on the device design, cells then form attached layers, spheroids, or organoids in either open or chambered systems where they have exposure to perfused media in defined environments.\textsuperscript{57} For example, the open-chamber device designed to mimic the structure of a single acinus by Prodanov et al.\textsuperscript{201} with iPSC-hepatocytes differs from the scaffolds developed by Domansky et al.\textsuperscript{200} to drive the self-assembly of iPSC-hepatocytes into arrays of 3D microtissues. For overall reference, Cotovio and Fernandes\textsuperscript{19} reviewed current methods for the formation of iPSC-derived liver organoids.

Engineering cellular microsystems for improving differentiations can offer many advantages, and these systems can be miniaturized for using low cell numbers within microfluidic chambers (see Figure 1(a)). In addition, image-based and other analytical non-destructive assays can examine the cellular secretome within the perfusate to provide quality control criteria for evaluating the progress of differentiations. As the field evolves toward leveraging microengineered platforms for iPSC differentiations, we will increase our understanding of how the microenvironment impacts differentiation.\textsuperscript{202} In the rest of this section, we discuss how specific features of microfluidic cellular systems (programmed media changes and formulation, perfusion flow settings to maintain homeostasis, set specified cell-to-media ratio, and establishment of shear force) can be used to mimic \textit{in vivo} microenvironments for the enhancement of differentiation outcomes and improvement of analysis methods.

Overall, programmable microfluidic devices with automated capabilities for media formulation and media change can considerably reduce human-induced error and variability and allow for higher standards of consistency during often extended and sensitive experimental procedures. In the context of differentiations, media changes are critical and rely on careful optimization and standardization of timing and factor concentrations. Culture media often needs to be changed during brief time windows of developmental competence to progress cell fate through developmental intermediates. Competent stages can last mere hours, which can create challenges in optimizing
protocols and keeping differentiations standardized due to user-to-user variability. Media formulations are also critical and can contain highly sensitive concentrations of growth factors affected by lot number, aliquot age, and media temperature or storage conditions. For example, the WNT inhibitor CHIR99021 in cardiomyocyte differentiation protocols needs finely tuned optimization for different cell lines.\footnote{203,204} Programmable microfluidic devices, such as the MultiWell MicroFormulator designed to deliver customized reagent mixtures over 96-well culture plates,\footnote{205} have the potential to assist in optimization of timing and formulation as well as standardization of procedures across users and sites. However, media components and small molecules used in differentiation can be temperature sensitive and the development and use of programmed automatic media handling and formulation should incorporate solutions to protect perishable solutions.

In traditional cell culture platforms, media remain static between changes, which can result in temporal gradients of nutrient and oxygen depletion or build-up of cellular waste products. In addition, over the course of differentiations, cells are exposed to exogenous signaling factors (small molecules or growth factors) that guide sensitive developmental decisions, and many of these factors can be unstable in culture conditions.\footnote{206} Microsystems featuring continuous media exchange can address these hurdles by providing a constantly refreshed soluble microenvironment. Continual flow without media recirculation offers the advantage of a consistent and defined soluble microenvironment to minimize culture-based variability for cells whose metabolic activity can affect their microenvironment.\footnote{207} In systems with static culture relying on manual maintenance, intermittent media changes can cause biological fluctuations in the microenvironment that significantly impact the homeostasis of cellular function. While continuous flow can provide constant renewal of microenvironmental conditions, in some applications it also allows the concentration of excreted factors to reach a natural equilibrium by avoiding media changes. For example, hepatocytes have been shown to establish steady states of glucose and lactate concentrations under conditions of continuous but slow media supply.\footnote{208} Additionally, microfluidic systems can be designed to create signaling gradients within a culture to spatially influence cell response or development. For example, Cui et al.\footnote{209} demonstrated regionalized formation of the primitive streak from pluripotent stem cells within a device using controlled microfluidics. This same approach could be applied to developmental models to optimize differentiation or maturation conditions, differentiate co-cultures within a device, or for downstream evaluation of various drug concentration gradients.

Maintaining a low ratio between cellular mass and media volume in culture systems can potentially increase assay sensitivity and therefore enable either quantification of low-level factors or detection of subtle changes in soluble markers. The use of devices designed with low cell-to-media ratios maintained by slow consistent media exchange allowing for high concentrations of secreted factor can enable studies that are dependent on such variables. By decreasing the cellular volume density of microfluidic extracellular environments, analysis of the secretome of cultured cells in the perfusate of microengineered devices\footnote{210} could enable a more detailed analysis of maturation stages from bioanalytical assays.

Research with primary hepatocytes in low-volume systems has indicated that secreted factors such as HGF and TGF-β1 play a role in maintaining functional hepatocyte identity in culture,\footnote{211} further indicating the need to understand the effects of stabilizing the microenvironment at a low cell-to-media ratio. This technique has not yet been explored in directed differentiation methods. These cases highlight the need for carefully designed strategies tailoring the extracellular environment to the biological properties of the research.

In addition to slowly exchanging cell culture media and maintaining the homeostasis of the microenvironment, microfluidics can be used to recreate the extracellular physiology of cells exposed to shear stress caused by fluid flow.\footnote{212} Although cardiomyocytes are not directly exposed to shear forces in the beating heart, hepatocytes are exposed to fluid flow in the liver.\footnote{213} Recreating this native property of shear force exposure \textit{in vitro} for hepatocyte cultures has been shown to enhance mature properties of iPSC-hepatocytes\footnote{214} and could also be used to facilitate differentiations by increasing efficiency and functional properties.\footnote{215} Most liver-on-a-chip devices on the market use controlled microfluidic flow to enhance and maintain hepatocyte function.\footnote{216,217} Further research should evaluate how microfluidic systems can benefit differentiations and how to optimize flow conditions for developmental intermediates.

Many early differentiation methods used cell aggregate methods such as embryoid body formats, either free-floating or through hanging drop formation. Transitioning to 2D methods (monolayer culture) offered the advantage of a defined and homogenous environment, but it can limit unknown cell–cell interactions and structural heterogeneity. Microfluidic settings are ideal for recreating 3D microenvironments for differentiating iPSCs. One major approach is the containment of cells within a device, providing an enclosure for aggregates that minimizes inadvertent loss of embryoid bodies, organoids, or spheroids during handling, testing of compounds, or media changes that can occur with open culture platforms.

The method of culturing spheroids with hanging drops has been used for both hepatocytes and cardiomyocytes.\footnote{215,218,219} Despite their improvements over monolayer cultures, these methods often remain subject to low-throughput limitations and influences including media exposure, aggregate size, and undefined cell–cell interactions. Currently, the challenge is to integrate similar tissue-building methods in more robust and highly defined microenvironments. The use of microfluidics in such systems can control for variables such as media flow, volume, oxygen tension and can provide opportunities for connected multi-lineage 3D systems.

**Future challenges and opportunities**

As an increasing number of cellular systems become implemented in iPSC fields, the next steps include connecting
organ systems to further replicate in vivo physiology. Integrating organ systems through connected devices provides unique opportunities for studying drug effects that rely on multi-organ interactions. Generally, organ lineages are cultured within respective devices, and these devices can be connected via microfluidic systems to interchange soluble factors for experimental analysis.\textsuperscript{201,220} Cardiac–hepatic interactions are of interest in drug development due to the prevalence of drug metabolites produced in the liver that can affect cardiac function.\textsuperscript{221} Development of connected systems reliably replicating such in vivo interactions can provide a platform for predicting drug adverse effects and allow for mechanistic studies of organ physiology. For these systems to reliably mimic in vivo interactions, connections between tissues need to be carefully designed and monitored to have appropriate flow parameters and pharmacokinetic properties. When iPSC-derived tissues are used, all lineages should be assayed for appropriate levels of functional maturity and robustness (Figure 1(b) and (g)).

As the field moves forward, there is increasing potential for fully integrated systems that can accommodate start-to-finish experiments, from initial seeding of iPSCs to monitoring of differentiations to end-point functional assays (Figure 1). Developing and integrating higher throughput, non-destructive, minimally invasive phenotyping analysis methods is of interest. Image-based machine learning platforms lend themselves to such higher throughput capacities in addition to bioanalytical methods. The next challenge is to incorporate different capabilities for maturing cells, while assaying their function, in miniaturized cellular systems. Given the high sensitivity of iPSCs to experimental handling, it can also be assumed that loading these cells in microfluidic devices without affecting their quality will be a constant challenge in the field.

In summary, we include a list of recommendations (Table 1) for the field to consider as research continues to integrate iPSC-derived lineages with increasingly sophisticated cellular systems toward the goal of predicting clinical drug effects.

**Authors’ contributions:** KD and AJSR contributed equally to this manuscript.

**ACKNOWLEDGEMENTS**

The authors acknowledge the members of the Division of Applied Regulatory Science and of the Office of Clinical Pharmacology at FDA for valuable discussions.

**DECLARATION OF CONFLICTING INTERESTS**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**FUNDING**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported via the Food and Drug Administration – Medical Countermeasures Initiative Intramural Challenge Grants funding.
REFERENCES

1. Schuster D, Laggner C, Langer T. Why drugs fail - a study on side effects in new chemical entities. Curr Pharm Des 2005;11:3545–59
2. Brown GE, Khetani SR. Microfabrication of liver and heart tissues for drug development. Phil Trans R Soc Lond B Biol Sci 2018;373:20170225
3. Choi J, Lee EK, Choo J, Yuh J, Hong JW. Micro 3D cell culture systems for cellular behavior studies: culture matrices, devices, substrates, and in-situ sensing methods. Biotechnol J 2015;10:1682–8
4. Dai G, Neugebauer M, Stein M, Bütefisch S, Neuschafer-Rube U. Overview of 3D micro and nanocoordinate metrology at PTB. Appl Sci 2016;6:257
5. Gnenzi G, Bertocci V, Ravasio A. Integrating microfabrication into biological investigations: the benefits of interdisciplinarity. Micromachines 2019;10:252
6. Paik DT, Tian L, Lee J, Sayed N, Chen Y, Rhee S, Rhee JW, Kim Y, Wirk RC, Buikema JW, Wu WM, Red-Horse K, Quertermous T, Wu JC. Large-scale single-cell RNA-Seq reveals molecular signatures of heterogeneous populations of human induced pluripotent stem cell-derived endothelial cells. Circ Res 2018;123:443–50
7. Friedman CE, Nguyen Q, Lukowski SW, Helfer A, Chiu HS, Miklas J, Levy S, Suo S, Han JJ, Ostell P, Peng G, Jing N, Baillie GJ, Senabouth A, Christ AN, Bruxner TJ, Murry CE, Wong ES, Ding J, Wang Y, Hudson J, Ruohola-Baker H, Bar-Joseph Z, Tam PPL, Powell JE, Falpant NJ. Single-cell transcriptomic analysis of cardiac differentiation from human PSCs reveals HOPX-dependent cardiomyocyte maturation. Cell Stem Cell 2018;23:896–903
8. Viiri LE, Rantapero T, Kämmer M, Alexanova A, Oittinen M, Viiri K, Niskanen H, Niikko M, Kaikkonen MU, Aalto-Selkä K. Extensive reprogramming of the nascent transcriptome during iPSC to hepatocyte differentiation. Sci Rep 2019;9:5562
9. Hwang NS, Varghese S, Eliseeff J. Controlled differentiation of stem cells. Adv Drug Deliv Rev 2008;60:199–214
10. Lu HF, Lim SX, Leong MF, Naranayan K, Toh RP, Gao S, Wan AC. Efficient neuronal differentiation and maturation of human pluripotent stem cells encapsulated in 3D microfibrous scaffolds. Biomaterials 2012;33:9179–87
11. Meier F, Freyer N, Brzeszczynska J, Knolle F, Leupold F, Langenbrand A. The impact of iPSC polymerization on its use for cardiac tissue engineering. J Mol Cell Cardiol 2017;104:121–33
12. Ong LJY, Chong LH, Jin L, Singh PK, Lee PS, Yu H, Ananthanarayanan A, Leo HL, Toh YC. A pump-free microfluidic 3D perfusion platform for the efficient differentiation of human hematopoietic-like cells. Biotechnol Bioeng 2017;114:2360–70
13. Simao D, Silva MM, Terrasso AP, Arez F, Sousa MFQ, Mehrjardi NZ, Sarie T, Gomes-Alves P, Raimundo N, Alves PM, Brito C. Recapitulation of human neural microenvironment signatures in iPSC-derived NPC 3D differentiation. Stem Cell Rep 2018;11:552–64
14. Teshigawara R, Cho J, Kameda M, Tada T. Mechanism of human pluripotent stem cell-derived hepatic cell lineages and liver organoids: current status and potential applications. Bioengineering 2020;7:36
15. Weaver RJ, Blomme EA, Chadwick AE, Copple IM, Gerets HHJ, Goldring CE, Guillouzo A, Hewitt PG, Ingelman-Sundberg M, Jensen KG, Juhila S, Klingmuller U, Labbe G, Liguori MJ, Lovatt CA, Morgan P, Naisbitt DJ, Pieters RHH, Snoeys J, van de Water B, Williams DP, Park BK. Managing the challenge of drug-induced liver injury: a roadmap for the development and deployment of preclinical predictive models. Nat Rev Drug Discov 2020;19:131–48
16. Yang X, Pabon L, Murry CE. Engineering adolescence: maturation of human pluripotent stem cell-derived cardiomyocytes. Circ Res 2014;114:511–23
17. Meseguer-Ripolles J, Luuendo-Villarín B, Wang Y, Hay DC. Semi-automated production of hepatocyte-like cells from pluripotent stem cells. J Vis Exp 2018;137:e57995
18. Ahamed RE, Anzai T, Chantra N, Uosaki H. A brief review of current maturation methods for human induced pluripotent stem cells-derived cardiomyocytes. Front Cell Dev Biol 2020;8:178
19. Baxter M, Wilhey S, Harrison S, Segeritz C-P, Zhang F, Atkinson-Dell C, Row E, Gerrard DT, Sison-Young R, Jenkins R. Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes. J Hepatol 2015;62:581–9
20. Kamakura T, Makiyama T, Sasaki K, Yoshida Y, Wurtzhaighi Y, Chen J, Hattori T, Ohno S, kita T, Horie M. Ultrastructural maturation of human-induced pluripotent stem cell-derived cardiomyocytes in a long-term culture. Circ J 2013;77:1307–14
21. Lewandowski J, Rozwadowska N, Kolanojski T, Malcher A, Zimna A, Rugowska A, Fiedorowicz K, Labdz W, Kabiszewski L, Chojnacka K, Bednarek-Rajewska K, Majewski P, Kurpisz M. The impact of in vitro cell culture duration on the maturation of human cardiomyocytes derived from induced pluripotent stem cell-derived cardiomyocytes of myogenic origin. Cell Transplant 2018;27:1047–67
22. Jung C, Fajardo C, Ribeiro AJ, Kooiker KB, Coronado M, Zhao M, Hu DQ, Reddy S, Kodo K, Sriman K, Insel PA, Wu JC, Pruitt BL, Bernstein D. Time-dependent evolution of functional vs. remodeling signaling in induced pluripotent stem cell-derived cardiomyocytes and induced maturation with biomechanical stimulation. FASEB J 2016;30:1464–79
23. Yang X, Rodriguez M, Pabon L, Fischer KA, Reinecke H, Regnier M, Sniadecki NJ, Ruohola-Baker H, Murry CE. Tri-iodo-thyronine promotes the maturation of human cardiomyocytes derived from induced pluripotent stem cells. J Mol Cell Cardiol 2014;72:296–304
24. Hu D, Linders A, Yamak A, Correia C, Kijlstra JD, Garakani A, Xiao L, Milan DJ, van der Meer P, Serra M, Alves PM, Domian IJ. Metabolic maturation of human pluripotent stem cell-derived cardiomyocytes by inhibition of HIF1alpha and LDHA. Circ Res 2018;123:1066–79
25. Parikh SS, Blackwell DJ, Gomez-Hurtado N, Frisk M, Wang L, Kim K, Dahl CP, Fiane A, Tonnessen T, Kryshtal DO, Louch WE, Knollmann BC. Thyroid and glucocorticoid hormones promote functional T-Tubule development in Human-Induced pluripotent stem cell-derived cardiomyocytes. Circ Res 2017;121:1332–30
26. Wang B, Jakus AE, Baptista PM, Soker S, Soto-Gutierrez A, Becacis MM, Shah RN, Wertheim JA. Functional maturation of induced pluripotent stem cell hepatocytes in extracellular matrix - a comparative analysis of bioartificial liver microenvironments. Stem Cells Transl Med 2016;5:1257–67
27. Zuppinger C. 3D cardiac cell culture: a critical review of current technologies and applications. Front Cardiovasc Med 2019;6:87
28. Yamada KM, Cukierman E. Modeling tissue morphogenesis and cancer in 3D. Cell 2007;130:601–10
29. Huh D, Torisawa YS, Hamilton GA, Kim HJ, Ingber DE. Microengineered physiological biomimicry: organs-on-chips. Lab Chip 2012;12:2156–64
36. Asthana A, Kisaalita WS. Biophysical microenvironment and 3D cell culture physiological relevance. Drug Discov Today 2013;18:533–40

37. Yamaguchi T, Matsuzaki J, Katsuda T, Saito Y, Saito H, Ochiya T. Regeneration Generation of functional human hepatocytes in vitro: current status and future prospects. Innnam Regen 2019;39:13

38. Baxter M, Wilhey S, Harrison S, Segeritz C-P, Zhang F, Atkinson-Dell R, Rowe C, Gerrard DT, Siison-Young R, Jenkins RJ. Phenotypic and functional analyses show stem cell-derived hepatocyte-like cells better mimic fetal rather than adult hepatocytes. J Hepatol 2015;62:581–9

39. Zabulica M, Srinivasan VC, Vosough M, Hammarstedt C, Wu T, Gramignoli R, Ellis E, Kannisto K, Collins de l'Hortet A, Takeishi K. Guide to the assessment of mature liver gene expression in stem cell-derived hepatocytes. Hepatocyte cell lines: their use, scope and limitations in drug metabolism studies. Expert Opin Drug Metab Toxicol 2006;2:183–212

40. Godoy P, Hewitt NJ, Albrecht U, Andersen ME, Ansari N, Bhattacharya S, Bode JG, Bolley J, Boettger J. Recent advances in 2D and 3D in vitro systems using primary hepatocytes, alternative hepatocyte sources and non-parenchymal liver cells and their use in investigating mechanisms of hepatotoxicity, cell signaling and ADME. Arch Toxicol 2013;87:1315–530

41. Gómez-Lechón MJ, Tolosa L, Conde I, Donato MT. Competency of different cell models to predict human hepatotoxic drugs. Expert Opin Drug Metab Toxicol 2014;10:1553–68

42. Lu J, Einhorn S, Venkataraman L, Höller M, Mann DA, Watkins PB, LeChuyse E. Morphological and functional characterization and assessment of iPSC-Derived hepatocytes for in vitro toxicity testing. Toxicol Sci 2015;147:39–54

43. Li J, Li T. Bile acid receptors link nutrient sensing to metabolic regulation. Liver Res 2017;1:17–25

44. Corbett JL, Duncan SA. iPSC-Derived hepatocytes as a platform for disease modeling and drug discovery. Front Med 2019;6:265

45. Gieseck III RL, Hannan NR, Bort R, Hanley NA, Drake RA, Cameron GW, Wynn TA, Vallier L. Maturation of induced pluripotent stem cell derived hepatocytes by 3D-culture. PLoS One 2014;9:e86372

46. Knöpfl F, Jacobs F, Freyer N, Damm G, De Bondt A, Van den Wyngaert I, Snoeys J, Monshouwer M, Richter M, Strahl N. In vitro model for hepatotoxicity studies based on primary human hepatocyte cultivation in a perfused 3D bioreactor system. Int J Mol Sci 2016;17:584

47. Akbari S, Sevinç ÇG, Ersoy N, Basak O, Kaplan K, Sevinç K, Oxel E, Sengun B, Enustun E, Ozçimen B. Robust, long-term culture of human mesenchymal stem cells. Stem Cells Dev 2018;27:907–19

48. Daney M, Bernier ML, Kimura K, Poulin S, Kato S, Mori D, Kido C, Plessy C, Kusuhara H, Miyajima A. An in vitro human liver model by iPS-derived parenchymal and non-parenchymal cells. Stem Cell Rep 2017;9:490–8

49. Besser RR, Ishahak M, Mayo V, Carbonero D, Clauwe I, Agarwal A. Engineered microenvironments for maturation of stem cell derived cardiac myocytes. Theranostics 2018;8:124–40

50. Ortega-Prieto AM, Shelton JK, Cherry C, Briones-Orta MA, Hately CA, Dorner M. Liver-on-a-chip cultures of primary hepatocytes and kupffer cells for hepatitis B virus infection. J Vis Exp 2019;144:e58333

51. Takebe T, Sekine K, Kimura M, Yoshizawa E, Ayano S, Koido M, Funayama S, Nakanishi N, Hisai T, Kobayashi T. Massively and reproducible production of liver buds entirely from human pluripotent stem cells. Cell Rep 2017;21:2661–70

52. Wu F, Wu D, Ren Y, Huang Y, Feng B, Zhao N, Zhang T, Chen X, Chen S, Xu A. Generation of hepatobiliary organoids from human induced pluripotent stem cells. J Hepatology 2019;70:1145–58

53. Ouchi R, Togo S, Kimura M, Shinozawa T, Koido M, Kioke H, Thompson W, Karns RA, Mayhew CN, McGrath PS. Modeling steatothipatitis in humans with pluripotent stem cell-derived organoids. Cell Metab 2019;30:374–84.e6

54. Underhill GH, Khetani SR. Bioengineered liver models for drug testing and cell differentiation studies. Cell Mol Gastroenterol Hepatol 2018;5:426–39.e1

55. Ribeiro AJS, Yang X, Patel V, Madabushi R, Strauss DG. Liver microphysiological systems for predicting and evaluating drug effects. Clin Pharmacol Ther 2019;106:139–47

56. Raasch M, Fritsche E, Kurtz A, Bauer M, Mosig AS. Microphysiological systems meet hiPSC technology – new tools for disease modeling of liver infections in basic research and drug development. Adv Drug Deliv Rev 2019;140:51–67

57. Goral VN, Hsieh Y-C, Petzold ON, Clark JS, Yuen PK, Faris RA. Perfusion-based microfluidic device for three-dimensional dynamic analysis of human hepatocyte cell culture in the absence of biological or synthetic matrices or coaguants. Lab Chip 2010;10:3380–6

58. Kostadinova R, Boess F, Applegate D, Suter L, Weiser T, Singer T, Naughton B, Roth A. A long-term three dimensional liver co-culture system for improved prediction of clinically relevant drug-induced hepatotoxicity. Toxicol Appl Pharmacol 2013;268:1–16

59. Nakao Y, Kimura H, Sakai Y, Fujii T. Bile canaliculi formation by aligning rat primary hepatocytes in a microfluidic device. Biomicrofluidics 2011;5:022212

60. Ma X, Qu X, Zhu W, Li YS, Yuan S, Zhang H, Liu J, Wang P, Lai CS, Fanella F, Feng GS, Sheikh F, Chien S, Chen S. Deterministically patterned biomimetic human iPSC-derived hepatic model via rapid 3D bioprinting. Proc Natl Acad Sci U S A 2016;113:2206–11

61. Fearnley C, Roderick HL, Bootman MD. Calcium signaling in cardiac myocytes. Cold Spring Harb Perspect Biol 2011;3:a002424

62. Lyon RC, Fanella F, Omens JH, Sheikh F. Mechanotransduction in hepatic hypertrophy and failure. Circ Res 2015;116:1462–76

63. Taegmeyer H, Young ME, Lopashuk GD, Abel ED, Brunenger HB, Darley-Usmar V, Des Rosiers C, Gerszten R, Glatz JF, Griffin JL, Gropler RJ, Holzhuetter HG, Kizer JR, Lewandowski ED, Malloy CR, Neubauer S, Peterson LR, Portman MA, Recchia FA, Van Eyk JE, Wang TJ. Assessing cardiac metabolism: a scientific statement from the american heart association. Circ Res 2016;118:1690–701

64. Eisner DA, Caldwell JL, Kistamas K, Trafford AW. Calcium and cardiac hypertrophy and failure. Circ Res 2017;121:181–95

65. Jiang Y, Park P, Hong SM, Ban K. Maturation of cardiomyocytes derived from human pluripotent stem cells: current strategies and limitations. Mol Cells 2018;41:613–21

66. Tu C, Chao BS, Wu JC. Strategies for improving the maturity of human induced pluripotent stem cell-derived cardiomyocytes. Circ Res 2018;123:512–4

67. Machiraju P, Greenway SC. Current methods for the maturation of induced pluripotent stem cell-derived cardiomyocytes. World J Stem Cells 2019;11:33–43

68. Mills RJ, Hudson JE. Biengineering adult human heart tissue: how close are we? APL Bioeng 2019;3:010901

69. Eder A, Vollert I, Hansen A, Eschenhagen T. Human engineered heart tissue as a model system for drug testing. Adv Drug Deliv Rev 2016;96:214–24

70. Ulmer BM, Eschenhagen T. Human pluripotent stem cell-derived cardiomyocytes for studying energy metabolism. Biochem Biophys Acta Mol Cell Res 2020;1867:118471

71. Ronaldson-Bouchard K, Yeager K, Teles D, Chen T, Song L, Ohashi F, Toyofuku T, Koda K, Sawa Y. Maturation of human induced pluripotent stem cell-derived cardiomyocytes by soluble factors from human mesenchymal stem cells. Mol Ther 2018;26:2681–95

72. Wolling H, Konze SA, Hofer A, Erdmann J, Pich A, Zweigerdt R, Buettner FF. Quantitative secretomics reveals extrinsic signals involved in human pluripotent stem cell cardiomyogenesis. Proteomics 2018;18:1800102
77. Drasenel FM, Boccardo S, Prummer M, Delobel F, Graff A, Weber M, Gerard R, Badi L, Kam-Thong T, Bu L, Jiang X, Hoflack JC, Kiilalaiven A, Jaworutzki E, Aoyama N, Carlson C, Burcin M, Gromo G, Boehringer M, Stahlberg H, Hall BJ, Magnone MC, Kolaja K, Chien KR, Bailly J, Iacone R. Disease modeling and phenotypic drug screening for diabetic cardiomyopathy using human induced pluripotent stem cells. Cell Rep 2014;9:810–21

78. Correia C, Koshkin A, Duarte P, Hu D, Teixeira A, Domian I, Serra M, Alves PM. Distinct carbon sources affect structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Sci Rep 2017;7:8590

79. Li M, Kanda Y, Ashihara T, Sato T, Nakay Y, Kodama M, Hayashi E, Sekino Y, Furukawa RT, Kurokawa J. Overexpression of KCNJ2 in induced pluripotent stem-cell-derived cardiomyocytes for the assessment of QT-prolonging drugs. J Pharmacol Sci 2017;134:75–85

80. Guo F, Sun Y, Wang X, Wang H, Wang J, Gong T, Chen X, Zhang P, Su L, Fu G, Su J, Yang S, Lai R, Jiang C, Liang P. Patient-specific and gene-corrected induced pluripotent stem-cell-derived cardiomyocytes elicit single-cell phenotype of short QT syndrome. Circ Res 2019;124:66–78

81. Liu J, Lieu DK, Siu CW, Fu JD, Tse HF, Li RA. Facilitated maturation of Ca2+- handling properties of human embryonic stem cell-derived cardiomyocytes by calsequestrin expression. Am J Physiol Cell Physiol 2009;297:C152–9

82. Kuppusamy KT, Jones DC, Badi L, Hersee M, Fischer KA, Rodriguez ML, Pabon L, Zhu WZ, Talloch NL, Yang S, Snodgrass NJ, Altman J, Ruzzo WL, Stolz CE, Rouhola-Baker H. Let-7 family of microRNA is required for maturation and adult-like metabolism in stem-cell-derived cardiomyocytes. Proc Natl Acad Sci USA 2015;112:E2785–94

83. Cyr KJ, Avaldi OM, Wikswo JP. Circadian hormone control in a human-on-a-chip: in vitro biology’s ignored component? Exp Biol Med 2017;242:1714–31

84. Ronaldson-Bouchard K, Ma SP, Yeager K, Chen T, Song L, Sirabella D, Morikawa K, Teles D, Yazawa M, Vunjak-Novakovic G. Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature 2018;556:239–43

85. Abilez OJ, Tzatzalos E, Yang H, Zhao MT, Jung G, Zollner AM. Distinct carbon sources affect structural and functional maturation of engineered heart muscle as predicted by computational modeling. Stem Cells 2018;36:265–77

86. Lundy SD, Zhu WZ, Regnier M, Laflamme MA. Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. Stem Cells Dev 2013;22:1991–2002

87. Ribeiro MC, Tertoolen LG, Guadix JA, Bellin M, Kmosidis G, D’Aniello C, Monshouwer-Kloots J, Goumans MJ, Wang YL, Feinberg AW, Mummery CL, Passier R. Functional maturation of human pluripotent stem cell-derived cardiomyocytes in vitro – correlation between contraction force and electrophysiology. Biomaterials 2015;51:138–50

88. Feaster TK, Cadar AG, Wang L, Williams CH, Chun YW, Hempel JE, Bloodworth N, Merryman WD, Lim CC, Wu JC, Klos M, Conradi L, Eschenhagen T. Cooperative coupling of cell-matrix and cell-cell adhesions in cardiac muscle. Proc Natl Acad Sci USA 2012;109:9881–6

89. Liao B, Jackman CP, Li Y, Bursac N. Developmental stage-dependent effects of cardiac fibroblasts on function of stem cell-derived engineered cardiac tissues. Sci Rep 2017;7:42290

90. Zuppinger C. 3D culture for cardiac cells. Biochim Biophys Acta 2016;1863:1873–81

91. Lind JU, Busbee TA, Valentine AD, Pasqualini FS, Yuan H, Yadid M, Park S-J, Kotikian A, Nesmith AP, Campbell PF. Instrumented cardiomyological devices via multimaterial three-dimensional printing. Nat Mater 2017;16:303–8

92. Hanssen A, Eder A, Bonstrup M, Flato M, Polla M, Elliott DA, Drowley LC, Clausen M, Plovright AT, Barrett RP, Wang QD, James DE, Perrollo ER, Hudson JE. Drug screening in human PSC-cardiac organoids identifies pro-proliferative compounds acting via the mevalonate pathway. Cell Stem Cell 2019;24:895–907.e6

93. Laurila E, Ahola A, Hyttinen J, Aalto-Setälä K. Methods for in vitro functional analysis of iPSC derived cardiomyocytes – special focus on analyzing the mechanical beating behavior. Biochim Biophys Acta 2016;1863:1864–72

94. Hansen A, Ender A, Bonstrup M, Flato M, Meve M, Schaaf S, Aschirlikoglou B, Schweizer AP, Uebeler J, Eschenhagen T. Development of a drug screening platform based on engineered heart tissue. Circ Res 2010;107:35–44

95. Schaaf S, Shibayama A, Meve M, Ender A, Stöhr A, Hirt MN, Rau T, Zimmermann W-H, Conradi L, Eschenhagen T. Human engineered
heart tissue as a versatile tool in basic research and preclinical toxicology. *PloS One* 2011;6:e26397.
110. Franko A, Hartwig S, Kotzka J, RoUö M, Nüssler AK, Königrainer A, Haring H-U, Lehr S, Peter A. Identification of the secreted proteins originated from primary human hepatocytes and HepG2 cells. *Nutrients* 2019;11:1795.
111. Waisman A, La Greca A, Möbb MS, Scarafia MA, Velazque NLS, Neiman G, Moro LN, Luzzami C, Seveliere GE, Gubernman AS. Deep learning neural networks highly predict very early onset of pluripotent stem cell differentiation. *Stem Cell Rep* 2019;12:845–59.
112. Wakis T, Matsumoto T, Matsukasa K, Kawasaki T, Yamaguchi H, Akutsu H. Method for evaluation of human induced pluripotent stem cell quality using image analysis based on the biological morphology of cells. *J Med Imaging* 2017;4:044003.
113. Brackman DJ, Giacomini KM. Reverse translational research of ABCG2 (BCRP) in human disease and drug response. *Clin Pharmacol Ther* 2018;103:233–42.
114. Roberts B, Haupt A, Tucker A, Grancharova T, Arakaki J, Fuqua MA, Haupt A, Tucker A, Grancharova T, Arakaki J, Fuqua MA. Blinded contractility analysis in human liver coculture platform. *Exp Biol Med* 2016;241:121–29.
115. Chen T-W, Wardill TJ, Sun Y, Pulver SR, Renninger SL, Baohan A, Simian M, Bissell MJ. Organoids: a historical perspective of thinking in three dimensions. *J Cell Biol* 2017;216:31–40.
116. Weaver RJ, Valentin JP. Today’s challenges to de-risk and predict drug safety in human mind-the-Gap. *Toxicol Sci* 2019;167:307–21.
117. Solotke MT, Dhruba VS, Downing NS, Shah ND, Ross JS. New and incremental FDA black box warnings from 2008 to 2015. *Expert Opin Drug Saf* 2018;17:117–23.
118. Ebrahimkhani MR, Neiman JA, Raredon MS, Hughes DJ, Griffith LG. Bioreactor technologies to support liver function in vitro. *Adv Drug Deliv Rev* 2014;69:70–132–57.
119. Bell CC, Hendriks DF, Moro SM, Ellis E, Walsh J, Renblom A, Fredriksson Puigvert L, Dankers AC, Jacobs F, Snoeys J, Sison-Young RL, Jenkins RE, Nordling A, Mertschin S, Park BK, Kittingham NR, Goldring CE, Lautshe VM, Ingelman-Sundberg M. Characterization of primary human hepatocyte spheroids as a model system for drug-induced liver injury, liver function and disease. *Sci Rep* 2016;6:25187.
120. Kim YS, Ahn Y. Functional relevance of macrophage-mediated inflammation to cardiac regeneration. *Chonnam Med J* 2018;54:10–16.
121. Psarras S, Beis D, Nikouli S, Tsikitis M, Capetanaki Y. Three in a box: understanding cardiomyocyte, fibroblast, and innate immune cell interactions to orchestrate cardiac repair processes. *Front Cardiovasc Med* 2019;6:32.
122. Long TJ, Cosgrove PA, Dunn RT 2nd, Stolz DB, Hamadeh H, Afshari C, McBride H, Griffith LG. Modeling therapeutic antibody-small molecule drug-drug interactions using a three-dimensional perfusible human liver coculture platform. *Drug Metab Dispos* 2016;44:1940–8.
123. Ewart L, Fabre K, Chakilam A, Dragoi Y, Diugnan DB, Eswaraka J, Gian J, Guazzie-Fech P, Otieno M, Jeeun GC, Kelker DA, de Morais SM, Phillips JA, Proctor W, Sura R, Van Vleet T, Watson D, Will Y, Ruoß M, Nuessler AK, Kotzka J, Ruoß M, Nußler AK, Hansla A. Blinded contractility analysis in human liver coculture platform for generation of chamber-specific cardiac tissues and disease modeling. *Cell* 2019;176:913–27.e18.
124. Usta OB, McCarty WJ, Bale S, Hegde M, Jindal R, Bhushan A, Golberg I, Yarmush ML. Microengineered cell and tissue systems for drug screening and toxicology applications: evolution of in-vitro liver technologies. *Technology* 2015;3:1–26.
125. Kileen MJ. Drug-induced arrhythmias and sudden cardiac death: implications for the pharmaceutical industry. *Drug Discov Today* 2009;14:589–97.
126. Varga ZV, Ferdinandy P, Laiudet L, Pacher P. Drug-induced mitochondrial dysfunction and cardio toxicity. *Am J Physiol Heart Circ Physiol* 2015;309:H1453–67.
127. Mladenka P, Appllova L, Patocka J, Costa VM, Remiao F, Pourouva J, Mladenka A, Karlickova J, Jahodar L, Vopralova M, Varner KJ, Sibera M. Comprehensive review of cardiovascular toxicity of drugs and related agents. *Med Res Rev* 2018;38:1332–403.
128. Cuomo A, Rodolico A, Galdieri A, Russo M, Campi G, Franco R, Bruno D, Aran L, Carannante A, Attanasio U, Ticchetti CG, Varricchi G, Mercurio V. Heart failure and cancer: mechanisms of old and new cardiotoxic drugs in cancer patients. *Card Fail Rev* 2019;5:112–8.
129. Hants P. Mechanisms of toxic cardiomyopathy. *Clin Toxicol* 2019;57:1–9.
130. Gambardella J, Trimarco B, Iarcino G, Sorriento D. Cardiac nonmyocyte cell functions and crosstalks in response to cardiotoxic drugs. *Oxid Med Cell Longev* 2017;2017:doi: 10.1155/2017/1089359.
131. Ferdinandy P, Baczko I, Bencsik P, Gierz Z, Gorbe A, Pacher P, Varga ZV, Varro A, Schulz R. Definition of hidden drug cardiotoxicity: paradigm change in cardiac safety testing and its clinical implications. *Eur Heart J* 2019;40:1771–7.
132. Bauman JL. The role of pharmacokinetics, drug interactions and pharmacogenetics in the acquired long QT syndrome. *Eur Heart J* 2001;3:K9–K10.
133. Oeleaga C, Riu A, Rothemund S, Lavado A, McAleer CW, Long CJ, Persaud K, Narasimhan NS, Tran M, Roles J, Carmona-Moran CA, Sasserath T, Elbrecht DH, Kumanichik, Bridges LR, Martin C, Schneppef MT, Ekman G, Jackson M, Wang YI, Note R, Langer J, Teissier S, Hickman J. Investigation of the effect of hepatic metabolism on off-target cardiotoxicity in a multi-organ human-on-a-chip system. *Bionanomaterials* 2018;182:176–90.
134. Vernetti L, Gough A, Baetz N, Blutt S, Broughtman JR, Brown JA, Foulke-Abel J, Hasan N, J, Kelly E. Functional coupling of human microbiology systems: intestine, liver, kidney proximal tubule, blood-brain barrier and skeletal muscle. *Sci Rep* 2017;7:42296.
135. Colatsky T, Fennbini M, Gaitnt G, Pierson JB, Sager P, Sekino Y, Strauss DG, Stockbridge N. The comprehensive in vitro proarrhythmia assay (CIPA) initiative - Update on progress. *J Pharmacol Toxicol Methods* 2016;81:15–20.
136. Wang H, Sheehan RP, Palmer AC, Avery LR, Boswell SA, Ron-Harel N, Ringel AE, Holton KM, Jacobson CA, Erickson AR, Maliszewski L, Haics MC, Sorgor PK. Adaptation of human iPSC-Derived cardiomyocytes to tyrosine kinase inhibitors reduces acute cardiotoxicity via metabolic reprogramming. *Cell Syst* 2019;8:412–26.e7.
137. Kaplowitz N. Idiosyncratic drug hepatotoxicity. *Nat Rev Drug Discov* 2005;4:489–99.
138. Daly AK. Pharmacogenomics of adverse drug reactions. *Genome Med* 2013;5:5.
139. Reichel A, Lienau P. Pharmacokinetics in drug discovery: an exposure-centred approach to optimising and predicting drug efficacy and safety. *Handb Exp Pharmacol* 2016;232:235–60.
140. Morgan P, Van Der Graaf PH, Arrowsmith J, Feltner DE, Drummond KS, Wegner CD, Street SD. Can the flow of medicines be improved? *Drug Discov Today* 2012;17:419–24.
141. Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, Pangalos MN. Lessons learned from the fate of AstraZeneca’s drug pipeline: a five-dimensional framework. *Nat Rev Drug Discov* 2014;13:419–31.
146. Fan J, de Lannoy IA. Pharmacokinetics. Biochem Pharmacol 2014;87:93–120
147. Prantil-Baun R, Novak R, Das D, Somayaji MR, Przekwas A, Inger DE. Physiologically based pharmacokinetic and pharmacodynamic analysis enabled by microfluidically linked organs-on-chips. Annu Rev Pharmacol Toxicol 2018;58:37–64
148. Verbeck RK. Pharmacokinetics and dosage adjustment in patients with hepatic dysfunction. Eur J Clin Pharmacol 2008;64:1147–61
149. Michel V, Frappier M, Dumas MC, Turgeon J. Metabolic activity and mRNA levels of human cardiac CYP450s involved in drug metabolism. PLoS One 2010;5:e15666
150. Kolwicz SC, Jr., Purushot S, Tian R. Cardiac metabolism and its interactions with contraction, growth, and survival of cardiomyocytes. Circ Res 2013;113:603–16
151. He F, Li Y, Zeng C, Xia C, Xiong Y, Zhang H, Huang S, Liu M. Contribution of cytochrome P450 isozymes to glidoidine metabolism in rats and human. Xenobiotica 2014;44:229–34
152. Lu C, Di L. In vitro and in vivo methods to assess pharmacokinetic drug–drug interactions in drug discovery and development. Biopharm Drug Dispos 2020;41:3–31
153. Zeilinger K, Freyer N, Damm G, Seehofer D, Knapp M, Anklam E. Contribution of cytochrome P450 isoforms to glidoidine metabolism in rats and human. Xenobiotica 2014;44:229–34
154. Schütz EG, Schinkel AH, Relling MV, Schuetz JD. P-glycoprotein: a critical drug efflux pump in cancer. Nat Rev Cancer 2008;8:71–83
155. Frishman WH, Alwarshetty M. Beta-adrenergic blockers in systemic hypertension: pharmacokinetic considerations related to the current guidelines. Clin Pharmacokinet 2002;41:505–16
156. Luo Y, Lou C, Zhang S, Zhu Z, Xing Q, Wang P, Liu T, Liu H, Li C, Shi W, Du Z, Gao Y. Three-dimensional hydrogel culture conditions promote the differentiation of human induced pluripotent stem cells into hepatocytes. Cytotherapy 2018;20:95–107
157. Goulart E, de Caires-Junior LC, Telles-Silva KA, Araujo BHS, Matos AL, de Oliveira AG, Cunha IA, Costa AP, Egerer K. Adult and iPSC-derived non-parenchymal cells regulate liver organoid development through differential modulation of Wnt and TGF-β. Stem Cell Res Ther 2019;10:258
158. Ahmed S, Zhou Z, Zhou J, Chen SQ. Pharmacogenomics of drug metabolizing enzymes and transporters: relevance to precision medicine. Xenobiotica 2014;44:298–313
159. Peng KW, Bacon J, Zheng M, Guo Y, Wang MZ. Ethnic variability in drug CYP450s involved in drug metabolism. Pharmacogenomics 2013;14:93–101
160. Sinz M, Wallace G, Sahi J. Current industrial practices in assessing CYP450 enzyme induction: preclinical and clinical. AAPS J 2010;12:391–400
161. Kim JH, Jang YJ, An SY, Son J, Lee J, Lee G, Park JY, Park HJ, Hwang HY, Kim JH, Han J. Enhanced metabolizing activity of human ES Cell-Derived hepatocytes using a 3D culture system with repeated exposures to xenobiotics. Toxicol Sci 2015;147:190–206
162. Pettinato G, Ramanathan R, Fisher RA, Mangino MJ, Zhang N, Wen X, Fischer RA. Generation of fully functional hepatocyte-like organoids from human induced pluripotent stem cells mixed with endothelial cells. Sci Rep 2019;9:8920
163. Frishman WH, Alwarshetty M. Beta-adrenergic blockers in systemic hypertension: pharmacokinetic considerations related to the current guidelines. Clin Pharmacokinet 2002;41:505–16
164. Blumenthal DK, Garrison JC. Pharmacodynamics: molecular mechanisms of drug action, Goodman and Gilman’s the pharmacological basis of therapeutics. San Diego: The McGraw-Hill Companies, 2011, pp.41–71
165. Wright DF, Winter HR, Duffull SB. Understanding the time course of pharmacological effect: a PKPD approach. Br J Clin Pharmacol 2011;71:815–23
166. van den Brink L, Grandela C, Mummery CL, Davis RP. Inherited cardiac diseases, pluripotent stem cells, and genome editing combined - the past, present, and future. Stem Cells 2019;38:174–86
167. Burridge PW, Li YE, Matsa E, Wu H, Ong SC, Sharma A, Holmstrom A, Chang AC, Coronado MJ, Ebert AD, Knowles JW, Telli ML, Witteles RM, Blau HM, Bernstein D, Altman RB, Wu JC. Human induced pluripotent stem cell-derived cardiomyocytes recapitulate the predilection of breast cancer patients to doxorubicin-induced cardiotoxicity. Nat Med 2016;22:547–56
168. Knowles DA, Burrows CK, Blishak JD, Patterson KM, Serie DJ, Norton N, Ober C, Pritchard JK, Gilad Y. Determining the genetic basis of anthracycline-cardiotoxicity by molecular response QTL mapping in induced cardiomyocytes. Elife 2018;7:e33480
169. Lauschaie VM, Ingelman-Sundberg M. The importance of patient-specific factors for hepatic drug response and toxicity. Int J Mol Sci 2016;17:1714
170. Kitzemann T. Metabolic zonation of the liver: the oxygen gradient revisited. Redox Biol 2017;11:622–30
171. White WB, Rodman MD. The effects of cardiovascular drugs on exercise performance. J Cardiaca Nurs 1998;2:320–9
172. Abi-Samra F, Gutterman D. Cardiac contractility modulation: a novel basis of anthracycline-cardiotoxicity by molecular response QTL mapping in induced cardiomyocytes. Elife 2018;7:e33480
173. Jovanovic A. Cardioprotective signalling: past, present and future. Eur J Pharmacol 2018;833:314–9
174. Marques-Aleixo I, Santos-Alves E, Oliveira PJ, Moreira PI, Magalhaes J, Ascensoa A. The beneficial role of exercise in mitigating doxorubicin-induced mitochonronopathy. Biochim Biophys Acta Rev Cancer 2018;1869:189–99
175. Buchanan CA, Todo Y, Durante M. Heart in space: effect of the extraterrestrial environment on the cardiovascular system. Nat Rev Cardiol 2018;15:167–80
176. Tzatzalos E, Abilez OJ, Shukla P, Wu JC. Engineered heart tissues and induced pluripotent stem cells: macro- and microstructures for disease modeling, drug screening, and translational studies. Adv Drug Deliv Rev 2016;81:314–9
177. Lian X, Zhang J, Azarin SM, Zhu K, Hazeltine LB, Bao X, Hsiao C, Kamp TJ, Palecek SP. Directed cardiomyocyte differentiation from human pluripotent stem cells by modulating wnt/β-catenin signaling under fully defined conditions. Nat Protoc 2013;8:162
178. D’Amour KA, Agulnick AD, Eliazer S, Kelly GC, Kroon E, Baetje EE. Efficient Differentiation of human embryonic stem cells to definitive endoderm. Nat Biotechnol 2005;23:1534
179. Si-Tayeb K, Noto FK, Nagaoka M, Li J, Battle MA, Duris C, North PE, Dalton S, Duncan SA. Highly efficient generation of human hepatocyte-like cells from induced pluripotent stem cells. Hepatology 2010;51:297–305
202. Lenzini S, Turnbull IC, Adjayi V, Kim J, Seliktar D, Easley C, Costa KD, Lipke EA. Direct hydrogel encapsulation of pluripotent stem cells enables oncomimetic differentiation and growth of engineered human heart tissues. *Biomaterials* 2016;83:383–95

203. Kerscher P, Kaczmarek JA, Head SE, Ellis ME, Seeto WJ, Kim J, Thompson JA, Chen G. Passaging and colony expansion of human pluripotent stem cells by enzyme-free dissociation in chemically defined culture conditions. *Nat Protoc* 2012;7:2029

204. Beers J, Gullbranson DR, George N, Siniscalchi LI, Jones J, Thomson JA, Chen G. Fusing cell colonies in human pluripotent stem cells for oncomimetic differentiation and growth of engineered human heart tissues. *Biomaterials* 2017;3:1499–509

205. Kolnik M, Tsimring LS, Hasty J. Vacuum-assisted cell loading enables shear-free mammalian microfluidic culture. *Lab Chip* 2012;12:4732–7

206. Wang L, Ni X-F, Luo C-X, Zhang Z-L, Pang D-W, Chen Y. Self-loading and cell culture in one layer microfluidic devices. *Biomed Microdevices* 2009;11:679–84

207. Branco MA, Cotovio JP, Rodrigues CA, Vaz SH, Fernandes TG, Moreira LM, Cabral JM, Diogo MM. Transcriptomic analysis of 3D cardiac differentiation of human induced pluripotent stem cells reveals faster cardiomyocyte maturation compared to 2D culture. *Sci Rep* 2019;9:1–13

208. Zahir N, Weaver VM. Death in the third dimension: apoptosis regulation and tissue architecture. *Curr Opin Genet Dev* 2004;14:71–80

209. Holloway EM, Capeling MP, Spence JR. Biologically inspired approaches to enhance human organoid complexity. *Development* 2019;146:dev16173

210. Starokozhko V, Hemmingsen M, Larsen L, Mohanty S, Merema M, Pimentel RC, Wolff A, Emnés J, Aspegren A, Gheibi P, Gao Y, Foster E, Son KJ, You J, Stybayeva G, Patel D, Revzin A. Cell biology is different in small volumes: endogenous signals shape phenotype of primary hepatocytes cultured in microfluidic channels. *Sci Rep* 2016;6:33980

211. Niiya T, Murakami M, Aoki T, Murai N, Shimizu Y, Kusano M. Immediate increase of portal pressure, reflecting sinusoidal shear stress, induced liver regeneration after partial hepatectomy. *J Hepatobiliary Pancreat Surg* 1999;6:275–80

212. Rashidi H, Alhaque S, Szolnicka D, Flint O, Hay DC. Fluid shear stress modulation of hepatocyte-like cell function. *Arch Toxicol* 2016;90:1757–61

213. Kehtari M, Zeynali B, Soleimani M, Kabiri M, Seyedjafari E. Fabrication of a co-culture micro-bioreactor device for efficient hepatic differentiation of human induced pluripotent stem cells (hiPSCs). *Artif Cell Nanomed Biotechnol* 2018;46:161–70

214. Zhang B, Radisic M. Organ-on-a-chip devices advance to market. *Lab Chip* 2017;17:2395–420

215. Berg KJ, Otieno MA, Ronski J, Lim H-K, Ewart L, Kodella KR, Petrilis DP, Kulkarni G, Rubins JE, Conneligano M, Reuveny S, Chen A, Oh S. Unraveling the inconsistencies of cardiac differentiation efficiency induced by the GSK3 inhibitor CHIR99021 in human pluripotent stem cells. *Stem Cell Rep* 2018;10:1851–66

216. Hallon C, Schwanke K, Lelwe, Franke A, Szepes M, Biswanath S, Wunderlich S, Merkert S, Weber N, Osten F. Continuous WNT control enables advanced hiPSC cardiac processing and prognostic surface marker identification in chemically defined suspension culture. *Stem Cell Rep* 2019;13:366–79

217. Initiative NAKF. Collaborations of consequence: NAKFI’s 15 years igniting innovation at the intersections of disciplines. Washington, DC: National Academies Press, 2018

218. Andreopoulos FM, Persaud I. Delivery of basic fibroblast growth factor (bFGF) from photore sponsive hydrogel scaffolds. *Biomaterials* 2006;27:2468–76

219. Shin W, Hinojosa CD, Ingber DE, Kim HJ. Human intestinal morphogenesis controlled by transepithelial morphogen gradient and flow-dependent physical cues in a microengineered gut-on-a-chip. *iScience* 2019;15:391–406

220. Maas C, Dallas ME, LaBarge ME, Shockley M, Valdez J, Geishecker E, Stokes CL, Griffith LG, Cirit M. Establishing Quasi-steady state operations of microphysiological systems (MPS) using tissue-specific metabolic dependences. *Sci Rep* 2018;8:1–13

221. Cui KW, Engel L, Dunse CE, Nguyen TC, Loh KM, Dunn AR. Spatially controlled stem cell differentiation via morphogen gradients: a comparison of static and dynamic microfluidic platforms. *J Vac Sci Technol* 2020;38:033205

222. Hu Q, Luni C, Elvassore N. Microfluidics for secretome analysis under enhanced endogenous signaling. *Biochem Biophys Res Commun* 2018;497:480–4

223. Haque A, Gheibi P, Gao Y, Foster E, Son KJ, You J, Stybayeva G, Patel D, Revzin A. Cell biology is different in small volumes: endogenous signals shape phenotype of primary hepatocytes cultured in microfluidic channels. *Sci Rep* 2016;6:33980

224. Naka R, Takahara O, Kasai M, Kohri H, Kusano M. Rapid assembly of 3D human and cross-species drug toxicities using a liver-chip. *Stem Cells Transl Med* 2019;8:1577–61

225. Ronaldson-Bouchard K, Vunjak-Novakovic G. Organs-on-a-chip: a fast track for engineered human tissues in drug development. *Cell Stem Cell* 2018;22:310–24

226. Correale M, Tarantino N, Petrucci R, Tricario L, Laonigro I, Di Biase M, Brunetti ND, Liver disease and heart failure: back and forth. *Eur J Intern Med* 2018;48:25–34