

High-Content Assessment of Cardiac Function Using Heart-on-a-Chip Devices as Drug Screening Model

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Abstract Drug discovery and development continues to be a challenge to the pharmaceutical industry despite great advances in cell and molecular biology that allow for the design of better targeted therapeutics. Many potential drug compounds fail during the clinical trial due to inefficacy and toxicity that were not predicted during preclinical stages. The fundamental problem lies with the use of traditional drug screening models that still largely rely on the use of cell lines or animal cell monolayers, which leads to lack of predictive power of human tissue and organ response to the drug candidates. More physiologically relevant systems are therefore critical in relieving the burden of high failure rates. Emerging knowledge and techniques in tissue engineering and microfabrication have enabled the development of micro-engineered systems — collectively known as organs-on-chips — that may lead to a paradigm shift in preclinical drug screening assays. In this review we explore the technological advances and challenges in the development of heart-on-a-chip models, by addressing current assessment methods for drug-induced cardiotoxicity and providing a perspective on the modifications that should be implemented to realize the full potential of this system.

Keywords Drug screening · Heart-on-a-chip · Organ-on-a-chip · Cardiotoxicity · Kinase

Introduction

Drug development is a labor intensive and expensive process. The average time from discovery to development to market approval ranges between 10 and 15 years at the cost of more than 1.5 billion USD [1]. Despite these efforts and the staggering costs invested in pharmaceutical research and development (R&D) activities, there appears to be a significant decrease in the number of new chemical compounds that are being approved by the U.S. Food and Drug Administration (FDA). In 2016, only 22 new molecular entities have been approved and that number has dropped considerably in the last few years [2, 3].

Assurance of drug safety has always been an explicit regulatory objective. Most new drug candidates are often rejected due to efficacy and off-target toxicity during clinical trials [4]. If, during or after clinical trials, it is determined that a compound has adverse effects on non-target organs, including the heart, the drug will be recalled and millions of dollars will have been wasted. The challenges of the current business model arise primarily from growing concerns regarding safety and efficacy [5] of new chemical entities, the worry of a decrease in profitability [6] and high compound attrition rates. To maintain competitiveness while remaining profitable, some pharmaceutical R&D scientists suggest a shift from the traditional drug discovery process to a “fast fail” model. By addressing inefficiencies during discovery phase (Fig. 1), pre-clinical trials and Phase I clinical trials where technical uncertainty could be resolved early in the pipeline, pharmaceutical companies may prevent significant losses during the expensive Phase II and Phase III development stages [6]. However,

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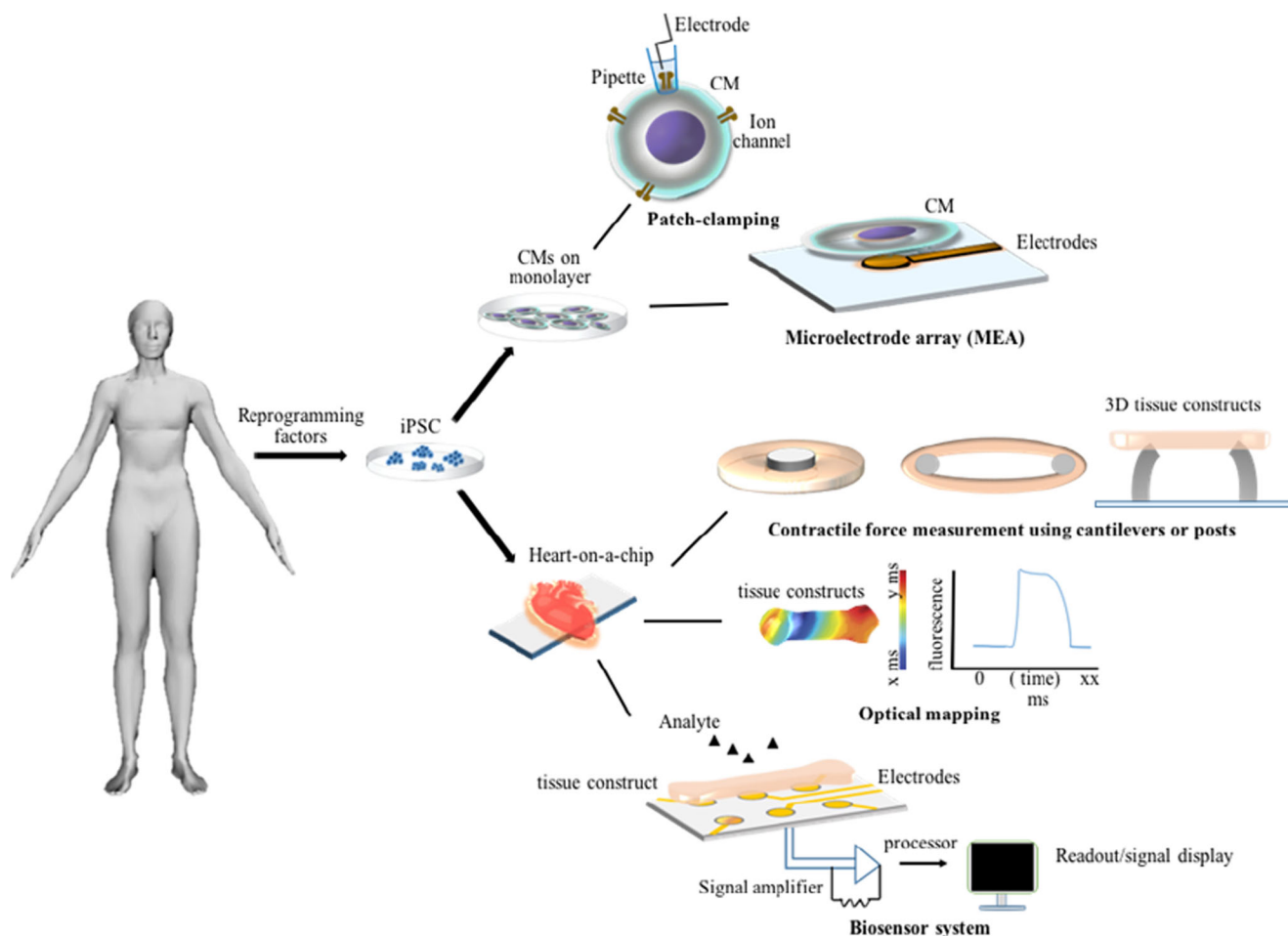


Fig. 1 hiPSC-based monolayer and heart-on-a-chip platforms for cardiac functional assessment

many of the disparities observed for the drug candidates between preclinical and clinical trials are directly related to the inadequacy of current drug screening models.

Evaluation of a new drug's efficacy and toxicity during the preclinical stages relies heavily on the combination of *in vivo* animal studies and *in vitro* cell culture analyses. For decades, animal models have served as the gold standard of preclinical testing (Fig. 2). However, there are several drawbacks to animal models including high cost, time-consuming analysis, and species-species differences that lead to a limited ability to reproduce similar drug responses in humans and for human diseases. Traditional *in vitro* two-dimensional (2D) cell culture models based on human cells still lack the three-dimensional (3D) architecture of a tissue and organ. The absence of 3D cell-cell and cell-matrix interactions can affect the prediction of dose effectiveness and dose toxicity as well as hamper the functionality of cells. Additionally, the preclinical predictability with 2D cell culture models is restricted due to the use of animal-derived cells or immortalized human cell lines. These cells do not exhibit phenotypical similarities to the cells of human tissue. Most importantly, the 2D system is a reductionist method, as it does not take into account a whole-

body system. The diffusion kinetics of drugs to the target organ and toxicity of drug metabolites to non-target human tissue are some of many examples that are not considered in the 2D model. Thus, there is considerable need for a more accurate validation system to screen the effect of new drug compounds on their target site as well as on non-target organs.

In recent years, advances in cell engineering have revolutionized the drug discovery field, into the realization of tailoring medical compounds for individual patients. The worries of using inter-species cells or immortalized cell lines in screening potential drug compounds can be resolved through the use of human induced pluripotent stem cells (hiPSCs). Since Yamanaka first introduced iPSCs by reprogramming mouse fibroblasts through forced expression of four transcription factors [7], extensive progress has been made into harnessing iPSC potential into forming physiologically relevant tissues. Protocols for hiPSC differentiation into liver, lung, kidney and heart-like cells are now well developed and the hiPSC-derived progeny are already being used for preliminary drug toxicity screening. Although there is a recognized concern that these hiPSC-derived cells are still in their fetal state and do not fully recapitulate the adult phenotype, the stem cell field is actively


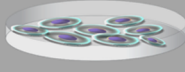

	Animal model 	Monolayer cell culture 	Heart-on-a-chip 
Pros	<ul style="list-style-type: none"> • Provides whole body assay systems to study drug response and its subsequent metabolites 	<ul style="list-style-type: none"> • Low cost • High-throughput • Easy to maintain and analyze 	<ul style="list-style-type: none"> • Accurate representation of topographical, electromechanical and biochemical cues of native tissue • Realistic cell function and behavior akin to in vivo conditions • High-content readouts
Cons	<ul style="list-style-type: none"> • High cost • Time-consuming • Less predictive of drug response due to interspecies variation • Ethical issues 	<ul style="list-style-type: none"> • Unable to represent the 3D architecture of native tissue • Unable to predict the impact of subsequent metabolites on the whole organism • Uncertain drug sensitivity and toxicity 	<ul style="list-style-type: none"> • Lower throughput than monolayer • Off-lines analysis of responses still needed

Fig. 2 Comparison of different models for preclinical drug screening

addressing this limitation by examining different approaches to accelerate the cell maturation process. In the case of hiPSC-differentiated cardiomyocytes, chemical factors, physical cues and electro-mechanical stimulation have all shown great promise for promoting maturation of cells into more adult cardiomyocyte phenotype [8–11]. For more details about differentiation and maturation of hiPSC-derived cardiomyocytes (hiPSC-CMs), the authors would like to refer to an excellent review by Hartman and colleagues [12].

In addition to the discovery of hiPSCs, advanced microfabrication techniques have also drawn a lot of interest in the past few years. Engineering of miniaturized cell interfaces and organoids (i.e. organ-on-a-chip engineering) is an emerging technology that may help bridge the gap between in vitro 2D cell culture and in vivo animal models. These platforms have transitioned the drug screening process from a static 2D basic analysis to a dynamic 3D construct where drug diffusion and other in vivo physiological events may be controlled and mimicked. Besides providing topographical, mechanical and biochemical cues that are physiologically relevant, these platforms are miniaturized and require only a small volume of culture medium and a small number of cells to mimic an in vivo microenvironment. Thus, appealing

preclinical screening options are generated when these platforms are integrated with the hiPSC-derived cells.

In this article we will highlight the drug screening process with kinase inhibitors as an example, due to their potential as cancer treating therapeutics but also due to the toxicity they may induce on cardiac tissue. Thereafter, we will review the current means of assessing cardiac function on different cardiac constructs. Finally, we will address the need to change current cardiac assessments to avoid analytical uncertainties and discuss the challenges that must be overcome in generating a more physiologically relevant high-throughput and high-content drug discovery platform.

Kinases as Cancer Treatment Targets

Cancers are known for their heterogeneity. They arise from normal cells that have undergone numerous mutations to acquire the capabilities that Hanahan and Weinberg, in 2000, proposed to be “hallmarks of cancer” [13]. These six requirements are: (i) self-sufficiency in growth signals, (ii) insensitivity to anti-growth signals, (iii) evasion of apoptosis, (iv) limitless replicative potential, (v) sustaining angiogenesis,

and (vi) ability to invade and metastasize. This paradigm was revised in 2011 by Hanahan and Weinberg and has recognized the emergent role of tumor microenvironment in leading to the tumorigenesis of the cancerous cells [14].

When the human genome was plotted in the 2000s, over 500 protein kinases were identified [15]. Protein kinases, specifically tyrosine kinases (TK), are heavily implicated in the regulation of the normal life cycle of human cells. By catalyzing the transfer of the γ -phosphate group from adenosine triphosphate (ATP) to tyrosine residues on other proteins, TKs are essential in activating signaling pathways that regulate basic cellular function, such as cell growth, differentiation, metabolism, migration, and programmed cell death [16].

Mutation of the gene of a certain regulatory pathway can lead to either overexpression or silencing of the regulatory component which eventually results in uncontrolled mass outgrowth [17]. For example, the overexpression of epidermal growth factor receptor (EGFR) is implicated in several solid tumors, such as non-small cell lung cancer, and cancers of the breast, prostate, and colon [18], the overexpression of vascular endothelial growth factor receptor (VEGFR) is commonly found in renal cell carcinoma [19], and the overexpression of b-RAF is commonly found in malignant melanomas due to a point mutation in the b-RAF-coding gene [20]. In addition to their implication in tumor growth, kinases have been linked to non-tumorigenic cancers, such as the kinase complex Bcr-Abl's anti-apoptotic properties in chronic myeloid leukemia (CML) [21], and the role of anaplastic lymphoma kinase in anaplastic large cell lymphoma [22]. These are merely a glimpse at the impact that kinase molecules have in regulation of cells and cancer.

In the past several years there has been enormous progress in the field of cancer treatment due to the development of targeted therapeutics, where anti-cancer drugs are designed to target specific tumor cells, while ignoring healthy cells in the body [23]. A significant emerging trend in targeted cancer therapeutics is the use of compounds that inhibit TK activity in cells. Both monoclonal antibody-based kinase inhibitors (KIs) and small molecule KIs have been approved for clinical treatment of a variety of cancers. There are over 12 clinically approved KI-based cancer treatments on the market, from pharmaceutical companies such as Pfizer, AstraZeneca, Genentech, Bayer, Novartis and GlaxoSmithKline [24]. A selection of these approved kinase-inhibiting cancer treatments can be seen in Table 1.

Clinical Cardiotoxicity of Kinase Inhibitors

The contractile mechanism of cardiomyocytes requires a constant supply of ATP for normal function. Disruption to mitochondrial function in cardiomyocytes, resulting in the interruption of the ATP supply, can have drastic effects on

metabolically active cardiac tissue [25]. In addition, several kinases have been implicated in cardiomyocyte proliferation, survival, regulating hypertrophy, and prevention of degenerative cardiac diseases such as cardiomyopathy [26–31]. For example, the ErbB signaling in cardiomyocytes can have an effect on apoptosis and cardiac cell contraction, when dimerized with neuregulin-1, a member of the epidermal growth factor family [32]. In addition, many signaling cascades in cardiomyocytes are regulated by glycogen synthase kinase-3 (GSK-3). GSK-3 system has been shown to promote gene expression in cardiac cells through β -catenin and serve as a key cell cycle regulator in cardiomyocyte proliferation [33]. Thus inhibition of various kinases could potentially have detrimental effects on cardiomyocyte health and function, such as reduction in the left ventricular ejection fraction (LVEF), myocardial infarction (MI), and congestive heart failure (HF) [34].

As stated previously, KI-based cancer therapies have been very successful at reducing tumor size and preventing metastasis. KI-based cancer treatment methods can vary between single-kinase inhibitors, such as trastuzumab, or multi-kinase inhibitors, aiming to inhibit vascular growth and tumor cell proliferation and survival, such as sunitinib. However, repeated and prolonged use of these drugs has been linked to increased incidences of cardiovascular complications [23, 34, 35]. The first reported incidence of cardiotoxicity in KI use was with trastuzumab, the monoclonal antibody that targets ErbB2. In 4 of 5 randomized trials of trastuzumab, there was an increase of 5–17% in the frequency of asymptomatic LVEF decrease and an increase of 1–3% in the incidence of chronic heart failure [36–39]. Due to its multiple target nature, targeting the VEGFR1–3, PDGFR, KIT, FLT3, CSF1R, and RET kinases, Sunitinib has also been linked to a higher risk of off-target activity and cardiotoxicity. These fears were confirmed when researchers such as Telli et al. reported that 15% of patients undergoing Sunitinib treatment developed symptomatic heart failure after prolonged use [40]. Several other tyrosine kinase inhibitors (TKI) approved by the FDA have also been shown to induce a variety of cardiovascular complications, ranging from QT prolongation to LVEF depression (as identified in Table 1), and their symptoms can range from mild to severe [23]. Although the causes of these toxicities are not well understood, it has been suggested that lack of target specificity and unintended mitochondrial toxicity are responsible for the cardiomyocyte damage during TKI treatment [25, 41].

The silver lining to these findings is that with careful monitoring and cardiac treatment, TKI-induced cardiac toxicity can be mediated and the effect is reversible [42]. However, due to the limitations of clinical trials and the ambiguity regarding the causes of cardiovascular disease (CVD), the effect of these drugs could not be determined before being approved for use with patients [24, 35]. Thus, identifying these adverse effects before

Table 1 Selection of clinically available KI-based cancer therapies, their kinase target, and their observed cardiotoxicities

Drug	Molecule type	Kinase Target	Cancer Type	Cardiac Toxicity	Reference
Trastuzumab (Genentech, 1998)	Monoclonal antibody	ERBB2	Breast cancer	Congestive heart failure and depression of LVEF	[38, 39]
Sunitinib (Pfizer, 2006)	Small molecule	VEGFR	Renal cell carcinoma	Acute coronary symptoms (MI), HF, LV dysfunction	[42, 107]
Lapatinib (GlaxoSmithKline, 2007)	Small molecule	ERBB2	Breast cancer (HER2 Positive)	Rare occurrences of decreased LVEF (reversible)	[108, 109]
Imatinib (Novartis, 2001)	Small molecule	Bcr-Abl	Myeloid leukemia	Isolated instances of HF, mitochondrial corruption	[110, 111]
Sorafenib (Bayer, 2005)	Small molecule	VEGFR, KIT, RAF	Renal cell carcinoma, liver cancer	Acute coronary symptoms (MI), HF. Increased incidences after treatment with sunitinib	[42, 112]
Gefitinib (Astrazeneca, 2003)	Small molecule	EGFR	Lung cancer and other solid malignancies	No evidence of toxicity (one case of a patient with recurring MI, no link to gefitinib)	[113]
Erlotinib (OSIPharma, 2004)	Small molecule	EGFR	Non-small-cell lung cancer, pancreatic cancers	Evidence of cardiac damage when administered in rats	[114]
Nilotinib (Novartis, 2007)	Small molecule	Bcr-Abl	Myeloid leukemia	QT prolongation	[115, 116]

clinical trials is imperative to conserving costs and reducing the negative side effects of anti-cancer drugs on patients.

High Throughput Screening of Cardiotoxicity

High throughput screening is widely demanded by the pharmaceutical industry in order to perform expedited screening of large libraries of compounds while minimizing costs of experimentation. There are several approved drugs that began as part of high-throughput screening initiatives, many of which are the KI molecules we have discussed previously [43]. Unfortunately, preclinical high throughput screening in cardiovascular research is still difficult to perform on an industrially relevant level due to the difficulty of acquiring reliable cardiac cells and tissues in a cost-effective manner. Cardiomyocytes are inherently contractile cells, and few existing protocols measure cardiomyocyte function (i.e. contractility) reliably. As such, researchers need to maximize the amount of information generated from each test performed and minimize the amount of resources consumed.

When assessing the potential cardiotoxicity of a new chemical entity, there are certain parameters one must evaluate. Initial viability testing must be performed to eliminate compounds that are inherently cytotoxic. Following confirmation that the compound is not cytotoxic, we must assess its effects on both cardiomyocyte electrophysiology and contractility. As a safety measurement for cardiotoxicity, the FDA requires researchers to evaluate whether a new drug candidate may inhibit the cardiac human ether-a-go-go (hERG) channel, an ion channel that is primarily responsible for the electrical activity of the heart. Prolongation of the QT interval of the

repolarization event in the heart's electrical cycle is indicative of alterations to the hERG channel, and can be viewed as an indicator of early cardiotoxicity [44].

Currently, immortalized animal or tumor-based cell lines are used as screening tools for cardiotoxicity. Chinese hamster ovary (CHO) and human embryonic kidney (HEK) cell lines have been engineered to express the hERG channel to assess cardiotoxicity, and meet FDA requirements [45]. In addition, neonatal rat cardiomyocytes, and other animal models, can be cost-effective alternatives to engineered cells. But, as Lu et al. has demonstrated, there is significant interspecies variation in the ion channel currents, which contribute to the repolarization of cardiomyocytes, and thus the sensitivity of cells to various drugs will be different [46]. Primary cardiomyocytes from human donors should provide a more accurate means of assessing the effects of cardiotoxic compounds. Complications such as low donor availability, problematic isolation techniques, and poor viability after isolation are common factors that limit their usage as a screening tool. hiPSC-CM and human embryonic stem cell-derived cardiomyocytes (hESC-CM) are exciting alternatives to donor and animal-based cardiomyocytes, as they display many of the characteristics of normal *in vivo* cardiomyocytes [47]. In addition to the ability to derive functionally relevant and viable cardiomyocytes from hiPSC, Liang et al. also demonstrated the ability to derive a library of hiPSC-CM from patients suffering hereditary cardiac disorders, such as long QT syndrome (LQT), familial hypertrophic cardiomyopathy (HCM), and familial dilated cardiomyopathy (DCM) [48]. With these disease models, Liang and colleagues demonstrated that patient-derived hiPSC-CM detect susceptibility to cardiotoxic compounds more accurately than standard healthy hiPSC-CM controls [48].

Assessment of Cardiac Function Using Monolayers of Cardiomyocytes

To meet the pharmaceutical industry's requirement for highthroughput and cost effective screening on cardiomyocytes, monolayer culture of cardiac cells is used for assessing cardiac function. One significant aspect of evaluating cardiac function is measuring changes to electrophysiological properties. Ion channels are involved in a variety of fundamental physiological processes, and their malfunction may cause a plethora of human diseases [49–51]. In general, ion channel activity can be measured using ligand binding assays, flux-based assays, fluorescent-based assays, voltage-sensitive dyes and ion specific assays, but each method has its shortcomings [52]. Traditionally, the gold standard for studying ion channels and cardiomyocyte electrophysiology is to use patch-clamping techniques (Fig. 1). However, conventional patch clamp techniques using glass micropipettes are labor intensive, and the chance of cellular fluid mixing with the solution inside the recording electrode may result in the loss of some key biological activities due to the dilution of the intracellular fluid. It is, therefore, still difficult to assess changes to cardiomyocyte electrophysiology in an automated, high throughput fashion.

Another means of examining extracellular field potential of cardiac cells is using multi-well microelectrode array (MEA) systems whereby cardiomyocytes are seeded directly onto an integrated micro electrode substrate (Fig. 1). This system provides a simple and cost effective means of measuring impulse propagation in cardiomyocytes, non-invasively. Braam et al., Caspi et al., and Harris et al. have all successfully demonstrated using MEA methods that dose-dependent QT-prolongation can be detected in cardiomyocytes using hERG channel blocking compounds [53–55]. Natarajan et al. improved on the MEA-based action potential (AP) platform by patterning cardiomyocyte monolayers onto the MEA surface for guided AP propagation [56]. The advantages of this system are its multi-well and automated capabilities, leading MEA to be a medium throughput alternative to the traditional patch-clamp method of measuring cardiomyocyte electrophysiology [55].

An aspect of cardiac function that has yet to be addressed on monolayer culture is the measurement of physical contraction of cardiomyocytes. In the early 1990s, Spurgeon et al. have demonstrated simultaneous measurement of contraction and calcium ($[Ca^{2+}]$) flux in single cell cardiomyocytes. They were able to validate the use of calcium flux measurement as a surrogate measurement of contraction in a cardiomyocyte monolayer [57]. With this knowledge of measuring calcium flux across the cardiomyocyte membrane, various groups have developed high throughput screening techniques to classify a vast library of compounds quickly using cardiomyocyte monolayers in a multi-well setup with non-invasive measurement techniques and readily available equipment, such as the

FLIPR Tetra plate reader. Sirenko et al. have demonstrated the use of fast kinetic fluorescent imaging of intracellular $[Ca^{2+}]$ to classify whether the compounds are cardiotoxic or cardiosafe [58]. Poynton et al. demonstrated the relationship between fast kinetic fluorescent imaging of intracellular $[Ca^{2+}]$ and cardiomyocyte contractility to observe both positive and negative chronotropic effects, and to determine IC_{50} values of various compounds [59]. The advantage of calcium flux measurement to measure contraction is that it is a non-invasive measurement technique and allows researchers to subsequently measure cell viability by a variety of means, such as measurement of ATP content or cell imaging [59, 60].

Although understanding the change in electrophysiological responses of cardiomyocytes induced by drug candidates is important in determining cardiotoxicity, we must also understand the effects of drugs on cardiomyocyte contractility, as changes in contraction can be a better indicator for ventricular dysfunction. However, a challenge arises when trying to measure the contractile force of cardiomyocyte monolayer, as monolayers are typically cultured on hard and inelastic polystyrene dishes. Park and colleagues have tried to navigate this problem by incorporating a flexible polymeric microcantilever substrate and culturing cardiomyocytes around it. They demonstrated that this polymeric substrate can be used to measure contractile forces in cardiomyocyte monolayers [61]. Similarly, Grosberg et al. developed a platform they termed “heart-on-a-chip”, whereby cardiomyocytes could be seeded onto polymeric thin films to measure contractility and stained with voltage sensitive dye to measure action potential propagation [62]. Although these platforms have combined the ability to measure contractility and electrophysiology, we still encounter the issue of lack of high throughput capability due to the difficulty of manufacturing the platforms and their inability to screen multiple compounds simultaneously.

However, recurring limitations of these monolayer studies are the immature nature of hiPSC-CM and hESC-CM compared to the adult and even fetal primary cardiomyocytes [63], as well as the lack of similarities between monolayer cells and 3D native tissues. To better mimic native adult myocardial physiology, we need to turn to 3D engineered heart tissue constructs that are physiologically relevant.

Assessment of Cardiac Function Using Engineered 3D Cardiac Tissues

It is widely accepted that 3D tissue cultures have greater physiological relevance when compared to 2D monolayer cultures, in such areas as gene and protein expression, cell-cell signaling pathways, and cell-ECM interactions [64, 65]. Engineered heart tissue constructs have been developed to address many facets of the treatment of CVD, such as cell-based cardiac

repair and predictive toxicology on cardiac tissues. They have an advantage over monolayer 2D cultures, as they are a better representation of physiological conditions. Several platforms already exist to test the effects of drugs on cardiac tissues *in vitro*.

A variety of techniques have been reported as effective means of measuring tissue contractility (Fig. 1). Many of the current methods of engineering 3D cardiac models involve the formation of cardiac tissue around supporting structures, such as cantilevers [66], micro-posts [67], or thin films [68, 69]. As the cardiac tissue moves the support structure on the platform, the structure displacement can be translated into contraction force and beating frequency using mathematical modeling or empirical relationships. For example, when cardiac tissue is grown around two flexible silicone posts, the force of contraction can be calculated using the beam bending theory from the deflection of the posts, the elastic modulus of the post material, and the dimensions of the post itself. Stiffness and elastic moduli of these cantilevers can be varied to alter cardiomyocyte contraction [70]. Post-based platforms have already been used by several groups as a model of cardiac tissue contraction for drug screening [70–73]. Despite their robustness, it is difficult to reproducibly measure the small forces that are generated by miniature contractile tissue in a high throughput manner due to the computer intensive nature of edge detection and its subjectivity [74].

Cardiomyocyte Source

One major limitation of current approaches irrespective of the platform comes from the source of cardiomyocytes. Certain groups have used rat cardiomyocytes as their cell source [70–72], and have used their tissues to test the effects of clinically available KIs, such as sunitinib [75]. Jacob et al. were able to detect a reduction in contractile force in their engineered rat heart tissues after 96 h of inhibitor exposure [75]. Since rat cardiomyocytes do not represent a high fidelity model of human tissues, due to the differences in electrophysiological properties, other groups have used hESC-CMs in their post platforms to measure contractility [73]. However, this raises another significant variability in existing cardiac models, due to the cell line-to-line and batch-to-batch variability. Immaturity of cells coming out of the directed differentiation protocols may also lead to inaccurate results in determining the toxic doses of the test compounds.

It has been well documented that the application of electrical stimulation during cultivation of cardiomyocytes and cardiac tissues is essential for cell maturation [76]. Nunes et al. have developed a platform termed Biowire™, to engineer mature 3D heart tissues through electrical stimulation of hESC-derived cardiomyocytes during culture [11]. More recently, Zhao and colleagues have developed a modified

Biowire platform that is able to analyze contractile force [77]. This platform enables relatively easy analysis of contractile force through the use of fluorescent microscopy without the need for additional cell-monitoring assays [77].

Emerging Approaches

However, once the 3D cardiac tissue has been engineered, *in vitro* assessment of the aforementioned properties without affecting their performance remains challenging. For example, it is a common practice to immobilize cardiac tissues using such compounds as blebbistatin during assessment of electrophysiology to eliminate movement artifacts [10, 78, 79]. Other groups have measured tissue contractility by restricting movement of one end of the cardiac tissue and assessed force of contraction using an optical force transducer [80]. These analysis techniques introduce unnatural factors to the tissue, which can have negative implications on tissue function.

Hence, it is of utmost importance to develop the “next-generation” platforms that include in-line sensors to monitor overall tissue performance in real-time while maintaining its highthroughput capability. Extensive effort has been invested into developing new techniques to integrate robust sensory systems in heart-on-a-chip platforms to improve electrophysiological characterization of cardiac tissues. One of the promising methods to achieve this goal is to couple nanoelectronics with tissue constructs. Tian et al. and Feiner et al. have demonstrated the concept of integrating nanoelectronic biomaterials and their capability to monitor extracellular electrical potential within their cardiomyocyte constructs to known drugs [81, 82]. Additionally, recent advances in developing printable conductive elastomers and biocompatible soft materials enable us to integrate electronic sensors while fabricating complex 3D functional living tissues using 3D printers [83, 84]. Lind et al. have developed a fully 3D printed heart-on-a-chip platform integrated with soft strain gauge sensors to provide non-invasive readouts of tissue contractile stress and have applied these devices to study drug responses [85]. This 3D printed heart-on-a-chip can be quickly fabricated in a desired form, allowing researchers to do rapid prototyping and to collect reliable data.

In addition to directly integrating electrodes into a platform, optogenetics is gaining ground as an exciting technology for production of high-throughput drug testing models. By transfecting cardiomyocytes with an optogenetic stimulator (actuator) such as channelrhodopsin to express the light-sensitive ion channel, tissue pacing can be achieved by applying short pulses of light. Utilizing an optogenetic sensor such as voltage-sensitive fluorescence protein 2.3 (VSFP 2.3), cardiomyocyte activities such as action potential or calcium transient generation can be monitored in a spatiotemporal-specific manner [86]. Kilmas et al. have since developed OptoDyCE, an

automated all-optical system, to experiment and track the drug dose dependent effect of nifedipine and dofetilide on cardiac electrophysiology [87].

The Need for a Multi-Organ Platform Device

It is clear and intuitive that within the human body, organ systems rely on each other. Different cell and tissue types are co-dependent on each other for their proper growth and development. In the immediate vicinity, different cell types rely on one another for the exchange of secreted growth factors and signaling molecules, for cell-ECM interaction, and for direct cell-cell contact [88]. Even in single-tissue on-a-chip models, cell co-cultures are the key in achieving high-fidelity tissue function. For instance, human cardiac tissue constructs grown with the support of endothelial cells showed enhanced cardiomyocyte proliferation [89]. Co-culture of cardiomyocytes with cardiac fibroblasts yielded optimized tissue remodeling and enhanced structural and functional properties [90] of cardiac microtissues.

In the context of drug testing, it is important to consider that a compound is never delivered in isolation to the target tissue. The drug compound is metabolized and passes through several organ systems before it or its metabolites may execute its intended action. Drug interactions within the cardiovascular system are especially complex, as they include effects on the heart, blood vessels, and blood constituents, as well as the roles of the nervous and renal systems on moderating cardiovascular function [91]. Thus, in spite of major advances achieved from aforementioned cardiac drug testing platforms, *in vitro* model cardiotoxicity results do not always correlate with clinical risks [92]. Structural aspects, organ-organ interactions, cell mobilization, cellular composition, signaling features and metabolic gradients, as well as dynamic forces from blood circulation, are difficult to recapitulate in a platform with only one tissue type [93].

To achieve the most accurate drug screening models, it is becoming apparent that the integration of multiple organ types into a single platform is vital. A few early platforms have been developed with the aim of integrating complex tissues into a platform for predictive drug testing applications. For example, HeLiVa integrates heart, liver, and vascular tissues using an interlocking culture chamber approach [93]. A four-organ-chip was also recently developed with pulsatile flow maintained between intestine, liver, skin and kidney compartments, where each compartment had a unique setup [94]. Another creative approach draws inspiration from Lego blocks, where different organs are cultured on standard microfluidic modules, which may then be built into various patterns [95, 96]. Several platforms have also been developed that mimic the ingestion of a compound by utilizing a model gastrointestinal

tract compartment in line with other tissue type compartments downstream [97–100].

A great deal of work has been done in the quest to create integrated tissue models. However, the future of integrated multi-organ platforms will need to establish optimized maturation protocols, suitable universal culture media, and functional readout capability, which could be especially difficult with complex tissues that have different functional purposes [101–104]. For instance, cardiac tissue requires force of contraction measurements [105], while liver tissue functionality is assessed by enzyme kinetics [106]—two parameters that require very different engineering approaches.

Conclusions and Future Studies

Fast and effective drug discovery primarily depends on multiple levels of qualitative and quantitative results: from simple “yes” or “no” characterization to complex analysis. Numerous innovative organ-on-a-chip platforms have opened new avenues for *in vitro* tissue engineering as they recapitulate mechanics and physiological responses of tissues in the 3D manner [11, 71]. Through the measurement of changes in force of contraction, ion flux and electrical activities, drug toxicity to the cardiac tissue can be assessed accurately. However, it becomes more arduous to carry out high-resolution imaging and analysis with these platforms.

Therefore, despite these recent groundbreaking innovations, several central requirements remain to be addressed. First, multiple parameter readouts place a heavy burden on sample analysis, as drug screening typically requires detecting cellular responses and monitoring the quality of the micro-environment of many different chemical combinations. Fabrication and testing in organ-on-a-chip platforms is costly, and if their improvement in accuracy cannot justify their added expenses over the pre-existing 2D platforms and animal models, they are unlikely to obtain wide acceptance from pharmaceutical industry. In addition, extensive engineering efforts have been applied to develop novel microdevices to analyze tissue integrity and extracellular metabolites, but recapitulating its readouts to meet their clinical counterpart’s demand is still challenging due to the major differences in measurement techniques. Overall, organ-on-a-chip devices will have a pivotal role in drug discovery in the future, but much work remains to be done, such as improving the capability for high-throughput analysis, ability to generate high quality data, and manufacturability (Fig. 2).

Current research goals in the drug screening industry are to develop cost effective, miniaturized, automated, parallelized, and non-invasive microsystems that enable high resolution imaging and data acquisition without hindering tissue function or causing tissue damage for acute and chronic studies. It is imminent that 3D printing will replace conventional soft

lithography techniques as it allows the production of 3D structures with complex geometries, and may be applied in both rapid prototyping and manufacturing. Preclinical analysis of drugs, using cost effective high fidelity models of cardiac function have the potential to greatly reduce late stage drug attrition, and save money in the overall drug development process. Researchers have already begun to explore and identify alternative low cost materials and fabrication techniques that will unlock the potential of high-fidelity cardiac tissue models as accessible, mass-produced tools in the healthcare industry.

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Compliance with Ethical Standards

Conflict of Interest M.R holds equity in TARA Biosystems Inc.

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