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Review

Heart-on-Chip for Combined Cellular Dynamics Measurements and Computational Modeling Towards Clinical Applications

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Abstract—Organ-on-chip or micro-engineered three-dimensional cellular or tissue models are increasingly implemented in the study of cardiovascular pathophysiology as alternatives to traditional *in vitro* cell culture. Drug induced cardiotoxicity is a key issue in drug development pipelines, but the current *in vitro* and *in vivo* studies suffer from interspecies differences, high costs, and lack of reliability and accuracy in predicting cardiotoxicity. Microfluidic heart-on-chip devices can impose a paradigm shift to the current tools. They can not only recapitulate cardiac tissue level functionality and the communication between cells and extracellular matrices but also allow higher throughput studies conducive to drug screening especially with their added functionalities or sensors that extract disease-specific phenotypic, genotypic, and electrophysiological information in real-time. Such electrical and mechanical components can tailor the electrophysiology and mechanobiology of the experiment to better mimic the *in vivo* condition as well. Recent advancements and challenges are reviewed in the fabrication, functionalization and sensor assisted mechanical and electrophysiological measurements, numerical and computational modeling of cardiomyocytes' behavior, and the clinical applications in drug screening and disease modeling. This review concludes with the current challenges and perspectives on the future of such organ-on-chip platforms.

Keywords—Microfluidics, Heart-on-chip, Computational modeling, Drug screening, Cardiovascular disease modeling.

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INTRODUCTION

Known as the "engine of life", the heart pumps blood throughout the body to transport nutrients and wastes from other organs, a critical function of the human body to maintain normal physiological activities. Although cardiovascular diseases are the leading causes of death every year in the United States according to the Center for Disease Control and Prevention, cardiovascular research and regenerative studies have been significantly limited by the lack of relevant and effective *in vitro* models. The majority of phase I drug failures and post-approval withdrawal of medicinal products are attributed to cardiovascular toxicity. Almost half of the drugs in the pharmacology market since the 1990s have been retracted due to cardiovascular complications.[123] *In vitro* static cell culture models and animal studies have been long employed as standard methods to study and develop therapeutic strategies, but conventional 2D static cardiac cell models do not accurately recapitulate the heart physiology or microenvironment. The *in vivo* orthotopic animal models are ineffective in predicting human responses. In addition, the lack of temporal resolution and sensitivity in animal models makes it difficult to comprehend the cellular interactions and physiological processes, such as how specific cells behave over time and interact locally with other cell types, or how specific cell or tissue components influence the progression of a specific disease. Hence, there is an urgent need for a novel and physiologically relevant *in vitro* model of the human heart.

The advent of human induced pluripotent stem cell derived cardiomyocytes and advances in cardiac tissue engineering have led to building miniature human heart tissues and organoids *in vitro*.[15,29,58] Such stem cell-based tissue engineering combined with advances in microfluidic technology have pushed forward the development of heart-on-chip platforms. Heart-on-chips, or largely speaking, organ-on-chips are mainly microfluidic cell culture devices created with microchip manufacturing methods. Organ-on-chip systems can mimic the biomechanical and biochemical microenvironment of cells and tissues, and the interactions between the microenvironment and cells. They also recapitulate the vascular perfusion of the organ and produce tissue and organ functionality not possible with conventional *in vitro* 2D culture systems. Organ-on-chip platforms have been receiving promising attention for their applications in drug screening, drug delivery, and tissue engineering. They can be used as an alternative *in vitro* platform to provide cell-, tissue-, and organ level characterization, as well as address patient-to-patient variations of drug response. In particular, the human heart-on-chip has great potential to transform many avenues of basic research and drug development. It can address questions of how the microenvironment regulates cell and tissue development and disease progression, and how different molecular factors and immune cells contribute to toxicity, inflammation, and infection. Once combined with patient-specific primary or induced pluripotent stem cells, these chips can provide personalized solutions for patients and revolutionize drug screening platforms.

This review starts by describing the human heart biology and physiology that are relevant to designing a heart-on-chip platform. Then we will discuss heart-on-chip platforms with respect to their fabrication, functionalization, computational modeling, and applications in drug screening and disease modeling (Fig. 1).

BIOLOGICAL BACKGROUND OF THE HEART

The human heart is a specialized four-chamber muscular organ that rhythmically coordinates efficient forward flow of blood from the low-pressure venous side to the high-pressure arterial side of the pulmonary and systemic circulation systems. The heart does this *via* dynamically coordinated synchronous electrical activation of the muscle tissues that comprise each of the chambers. During human cardiogenesis, various cell types differentiate during the maturation process, and they populate and develop within different parts of the heart. The myocardium, primarily composed of cardiomyocytes, directly generates the mechanical force necessary for moving blood. Cardiomyocytes are connected by desmosomes, adherens junctions, and gap junctions, and these also combine to comprise intercalated disc structures at the longitudinal ends of cardiomyocytes, which connect those cells to form myocardial fibers. The myocardial fibers transmit electrical impulses between cells primarily along the longitudinal axis of the fibers to generate concerted contractile activity.[140] During cardiogenesis, in addition to the development of atrial and ventricular cardiomyocytes, specialized nodal cells and His-Purkinje fibers develop, which facilitate coordinated electrical activation of spatially organized syncytial groupings of myocardial fibers. The spatiotemporal interaction of these different cell types enables electromechanical coupling that results in efficiently coordinated myocardial contraction. Approximately half of the entire cell population of the heart is comprised of non-muscular cardiac fibroblasts, which serve to maintain the structural and mechanical integrity of the heart, particularly through their role in maintaining the extracellular matrix (ECM) of the heart wall.[116] Another type of differentiated non-muscular heart cells are the endothelial cells that populate the cardiac endocardium, cardiac valves, pericardium, and coronary vasculature. Differentiated cells comprising the epicardial adipose tissue and cells of the immune system, such as macrophages and mast cells, also have a role in maintaining the ECM, and impact cardiac function in both health and disease. For instance, the epicardial and pericardial adipose tissue have a connection between type 2 diabetes and cardiovascular disease, and that these adipose tissues play a critical role in accelerating heart failure.[26]

There are three core components to the heart's electrical system: the sinoatrial node (SA node), the atrioventricular node (AV node) and the His-Purkinje system.[17] The electrical impulses that initiate coordinated myocardial mechanical action are generated in nodal tissue, with the SA node in the right atrial wall normally having the fastest rate of spontaneous depolarization, which thereby allows it to function as the rate-determining 'pacemaker' of the heart. The electrical impulse from the SA node rapidly expands and propagates across atrial tissue syncytia, which then results in the coordinated contraction of all atrial cardiomyocytes within 120 ms in healthy young human adults. Electrical activation of the AV node introduces a dynamic delay in electrical propagation between the atria and ventricles, and primarily is modulated by the underlying heart rate and autonomic neuroendocrine inputs. Conduction delay during propagation through the AV node helps to optimize ventricular filling in response to atrial contraction, since simultaneous atrial and ventricular contraction would both (1) shorten

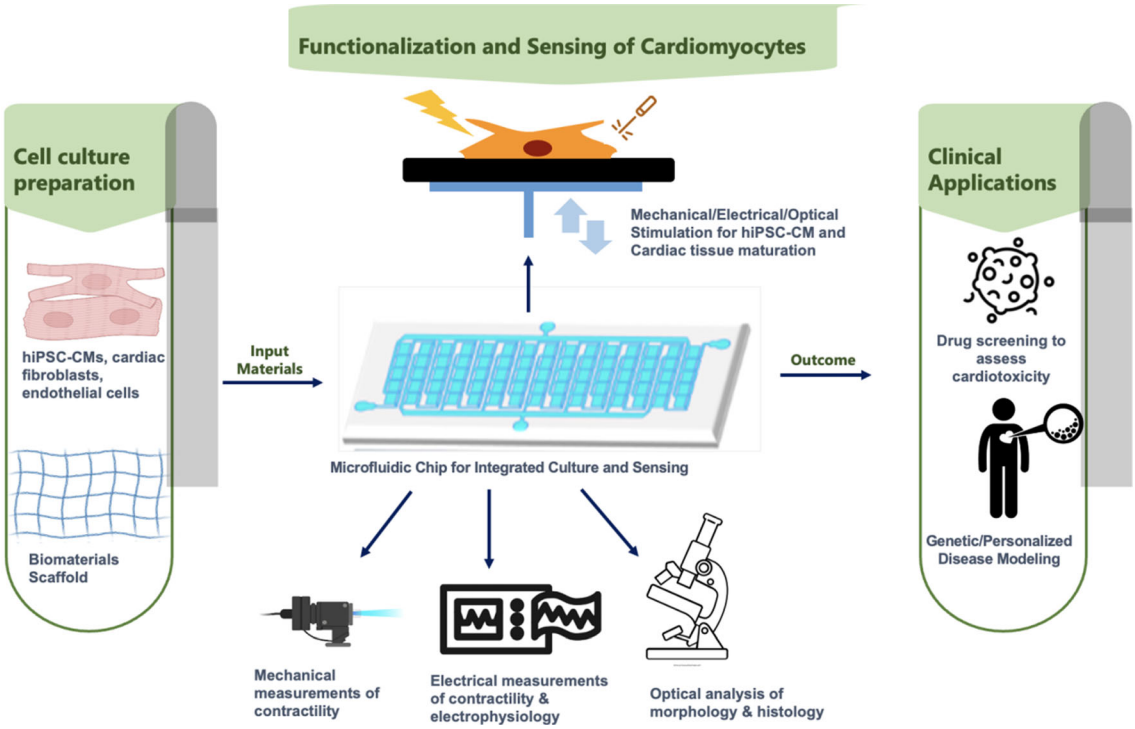

FIGURE 1. Design and fabrication of heart-on-chip platforms driven by clinical vision in cardiotoxicity screening assays and personalized or rare genetic disease modeling. Human induced stem cell derived cardiomyocytes (hiPSC-CMs), cardiac fibroblast and/or endothelial cells that constitute the cardiac tissue can be cultured in the microfluidic chip. Biomaterials or polymer scaffolds assist the cell culture for enhanced cell adhesion and cell to cell interactions. Various modes of stimulation techniques help with functionalization and maturation of cardiac cells and tissues. In these microfluidic chips that enable continuous culture media perfusion and precise alignment of cells, various sensing modes are applied to measure the mechanical or electrophysiological properties of cardiomyocytes. This integrated culture and sensing ability can be readily applied in clinical settings.

ventricular filling time, reducing subsequent ventricular output per heart cycle, and (2) waste mechanical energy due to early obliteration of the atrial to ventricle pressure gradient that enables forward flow from atrium to ventricle during atrial contraction. Once the electrical impulse passes through the AV node, the reach of the His-Purkinje system extends into the ventricles, broadly branching out across the endocardial surfaces to enable rapid propagation into all ventricular myocardial tissue. The His-Purkinje tissue is uniquely differentiated cardiac tissue that serves to amplify the spatial footprint of the electrical wavefront initiated by the relatively focal impulse transmitted out from the AV node, and it then substantially accelerates propagation of that impulse to achieve activation of all ventricular myocardial fibers within 80 ms in a healthy young adult.

The heart wall is composed of three layers as shown in the right image of Fig. 2. The heart is situated on the pericardium, which is composed of the parietal and the visceral layer. The visceral pericardium is also known as the epicardium, the outermost and thin membrane of the heart wall. Then the myocardium consists of cardiomyocytes, the extracellular matrix, and capillaries. The extracellular matrix provides a viscoelastic scaffold for cardiomyocytes as they are made up of type I and type III collagen and fibroblasts. The endocardium is the innermost layer of the heart that lines the chambers. It plays a complex role in heart development, which involves the development of Purkinje fibers, cardiac valves, *etc.*

PLATFORM DESIGN AND FABRICATION

Biomaterials

Heart-on-chips are employed for uniform cell capture, culture, alignment, maturation, and functionalization. To do so, heart-on-chips focus on reconstructing the blood vessel network, structurally organizing and aligning cardiomyocytes, and facilitating cell-to-cell and cell-to-microenvironment interactions. The combination of microfluidic techniques and biomaterials allows for the mimicry of native extracellular environment with physiochemical aspects, thereby facilitating the investigation of biological phenomena with a higher level of complexity and

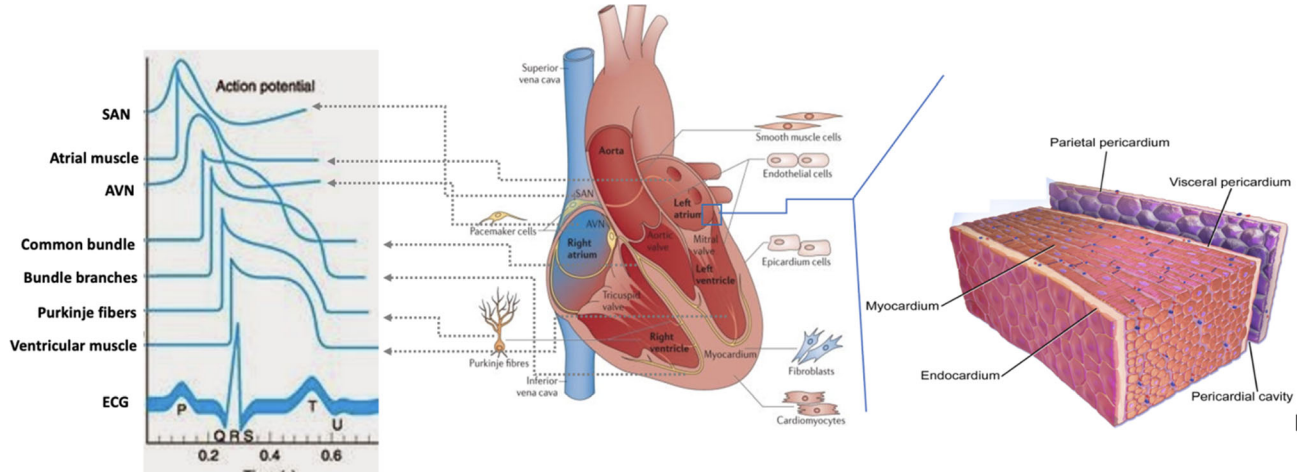

FIGURE 2. Schematics showing the anatomy and cell populations of a mature heart, and a histological representation of the heart wall. Reprinted with permission from Blausen.com staff,[14] Jalife,[17] and Xin *et al.*[140]

physiological relevance. Choosing the appropriate biomaterial for heart-on-chip systems is the major priority when it comes to the fabrication process. Biomaterials in microfluidic systems serve mainly two functions: substrate/scaffold and surface coating. Table 1 summarizes the variety of biomaterials currently applied in heart-on-chip platforms.

The substrate serves as the main body of heart-on-chips. Ideal substrate materials exhibit characteristics including mechanical robustness, biocompatibility, and ease of fabrication. Polydimethylsiloxane (PDMS) is the most popular polymer for microfluidic devices. Ease of fabrication *via* soft lithography and low cost make PDMS an attractive choice for organ-on chips. The surface of PDMS is biocompatible and gas permeable, which plays a vital role in the long-term culture of cells in microfluidic systems. The flexibility of PDMS is another attractive trait that enables facile integration of mechanical stimuli on chip. Due to these traits, PDMS has been broadly employed in microfluidic devices for cell culture and drug screening.[41] Poly(methyl methacrylate) (PMMA) is another commonly used polymer notable for its low cost and method of hot embossing for fabrication.[22] It is also an ecofriendly material for its ability to be decomposed and reused. In contrast to PDMS, PMMA is rigid and impermeable, which allows for a different microenvironment. Therefore, PMMA is often combined with PDMS to form a hybrid chip to generate complex environments and multiple purposes.[121]

Polymer based microfluidic substrates, however, limit the cells' potential to form thick tissues. Thus, the surface of microfluidic substrates is modified with hydrogels to provide a biological microenvironment for cell culture. Photo-crosslinkable hydrogels can be crosslinked under exposure to ultra-violet light within a few seconds. Due to this fast and convenient solidification process, photo-crosslinkable hydrogels offer an easy fabrication solution for coating substrates.[25,32,73,120] Alginate is a commonly used hydrogel that is solidified through ionic-crosslinking.[118] Recent development in 3D bioprinting has led to the generation of bioinks from cell-seeded hydrogels and printing of out-of-plane scaffolds for 3D culture of cells. Bioprinting can be particularly advantageous for its precise control over the geometry and spatial distribution of cells, high reproducibility, and the possibility for customized geometries. However, higher resolution, deposition speed, proper storage of cells inside bioinks, and the tradeoff between high precision and high shear stress on cells with smaller nozzles are some of the challenges that still remain to be optimized.

Fabrication Methods

Soft lithography is the most broadly used fabrication method for microfluidics organ-on-chip devices due to their low cost, high throughput and microscale resolution. In this process, computer aided design (CAD) software is used to create a film mask, which is used to pattern a silicon wafer that serves as the mold for a pre-crosslinked elastomer. The patterning of a silicon wafer is done *via* photolithographic etching. The patterned silicon wafer is then etched to form the microfluidic channels and microstructures. A liquid polymer, such as polydimethylsiloxane (PDMS), is poured on the etched silicon substrate and allowed to polymerize into an optically clear and elastic material. Optically transparent PDMS chips offer real-time, high resolution optical imaging of cellular responses. Organ-on-chips are also fabricated out of different

TABLE 1. Biomaterials used in heart-on-chip platforms.

Material	Function	Properties	Fabrication/crosslink	References
Polydimethylsiloxane (PDMS)	Template, substrate	Flexible, permeable	Photolithography, soft lithography	41
Polymethylmethacrylate (PMMA)	Template, substrate	Rigid, impermeable	Hot embossing, room temperature imprinting	22, 121
Poly(ethylene glycol) (PEG)	Scaffold, coating	Low permeability, biocompatible, non-cell adhesive	Photolithography, Photo-crosslink	32, 73
Gelatin methacrylate (GelMA)	Scaffold, coating	Biocompatible, cell-adhesive	Photo-crosslink	120
Methacrylated hyaluronic acid (MeHA)	Scaffold, coating	Biocompatible, cell-adhesive, tumor targeting	Photo-crosslink	25
Alginate gel	Scaffold, coating	Biocompatible, high swelling rate	Ionic-crosslink	118

materials, including silicon, plastic, glass, and silk using micro-molding, laser etching, injection molding, photopolymerization, and other microscale manufacturing methods. Soft lithography based microfluidic devices are easy to incorporate diverse micropatterns for cell alignment and culture. Deutsch *et al.* utilized soft lithography to fabricate films with topological texture of microgrooves for the culture of cardiomyocytes.[33] Their study showed that cardiomyocytes tend to be directed by the microgrooves and therefore improve their expression. Similar approaches of using microgroove or microposts have been used for the enhanced alignment of various cardiac cells.

The common workflow of heart-on-chips goes by capturing cardiomyocytes in a uniform manner, culturing them *via* continuous media perfusion, and using integrated sensors or stimuli to functionalize the cardiac cells and analyze their response. A design conducive to effective cell capture is reported by Plouffe *et al.*[106] In this microfluidic chip, the peptide-functionalized alginate gels coated on microfluidic channels enabled controlled capture and release of cardiac fibroblasts. Kim *et al.* designed surface treated microwells with polyethylene glycol (PEG) that foster a spontaneous and synchronized beating environment for cardiomyocytes.[69] This system particularly highlights the importance of surface topography in inducing cell growth and maturation. In similar studies, Beussman *et al.* introduced ECM proteins to functionalize the microarrays, which helped measure the contractility of human iPSC-CMs.[13] Others modified the surface of microposts or pillars to achieve enhanced signals or better control on cell alignment.[68,69,101,114]

While soft lithography allows for diverse 2D microfluidic designs, the technology of 3D printing offers an alternative for organ-on-chips with complex out-of-plane microstructures. 3D printing technique was first invented by Charles Hull in 1986 and became broadly employed in tissue engineering and regenerative medicine.[132] In contrast to soft lithography which usually involves multiple steps in the fabrication process, 3D printing is much more flexible to handle. Microfluidic devices can be built in one step or in multiple steps *via* 3D printing.[132] Digital data input for printing can come from scanning an existing object or designed from CAD programs. After printing, devices undergo treatment such as cleaning and surface modification.

Due to the simple manufacturing process, 3D printing can readily incorporate biomaterials to microfluidic devices. In contrast to the laborious process of creating elaborate designs in soft lithography, 3D printing allows direct, one-step production of scaffolds embedded with cells and growth factors.[89,91,97] Bertassoni *et al.* bioprinted 3D microchannel networks out of photocrosslinkable hydrogel with various architecture features that support vascularization.[12] Dvir *et al.* 3D printed vascularized, perfusable, patient-specific, and immune-compatible heart patches from patient's own cells and biological materials. They successfully printed an entire heart replete with cells, blood vessels, ventricles, and chambers, demonstrating the importance of using "native" patient specific material in successfully engineering tissues and organs.[35]

3D printing also enables facile fabrication of instrumented heart-on-a-chip systems. The Parker group utilized 3D printing for the fabrication of cardiac microphysical devices embedded with soft strain gauge sensors.[80] The integrated device provides non-invasive electronic readouts of contractile stress and simplifies data acquisition for long term functional studies.

A challenge for generating a functional cardiac tissue is to form the interaction between different types of cardiac cells, which requires specific biomaterials to promote the hetero-cellular coupling at their inter-

faces. 3D bioprinting facilitates the manipulation of bio ink and cell solutions layer by layer, and thus is an ideal approach to promote the hetero-cellular crosstalk between different cardiac cells and recapitulate functional cardiac structures.[6] Cardiac 3D bioprinting offers promising opportunities to recreate the histological architecture of the native heart, but with considerable challenges. Current bioprinting materials, mostly hydrogels, are too soft to withstand physiologic pressures. However, simply increasing the stiffness can compromise the viability of cells. Since heart tissues are thick and complex, they also require adequate vascularization and enervation to allow for functionality and biocompatibility. Establishing an intrinsic vasculature with clinically relevant thickness and stiffness that can appropriately respond to electrical impulses and maintain a synchronized beating is a critical challenge to solve. In addition, incorporation of nano-electronic scaffolds in the bioink or biomaterial, such as carbon nanotubes in hydrogels, to provide electrical and mechanical stimulation can promote maturation of 3D printed hiPSC-CM tissues and significantly enhances their biomechanical behavior and survival.

FUNCTIONALIZATION AND MEASUREMENTS

An important criteria for the construction of *in vitro* cardiac models is the functionalization of cardiac tissues, which calls for not only an adequate number of anisotropically organized mature cardiac cells but also the incorporation of suitable stimulus for *in vivo*-like cell beating.[146] With the development of microfluidic technology, multiple stimuli can be incorporated to mature cells and functionalize tissues. Typically, mechanical, electrical and optical stimuli are applied to heart-on-chips to allow the complex mechanical and electrophysiological microenvironment of living tissues to be recapitulated *in vitro*. Most *in vitro* differentiated cardiomyocytes have fetal-stage phenotypes, featuring underdeveloped contractile performance, weak subcellular structural organization, and defective intracellular calcium ion uptake kinetics.[84] This maturation bottleneck severely limits the use of hiPSC-CMs in *in vitro* modeling for pharmacological or therapeutic applications. Several mechanical, electrical, and biochemical approaches have been developed to promote cardiomyocyte maturation.

Mechanical Stimulation

In each beating cycle, the myocardium withstands mechanical loads from its contracting and relaxing motion. The myocardium is composed of cardiomyocytes in a complex 3D assembly of endothelial and smooth muscle cells and collagen fibers in parallel. These cardiomyocytes contract synchronously with electrical stimulation, and the surrounding myocardial fibers are anisotropic both mechanically and electrically. Therefore, it is important to incorporate the complex mechanical cues to functionalize the *in vitro* cardiac models and accurately recapitulate the cardiac environment. To this end, a number of heart-on-a-chip platforms have been reported that stimulate the beating of 2D cardiomyocytes or 3D cardiac tissue *via* pneumatic actuation (Fig. 3). Ugolini *et al.* incorporated uniaxial cyclic strain to enhance functionality on human cardiac fibroblast monolayers.[128] They incorporated four stretching units in a single device, each actuated by a common pneumatic circuit and lower fluidic channels. 2 or 8% cyclic strain at 1Hz was applied to the culture chambers for 24 or 72 h, in addition to delivery of negative pressure through a standard laboratory vacuum line. Mechanical load dependent effects of cardiac fibroblast proliferation were observed, in which the 8% strain intensity induced higher myocardial contractility and proliferative behavior. Similarly, Giridharan *et al.* proposed a microfluidic cardiac cell culture model platform that mimics the *in vivo* pressure-volume changes in the left ventricle.[93] Chicken embryonic ventricular cardiomyocytes were cultured on a thin flexible PDMS membrane within a flow loop subjected to mechanical stimulation (13% stretch at a frequency of 2 Hz). This microfluidic system accelerated structural and functional maturation of chick embryonic ventricular CMs in comparison to those in static cultures, which addresses the importance of mechanical stimulation in maintaining intracellular calcium regulation and enhancing protein synthesis of embryonic chick CMs during embryogenesis. Mechanical stimulation is mostly applied on a two-dimensional plane, but the complex cell–cell and cell–matrix interactions are neglected in planar systems. For this reason, researchers try to seek strategies to apply mechanical stimulus in three dimensions. Marsano *et al.* presented a PDMS-based microfluidic device which reproduces the 3D *in vivo* mechanical environment of a native myocardium,[88] as shown in Fig. 3(i). With two separate compartments, the cell culture chamber and the actuation chamber, cardiac cells suspended in fibrin gel matrix generated micro tissues, and the bottom PDMS compartment compressed the 3D cell construct above. The openings between the posts in the bottom compartment allowed transduction of compression into a uniaxial strain. By controlling the cyclic strain directly on-chip, 3D cardiac constructs were generated along the proper anisotropic direction. The microfluidic device with 10% strain generated mature and highly

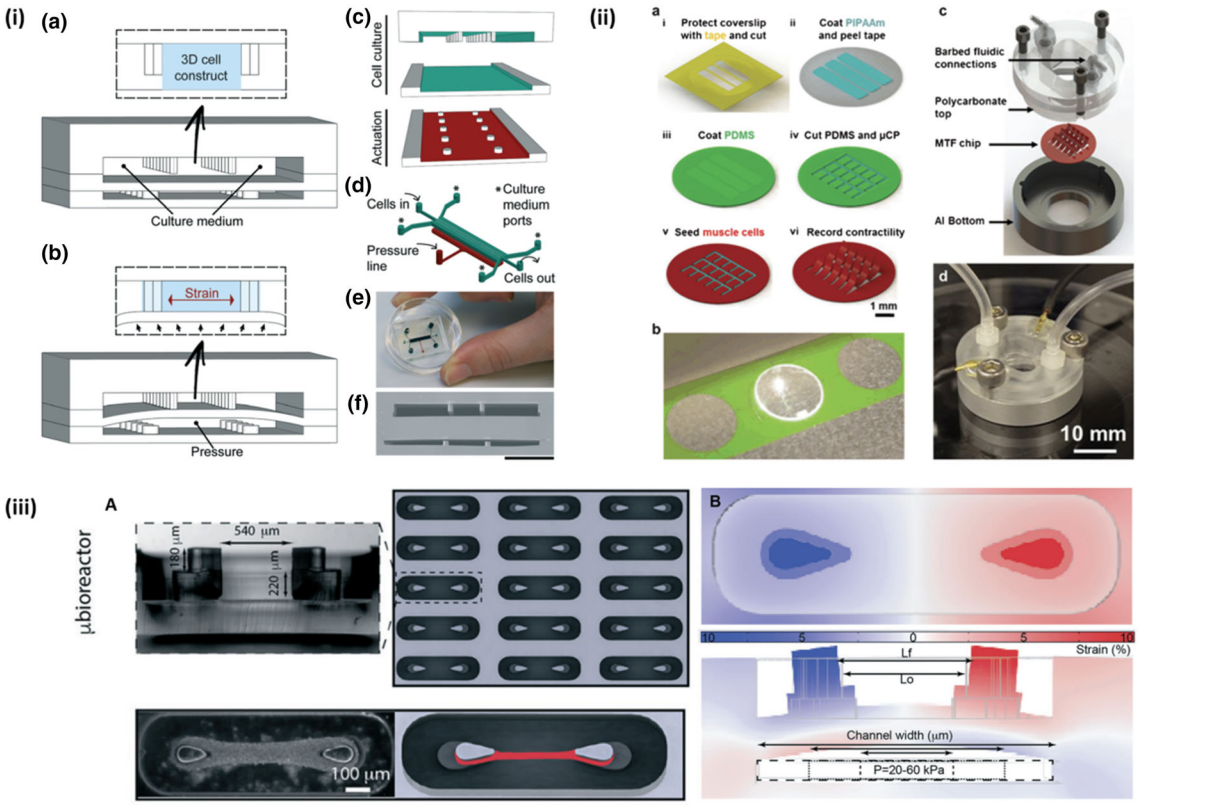

FIGURE 3. Illustration of mechanical stimulated heart-on-a-chip platforms. (i) The PDMS-based microfluidic platform to reproduce the mechanical environment of native myocardium, where functional 3D cardiac tissues can be generated. The microfabricated heart-like device uses two compartmentalized PDMS microchambers. By pressurizing the bottom compartment, the PDMS membrane deforms, generating a prominently uniaxial strain on the 3D cell construct. Reprinted with permission from Ref. 3. (ii) The MTF was fabricated through a semi-automated technique to engineer the cardiac microtissues, allows for the stress calculation. A one-channel microfluidic device embedded with electrodes was incorporated with the MTF for optical cardiac contractility measurements. Reprinted with permission from Ref. 88. (iii) The multiplayer platform consists of a top tissue culture layer and a bottom pneumatic actuated layer. The control layer is pressurized to deflect the culture layer and stretch the tissue. The effects of mechanical stress on cardiac hypertrophy was studied by real time on-chip analysis of the tissue phenotype. Reprinted with permission from Ref. 103.

functional cardiac tissues with spontaneous and synchronized cell beating and enhanced contractile capability.

Heart-on-a-chip platforms used for high throughput studies of cardiac mechanical physiology have been investigated in recent years. Agarwal *et al.* demonstrated a heart-on-a-chip that enables high throughput drug screening studies,[3] as shown in Fig. 3(ii). Submillimeter sized thin film cantilevers of soft elastomers termed muscular thin films (MTF) were embedded in the microfluidic chip to measure the stress generated by the engineered cardiac tissue. The fluidic microdevice was built from reusable, autoclavable materials. It consisted of a metallic base, transparent top for recording cantilever deformation, and electrodes for electric field stimulation of the tissue. An important application of the device was in isoproterenol dose response studies, which measured the contractile function of multiple tissues during pharmacological interventions. The closed chamber configuration of the device deemed especially advantageous in allowing complete flushing between incremental drug dosages. Parsa *et al.* also developed a pneumatic microfluidic platform for high-throughput drug screening studies of cardiac hypertrophy,[103] as shown in Fig. 3(iii). Their design was motivated by the need to reduce the number of cells per tissue and eliminate complicated setups of the previous cardiac tissue bioreactors. This microfluidic system consists of a cardiac tissue culture layer that contains arrays of microwells and pillars and a pneumatically actuated control layer. Cardiac microtissues were pneumatically loaded and coupled with real time on-chip analysis. By loading cardiac tissue in high density, the mechanical stress present in volume overload observed in cardiac hypertrophy was recapitulated and enabled study in a high throughput fashion.

Electrical Stimulation

Intracellular electrophysiology plays a significant role in the heart's function and thus, electrocardiosignals represent key physiological events of the heart.[31] A pair or an array of electrodes have been often used to electrically stimulate cardiomyocytes and to measure the intracellular electrical response.

The most common method of electrical stimulation uses a pair of electrodes to achieve a uniform electrical field between them. The resolution of the generated electrical field can be compromised when stimulation is applied to a bulk of cells. To address this limit, a microelectrode array (MEA) that consists of thousands of carbon or platinum microelectrodes was designed to generate thousands of localized electrical fields that can excite each individual cardiomyocyte. As shown in Fig. 4(ii), Zhang *et al.* proposed a microfluidic system embedded in an array of interdigitated–castellated microelectrodes.[142] The interdigitated–castellated microelectrodes served to introduce dielectrophoresis and electro-orientation in order to accumulate cardiomyocytes and form a tissue-like structure oriented along the AC electric field. A large orientation torque and force were achieved with appropriate frequency and low conductive medium. Basic structural and biophysical anisotropy of electro-oriented structure were validated with electromechanical experiments.

2D MEAs are broadly employed to provide electrical stimulation on a monolayer of cardiomyocytes to facilitate spontaneous and synchronized beating and functional maturation. 3D MEAs better suit the study of thicker cardiac tissues with substantial out-of-plane structures. Pavesi *et al.* demonstrated a facile method to embed 3D flexible electrodes within a microfluidic device[104] (Fig. 4(i)). This device fabricated *via* soft lithography consists of a single culture channel and sixteen vertical electrodes. The 3D electrodes were employed to produce uniform electrical field within the microfluidic channels that culture cells. In a similar study, Dvir *et al.* developed a 3D cardiac tissue culture platform that incorporated gold nanowires within alginate scaffolds[35] (Fig. 4(iii)). Electrically resistant alginate pore walls were bridged together to enhance electrical communication between adjacent cardiac cells. Cells were formed into 3D tissues that contract synchronously *via* electrical stimulation. Compared to those grown on pristine alginate, cardiac tissues grown on the 3D scaffold with gold nanowires are thicker and better aligned.

Electrodes are also used in heart-on-chips to monitor the physiological activity of cardiac tissues and extract quantitative data. Cheng *et al.* developed a microfluidic system integrated with five individually addressable microelectrodes that are used for field-stimulation or measurement of lactate secreted by cardiomyocytes.[23] *In-situ* microscopy can be interfaced with this microfluidic system to measure cell contractility, extracellular pH and intracellular Ca^{2+} levels in real time. Qian *et al.* reported a microfluidic platform that integrates two independent yet interpenetrating electrode arrays that simultaneously record cardiac tissue adhesion, electrophysiology, and contractility on the same chip[107] (Fig. 4(iv)). A microelectrode array (MEA) was used for field potential readouts and an interdigitated electrode array for impedance readouts. With the ability to provide real time and non-invasive data of both cardiac electrophysiology and contractility, this platform provides a quantitative and predictive assay system for detecting cardiotoxicity.

Optical Stimulation

Optical stimulation or optogenetic techniques provide novel means to interact with biological systems and induce excitation by genetically introducing light-gated ion channels and pumps. Optical stimulation is less damaging than electrical stimulation as the cell stimulated by light is not permeabilized under normal conditions and provides better resolution and spatial control over the stimulated region. Electrical stimulation can depolarize cells in the vicinity if the induced voltage amplitude exceeds the depolarization threshold, but optical stimulation can be performed specific to a certain cell.

Yakushenko *et al.* developed a micropixellated array of light emitting diodes with more than 4000 LEDs in an area of less than 11 m^2. This group cultured optogenetically modified cardiomyocyte-like cells on the chip and induced depolarization upon illumination with blue light from the micro-LED array.[141]

There are a number of groups that used genetically modified human iPSC-CM to conduct optogenetic pacing and measure robust extracellular field potential signals. Lapp *et al.* cultured genetically modified human iPSC-CMs that express Channelrhodopsin-2 on microelectrode arrays and applied global pulsed illumination at varying frequencies to evoke synchronized activation to gather robust field potential signals.[77] In principle, the optogenetic stimulation is well scalable for cardiotoxicity screening or developing personalized medicine for cardiac channelopathies.

Enhancing Maturation of Stem Cell Derived-Cardiomyocytes

Prolonged Culture Time

Cardiomyocytes *in vivo* take several years to fully mature,[131] so prolonged culture time can promote

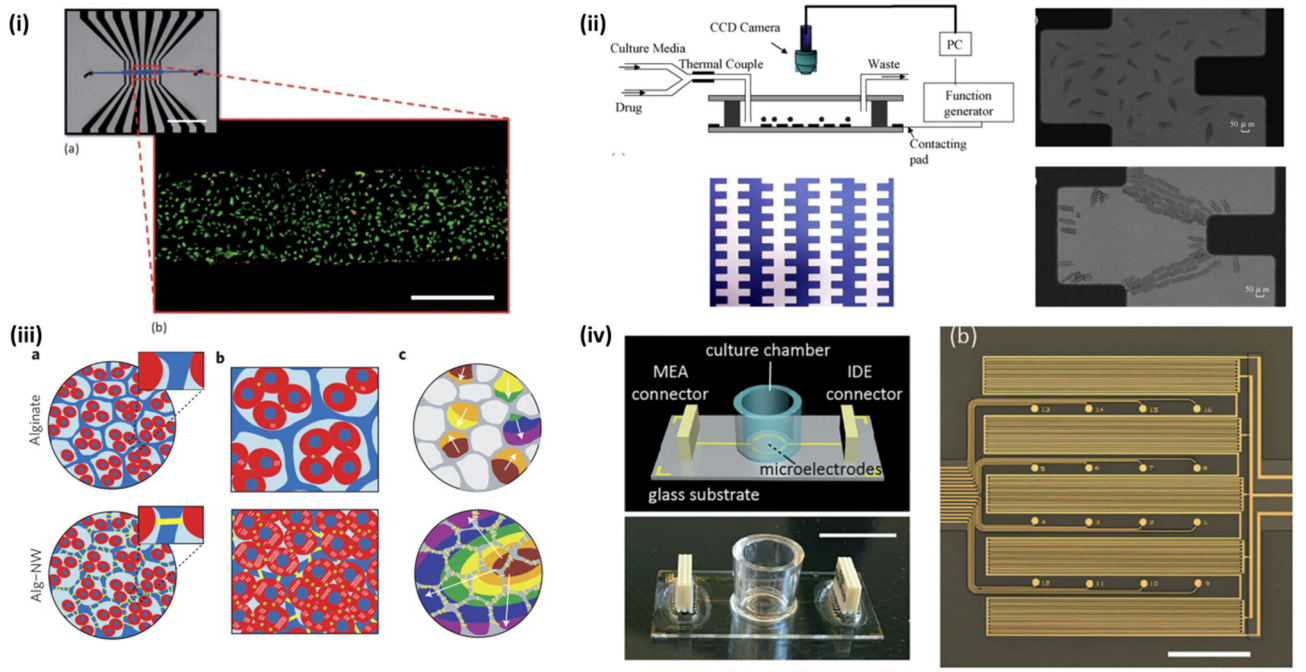

FIGURE 4. Illustrations of electrically stimulated heart-on-a-chip systems. (i) 3D flexible electrodes composed of PDMS and carbon nanotubes are incorporated in a microfluidic chip device and allows generation of uniform electric field. These 3D electrodes not only provide electrical stimulation, but their inherent flexibility allows concomitant application of mechanical stimulations, which drive key cellular processes such as matrix deposition, gene and protein expression and stem cell differentiation. Reprinted with permission from Ref. 35. (ii) Microfluidic device with interdigitated–castellated microelectrodes that induce dielectrophoresis and electro-orientation are shown. These microelectrodes stimulate cardiomyocytes to mature into a tissue-like structure with orientation along the AC electric field. Reprinted with permission from Ref. 104. (iii) Gold nanowires within alginate scaffolds for cardiac patches, cardiac cells (red), alginate pore walls (blue) and gold nanowires (yellow). Gold nanowires bridge the electrically resistant pore walls of alginate and improve electrical communication between adjacent cardiac cells. Tissues grown on these composite matrices were thicker, better aligned, have higher levels of proteins involved in muscle contraction and electrical coupling (bottom images of a-b) and show synchronized beating (bottom image of c) than those grown on pristine alginate matrices (top images of a–c). Reprinted with permission from Ref. 107. (iv) Microfluidic device consisting of interpenetrating microelectrode array and interdigitated electrodes that performs electrical simulation, field potential mapping, contraction recording, and optical observation of cardiac cells and tissues. A pair of stimulation electrodes connected to waveform generator delivered periodic voltage pulses to pace the cardiac cells, and the microelectrode array recorded voltage as a function of time and generated the activation map to reveal field potential. The interdigitated electrodes performed high speed impedance-time recording to measure cardiac contraction. Reprinted with permission from Ref. 142.

maturation of hiPSC-CMs to some degree. To date, hiPSC-CMs were cultured up to a full year, and they displaced more mature phenotypes in cell size, myofibril density and alignment, sarcomere maturity of all Z-, A-, H-, I-, and M-band and calcium handling physiology. Interestingly, hiPSC-CM cultured for 180 days did not exhibit mature M-bands, a key feature of sarcomere structure.[62] A critical downside of maturation *via* prolonged culture time is the unrealistic and financially inappropriate culture timeframe.

Biochemical Cues and Substrate Stiffness Modulation

Cells interact with each other through direct cellular contact *via* gap junctions or *via* indirect paracrine factors secreted by neighboring cells. Studies have implicated mimicking this cellular interaction *in vitro* promote cell maturation. Co-culture of hiPSC-CM with fibroblast and endothelial cells helped increase sarcomere length as the endothelial cells express extracellular matrices including collagens I and III, fibronectin, and thrombospondin-4.[1] Co-culture of hiPSC-CM with human mesenchymal stem cells mediated electrical coupling of hiPSC-CMs by secreting VEGF, bFGF, SDF-1 and GM-CSF.[144] Incorporation of extracellular matrices alone, such as fibronectin, gelatin, collagen, also promotes structural and functional maturation of hiPSC-CM.[100]

Biophysical Stimulation

Cardiomyocytes in a heart are regularly exposed to electrical stimulation from the action potential and mechanical hemodynamic stress, which are often absent under standard culture conditions. However, applying external electrical stimulation has proven to yield hiPSC-CM with enhanced cellular alignment, organized sarcomeres, and rod-like morphology.[20,98] In addition, combined and synchronized electrical and mechanical stimulation on hiPSC-CMs induced sar-

comere shortening and reduced transmembrane calcium currents, which align with mature phenotypes of cardiac cells.[72]

Controlled Microfluidic Environment with Hemodynamic Force

Appropriate modulation of cellular microenvironment and recreation of mechanical loading conditions are important to achieve hiPSC-CM maturation. hiPSC-CM culture in a dynamic microfluidic environment with provision of cyclic pulsatile hemodynamic forces that simulate the physiological shear stress has been shown to promote increased alignment, contractility, cell size and aspect ratio, and sarcomere length.[71] Another group developed a microfluidic cardiac cell culture model (uCCCM) that allows *in vitro* hemodynamic simulation of cardiomyocytes by directly coupling cell structure and function with fluid induced loading.[42] The microfluidic cell culture chamber was integrated with a pump, collapsible pulsatile valve, and an adjustable resistance element (hemostatic valve) to mimic hemodynamic conditions associated with the normal and failing heart. Cardiomyocytes that experienced physiologic levels of pressure and loads within the native ventricle showed mature phenotypes.

Influence of Chip Design on Measurement of Cardiac Contractility and Electrophysiology

In addition to functionalization and maturation of the cardiac cells *via* stimulation, contractility measurement is an important function of the heart-on-chip to monitor cardiac systolic functions, such as the frequency, force, and synchronization of cardiac contractions, which aid the understanding of fundamental mechanisms of heart functions. Various sensors are integrated in heart-on-chips, including optical, electrical, and mechanical detection. Select mechanical and electrophysiological measurement techniques and their translational applications are summarized in Table 2.

One of the primitive ways to obtain contractility measurement *via* optical observation is the design of micro post arrays on chip, as shown in Fig. 5. Contraction of cardiomyocytes that adhere to the top of each micro post causes deflection of micro posts, and the displacement of deflection is measured *via* video analysis[13] (Fig. 5(i)). A similar approach is the use of traction force microscopy and fluorescent beads immobilized within an elastic substrate on which cardiomyocytes are cultured. The contraction is measured by tracking the position of the fluorescent bead[28] (Fig. 5(ii)). Conclusively, optical microscopy and video analysis can provide precise measurements, but not suitable for drug screening applications as it is time consuming and can't be measured in real time. Beyond the use of optical microscopes, a colorimetric sensor integrated in an elastic chip was recently developed by Shang *et al.*[115] (Fig. 5(iii)). Periodically arranged macropores on hydrogel known as inverse opal was used to manipulate the propagation of photons in their photonic band gap, which gives rise to vivid structural color properties. Volume or shape change in the hydrogel on which cardiomyocytes are cultured leads to shifts in the photonic band gap spectra, which reflects the beating intensity. Another recent optical module developed to characterize cardiomyocytes mechanical behavior is the use of surface plasmon resonance (SPR) technology. Mozneb *et al.* cultured a monolayer of cardiomyocytes directly on fibronectin coated gold SPR chips, and the refractive index angle shifts due to cell beating were recorded[90] (Fig. 5(iv)). To calculate the contractile force, the refractive angle shift was calibrated against contractile force calculated from video analysis. The biggest advantage of this method can be attributed to its noninvasive, label free, real-time technique. Drug screening can be carried out in a microfluidic chip design and measure the kinetics of each drug interaction with the cardiomyocytes.

Some appealing, noninvasive mechanical approaches for force measurement at the tissue level leverage cantilevers, as shown in Fig. 6. Cantilevers with laser sensors have been introduced, in which the laser reflecting electrode on the cantilever surface gauges the displacement of the cantilever induced by cardiac contraction[79] (Fig. 6(i)). Cantilevers have been also combined with atomic force microscopy, which can record the interaction force between the beating cardiomyocytes and the AFM tip in real time.[83] Recently, a piezoelectric sensor was combined with a fibrin coated cantilever to measure the contraction force of a human cardiac tissue *in-situ* under microscope[130] (Fig. 6(iv)). The measurement hardware consisted of AD8691 from Analog Devices and an operational amplifier circuit, and an Arduino Due analog-to-digital converter through which the amplified signal was read. This piezoelectric cantilever was only able to measure the force at the tissue level, and not sensitive enough to measure at single cell level. Electrospun fibrous PVDF-TrFe scaffolds were also used to culture cardiomyocytes and induce tissue organization, and with its inherent mechanoresponsive ability, the scaffold itself also served as a sensor that outputs measurable voltage signals in response to the cardiac tissue beating[2] (Fig. 6(iii)).

Electrical signal-based contractility sensors also enable facile and real time recording of cardiac contractility. Impedance sensors can monitor cardiomyocyte contractility by recording changes in the impedance. The exposure of gap between the electrode

TABLE 2. Current modes of electrophysiology and contraction force measurements and applications in disease modeling/drug screening.

Measurement	Advantage	Limitation	Disease model/-drug type	Method	References
Electrophysiology					
Microelectrode array	Non-invasive Long term recording available Can measure conduction velocity and field potential duration	Cannot measure individual currents or action potentials High variability Limited scalability	Cardiac arrhythmia N/A Solatol, norepinephrine, verapamil	Planar electrode array Microelectrode array Patterned multielectrode array with multichannel systems amplifier	5, 49, 117
Nanopillar electrode	High yield of multiplexed readouts Wide applicability of conditions relevant to hypoxia or ischemia	Measures multicellular clusters	Ischemia	Measures intracellular calcium ion and extracellular potassium ion currents	82
Patch clamp	Gold standard with high resolution and accuracy Precise measurements of current and potential Only method to determine IC50 values of individual cardiomyocytes	Action potentials (measured by current clamp) and ion currents(measured by voltage clamp) can't be recorded in the same cell Low throughput Labor intensive	Type 3 long QT syndrome	Whole cell patch clamp recording	86
Contraction force					
Piezoelectric cantilever	Can combine measurement of contractility with voltage and calcium measurements	Low throughput	N/A	Combined with *in situ* imaging during force measurement	130
Cantilever	High thermal resistance material Enhanced contractility, maturation, and sarcomere length with nanotextured cantilever surface	Low throughput	Verapamil treatment	Nanotextured polyimide cantilever with laser vibrometer	79
Micropillar arrays	Combines measurement of beat force, rae, and cellular elasticity PDMS is stiffer and more cell-adhesive than acrylamie-based hydrogel	Requires cardiomyocyte to generate strong enough force to observe pillar displacement	N/A	PDMS based replica mold fabrication of micropillars	122
Surface plasmon resonance	Noninvasive, label-free, real-time monitoring High spatiotemporal sensitivity	Requires real time image processing method to calculate cell displacements Variations in signal from different flow rates	Blebbistatin and ATP screening	Analysis of contractile force, contraction and relaxation period, and beating rate	90
Intracellular calcium imaging	Rapid, automated method Vector fields can quantify spatial distribution and temporal correlations in motion within tissues	Requires fast recording camera Produces large amount of data that requires manual processing	Isoproterenol Long QT syndrome	Analysis of calcium flux and electromechanical coupling with optical flow analysis on cardiomyocyte beating	54
Magnetic beads	Noninvasive High sensitivity and large dynamic range Convenient and cost effective	Sensitive to small errors in bead displacement	Chronic ischemic cardiomyopathy model	Measurement of contractile force on a single cardiomyocyte Studied contractile properties under different external loading force	143

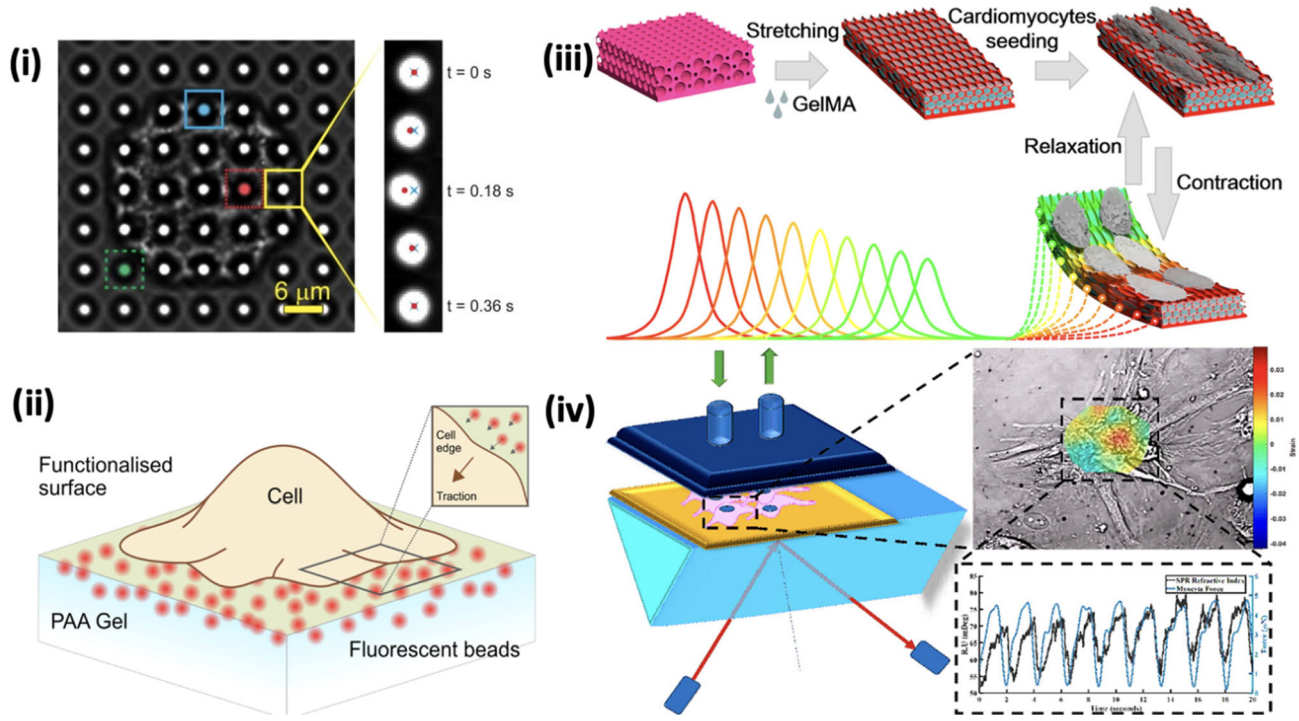

FIGURE 5. Optical measurement of cardiac contractility. (i) Cardiomyocytes are cultured on top of micro-post arrays, and the displacement of deflection due to contraction is measured *via* video analysis. Reprinted with permission from Ref. 13. (ii) Traction force microscopy that tracks the change in fluorescent bead positions to measure cardiac contraction. Reprinted with permission from Ref. 28. (iii) Volume or shape change in the hydrogel on which cardiomyocytes are cultured shift the photonic band gap spectra as a reflection of cardiac contraction intensity. Reprinted with permission from Ref. 90. (iv) Cardiomyocytes are cultured on fibronectin coated gold SPR chips, and the refractive angle shift reflects the cardiac contraction intensity. Reprinted with permission from Ref. 115.

and the cell layer changes the impedance of the electrodes, and the impedance fluctuation can be used to calculate the contractile force.[107] Strain sensors are also capable of measuring contraction. The strain caused by cardiomyocyte contraction can be converted into electrical signals, such as change in resistance. Carbon nanotube-based strain sensor developed by Wang *et al.* exhibited a higher magnitude of resistance measurements compared to impedance sensing[133] (Fig. 6(ii)). Lind *et al.* integrated a cantilever structure and strain sensor in a microgroove culture well and measured the contraction in real time.[80] Crack-induced strain gauge sensors developed by Kim *et al.* have shown the highest signal to noise ratio to date among electrical sensors and maintain the stability of sensing in culture medium for as long as 26 days[67] (Fig. 6(vi)).

Electrophysiology of cultured cardiomyocytes, such as depolarization and repolarization patterns and ionic currents magnitude, are particularly important for drug screening and regenerative medicine applications. Recent sensor development for electrophysiology detection mostly lies in the domain of multielectrode arrays (MEA) and field effect transistors (FET). Various parameters of extracellular potential (amplitude, firing rate, *etc.*) can be measured with MEA recordings without making invasive contact as patch clamps do. Cheng *et al.* developed a microfluidic system integrated with five individually addressable microelectrodes that are used for field-stimulation or measurement of lactate secreted by cardiomyocytes.[23] *In-situ* microscopy can be interfaced with this microfluidic system to measure cell contractility, extracellular pH and intracellular Ca^{2+} levels in real time. Qian *et al.* designed a culture chamber integrated with microelectrodes and impedance sensors that can record both electrophysiology and contractility on one chip[107] (Fig. 6(v)). The geometry design of the impedance sensors was chosen based on impedance baseline values. The microelectrode array geometry was designed by considering higher density integration to achieve better spatial resolution and lowering electrical impedance to achieve higher signal-to-noise ratio. Various micropatterned MEA designs with carbon nanotubes,[119] platinum black,[136] indium tin oxide,[119] and graphene[108] have been demonstrated to show improved cell-electrode coupling. Nanoscale FETs can identify the specific single cellular signal that are difficult to extract from MEAs, which typically yield signals from several cells. Silicon and graphene-based nanowire FET arrays have been explored from the Lieber group to record multiplexed electrical signals of embryonic

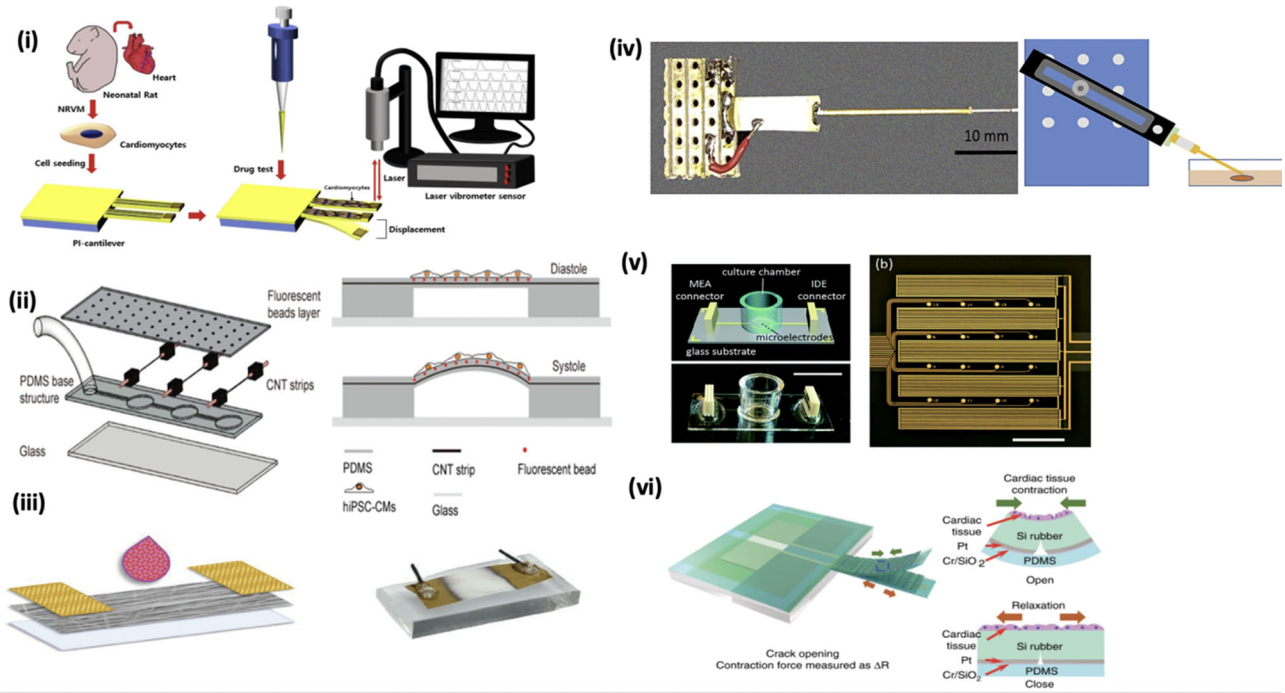

FIGURE 6. Mechanical and electrical measurements of cardiac contractility. (i) The laser reflecting electrode on the cantilever surface gauges the displacement of the cantilever induced by cardiac contraction. Reprinted with permission from Ref. 2. (ii) Microdevice array with a deformable PDMS membrane with embedded carbon nanotube-based strain sensors. Electrical resistance change induced by contraction of cardiomyocytes on membrane was measured continuously. Reprinted with permission from Ref. 67 (iii) Electrospun fibrous PVDF-TrFe that serves both as a culture scaffold for cardiomyocytes and a piezoelectric sensor that outputs voltage readouts induced by cardiomyocyte contraction. Reprinted with permission from Ref. 79 (iv) Piezoelectric sensor with fibrin coated cantilever that can measure contraction force of cardiac tissue *in situ*. Reprinted with permission from Ref. 107 (v) Use of microelectrode arrays and interdigitated electrodes that measure impedance change as an indirect reflection of cardiac contractile force. Reprinted with permission from Ref. 130 (vi) Strain sensor based on metal cracks formed on silicone rubber cantilever measures strain changes caused by contraction of cultured cardiomyocytes on the cantilever. Reprinted with permission from Ref. 133.

chicken cardiomyocytes with excellent signal to noise, spatial and temporal resolution.[27,126] When nanoscale FET arrays are integrated with a silicon dioxide nanotube with phospholipid modification, these vertical nanotubes can penetrate the cell membrane and record the intracellular transmembrane potential of a single cell or multicell network. Targeted intracellular recordings are available with a free-standing kinked silicon nanowire FET probe.[125] With the ability to provide real time and non-invasive data of both cardiac electrophysiology and contractility, these platforms provide a quantitative and predictive assay system to assess cardiotoxicity.

COMPUTATIONAL MODELING OF HEART AND APPLICATIONS OF HEART-ON-CHIP DEVICES

Computational Models of Heart

Computational modeling plays an important role to understand both the structural and functional properties of cardiac systems and is therefore valuable to predict the performance of a personalized cardiac system. With the fast development of cardiac sensing and measuring technology, various mathematical models arise and get refined according to the *in vivo* measurement, as shown in Fig. 7. In this section, we review the numerical models in cardiac electrophysiology and cardiac mechanics at various scales (cell-tissue-whole heart) and discuss their importance and applications.

Computational Model of the Mechanics and Electrophysiology at the Cellular Level

As one of the most fundamental primitives comprising a human heart, the interaction and collective motion of cardiomyocytes enable the contraction of the muscles to pump blood throughout the entire heart chambers. As the key component of the heart, cardiomyocytes are primarily involved in the contraction of the heart to serve pumping of blood throughout the circulatory system. The basic mechanical unit of car-

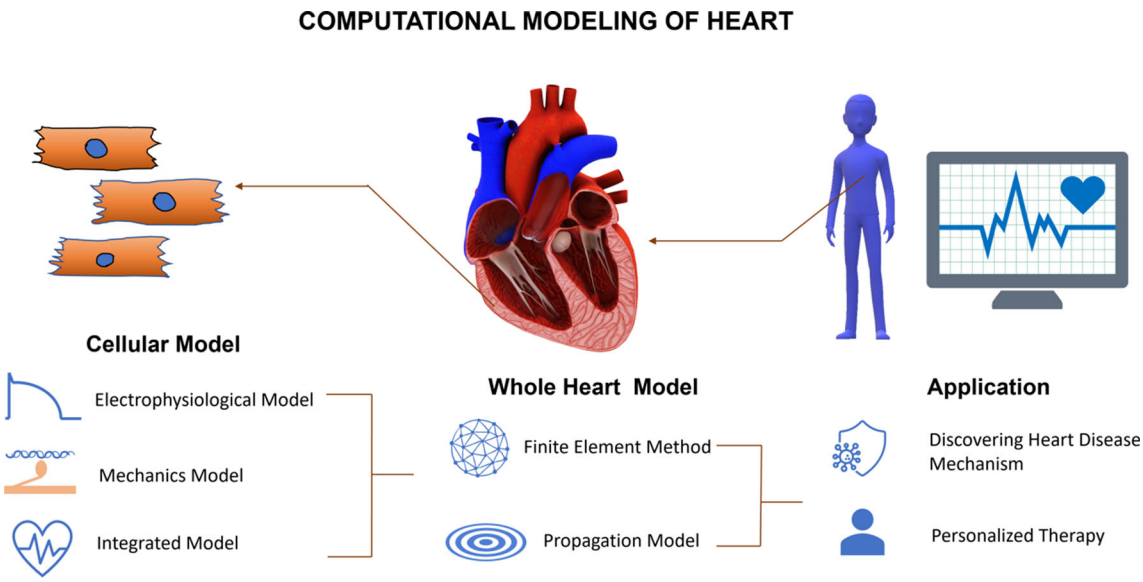

FIGURE 7. Computational models of heart and their applications. At the cellular level, electrophysiological models and mechanics models are presented to study cardiac cells' corresponding properties, as well as the integrated models to study the coupling of cardiac cells' electrical and mechanical events. The whole heart model employs finite element method to study how the electrical wave is propagated through the heart and its interaction with beating activities. The whole heart model is helpful to discover the heart disease mechanism and implement personal therapy.

diomyocytes is a sarcomere, which is composed of the filament actin and myosin. The thin actin filaments and thick myosin filaments are aligned and crosslinked by Z-disks inside the cardiomyocytes to enable the contractile function.[63,139]

Another significant mechanism of cardiomyocytes is the cardiac electrophysiology to regulate the cardiomyocyte contractility. Filaments are driven to contract through the interaction between the protein troponin C and the intracellular free calcium ions. In a cardiac action potential, human adult ventricular cardiomyocyte has five phases. In phase 0, the upstroke of action potential is generated by the inward sodium current. Then in phase 1, the sodium channels are inactivated, combined with a transient potassium efflux to form the early repolarization notch. In phase 2, calcium enters the cell through the L-type calcium channels to balance the efflux of potassium, leading to the so-called plateau phase. At the end of plateau, L-type calcium channels close while efflux of potassium remains, constituting the phase 3 as the rapid repolarization phase. Finally, in phase 4, which is also known as the diastole phase, influx and efflux of ions are balanced to maintain the resting potential.

So far various models have been developed to understand and simulate the mechanical properties and electrophysiology properties of cardiomyocytes, as shown in Fig. 8. A number of cardiac muscle contraction models are based on Hill's three-element muscle model.[50] Hill's model is constituted by a contractile element, a spring element in series and another parallel spring element. In this model, the contractile element stands for the active force due to activation; the series element represents the intrinsic elasticity of myofilaments and tendon; the parallel element indicates the passive force which comes from the connective tissues around the contractile elements. In recent years, Cansiz *et al.*[19] proposed a modified Hill model to describe the electro-visco-elasticity of the myocardium and showed that the viscous effects significantly altered the electromechanical response of cardiac tissue. With the purpose to provide insights at the microscopic level, Huxley *et al.* proposed the sliding filament theory.[56] In Huxley's theory, the dynamics of filaments and the actin-myosin cross-bridges possibilities are considered. The distribution of cross-bridges, i.e., the rate of actin-myosin connections, is modeled to show the dynamics inside sarcomeres. The model was then extended by Huxley and Simmons to take multiple bound configurations into consideration.[57] However, the distributions alone cannot reflect the behavior of muscles. Based on this observation, Zahalak *et al.*[145] proposed the distribution-moment model to describe the evolution of moments. By assuming a Gaussian underlying distribution, this model not only reduces the mathematical complexity but also provides reasonable approximation. The sliding filament model has inspired many cardiac muscle models since then.[36,51,138]

Cardiac electrophysiological models typically describe the cardiac action potential, which depend on the flow of ions across the cell membranes. Most of the

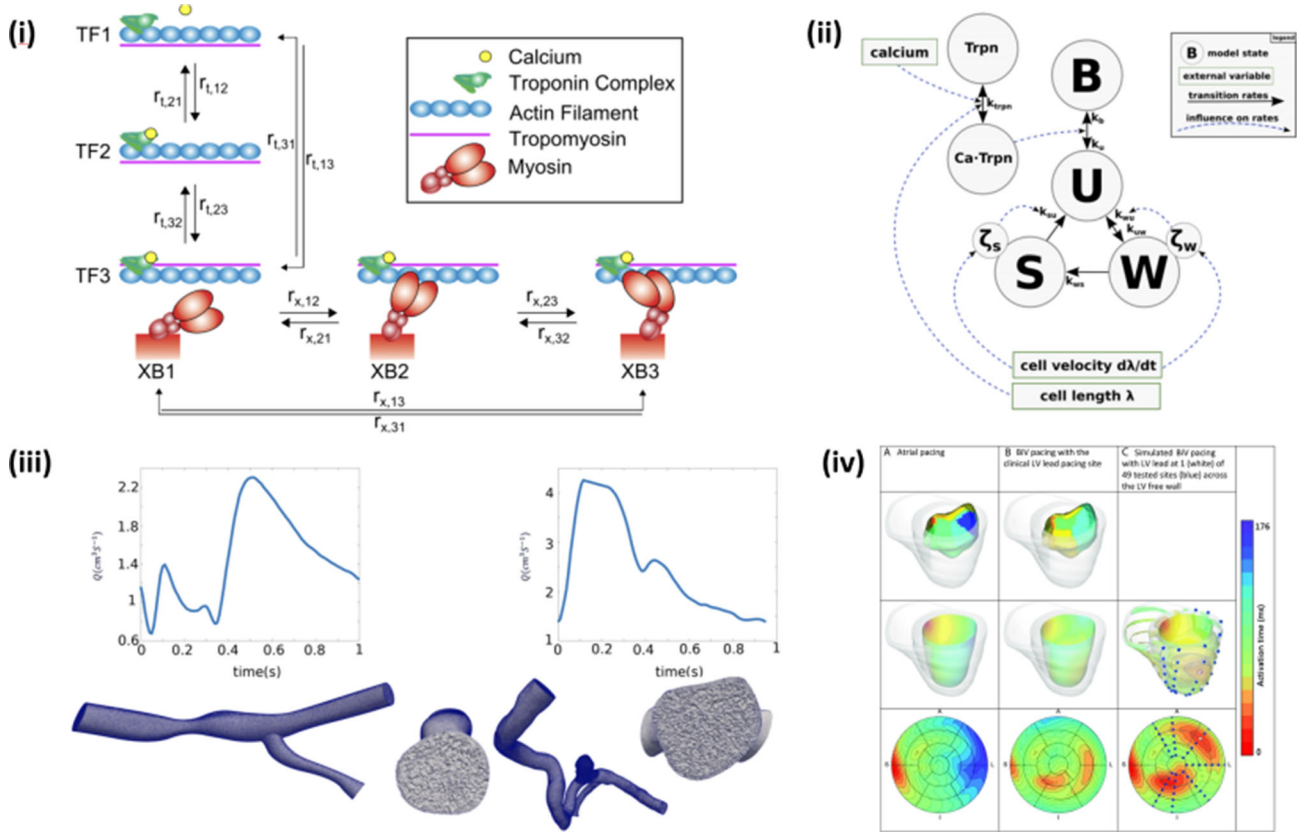

FIGURE 8. Computational cardiac models. (i) The model combines two three-state cycles to simulate how force production, energy utilization, and the number of bound cross-bridges are affected by dynamic changes in sarcomere length. Reprinted with permission from Ref. 39. (ii) Data-driven cardiac contraction model which includes troponin C kinetics, tropomyosin kinetics and a three-state crossbridge model. Reprinted with permission from Ref. 75. (iii)Data-driven dynamic mode decomposition to simulate the pulsatile blood flow physics. Reprinted with permission from Ref. 48. (iv) The cardiac model to predict the effects of chronic CRT on the optimal LV lead location and device timing settings over time with top row the clinical measurements, middle row the parameter fitting and the bottom row simulated results. Reprinted with permission from Ref. 78.

electrophysiology models[11,40,96] are based on Hodgkin and Huxley's ionic model of a squid giant axon.[52] In these models, the conductivity inside cells is typically described by the open probability of channels in the form of ordinary differential equations (ODE).[66] Up till now, ionic models have been developed for a variety of animal species, including rats,[102] rabbits[81,87] and canine.[24,55] Meanwhile, a number of models have been proposed to explain and predict human cardiac electrophysiology properties, including ventricular cells and atrial cells.[37,43,59,85,99] For example, Luo *et al.*[85] presented a cardiac ventricular action potential model which is based on the regulation and concentration of calcium ions. Iyer *et al.*[59] developed a computational model of the human left-ventricular epicardial myocyte. These ionic current models are formulated and validated by experiment data from recombinant human ion channels and single myocytes from the human left-ventricular subepicardium. Grandi *et al.*[43] developed a detailed mathematical model for calcium handling and ionic currents in the human ventricular myocytes based on a rabbit myocyte model.[43] The simulation results robustly described the excitation-contraction coupling and were validated against experiments on a broad range of human myocytes. Nygren *et al.*[99] developed a mathematical model of the human atrial myocyte based on averaged voltage-clamp data recorded from isolated single myocytes. By combining the Hodgkin-Huxley model and fluid compartments, their model provided reasonable simulation results and reconstructed the action potential data. Grandi *et al.*[37] proposed a novel human atrial action potential model derived from their human ventricular myocyte model and atrial experimental results. This model provides insights into the atrioventricular action potential difference and shows how abnormal atrial fibrillation abnormal repolarization is mediated by sodium and calcium homeostasis.

While many models describe the electrophysiology of cardiac cells without considering the contraction mechanics, a real cardiac system exhibits complex mechanical-electrical coupling. A line of pioneering

research has been devoted to exploring such coupling. Weber[135] first demonstrated that free intracellular calcium ions activate actin-myosin interaction and regulate the contraction cycle. From then on, mathematical models to incorporate the coupling of muscle activation and contraction have been intensively explored. To account for the effect of calcium ions concentration on cross-bridge formation, Landesberg and Sideman[76] published a model which couples the calcium binding to troponin C and cross-bridge cycling in skinned cardiac cells. In the following years, Rice *et al.*[111] developed a cardiac contraction model to explore the cooperative mechanisms in cardiac cells, including end-to-end troponin-tropomyosin interactions, neighboring cross-bridge interactions, and feedback on troponin affinity for calcium ions. Subsequently, Razumova *et al.*[109] developed a stiffness-distortion model to represent forces as a product of the stiffness of parallel cross-bridges and their distortion. Niederer and Smith[94] developed a mathematical model of the rat ventricular myocyte to analyze the influence of calcium dynamics on the slow force response upon changes in the muscle length. Recent models include Tewari *et al.*'s study[124] to explicitly account for the interaction between chemical events (ATP, inorganic phosphate release) and the mechanical process and Land *et al.*'s model[75] to address contraction and passive viscoelasticity in human cardiac myocytes (Fig. 8(ii)).

As models develop, there is a growing demand to validate the model parameters with experimental data. However, electromechanical models described by sets of ordinary differential equations often lack explicit representation of spatial interactions of regulatory proteins in cardiac cells. To explicitly incorporate the parameters of interest, Daniel *et al.*[30] first proposed a spatially explicit model to study the mechanical tuning at the molecular level. Later on, spatially explicit model of cardiac thin filament represented by neighboring regulatory units were developed by Rice *et al.*[110] and Campbell *et al.*[18] In their models, periodic boundary conditions are considered to reduce computation complexity. However, regulatory unit number is still limited in this approach. To overcome this drawback, Washio *et al.* developed a novel ODE model to approximate the cooperative interactions in cardiac sarcomere in a spatially explicit method.[134] This modified ODE model reduces computational cost but maintains the spatial integrity and co-operative effects, showing good agreement with the time-consuming Monte Carlo simulation. Other research groups also developed spatially explicit models to further reduce the computation cost[74] and to examine the consequences of filament compliance[16,39] (Fig. 8(i)).

Whole Heart Models

The previously mentioned models describe the cardiac mechanics from the bottom-up approach, starting from the cellular mechanics to the whole heart. An alternative method is to simulate from the view of whole heart, by describing the dynamics of ventricles. The heart is composed of four chambers: two upper chambers called the left /right atrium and two lower chambers named left/right ventricles. Composed with billions of cardiomyocytes, cardiac walls are able to contract to pump blood to the next chamber or propel blood out of the heart. Each cardiac cycle includes two periods, known as the diastole and systole. The cardiac cycle starts with diastole, in which both atria and ventricles are relaxed and receive blood from central veins. At the end of diastole, the atria contract to force blood into the lower ventricles. After the atrial contraction, the left and right ventricles contract to eject blood into the aorta and pulmonary artery, respectively. The repeating cardiac cycle enables the heart to pump blood through the body and maintain life activities, which is the theme of whole heart modeling. Whole heart models focus on the simulation of cardiac cycles to reflect both the mechanical and electrophysiological properties.

Current whole heart models typically involve the use of finite element method, which solves the governing equations on discretized computational mesh of the whole heart domain. Resolution and geometry of the mesh can make a significant difference in the simulated results, therefore should be carefully determined according to the natural cardiac properties.[127] In general, high-resolution mesh indicates accurate results whereas cost expensive computation. Thanks to the development of high-performance computing techniques, high precision calculation of whole heart models is possible while maintaining high computing speed.

Mechanical models of the whole heart are typically proposed to understand the distribution of stress and strain in the heart. Early whole heart models simplified the heart as a thick walled cylinder based on the orientation and sequential activation[7] of muscle fibers across the left ventricular wall and the material parameters[46] that account for the observed epicardial deformations in left ventricles. To solve the complex system, finite element method was introduced. Nash and Hunter[92] proposed a more comprehensive model of left and right ventricles with the finite element method, in which the measured fibrous-sheet structure during a cardiac cycle was incorporated. Later whole heart models combined the observed contraction with cardiac electrical activities by solving the mechanical equation and electrical propagation equation respec-

tively on meshes of the whole heart domain. Kerckhoffs *et al.*[65] proposed a simple integrated model by coupling the cardiac contraction with the depolarization wave which is described by the eikonal-diffusion equation. This weak coupled model compromises accuracy for computational cost by limiting the number of parameters to be known. Usyk *et al.*[129] developed an anatomically detailed ventricular model to investigate the relationship between regional electrical activation and the timing of fiber shortening. The electrical activities are simulated by modeling the ionic kinetics with the two-variable modified FitzHugh-Nagumo equations[40] and the impulse propagation by a monodomain formulation. With the development of high-performance computing technology, strong coupled electromechanics based whole heart models have been developed in recent years. Niederer *et al.*[95] constructed a multi-scale model to investigate the transduction of cellular work to the whole heart pump function. This model specifically studies the role of cellular length dependency regulators of tension generation, including the calcium sensitivity, filament overlap, tension velocity and calcium-Troponin C binding.

Considering the heart is a complex system that functions *via* the interaction of air, blood and cardiac cells, there is an emerging need for whole heart modeling to take full consideration of multi-physics interaction inside the heart. Peskin[105] proposed the immersed boundary method to effectively study flow patterns around heart valves, which has then been broadly applied to hydrodynamic models of cardiovascular systems.[9,44,53] Following the previous work, Riken group[113] developed a multi-scale, multi-physics heart simulator which is able to accurately model heart motions, valve actions and blood flows of the entire heart.

Whole heart models play a vital role in discovering the mechanisms of heart diseases. Jie *et al.*[60] developed a multiscale electromechanical model of rabbit ventricles to investigate the impact of ischemia on spontaneous arrythmias. This model studies how ischemia-induced mechanical dysfunction can induce ventricular premature beats and their subsequent degradation into ventricular arrythmias. Gurev *et al.*[47] developed a 3D model to study the distribution of electromechanical delay (EMD) in rabbit ventricles by simulating the EMD in both sinus rhythm and epicardial pacing.

Generation of patient-specific cardiovascular system models is a promising approach to implement personalized therapy and medicine, and thus, one of the high priorities of cardiac research. Aguado-sierra *et al.*[4] developed a case study to describe the methodology for generating a patient-specific model of the failing heart. More recently, Lee *et al.*[78] proposed a personalized biophysical model to explore the effects of chronic cardiac resynchronization therapy on the optimal left ventricle lead location and device settings (Fig. 8(iv)). While previous personalized models were developed on one or a few patients, Kayvanpour *et al.*[64] expanded the personalized model to 46 patients with a computationally efficient model and robust parameter estimation. This predictive model shows consistent results with cardiac imaging, lab tests and prognosis scores and thus, demonstrates its potential in clinical personalized resynchronization therapy.

Despite the development in experimental characterization of cardiovascular systems, there are still challenges with extracting critical and relevant information from the dataset, which is usually limited by its scale, resolution and noise. In addition, current computational models are limited in their complexity and physiological relevance as many cardiac mechanisms remain unexplored. The data-driven modeling approach offers new solution to these challenges and redefines modeling of the cardiovascular system. So far, data-driven models have been developed to tackle a variety of issues in the collected dataset of cardiovascular systems, including redundancy detection and reduction,[8,48] noise treatment[112] and reconstruction from sparse sampled data.[21,61]

Disease Modeling and Drug Screening with Heart-on-Chips

Disease Modeling Applications

The primary value of organ-on-chips stressed by pharmaceutical and biotechnology industries lies in validation of drug candidates, high-throughput screening, and studying molecular mechanisms of drug efficacy and toxicity. More sophisticated organ-on-chips model specific phenotypes or genotypes of a disease, identify biomarkers related to the disease mechanism and thus, provide valuable information for clinical trials. Organs-on-chips cultured with animal derived cells can be compared to those cultured with human cells to study species differences and refine the *in vitro* and *in vivo* correlations and predictions.

Microfluidics based organ-on-chips are highly versatile and suitable to provide continuous media perfusion, control of shear stress, and direct specific spatial distributions of different cell types, all of which are necessary to engineer appropriate drug screening and disease models. Recent advances in microfluidics-based organs-on-chips have been widely applied in pharmaceutical screening, cell/tissue characterization, implantation, and disease modeling.

Primary heart cells are hard to obtain and have short culture period, so they are not suitable for robust

disease models. However, human induced pluripotent stem cell derived cardiomyocytes offer unprecedented opportunities to generate patient-specific human cardiomyocytes, and theoretically provide unlimited supply of human cardiomyocytes. They can be functionally characterized to model specific cellular physiology tailored to each disease model and recapitulate the patients' genome to characterize genotype-phenotype relation, as shown in Fig. 9. Ellis *et al.* is one of the few groups that modeled the entire cardiovascular system *in vitro*.[38] They engineered a myocardium-on-chip using human iPSC-derived cardiomyocytes (iCMs) and endothelial cells (iECs) from the same cell line. The co-culture of iCMs and iECs with the inclusion of capillary-like side channels enabled creation of a 3D human cardiac muscle with the surrounding microvasculature. Conduction velocity and calcium transportation were measured with carbon nanotube-based microelectrode arrays and calcium flux assays respectively. Contractility of single iCMs were measured through image analysis of the cardiac muscle portion. Since hypoxia is a critical condition that can lead to many disorders, including ischemia and arrhythmia, heart-on-chip models that can monitor cardiac electrophysiology under acute hypoxia conditions was designed by Liu *et al.*[82] The microfluidic channel enabled rapid modulation of medium oxygenation to model hypoxia. Extracellular bioelectronics and platinum nanopillars provided continuous readouts on actional potential, demonstrating that hypoxic cells lead to tachycardia and eventually arrhythmia.

An important research question that needs to be addressed particularly when using stem cell sources for cardiovascular disease modeling is how to differentiate iPSC-CMs into developmentally mature cardiomyocytes. Since many heart diseases take years to manifest in patients, the relatively immature iPSC-CMs may not accurately reflect the risk or important phenotypes of disease types. In addition, heart-on-chip disease models need to incorporate the interactions of various cell types and the microenvironment. 3D co-culture of cardiomyocytes with endothelial cells and extracellular matrix including fibroblasts, collagen, and hydrogel can realize this complex microenvironment of the heart tissue. Culturing cardiomyocytes on heart-on-chips for longer term needs to be considered as well by integrating vessels or continuously perfusing the chips with nutrients to fully mature the stem-cell derived cardiomyocytes.

Drug Screening Applications

Microfluidics combined with electrical stimulation have been created for use as screening assays for drugs, as shown in Fig. 10. Klauke *et al.* fabricated an array of microchannels that can hold adult ventricular myocytes in individual microchambers with integrated electrodes for field stimulation.[70] Single adult cardiomyocytes were continuously field stimulated *via* planar electrodes within small volume, and the contractility in terms of sarcomere length and intracellular calcium ion transients were quantified. Werdich *et al.* designed a hybrid chip that allows trapping and maintaining of single cells in a restricted extracellular space and perfusion of drugs into each cell chamber.[137] The chip combines a microfluidic network fabricated in PDMS with planar microelectrodes to measure extracellular potentials associated with intracellular calcium waves in response to drug treatments. In a similar study, a heart-on-chip containing 20 rat cardiomyocyte thin films was stimulated electrically to

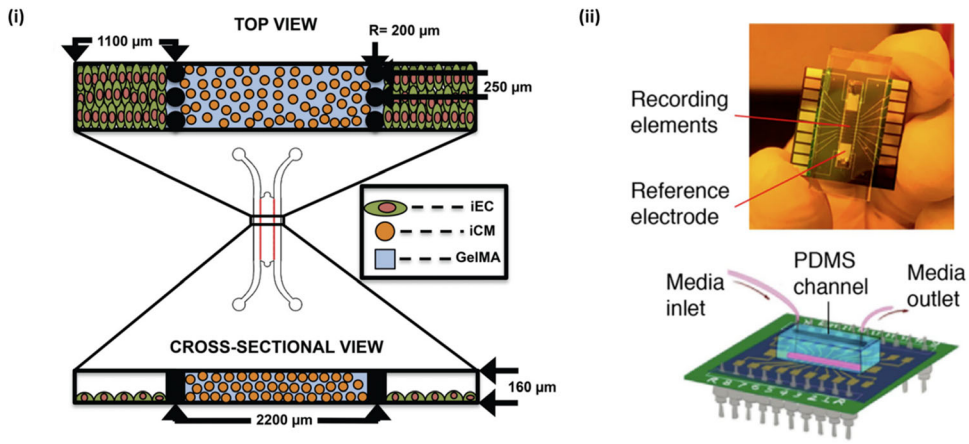

FIGURE 9. Disease modeling with heart on chip examples. (i) Chip that models the entire myocardium *via* co-culture of human induced stem cell derived cardiomyocytes and endothelial cells in capillary like side channels. Reprinted with permission from Ref. 38. (ii) Chip consisting of cell culture area, bioelectronic devices, and microfluidic channel, which enables temporal modulation of medium oxygenation to model cardiac electrophysiology under acute hypoxia. Reprinted with permission from Ref. 82.

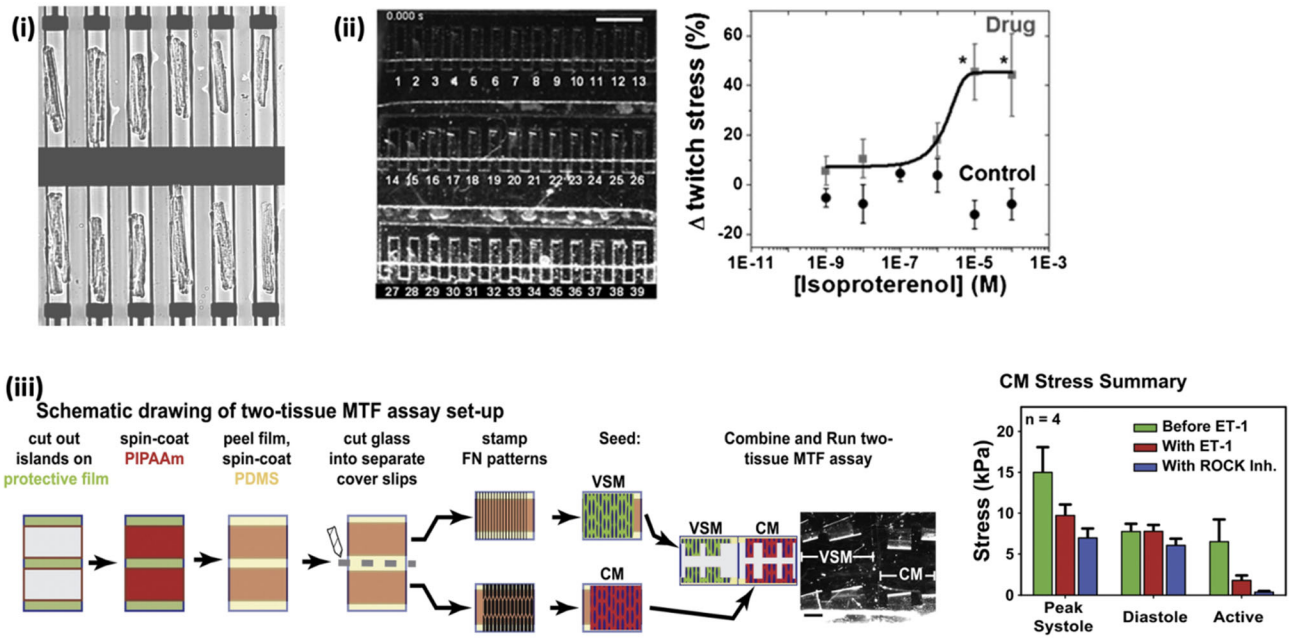

FIGURE 10. Drug screening with heart-on-chip examples. (i) A microfluidic network with an inlet for drugs/toxins with planar microelectrodes to measure extracellular potentials from single cardiomyocytes. Reprinted with permission from Ref. 3. (ii) A single channel fluidic microdevice with embedded electrodes for electrical field stimulation of cardiac tissue and measurement of cardiac contractility. The inotropic effect of isoproterenol on cardiac contractility was tested at varying concentrations with high throughput. Reprinted with permission from Ref. 45. (iii) Cardiac microtissue was cultured on elastic thin films patterned with microcontact printing. The change of stress generated by cardiac microtissue with injection of ET-1 and ROCK inhibitors were measured. Reprinted with permission from Ref. 70.

contract, and the drug response of the chip to isoproterenol showed similar results to the *in vivo* data.[3] The deflection of cantilevers on the chip allowed a simple method to calculate the diastolic and systolic stress of the cardiac tissue. This engineered platform has merits in its ability to image cell structure and function with high resolution and measure contractile function of multiple cardiac tissues at once in a single chip during pharmacology treatment. The same Parker group also combined such muscular thin film technology with fluidic chips.[45] The tissue could be paced by placing platinum electrodes in the inlet and outlet wells, and the systolic and diastolic behavior of the cardiomyocyte muscular thin film paced at 1 Hz was observed with a high-speed camera under a stereomicroscope. The effects of two different drugs were analyzed simultaneously on the same chip, which suggests the assay throughput is significantly higher than conventional cell culture studies. The Agarwal group also demonstrated heart-on-chip platform with high throughput drug screening studies.[3] Submillimeter sized thin film cantilevers of soft elastomers termed muscular thin films (MTF) were embedded in the microfluidic chip to measure the stress generated by the engineered cardiac tissue. The fluidic microdevice consisted of a metallic base, transparent top for recording cantilever deformation, and electrodes for electric field stimulation of the tissue. An important application of the device was in isoproterenol dose response studies, which measured the contractile function of multiple tissues during pharmacological interventions. The closed chamber configuration of the device deemed especially advantageous in allowing complete flushing between incremental drug dosages. Parsa *et al.* also developed a pneumatic microfluidic platform for high-throughput drug screening studies of cardiac hypertrophy.[103] Their design was motivated by the need to reduce the number of cells per tissue an eliminate complicated setups of the previous cardiac tissue bioreactors. This microfluidic system consists of a cardiac tissue culture layer that contains arrays of microwells and pillars and a pneumatically actuated control layer. Cardiac microtissues were pneumatically loaded and coupled with real time on-chip analysis. By loading cardiac tissue in high density, the mechanical stress present in volume overload observed in cardiac hypertrophy was recapitulated and enabled study in a high throughput fashion.

Integrating delivery of screening molecules with a microfluidic system is challenging as the introduction of cells itself can interrupt laminar flow by inducing unintended non-uniform shear stress. Careful design of the dynamic system can prevent turbulent flow and unintended stress. One of the major challenges in 3D

culture systems that model spatial events in pathobiology fail to address questions associated with temporal events. These may include the progress of tissue or organ development and aging, dynamics of tissue regeneration, sequential pathogenesis of inflammation and organ failure. Heart-on-chip platforms can overcome the temporally stagnant challenges inherent in 3D culture systems by integrating cardiac cells into a dynamic system *via* a circulating flow.

CHALLENGES OF HEART-ON-CHIP PLATFORMS AND AREAS FOR IMPROVEMENT

Heart-on-chip platforms combined with advanced microfluidic technologies have been developed with several different designs to replicate the cardiac tissue's function at varying levels. The perfusable microfluidics actively contribute to the functional maturation of cardiac tissues through the delivery of nutrients and biomolecules. Studies are shifting towards the use of iPSC-CMs derived in a patient-matched manner, making them a superb source to construct human heart models for personalized drug screening and for understanding patient-specific disease mechanisms. Indeed, for the specific cardiac functionality or pathophysiologic condition to be assessed, the iPSC-CM based heart-on-chip platform need to be developed at a scale which effectively recapitulates relevant biologic structure, function, and environment. Relevant scale may will be determined along a continuum that extends from across cellular, multicellular microtissue, syncytial tissue, and ever larger, more complex, differentiated regional scope. For instance, microscopic scope may suffice for the study of patient-specific channelopathies, an intermediate scope may be suitable to study mechanical and conduction properties (and cardiac electromechanical coupling), and a larger regional scope more likely would be expected as necessary for the study of fibrillatory reentry. Beyond this, there are disorders that inherently entail more expansive macro-scale design considerations, such as congenital heart disease (characterized by chronically disordered macro-physiology across multiple chambers and vessels) and ventricular tachycardia or flutter (infarct-related scar reentry). Modeling macro or organ level heart conditions may be more approachable as we gradually increase the complexity of the heart anatomy and incorporate the vascular microenvironment to these *in vitro* microfluidic chip platforms.

A major challenge for developing suitable *in vitro* cardiac models is obtaining relevant cell types in enough quantity for analysis and modeling of functional tissue models. iPSC-CMs offer a good compromise to primary cardiomyocytes because it is challenging to collect primary cardiomyocytes in large quantities, and removal from their native environment can result in rapid functional decline. iPSCs derived from patients with genetic mutations can refine drug response studies and iPSC-CM treated with gene editing technologies can generate multiple genetic cardiac diseases and produce relevant personalized disease models. However, the current differentiation protocols result in structurally and functionally immature cells, which raises the question of their validity for drug testing. Therefore, optimizing iPSC-CM culture protocols is required to generate mature cardiomyocytes that exhibit key phenotypes—aligned sarcomere, presence of transverse tubules, positive force-frequency relationships, *etc*. Furthermore, strategies for long term cell survival, robust and consistent cell seeding in microfluidic channels, and controlling cell–cell and cell–ECM interactions to generate precise tissue structure-function relationships are matters that warrant more research exploration.

In addition, alternative materials for heart-on-chip fabrication need further investigation. PDMS is the popular material of choice for microfabrication-based prototyping of heart-on-chip devices owing to its highly compliance, deformable property that makes it applicable for microfluidic handling, and its ability to be plasma functionalized to bind multiple layers and networks of microstructures. PDMS micropillars and microposts can further be applied to mechanically stimulate cardiac cells and enhance their maturation and beating properties. The optical transparency of PDMS enables imaging of fluid flow, and its biocompatibility and oxygen permeability make it suitable for biological applications. However, PDMS also has some disadvantageous properties. Its gas permeability results in its susceptibility to bubble formation, and its hydrophobic nature can absorb and react with hydrophobic drugs and compounds in culture medium, compromising the reliability of drug screening experiments. Novel materials that feature flexibility, optical transparency, and non-absorption should be developed to overcome the challenges associated with PDMS. There is active research on integrating hydrophilic materials such as hydrogels and polyesters to ensure good cell attachment, while otherwise preventing drug absorption.

Fluid handling is an essential component in microfluidic devices. Different perfusion techniques such as syringe, vacuum, or peristaltic pumps are incorporated into heart-on-chip devices for continuous media circulation. Controlling culture parameters including nutrient and oxygen supply, cellular waste removal, and diffusion of metabolic or angiogenic factors is crucial to maintain a physiologically relevant cellular microenvironment. In support of this, external

tubing connections and large media reservoir can work at the laboratory scale as academic proof-of-concept prototypes, but built-in micropumps integrated into heart-on-chip platforms are better suited for commercialization and higher throughput. Heart-on-chips developed in academic institutions are mostly fabricated by soft lithography, which involves multiple assembly steps and lack reproducibility. Scalable manufacturing methods should also be further researched to enable large scale commercialization processes and to reduce manufacturing cost. 3D printing is one option currently being explored to automate and scale up the production of heart-on-chip devices.

Computational models of heart-on-chip systems require significant improvement in scale and reliability. Current multi-scale and multi-physics cardiac models are still at the initial stage, restricted by the computing capability. However, multi-physics simulation at various spatial and temporal levels are demanded, from nanoscale to macroscale and from nanoseconds to hours, to form a comprehensive understanding of cardiac mechanism and to establish the quantitative relationship between cardiac structure and function. In addition, current data-driven models are limited by modeling resolution, data-set scale and legibility, which limits immediate translation of computational modeling to be applied in benchtop. Computational models also have the potential to automate the efficient design of heart-on-chip platforms. While current heart-on-chip design processes follow the laborious top-bottom method, computational models that simulate the microfluidic system and solve the reverse problem can forge innovative paths toward automated and efficient design of chips that readily meets the researcher's needs.

Lastly, extraction of quantitative information through microsensors is a space that can be further refined. Current heart-on-chips rely on fluorescence microscopy to investigate electrophysiology and pharmacological modulation or use built-in electrochemical and optical sensors or force probes to monitor changes in cellular physiology and metabolism or measure biomechanical changes in cardiac cells and tissues. However, microscopic imaging and force probes constrain the throughput. To overcome this limitation, devices that can simultaneously track and output the functional changes of multiple tissues are needed. Higher throughput measurements at lower cost with fewer biological resources such as culture media and density of cells, noninvasive, and continuous sensing ability are some of the critical specifications to consider for novel sensor integrated heart-on-chip designs. Vibration based energy harvesters based on piezoelectric, triboelectric, electrostatic, or electromagnetic mechanisms have been applied in the *in vivo* heart to convert the mechanical beating into electrical energy, particularly for cardiac pacemakers and left-ventricular assist devices.[34] Smart materials and structures based on the four mechanisms offer highly sensitive sensing of low frequency vibrations. PZT or PVDF piezoelectric cantilever beams are some examples that can be mounted around the wrist or head and harvest energy from everyday movement.[10] There are also nanowire-based nanogenerators that can harvest mechanical energy from finger movements. Applications of these energy harvesters can expand into microfluidic heart-on-chips as embedded sensors that can accurately detect the low frequency beating motions of cardiomyocytes, which is a promising area of research.

CONCLUSIONS

To date, there has been significant strides in micro physiological devices or heart-on-chips that comprise biomaterials, tissue constructs, and specialized microenvironments housed in microfluidic hardware. These systems aim to recapitulate essential heart physiology and have potential for drug screening, disease modeling, and point-of-care diagnostics applications. The main fabrication methods and biomaterials applied in heart-on-chips are presented. Functionalization and/or maturation of cardiac cells and tissues *via* mechanical, electrical, and optical methods are compared, and advanced sensor technologies that measure the biomechanics and electrophysiology of the cardiac tissue are discussed extensively, particularly in the context of drug screening and disease modeling applications. However, *in vitro* experimental measurements and predictions are limited by reliability, throughput, production scalability. A framework that integrates both experimental heart-on-chip data and computational modeling and simulation can facilitate the mechanistic understanding of cardiac pathophysiology and further bridge the experimental and theoretical gaps. Particularly with respect to the drug development and screening pipeline to assess cardiotoxicity, testing all potential and relevant scenarios through clinical trials is simply not possible. Computational modeling based on data-driven simulations and advanced machine learning tools can not only enhance the predictive capability of *in vitro* models through a closed feedback loop but also expand the scope to testing numerous scenarios in virtual clinical trials.

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AUTHOR CONTRIBUTIONS

Conceptualization, JP, ZW; writing-original draft preparation, JP, ZW; writing-review and editing, JP, ZW, BZ, PS; supervision, JZ. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

[1]Abecasis, B., P. Gomes-Alves, S. Rosa, P. J. Gouveia, L. Ferreira, M. Serra, and P. M. Alves. Unveiling the molecular crosstalk in a human induced pluripotent stem cell-derived cardiac model. *Biotechnol. Bioeng.* 116(5):1245–1252, 2019. https://doi.org/10.1002/bit.26929.

[2]Adadi, N., M. Yadid, I. Gal, M. Asulin, R. Feiner, R. Edri, and T. Dvir. Electrospun fibrous PVDF-TrFe scaffolds for cardiac tissue engineering, differentiation, and maturation. *Adv. Mater. Technol.* 5(3):1900820, 2020. https://doi.org/10.1002/admt.201900820.

[3]Agarwal, A., J. A. Goss, A. Cho, M. L. McCain, and K. K. Parker. Microfluidic heart on a chip for higher throughput pharmacological studies. *Lab Chip.* 13(18):3599–3608, 2013. https://doi.org/10.1039/C3LC50350J.

[4]Aguado-Sierra, J., A. Krishnamurthy, C. Villongco, J. Chuang, E. Howard, M. J. Gonzales, J. Omens, D. E. Krummen, S. Narayan, R. C. P. Kerckhoffs, and A. D. McCulloch. Patient-specific modeling of dyssynchronous heart failure: a case study. *Prog. Biophys. Mol. Biol.* 107(1):147–155, 2011. https://doi.org/10.1016/j.pbiomolbio.2011.06.014.

[5]Alassaf, A., G. Tansik, V. Mayo, L. Wubker, D. Carbonero, and A. Agarwal. Engineering anisotropic cardiac monolayers on microelectrode arrays for non-invasive analyses of electrophysiological properties. *Analyst.* 145(1):139–149, 2020. https://doi.org/10.1039/C9AN01339C.

[6]Alonzo, M., S. AnilKumar, B. Roman, N. Tasnim, and B. Joddar. 3D bioprinting of cardiac tissue and cardiac stem cell therapy. *Transl. Res.* 211:64–83, 2019. https://doi.org/10.1016/j.trsl.2019.04.004.

[7]Arts, T., R. S. Reneman, and P. C. Veenstra. A model of the mechanics of the left ventricle. *Ann. Biomed. Eng.* 7(3):299–318, 1979. https://doi.org/10.1007/BF02364118.

[8]Arzani, A., G.-Y. Suh, R. L. Dalman, and S. C. Shadden. A longitudinal comparison of hemodynamics and intraluminal thrombus deposition in abdominal aortic aneurysms. *Am. J. Physiol.-Heart Circ. Physiol.* 307(12):H1786–H1795, 2014. https://doi.org/10.1152/ajpheart.00461.2014.

[9]Atzberger, P. J., P. R. Kramer, and C. S. Peskin. A stochastic immersed boundary method for fluid-structure dynamics at microscopic length scales. *J. Comput. Phys.* 224(2):1255–1292, 2007. https://doi.org/10.1016/j.jcp.2006.11.015.

[10]Bai, Y., P. Tofel, Z. Hadas, J. Smilek, P. Losak, P. Skarvada, and R. Macku. Investigation of a cantilever structured piezoelectric energy harvester used for wearable devices with random vibration input. *Mech. Syst. Signal Process.* 106:303–318, 2018. https://doi.org/10.1016/j.ymssp.2018.01.006.

[11]Belik, M. E.; Usyk, T. P.; McCulloch, A. D. Computational Methods for Cardiac Electrophysiology. In *Handbook of Numerical Analysis*; Computational Models for the Human Body; Elsevier, 2004; Vol. 12, pp 129–187. https://doi.org/10.1016/S1570-8659(03)12002-9.

[12]Bertassoni, L. E., M. Cecconi, V. Manoharan, M. Nikkhah, J. Hjortnaes, A. L. Cristino, G. Barabaschi, D. Demarchi, M. R. Dokmeci, Y. Yang, and A. Khademhosseini. Hydrogel bioprinted microchannel networks for vascularization of tissue engineering constructs. *Lab Chip.* 14(13):2202–2211, 2014. https://doi.org/10.1039/C4LC00030G.

[13]Beussman, K. M., M. L. Rodriguez, A. Leonard, N. Taparia, C. R. Thompson, and N. J. Sniadecki. Micropost arrays for measuring stem cell-derived cardiomyocyte contractility. *Methods.* 94:43–50, 2016. https://doi.org/10.1016/j.ymeth.2015.09.005.

[14]Blausen com. Medical Gallery of Blausen Medical 2014. *WikiJournal of Medicine* **2014**, *1* (2). https://doi.org/10.15347/WJM/2014.010.

[15]Burridge, P. W., A. Holmström, and J. C. Wu. Chemically defined culture and cardiomyocyte differentiation of human pluripotent stem cells. *Curr. Protoc. Human Genet.* 87(1):10, 2015. https://doi.org/10.1002/0471142905.hg2103s87.

[16]Campbell, K. S. Filament compliance effects can explain tension overshoots during force development. *Biophys. J.* 91(11):4102–4109, 2006. https://doi.org/10.1529/biophysj.106.087312.

[17]Cardiac Electrophysiology: From Cell to Bedside - 7th Edition https://www.elsevier.com/books/cardiac-electrophysiology-from-cell-to-bedside/zipes/978-0-323-44733-1 (accessed 2021 -07 -22).

[18]Campbell, S. G., F. V. Lionetti, K. S. Campbell, and A. D. McCulloch. Coupling of adjacent tropomyosins enhances cross-bridge-mediated cooperative activation in a Markov model of the cardiac thin filament. *Biophys. J.* 98(10):2254–2264, 2010. https://doi.org/10.1016/j.bpj.2010.02.010.

[19]Cansız, B., H. Dal, and M. Kaliske. Computational cardiology: a modified hill model to describe the electro-visco-elasticity of the myocardium. *Comput. Methods Appl. Mech. Eng.* 315:434–466, 2017. https://doi.org/10.1016/j.cma.2016.10.009.

[20]Chan, Y.-C., S. Ting, Y.-K. Lee, K.-M. Ng, J. Zhang, Z. Chen, C.-W. Siu, S. K. W. Oh, and H.-F. Tse. Electrical stimulation promotes maturation of cardiomyocytes derived from human embryonic stem cells. *J. Cardiovasc. Trans. Res.* 6(6):989–999, 2013. https://doi.org/10.1007/s12265-013-9510-z.

[21]Chen, G.-H., J. Tang, and S. Leng. Prior image constrained compressed sensing (PICCS): a method to accurately reconstruct dynamic CT images from highly

undersampled projection data sets. *Med. Phys.* 35(2):660–663, 2008. https://doi.org/10.1118/1.2836423.
[22]Chen, Y., L. Zhang, and G. Chen. Fabrication, modification, and application of poly(methyl methacrylate) microfluidic chips. *Electrophoresis*. 29(9):1801–1814, 2008. https://doi.org/10.1002/elps.200700552.
[23]Cheng, W., N. Klauke, H. Sedgwick, G. L. Smith, and J. M. Cooper. Metabolic monitoring of the electrically stimulated single heart cell within a microfluidic platform. *Lab Chip*. 6(11):1424–1431, 2006. https://doi.org/10.1039/B608202E.
[24]Cherry, E. M., J. R. Ehrlich, S. Nattel, and F. H. Fenton. Pulmonary vein reentry—properties and size matter: insights from a computational analysis. *Heart Rhythm*. 4(12):1553–1562, 2007. https://doi.org/10.1016/j.hrthm.2007.08.017.
[25]Choi, J. R., K. W. Yong, J. Y. Choi, and A. C. Cowie. Recent advances in photo-crosslinkable hydrogels for biomedical applications. *BioTechniques*. 66(1):40–53, 2019. https://doi.org/10.2144/btn-2018-0083.
[26]Christensen, R. H., B. J. von Scholten, L. L. Lehrskov, P. Rossing, and P. G. Jørgensen. Epicardial adipose tissue: an emerging biomarker of cardiovascular complications in type 2 diabetes? *Therap. Adv. Endocrinol.* 2020. https://doi.org/10.1177/2042018820928824.
[27]Cohen-Karni, T., Q. Qing, Q. Li, Y. Fang, and C. M. Lieber. Graphene and nanowire transistors for cellular interfaces and electrical recording. *Nano Lett*. 10(3):1098–1102, 2010. https://doi.org/10.1021/nl1002608.
[28]Colin-York, H., and M. Fritzsche. The future of traction force microscopy. *Curr. Opin. Biomed. Eng*. 5:1–5, 2018. https://doi.org/10.1016/j.cobme.2017.10.002.
[29]Correia, C., A. Koshkin, P. Duarte, D. Hu, M. Carido, M. J. Sebastião, P. Gomes-Alves, D. A. Elliott, I. J. Domian, A. P. Teixeira, P. M. Alves, and M. Serra. 3D aggregate culture improves metabolic maturation of human pluripotent stem cell derived cardiomyocytes. *Biotechnol. Bioeng*. 115(3):630–644, 2018. https://doi.org/10.1002/bit.26504.
[30]Daniel, T. L., A. C. Trimble, and P. Bryant Chase. Compliant realignment of binding sites in muscle: transient behavior and mechanical tuning. *Biophys. J.* 74(4):1611–1621, 1998. https://doi.org/10.1016/S0006-3495(98)77875-0.
[31]Dasi, L. P., H. A. Simon, P. Sucosky, and A. P. Yoganathan. Fluid mechanics of artificial heart valves. *Clin. Exp. Pharmacol. Physiol*. 36(2):225–237, 2009. https://doi.org/10.1111/j.1440-1681.2008.05099.x.
[32]Decock, J., M. Schlenk, and J.-B. Salmon. In situ photopatterning of pressure-resistant hydrogel membranes with controlled permeabilities in PEGDA microfluidic channels. *Lab Chip*. 18(7):1075–1083, 2018. https://doi.org/10.1039/C7LC01342F.
[33]Deutsch, J., D. Motlagh, B. Russell, and T. A. Desai. Fabrication of microtextured membranes for cardiac myocyte attachment and orientation. *J. Biomed. Mater. Res.* 53(3):267–275, 2000. https://doi.org/10.1002/(SICI)1097-4636(2000)53:3%3c267::AID-JBM12%3e3.0.CO;2-J.
[34]Dong, L., A. B. Closson, C. Jin, I. Trase, Z. Chen, and J. X. J. Zhang. Vibration-energy-harvesting system: transduction mechanisms, frequency tuning techniques, and biomechanical applications. *Adv. Mater. Technol.* 4(10):1900177, 2019. https://doi.org/10.1002/admt.201900177.
[35]Dvir, T., B. P. Timko, M. D. Brigham, S. R. Naik, S. S. Karajanagi, O. Levy, H. Jin, K. K. Parker, R. Langer, and D. S. Kohane. Nanowired three-dimensional cardiac patches. *Nat. Nanotechnol.* 6(11):720–725, 2011. https://doi.org/10.1038/nnano.2011.160.
[36]Eisenberg, E., and T. L. Hill. Muscle contraction and free energy transduction in biological systems. *Science*. 227(4690):999–1006, 1985. https://doi.org/10.1126/science.3156404.
[37]Eleonora, G., P. V. Sandeep, V. Niels, W. J. Antony, D. Dobromir, and J. José. Human atrial action potential and Ca^{2+} model. *Circ. Res.* 109(9):1055–1066, 2011. https://doi.org/10.1161/CIRCRESAHA.111.253955.
[38]Ellis, B. W., A. Acun, U. I. Can, and P. Zorlutuna. Human IPSC-derived myocardium-on-chip with capillary-like flow for personalized medicine. *Biomicrofluidics*. 11(2):24105, 2017. https://doi.org/10.1063/1.4978468.
[39]Fenwick, A. J., A. M. Wood, and B. C. W. Tanner. Effects of cross-bridge compliance on the force-velocity relationship and muscle power output. *PLoS ONE*.12(12):e0190335, 2017. https://doi.org/10.1371/journal.pone.0190335.
[40]FitzHugh, R. Impulses and physiological states in theoretical models of nerve membrane. *Biophys. J.* 1(6):445–466, 1961. https://doi.org/10.1016/S0006-3495(61)86902-6.
[41]Friend, J., and L. Yeo. Fabrication of microfluidic devices using polydimethylsiloxane. *Biomicrofluidics*. 4(2):26502, 2010. https://doi.org/10.1063/1.3259624.
[42]Giridharan, G. A., M.-D. Nguyen, R. Estrada, V. Parichehreh, T. Hamid, M. A. Ismahil, S. D. Prabhu, and P. Sethu. Microfluidic cardiac cell culture model (μCCCM). *Anal. Chem.* 82(18):7581–7587, 2010. https://doi.org/10.1021/ac1012893.
[43]Grandi, E., F. S. Pasqualini, and D. M. Bers. A novel computational model of the human ventricular action potential and Ca transient. *J. Mol. Cell. Cardiol.* 48(1):112–121, 2010. https://doi.org/10.1016/j.yjmcc.2009.09.019.
[44]Griffith, B. E., X. Luo, D. M. McQUEEN, and C. S. Peskin. Simulating the fluid dynamics of natural and prosthetic heart valves using the immersed boundary method. *Int. J. Appl. Mech.* 01(01):137–177, 2009. https://doi.org/10.1142/S1758825109000113.
[45]Grosberg, A., A. P. Nesmith, J. A. Goss, M. D. Brigham, M. L. McCain, and K. K. Parker. Muscle on a chip: in vitro contractility assays for smooth and striated muscle. *J. Pharmacol. Toxicol. Methods*. 65(3):126–135, 2012.
[46]Guccione, J. M., A. D. McCulloch, and L. K. Waldman. Passive material properties of intact ventricular myocardium determined from a cylindrical model. *J. Biomech. Eng.* 113(1):42–55, 1991. https://doi.org/10.1115/1.2894084.
[47]Gurev, V., J. Constantino, J. J. Rice, and N. A. Trayanova. Distribution of electromechanical delay in the heart: insights from a three-dimensional electromechanical model. *Biophys. J.* 99(3):745–754, 2010. https://doi.org/10.1016/j.bpj.2010.05.028.
[48]Habibi, M., S. T. M. Dawson, and A. Arzani. Data-driven pulsatile blood flow physics with dynamic mode decomposition. *Fluids*. 5(3):111, 2020. https://doi.org/10.3390/fluids5030111.
[49]Hayes, H. B., A. M. Nicolini, C. A. Arrowood, S. A. Chvatal, D. W. Wolfson, H. C. Cho, D. D. Sullivan, J. Chal, B. Fermini, M. Clements, J. D. Ross, and D. C.

Millard. Novel method for action potential measurements from intact cardiac monolayers with multiwell microelectrode array technology. *Sci. Rep.* 9(1):11893, 2019. https://doi.org/10.1038/s41598-019-48174-5.

[50]Hill, A. V. The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond. Series B Biol. Sci.* 126(843):136–195, 1938. https://doi.org/10.1098/rspb.1938.0050.

[51]Hill, T. L., E. Eisenberg, Y. D. Chen, and R. J. Podolsky. Some self-consistent two-state sliding filament models of muscle contraction. *Biophys. J.* 15(4):335–372, 1975. https://doi.org/10.1016/S0006-3495(75)85823-1.

[52]Hodgkin (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. The Journal of Physiology - Wiley Online Library https://physoc.onlinelibrary.wiley.com/doi/abs/https://doi.org/10.1113/jphysiol.1952.sp004764 (accessed 2021 -04 -02).

[53]Huang, J., P. M. Carrica, and F. Stern. Semi-coupled air/water immersed boundary approach for curvilinear dynamic overset grids with application to ship hydrodynamics. *Int. J. Numer. Methods Fluids.* 58(6):591–624, 2008. https://doi.org/10.1002/fld.1758.

[54]Huebsch, N., P. Loskill, N. Deveshwar, C. I. Spencer, L. M. Judge, M. A. Mandegar, B. C. Fox, T. M. A. Mohamed, Z. Ma, A. Mathur, A. M. Sheehan, A. Truong, M. Saxton, J. Yoo, D. Srivastava, T. A. Desai, P.-L. So, K. E. Healy, and B. R. Conklin. Miniaturized IPS-cell-derived cardiac muscles for physiologically relevant drug response analyses. *Sci. Rep.* 6(1):24726–24726, 2016. https://doi.org/10.1038/srep24726.

[55]Hund, T. J., and Y. Rudy. Rate dependence and regulation of action potential and calcium transient in a canine cardiac ventricular cell model. *Circulation.* 110(20):3168–3174, 2004. https://doi.org/10.1161/01.CIR.0000147231.69595.D3.

[56]Huxley, A. F. Muscle structure and theories of contraction. *Prog. Biophys. Biophys. Chem.* 7:255–318, 1957.

[57]Huxley, A. F., and R. M. Simmons. Proposed mechanism of force generation in striated muscle. *Nature.* 233(5321):533–538, 1971. https://doi.org/10.1038/233533a0.

[58]Israeli, Y., M. Gabalski, K. Ball, A. Wasserman, J. Zou, G. Ni, C. Zhou, and A. Aguirre. Generation of heart organoids modeling early human cardiac development under defined conditions. *JAMA.* 2020. https://doi.org/10.1101/2020.06.25.171611.

[59]Iyer, V., R. Mazhari, and R. L. Winslow. A computational model of the human left-ventricular epicardial myocyte. *Biophys. J.* 87(3):1507–1525, 2004. https://doi.org/10.1529/biophysj.104.043299.

[60]Jie, X., V. Gurev, and N. Trayanova. Mechanisms of mechanically induced spontaneous arrhythmias in acute regional ischemia. *Circ. Res.* 106(1):185–192, 2010. https://doi.org/10.1161/CIRCRESAHA.109.210864.

[61]Jung, H., K. Sung, K. S. Nayak, E. Y. Kim, and J. C. Ye. K-t FOCUSS: a general compressed sensing framework for high resolution dynamic MRI. *Magn. Reson. Med.* 61(1):103–116, 2009. https://doi.org/10.1002/mrm.21757.

[62]Kamakura, T., T. Makiyama, K. Sasaki, Y. Yoshida, Y. Wuriyanghai, J. Chen, T. Hattori, S. Ohno, T. Kita, M. Horie, S. Yamanaka, and T. Kimura. Ultrastructural maturation of human-induced pluripotent stem cell-derived cardiomyocytes in a long-term culture. *Circ. J.* 77(5):1307–1314, 2013. https://doi.org/10.1253/circj.CJ-12-0987.

[63]Kaushik, G., and A. J. Engler. Chapter nine - from stem cells to cardiomyocytes: the role of forces in cardiac maturation, aging, and disease. In: Progress in Molecular Biology and Translational Science. Mechanotransduction, edited by A. J. Engler, and S. Kumar. Academic Press: Boca Raton, 2014.

[64]Kayvanpour, E., T. Mansi, F. Sedaghat-Hamedani, A. Amr, D. Neumann, B. Georgescu, P. Seegerer, A. Kamen, J. Haas, K. S. Frese, M. Irawati, E. Wirsz, V. King, S. Buss, D. Mereles, E. Zitron, A. Keller, H. A. Katus, D. Comaniciu, and B. Meder. Towards personalized cardiology: multi-scale modeling of the failing heart. *PLos ONE.*10(7):e0134869, 2015. https://doi.org/10.1371/journal.pone.0134869.

[65]Kerckhoffs, R. C. P., O. P. Faris, P. H. M. Bovendeerd, F. W. Prinzen, K. Smits, E. R. McVEIGH, and T. Arts. Timing of depolarization and contraction in the paced canine left ventricle: model and experiment. *J. Cardiovasc. Electrophysiol.* 14(s10):S188–S195, 2003. https://doi.org/10.1046/j.1540.8167.90310.x.

[66]Kerckhoffs, R. C. P., S. N. Healy, T. P. Usyk, and A. D. McCulloch. Computational methods for cardiac electromechanics. *Proc. IEEE.* 94(4):769–783, 2006. https://doi.org/10.1109/JPROC.2006.871772.

[67]Kim, D.-S., Y. W. Choi, A. Shanmugasundaram, Y.-J. Jeong, J. Park, N.-E. Oyunbaatar, E.-S. Kim, M. Choi, and D.-W. Lee. Highly durable crack sensor integrated with silicone rubber cantilever for measuring cardiac contractility. *Nat. Commun.* 11(1):535–535, 2020. https://doi.org/10.1038/s41467-019-14019-y.

[68]Kim, D.-H., P. Kim, I. Song, J. M. Cha, S. H. Lee, B. Kim, and K. Y. Suh. Guided three-dimensional growth of functional cardiomyocytes on polyethylene glycol nanostructures. *Langmuir.* 22(12):5419–5426, 2006. https://doi.org/10.1021/la060283u.

[69]Kim, D.-H., J. Park, K. Y. Suh, P. Kim, S. K. Choi, S. Ryu, S. Park, S. H. Lee, and B. Kim. Fabrication of patterned micromuscles with high activity for powering biohybrid microdevices. *Sens. Actuat. B.* 117(2):391–400, 2006. https://doi.org/10.1016/j.snb.2005.11.051.

[70]Klauke, N., G. L. Smith, and J. Cooper. Stimulation of single isolated adult ventricular myocytes within a low volume using a planar microelectrode array. *Biophys. J.* 85(3):1766–1774, 2003. https://doi.org/10.1016/S0006-3495(03)74606-2.

[71]Kolanowski, T. J., M. Busek, M. Schubert, A. Dmitrieva, B. Binnewerg, J. Pöche, K. Fisher, F. Schmieder, S. Grünzner, S. Hansen, A. Richter, A. El-Armouche, F. Sonntag, and K. Guan. Enhanced structural maturation of human induced pluripotent stem cell-derived cardiomyocytes under a controlled microenvironment in a microfluidic system. *Acta Biomater.* 102:273–286, 2020. https://doi.org/10.1016/j.actbio.2019.11.044.

[72]Kroll, K., M. Chabria, K. Wang, F. Häusermann, F. Schuler, and L. Polonchuk. Electro-mechanical conditioning of human IPSC-derived cardiomyocytes for translational research. *Prog. Biophys. Mol. Biol.* 130:212–222, 2017. https://doi.org/10.1016/j.pbiomolbio.2017.07.003.

[73]Lai, J. H., G. Kajiyama, R. L. Smith, W. Maloney, and F. Yang. Stem cells catalyze cartilage formation by neonatal articular chondrocytes in 3D biomimetic hydrogels. *Sci. Rep.* 3(1):3553, 2013. https://doi.org/10.1038/srep03553.

[74]Land, S., and S. A. Niederer. A spatially detailed model of isometric contraction based on competitive binding of troponin I. Explains cooperative interactions between tropomyosin and crossbridges. *PLoS Comput. Biol.* 11(8):e1004376, 2015. https://doi.org/10.1371/journal.pcbi.1004376.

[75]Land, S., S.-J. Park-Holohan, N. P. Smith, C. G. dos Remedios, J. C. Kentish, and S. A. Niederer. A model of cardiac contraction based on novel measurements of tension development in human cardiomyocytes. *J. Mol. Cell. Cardiol.* 106:68–83, 2017. https://doi.org/10.1016/j.yjmcc.2017.03.008.

[76]Landesberg, A., and S. Sideman. Coupling calcium binding to troponin C and cross-bridge cycling in skinned cardiac cells. *Am. J. Physiol.-Heart Circ. Physiol.* 266(3):H1260–H1271, 1994. https://doi.org/10.1152/ajpheart.1994.266.3.H1260.

[77]Lapp, H., T. Bruegmann, D. Malan, S. Friedrichs, C. Kilgus, A. Heidsieck, and P. Sasse. Frequency-dependent drug screening using optogenetic stimulation of human IPSC-derived cardiomyocytes. *Sci. Rep.* 7(1):9629–9629, 2017. https://doi.org/10.1038/s41598-017-09760-7.

[78]Lee, A. W. C., A. Crozier, E. R. Hyde, P. Lamata, M. Truong, M. Sohal, T. Jackson, J. M. Behar, S. Claridge, A. Shetty, E. Sammut, G. Plank, C. A. Rinaldi, and S. Niederer. Biophysical modeling to determine the optimization of left ventricular pacing site and AV/VV delays in the acute and chronic phase of cardiac resynchronization therapy. *J. Cardiovasc. Electrophysiol.* 28(2):208–215, 2017. https://doi.org/10.1111/jce.13134.

[79]Lee, S.-Y., D.-S. Kim, E.-S. Kim, and D.-W. Lee. Nano-textured polyimide cantilever for enhancing the contractile behavior of cardiomyocytes and its application to cardiac toxicity screening. *Sens. Actuat. B.*301:126995, 2019. https://doi.org/10.1016/j.snb.2019.126995.

[80]Lind, J. U., T. A. Busbee, A. D. Valentine, F. S. Pasqualini, H. Yuan, M. Yadid, S.-J. Park, A. Kotikian, A. P. Nesmith, P. H. Campbell, J. J. Vlassak, J. A. Lewis, and K. K. Parker. Instrumented cardiac microphysiological devices via multimaterial three-dimensional printing. *Nat. Mater.* 16(3):303–308, 2017. https://doi.org/10.1038/nmat4782.

[81]Lindblad, D. S., C. R. Murphey, J. W. Clark, and W. R. Giles. A model of the action potential and underlying membrane currents in a rabbit atrial cell. *Am. J. Physiol. Heart Circ. Physiol.* 271(4):H1666–H1696, 1996. https://doi.org/10.1152/ajpheart.1996.271.4.H1666.

[82]Liu, H., O. A. Bolonduro, N. Hu, J. Ju, A. A. Rao, B. M. Duffy, Z. Huang, L. D. Black, and B. P. Timko. Heart-on-a-chip model with integrated extra- and intracellular bioelectronics for monitoring cardiac electrophysiology under acute hypoxia. *Nano Lett.* 20(4):2585–2593, 2020. https://doi.org/10.1021/acs.nanolett.0c00076.

[83]Liu, J., N. Sun, M. A. Bruce, J. C. Wu, and M. J. Butte. Atomic force mechanobiology of pluripotent stem cell--derived cardiomyocytes. *PLoS ONE.*7(5):e37559, 2012. https://doi.org/10.1371/journal.pone.0037559.

[84]Lundy, S. D., W.-Z. Zhu, M. Regnier, and M. A. Laflamme. Structural and functional maturation of cardiomyocytes derived from human pluripotent stem cells. *Stem Cells Dev.* 22(14):1991–2002, 2013. https://doi.org/10.1089/scd.2012.0490.

[85]Luo, C. H., and Y. Rudy. A dynamic model of the cardiac ventricular action potential. I. Simulations of ionic currents and concentration changes. *Circ. Res.* 74(6):1071–1096, 1994. https://doi.org/10.1161/01.RES.74.6.1071.

[86]Ma, D., H. Wei, Y. Zhao, J. Lu, G. Li, N. B. E. Sahib, T. H. Tan, K. Y. Wong, W. Shim, P. Wong, S. A. Cook, and R. Liew. Modeling type 3 long QT syndrome with cardiomyocytes derived from patient-specific induced pluripotent stem cells. *Int. J. Cardiol.* 168(6):5277–5286, 2013. https://doi.org/10.1016/j.ijcard.2013.08.015.

[87]Mahajan, A., Y. Shiferaw, D. Sato, A. Baher, R. Olcese, L.-H. Xie, M.-J. Yang, P.-S. Chen, J. G. Restrepo, A. Karma, A. Garfinkel, Z. Qu, and J. N. Weiss. A rabbit ventricular action potential model replicating cardiac dynamics at rapid heart rates. *Biophys. J.* 94(2):392–410, 2008. https://doi.org/10.1529/biophysj.106.98160.

[88]Marsano, A., C. Conficconi, M. Lemme, P. Occhetta, E. Gaudiello, E. Votta, G. Cerino, A. Redaelli, and M. Rasponi. Beating heart on a chip: a novel microfluidic platform to generate functional 3D cardiac microtissues. *Lab Chip.* 16(3):599–610, 2016. https://doi.org/10.1039/C5LC01356A.

[89]Mironov, V., R. P. Visconti, V. Kasyanov, G. Forgacs, C. J. Drake, and R. R. Markwald. Organ printing: tissue spheroids as building blocks. *Biomaterials.* 30(12):2164–2174, 2009. https://doi.org/10.1016/j.biomaterials.2008.12.084.

[90]Mozneb, M., A. Mirza, and C.-Z. Li. Non-invasive plasmonic based real time characterization of cardiac drugs on cardiomyocytes functional behavior. *Anal. Chem.* 92(2):2244–2250, 2019. https://doi.org/10.1021/acs.analchem.9b04956.

[91]Murphy, S. V., and A. Atala. 3D bioprinting of tissues and organs. *Nat. Biotechnol.* 32(8):773–785, 2014. https://doi.org/10.1038/nbt.2958.

[92]Nash, M. P., and P. J. Hunter. Computational mechanics of the heart. *J. Elast.* 61(1):113–141, 2000. https://doi.org/10.1023/A:1011084330767.

[93]Nguyen, M.-D., J. P. Tinney, F. Yuan, T. J. Roussel, A. El-Baz, G. Giridharan, B. B. Keller, and P. Sethu. Cardiac cell culture model as a left ventricle mimic for cardiac tissue generation. *Anal. Chem.* 85(18):8773–8779, 2013. https://doi.org/10.1021/ac401910d.

[94]Niederer, S. A., and N. P. Smith. A mathematical model of the slow force response to stretch in rat ventricular myocytes. *Biophys. J.* 92(11):4030–4044, 2007. https://doi.org/10.1529/biophysj.106.095463.

[95]Niederer, S. A., and N. P. Smith. The role of the frank-starling law in the transduction of cellular work to whole organ pump function: a computational modeling analysis. *PLoS Comput. Biol.*5(4):e1000371, 2009. https://doi.org/10.1371/journal.pcbi.1000371.

[96]Noble, D., and Y. Rudy. Models of cardiac ventricular action potentials: iterative interaction between experiment and simulation. *Philos. Trans. R. Soc. Lond. Series A.* 359(1783):1127–1142, 2001. https://doi.org/10.1098/rsta.2001.0820.

[97]Norotte, C., F. S. Marga, L. E. Niklason, and G. Forgacs. Scaffold-free vascular tissue engineering using bioprinting. *Biomaterials.* 30(30):5910–5917, 2009. https://doi.org/10.1016/j.biomaterials.2009.06.034.

[98]Nunes, S. S., J. W. Miklas, J. Liu, R. Aschar-Sobbi, Y. Xiao, B. Zhang, J. Jiang, S. Massé, M. Gagliardi, A. Hsieh, N. Thavandiran, M. A. Laflamme, K. Nanthakumar, G. J. Gross, P. H. Backx, G. Keller, and M. Radisic. Biowire: a platform for maturation of human pluripotent

stem cell-derived cardiomyocytes. *Nat. Methods*. 10(8):781–787, 2013. https://doi.org/10.1038/nmeth.2524.
[99]Nygren, A., C. Fiset, L. Firek, J. W. Clark, D. S. Lindblad, R. B. Clark, and W. R. Giles. Mathematical model of an adult human atrial cell. *Circ. Res*. 82(1):63–81, 1998. https://doi.org/10.1161/01.RES.82.1.63.
[100]Ogasawara, T., S. Okano, H. Ichimura, S. Kadota, Y. Tanaka, I. Minami, M. Uesugi, Y. Wada, N. Saito, K. Okada, K. Kuwahara, and Y. Shiba. Impact of extracellular matrix on engraftment and maturation of pluripotent stem cell-derived cardiomyocytes in a rat myocardial infarct model. *Sci. Rep*. 7(1):8630, 2017. https://doi.org/10.1038/s41598-017-09217-x.
[101]Oyunbaatar, N.-E., D.-H. Lee, S. J. Patil, E.-S. Kim, and D.-W. Lee. Biomechanical characterization of cardiomyocyte using PDMS pillar with microgrooves. *Sensors*. 2016. https://doi.org/10.3390/s16081258.
[102]Pandit, S. V., R. B. Clark, W. R. Giles, and S. S. Demir. A mathematical model of action potential heterogeneity in adult rat left ventricular myocytes. *Biophys. J*. 81(6):3029–3051, 2001. https://doi.org/10.1016/S0006-3495(01)75943-7.
[103]Parsa, H., B. Z. Wang, and G. Vunjak-Novakovic. A microfluidic platform for the high-throughput study of pathological cardiac hypertrophy. *Lab Chip*. 17(19):3264–3271, 2017. https://doi.org/10.1039/C7LC00415J.
[104]Pavesi, A., F. Piraino, G. B. Fiore, K. M. Farino, M. Moretti, and M. Rasponi. How to embed three-dimensional flexible electrodes in microfluidic devices for cell culture applications. *Lab Chip*. 11(9):1593–1595, 2011. https://doi.org/10.1039/C1LC20084D.
[105]Peskin, C. S. The immersed boundary method. *Acta Numer*. 11:479–517, 2002. https://doi.org/10.1017/S0962492902000077.
[106]Plouffe, B. D., M. A. Brown, R. K. Iyer, M. Radisic, and S. K. Murthy. Controlled capture and release of cardiac fibroblasts using peptide-functionalized alginate gels in microfluidic channels. *Lab Chip*. 9(11):1507–1510, 2009. https://doi.org/10.1039/B823523F.
[107]Qian, F., C. Huang, Y.-D. Lin, A. N. Ivanovskaya, T. J. O'Hara, R. H. Booth, C. J. Creek, H. A. Enright, D. A. Soscia, A. M. Belle, R. Liao, F. C. Lightstone, K. S. Kulp, and E. K. Wheeler. Simultaneous electrical recording of cardiac electrophysiology and contraction on chip. *Lab Chip*. 17(10):1732–1739, 2017. https://doi.org/10.1039/C7LC00210F.
[108]Rastogi, S. K., J. Bliley, D. J. Shiwarski, G. Raghavan, A. W. Feinberg, and T. Cohen-Karni. Graphene microelectrode arrays for electrical and optical measurements of human stem cell-derived cardiomyocytes. *Cell. Mol. Bioeng*. 11(5):407–418, 2018. https://doi.org/10.1007/s12195-018-0525-z.
[109]Razumova, M. V., A. E. Bukatina, and K. B. Campbell. Stiffness-distortion sarcomere model for muscle simulation. *J. Appl. Physiol*. 87(5):1861–1876, 1999. https://doi.org/10.1152/jappl.1999.87.5.1861.
[110]Rice, J. J., G. Stolovitzky, Y. Tu, and P. P. de Tombe. Ising model of cardiac thin filament activation with nearest-neighbor cooperative interactions. *Biophys. J*. 84(2):897–909, 2003. https://doi.org/10.1016/S0006-3495(03)74907-8.
[111]Rice, J. J., R. L. Winslow, and W. C. Hunter. Comparison of putative cooperative mechanisms in cardiac muscle: length dependence and dynamic responses. *Am. J. Physiol. Heart Circ. Physiol*. 276(5):H1734–H1754, 1999. https://doi.org/10.1152/ajpheart.1999.276.5.H1734.
[112]Scherl, I., B. Strom, J. K. Shang, O. Williams, B. L. Polagye, and S. L. Brunton. Robust principal component analysis for modal decomposition of corrupt fluid flows. *Phys. Rev. Fluids*.5(5):054401, 2020. https://doi.org/10.1103/PhysRevFluids.5.054401.
[113]SCLS-Channel. Multi-scale multi-physics heart simulator UT-heart.
[114]Seo, H.-R., H. J. Joo, D. H. Kim, L.-H. Cui, S.-C. Choi, J.-H. Kim, S. W. Cho, K. B. Lee, and D.-S. Lim. Nanopillar surface topology promotes cardiomyocyte differentiation through Cofilin-mediated cytoskeleton rearrangement. *ACS Appl. Mater. Interfaces*. 9(20):16803–16812, 2017. https://doi.org/10.1021/acsami.7b01555.
[115]Shang, Y., Z. Chen, F. Fu, L. Sun, C. Shao, W. Jin, H. Liu, and Y. Zhao. Cardiomyocyte-driven structural color actuation in anisotropic inverse opals. *ACS Nano*. 13(1):796–802, 2019. https://doi.org/10.1021/acsnano.8b08230.
[116]Souders, C. A., S. L. K. Bowers, and T. A. Baudino. Cardiac fibroblast: the renaissance cell. *Circ. Res*. 105(12):1164–1176, 2009. https://doi.org/10.1161/CIRCRESAHA.109.209809.
[117]Stancescu, M., P. Molnar, C. W. McAleer, W. McLamb, C. J. Long, C. Oleaga, J.-M. Prot, and J. J. Hickman. A phenotypic in vitro model for the main determinants of human whole heart function. *Biomaterials*. 60:20–30, 2015. https://doi.org/10.1016/j.biomaterials.2015.04.035.
[118]Sun, J., and H. Tan. Alginate-based biomaterials for regenerative medicine applications. *Materials*. 2013. https://doi.org/10.3390/ma6041285.
[119]Suzuki, I., M. Fukuda, K. Shirakawa, H. Jiko, and M. Gotoh. Carbon nanotube multi-electrode array chips for noninvasive real-time measurement of dopamine, action potentials, and postsynaptic potentials. *Biosens. Bioelectron*. 49:270–275, 2013. https://doi.org/10.1016/j.bios.2013.05.023.
[120]Takeuchi, M., T. Kozuka, E. Kim, A. Ichikawa, Y. Hasegawa, Q. Huang, and T. Fukuda. On-chip fabrication of cell-attached microstructures using photo-cross-linkable biodegradable hydrogel. *J. Funct. Biomater*. 11(1):18, 2020. https://doi.org/10.3390/jfb11010018.
[121]Tan, H. Y., W. K. Loke, and N.-T. Nguyen. A reliable method for bonding polydimethylsiloxane (PDMS) to polymethylmethacrylate (PMMA) and its application in micropumps. *Sens. Actuat. B*. 151(1):133–139, 2010. https://doi.org/10.1016/j.snb.2010.09.035.
[122]Tanaka, Y., K. Morishima, T. Shimizu, A. Kikuchi, M. Yamato, T. Okano, and T. Kitamori. Demonstration of a PDMS-based bio-microactuator using cultured cardiomyocytes to drive polymer micropillars. *Lab Chip*. 6(2):230–235, 2006. https://doi.org/10.1039/B512099C.
[123]Taylor, D. Fewer new drugs from the pharmaceutical industry. *BMJ*. 326(7386):408–409, 2003. https://doi.org/10.1136/bmj.326.7386.408.
[124]Tewari, S. G., S. M. Bugenhagen, B. M. Palmer, and D. A. Beard. Dynamics of cross-bridge cycling, ATP hydrolysis, force generation, and deformation in cardiac muscle. *J. Mol. Cell. Cardiol*. 96:11–25, 2016. https://doi.org/10.1016/j.yjmcc.2015.02.006.
[125]Tian, B., T. Cohen-Karni, Q. Qing, X. Duan, P. Xie, and C. M. Lieber. Three-dimensional, flexible nanoscale field-effect transistors as localized bioprobes. *Science*.

329(5993):830–834, 2010. https://doi.org/10.1126/science.1192033.
[126]Timko, B. P., T. Cohen-Karni, G. Yu, Q. Qing, B. Tian, and C. M. Lieber. Electrical recording from hearts with flexible nanowire device arrays. *Nano Lett*. 9(2):914–918, 2009. https://doi.org/10.1021/nl900096z.
[127]Trayanova, N. A., and R. Winslow. Whole-heart modeling. *Circ. Res*. 108(1):113–128, 2011. https://doi.org/10.1161/CIRCRESAHA.110.223610.
[128]Ugolini, G. S., M. Rasponi, A. Pavesi, R. Santoro, R. Kamm, G. B. Fiore, M. Pesce, and M. Soncini. On-chip assessment of human primary cardiac fibroblasts proliferative responses to uniaxial cyclic mechanical strain. *Biotechnol. Bioeng*. 113(4):859–869, 2016. https://doi.org/10.1002/bit.25847.
[129]Usyk, T. P., and A. D. McCulloch. Relationship between regional shortening and asynchronous electrical activation in a three-dimensional model of ventricular electromechanics. *J. Cardiovasc. Electrophysiol*. 14(s10):S196–S202, 2003. https://doi.org/10.1046/j.1540.8167.90311.x.
[130]Virtanen, J., M. Toivanen, T. Toimela, T. Heinonen, and S. Tuukkanen. Direct measurement of contraction force in human cardiac tissue model using piezoelectric cantilever sensor technique. *Curr. Appl. Phys*. 20(1):155–160, 2020. https://doi.org/10.1016/j.cap.2019.10.020.
[131]Vreeker, A., L. van Stuijvenberg, T. J. Hund, P. J. Mohler, P. G. J. Nikkels, and T. A. B. van Veen. Assembly of the cardiac intercalated disk during pre- and postnatal development of the human heart. *PLoS ONE*.9(4):e94722, 2014. https://doi.org/10.1371/journal.pone.0094722.
[132]Waldbaur, A., H. Rapp, K. Länge, and B. E. Rapp. Let there be chip—towards rapid prototyping of microfluidic devices: one-step manufacturing processes. *Analy. Methods*. 3(12):2681–2716, 2011. https://doi.org/10.1039/C1AY05253E.
[133]Wang, L., W. Dou, M. Malhi, M. Zhu, H. Liu, J. Plakhotnik, Z. Xu, Q. Zhao, J. Chen, S. Chen, R. Hamilton, C. A. Simmons, J. T. Maynes, and Y. Sun. Microdevice platform for continuous measurement of contractility, beating rate, and beating rhythm of human-induced pluripotent stem cell-cardiomyocytes inside a controlled incubator environment. *ACS Appl. Mater. Interfaces*. 10(25):21173–21183, 2018. https://doi.org/10.1021/acsami.8b05407.
[134]Washio, T., J. Okada, S. Sugiura, and T. Hisada. Approximation for cooperative interactions of a spatially-detailed cardiac sarcomere model. *Cel. Mol. Bioeng*. 5(1):113–126, 2012. https://doi.org/10.1007/s12195-011-0219-2.
[135]Weber, A. On the role of calcium in the activity of adenosine 5′-triphosphate hydrolysis by actomyosin. *J. Biol. Chem*. 234(10):2764–2769, 1959. https://doi.org/10.1016/S0021-9258(18)69777-7.
[136]Wei, X., C. Qin, C. Gu, C. He, Q. Yuan, M. Liu, L. Zhuang, H. Wan, and P. Wang. A novel bionic *in vitro* bioelectronic tongue based on cardiomyocytes and microelectrode array for bitter and umami detection. *Biosens. Bioelectron*.145:111673, 2019. https://doi.org/10.1016/j.bios.2019.111673.
[137]Werdich, A. A., E. A. Lima, B. Ivanov, I. Ges, M. E. Anderson, J. P. Wikswo, and F. J. Baudenbacher. A microfluidic device to confine a single cardiac myocyte in a sub-nanoliter volume on planar microelectrodes for extracellular potential recordings. *Lab Chip*. 4(4):357–362, 2004. https://doi.org/10.1039/B315648F.
[138]Wong, A. Y. K. Mechanics of cardiac muscle, based on Huxley's model: mathematical simulation of isometric contraction. *J. Biomech*. 4(6):529–540, 1971. https://doi.org/10.1016/0021-9290(71)90042-X.
[139]Woodcock, E. A., and S. J. Matkovich. Cardiomyocytes structure, function and associated pathologies. *Int. J. Biochem. Cell Biol*. 37(9):1746–1751, 2005. https://doi.org/10.1016/j.biocel.2005.04.011.
[140]Xin, M., E. N. Olson, and R. Bassel-Duby. Mending broken hearts: cardiac development as a basis for adult heart regeneration and repair. *Nat. Rev. Mol. Cell. Biol*. 14(8):529–541, 2013. https://doi.org/10.1038/nrm3619.
[141]Yakushenko, A., Z. Gong, V. Maybeck, B. Hofmann, E. Gu, M. D. Dawson, A. Offenhaeusser, and B. Wolfrum. On-chip optical stimulation and electrical recording from cells. *J. Biomed. Opt*. 18(11):1–8, 2013. https://doi.org/10.1117/1.JBO.18.11.111402.
[142]Yang, M., and X. Zhang. Electrical assisted patterning of cardiac myocytes with controlled macroscopic anisotropy using a microfluidic dielectrophoresis chip. *Sens. Actuat. A*. 135(1):73–79, 2007. https://doi.org/10.1016/j.sna.2006.06.071.
[143]Yin, S., X. Zhang, C. Zhan, J. Wu, J. Xu, and J. Cheung. Measuring single cardiac myocyte contractile force via moving a magnetic bead. *Biophys. J*. 88(2):1489–1495, 2005. https://doi.org/10.1529/biophysj.104.048157.
[144]Yoshida, S., S. Miyagawa, S. Fukushima, T. Kawamura, N. Kashiyama, F. Ohashi, T. Toyofuku, K. Toda, and Y. Sawa. Maturation of human induced pluripotent stem cell-derived cardiomyocytes by soluble factors from human mesenchymal stem cells. *Mol. Therapy*. 26(11):2681–2695, 2018. https://doi.org/10.1016/j.ymthe.2018.08.012.
[145]Zahalak, G. I. A distribution-moment approximation for kinetic theories of muscular contraction. *Math. Biosci*. 55(1):89–114, 1981. https://doi.org/10.1016/0025-5564(81)90014-6.
[146]Zhang, B., A. Korolj, B. F. L. Lai, and M. Radisic. Advances in organ-on-a-chip engineering. *Nat. Rev. Mater*. 3(8):257–278, 2018. https://doi.org/10.1038/s41578-018-0034-7.