Current Tissue Microenvironment Reports (2020) 1:61–72
https://doi.org/10.1007/s43152-020-00007-8

CELL BEHAVIOR MANIPULATION (S WILLERTH, SECTION EDITOR)

Modeling the Response of Heart Muscle to Mechanical Stimulation In Vitro

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Published online: 30 May 2020

Abstract

Purpose of Review This review summarizes current in vitro tools used for developing engineered heart muscle and studying the response of these tissues to mechanical loading changes that simulate the mechanical cues that heart muscle experiences in vivo.

Recent Findings Advances in stem cell technology allow researchers to model genetically inherited disease phenotypes in vitro. However, one major challenge for in vitro disease modeling and drug screening is that the cardiomyocytes in simple monolayer culture are normally not exposed to the mechanical stimuli that the heart experiences in vivo. Mechanical loading is critical to heart development and pathophysiology. In vitro models have shown that cells in culture can respond to mechanical conditioning, and in some cases, this condition is critical for maturing stem cell-derived cardiomyocytes.

Summary Tools from mechanobiology have broadened the view of how physical cues guide cardiomyocyte behavior and function. This review highlights in vitro technologies for developing cardiac models and studying cardiac responses to mechanical loading.

Keywords Induced pluripotent stem cell (iPSC) · Cardiomyocytes · Preload · Afterload

Introduction

The field of mechanobiology has advanced our understanding of how physical forces and mechanical properties of the microenvironment control cell development and behavior [1, 2]. Changes in mechanical cues such as extracellular matrix (ECM) rigidity affect the progression of diseases including fibrosis, cardiomyopathies, and cancer [3–6]. The heart is an especially interesting organ from a mechanobiology perspective because heart muscle cells (cardiomyocytes) not only interact with a mechanically complex ECM environment; they are subjected to passive stretch as the heart fills (preload) and must overcome systemic vascular resistance (afterload) during ejection. Postnatally, mammalian cardiomyocytes are almost exclusively post-mitotic [7]; therefore, as the heart enlarges during childhood, it is through individual cells becoming larger (hypertrophy). A significant part of this hypertrophy is due to a strong developmental cue: the mechanical stresses the growing heart experiences [8, 9]. Nonetheless, excess mechanical loading can cause heart failure [10, 11]. The relationship between increased loading and heart dysfunction makes intuitive sense—the heart becomes an overworked pump. However, the sophisticated mechanisms that cardiomyocytes use to sense and respond to different types and intensities of mechanical loading (which normally act to prevent such failure) are still unclear. Current developments in cardiac disease modeling should improve our integrative understanding of the roles of mechanical stimuli together with soluble chemical cues and genotypes in creating the cardiomyocyte phenotype and help us discover new therapeutic targets.

Cardiac mechanics and physiology have been studied for over a century. Ex vivo studies on animal heart muscle made tremendous contributions in establishing a direct link between preload (passive stretch on the heart, related to resting sarcomere length) and cardiac output (related to ejection fraction), which has been described as the Frank-Starling mechanism

This article is part of the Topical Collection on *Cell Behavior Manipulation*

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[12]. Similarly, in 1912, Anrep described the relationship between the pressure that heart must overcome during contraction (afterload) and cardiac output [13]. Since then, the effects of mechanical loading on heart muscle have been studied extensively with animal models, both in vivo and ex vivo. The extensive literature in this area is reviewed elsewhere [14]. To study how chronic changes in mechanical loading lead to heart remodeling and disease progression in vivo, scientists have developed surgical maneuvers to artificially increase afterload (e.g., transverse aortic constriction, TAC) and preload (e.g., shunt) in rodent and other animal models. These studies have shown that preload and afterload induce distinct phenotypes, such as different cardiac remodeling and gene expression [15••].

Despite the power of animal models in characterizing potential mechanisms for mechanical loading-induced disease and arrhythmia, there are two major limitations of these systems: First, the cardiovascular system works differently in order to meet the demands of different species [14]. Although mice are commonly used because of their short gestation time and genetic tractability, substantial differences in mouse versus human cardiac electrophysiology make it challenging to use these models to predict how specific mutations and drugs could cause arrhythmias in humans [16]. For example, drugs that cause heart rhythm disorders often tend to disrupt repolarizing currents through a potassium ion channel protein (K_V11.1) encoded by the human ether-à-go-go-related gene (hERG/KCNH2) [17]. Although the gene itself is expressed in rodents, the actual current contributed by K_V11.1 is negligible in these organisms [18]. Second, the surgical procedures used to manipulate preload and afterload affect other organs like the kidneys and cause systemic inflammation [19, 20], making it a challenge to determine cardiomyocyte-specific effects. Thus, developing in vitro human cardiomyocyte-based models to study mechanical loading effects is an essential, complementary approach to investigate cardiac function and pathophysiology.

Isolated human cardiomyocytes and tissue from donated non-transplantable human hearts are alternatives to animal models that allow studies on physiology and drug response in adult human heart cells. They are functionally mature and relatively intact, allowing analysis of signaling and tissue-level physiology. However, both isolated cardiomyocytes and heart slices lose critical morphological and functional characteristics (including conduction velocity) soon after their removal from the body [21]. Furthermore, because non-transplantable human hearts are a rare and precious resource, it is not feasible to use primary adult human heart muscle to study mechanisms of rare inherited diseases like cardiomyopathy, or for routine drug screening.

The availability of human pluripotent stem cells (PSC), together with the capability of differentiating these cells into cardiomyocytes [22, 23], overcomes many of the limitations of classic animal models and donated human heart tissue. The discovery of human-induced pluripotent stem cells (iPSC) [24, 25] has circumvented the ethical challenges involved with human embryonic stem cells (hESC) and allows PSC to be derived from patients harboring specific inherited diseases [26, 27]. Differentiation of PSC to cardiomyocytes in vitro involves mimicking the dynamic activation and deactivation of molecular signaling pathways (including the Wnt pathway) that occurs during heart development [28]. Protocol development and optimization require trade-offs among cost, reproducibility, and the quality of cardiomyocytes derived from the PSC. Widely used methods include: (i) monolayer differentiation using growth factors [29] or small molecule Wnt signaling agonists and antagonists [23, 30]; (ii) embryoid body-based suspension culture using growth factors/fetal bovine serum [31]; and (iii) co-culture of PSC with visceral endoderm-like cell lines (e.g., END-2), which produce chemical factors to induce differentiation [32]. Detailed comparisons between differentiation approaches are provided elsewhere [26, 33, 34].

Cardiomyocyte differentiation efficiency and the quality of cardiomyocytes derived from differentiation methods are characterized through molecular analysis of specific cardiac markers (e.g., cardiac muscle troponin T) and by functional assessment of PSC-derived cardiomyocytes (PSC-CM). PSC-CM function is characterized by assessing cellular physiology, for example, action potential [23]. Cell function is also assessed by measuring the response to drugs with known effects on the heart (e.g., isoproterenol) [35•]. A common challenge with hESC-derived cardiomyocytes (hESC-CM) and iPSC-derived cardiomyocytes (iPSC-CM) is that the cells have fetal cardiomyocyte like morphology (e.g., underdeveloped sarcomere and T-tubule networks) [33]. Although PSC-CM are developmentally immature compared with adult cardiomyocytes, the ability to derive these cells from a renewable iPSC source, coupled with the ability to control their genetic background, has made these cells extremely useful for disease modeling and some preclinical drug testing [36]. Meanwhile, progress has been made to force these cells to achieve a more mature, adult-like phenotype. Comprehensive reviews on addressing the challenge of maturing iPSC-CM are provided elsewhere [37, 38].

This article seeks to summarize current in vitro platforms involving iPSC-CM that leverage tools from mechanobiology to enhance our ability to model and understand disease processes of the heart. We will start with the in vitro studies establishing iPSC-CM disease models and review the microenvironment of the cardiomyocytes. Next, we will focus on the application of mechanobiology tools to mimic the mechanical cues that these cells receive in vivo. Finally, we will discuss technologies combining mechanical cues with functional readouts in analyzing cardiomyocyte behavior in vitro.

Current In Vitro Cardiomyocytes Models

Cardiovascular disease traces back to ancient times. For example, atherosclerosis was found in Egyptian mummies [39]. Today, heart disease is the leading cause of death worldwide [40]. Factors that lead to cardiac dysfunction can be broadly classified into three major categories (Fig. 1a): (i) genetics, which causes most congenial heart disease and is strongly associated with cardiomyopathy; (ii) soluble biochemical cues stemming from hormonal signaling, infections, drugs, and environmental pollution; and (iii) mechanical cues, including loading on the heart caused by blood pressure changes [41], and other reasons (e.g., physical activities) [42]. Importantly, many factors that are known to influence heart disease pathogenesis act through both mechanical and soluble means. For example, diet influences blood pressure (mechanical) but also provides cues (e.g., soluble fatty acids) that influence cardiomyocyte biology. Exercise provides mechanical loading but also regulates soluble levels of adrenergic stimuli.

Robust production of cardiomyocytes upon iPSC differentiation [23, 30] allows scientists to develop models of genetically linked heart disease in vitro and to study cardiotoxic effects of a variety of chemical compounds [43, 44]. Diseases studied include: (1) ion channelopathies arising patients within structurally normal hearts, such as long QT syndrome (LQTS) [45]; (2) intrinsic structure cardiomyopathies, for example, hypertrophic cardiomyopathy (HCM) [46, 47] and familial dilated cardiomyopathy (DCM) [48]; and (3) other cardiomyopathies, including mutations in intercellular desmosome protein such as arrhythmogenic right ventricular cardiomyopathy (ARVC) [49] and Barth syndrome [50, 51]. The use of patient-specific or genome edited iPSC to study genetically inherited diseases is a rapidly growing field, and comprehensive reviews on the disease models can be found elsewhere [33, 36].

Although iPSC-based heart disease models that used simplistic culture substrates were important to establish the possibility of studying inherited diseases in vitro (Fig. 1b), these models left out one key aspect of cardiovascular pathophysiology: mechanics. The heart is subjected to a complex set of mechanical cues, including constantly changing hemodynamic stresses, which play an essential role in heart behavior and function. For example, athletes' hearts undergo physiological hypertrophy because of chronic volume overload (preload), leading to larger diastolic dimension and thicker walls than normal hearts [52]. Interestingly, extreme levels of exercises are actually detrimental to heart function, reflecting an upper loading limit that the human heart is capable of adapting to [53]. The primary pumping function of the heart balances the hemodynamic pressure of the whole body; disturbing the hemodynamic balance can cause life-threatening disease. For example, hypertension (elevated afterload) was the most common cause of heart failure before the development of effective antihypertensive medicines and today remains a frequent cause of heart dysfunction [54]. iPSC-CM formed on tissue culture polystyrene are not exposed to the same mechanical cues as cardiomyocytes in a human heart. Therefore, understanding the mechanical environment of the heart and harnessing tools from mechanobiology to mimic in vivo-like iPSC-CM based models will better delineate the progression and underlying molecular causes of heart diseases. Based on current in vitro studies, it is not clear whether cardiomyocytes need mechanical stimulation to fully mature. However, electrically stimulated exercise of iPSC-CM based tissues against defined mechanical strains has led to some of the most convincing evidence to date of maturation in these cells [55•].

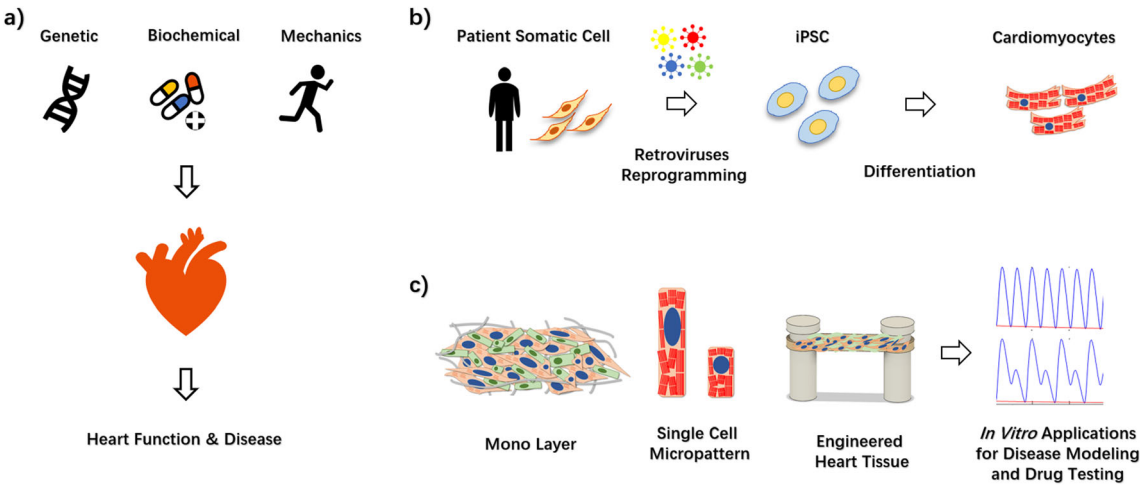


Fig. 1 Modeling cardiomyocyte pathophysiology in vitro. **a** Three major factors that influence heart function and disease progression: genetics, biochemical, and mechanical cues. **b** Cardiomyocytes production using human iPSC reprogrammed from somatic cells. **c** Human iPSC-derived cardiomyocytes in different cellular configurations (2D monolayer; single cardiomyocytes or 3D engineered cardiac tissue) for in vitro disease modeling and drug testing applications

Microenvironment of the Cardiomyocytes

Mechanical cues are an essential part of cardiomyocytes' microenvironment. Two main mechanical cues are (i) the external loadings that come from outside of the heart, primarily preload and afterload as described earlier, which will be the main focus of this section; and (ii) the passive viscoelastic properties (elasticity, shear modulus) of the tissue itself, which is determined by cells and ECM, which will be discussed in the later section.

Preload is defined by resting sarcomere length in mechanically relaxed cardiomyocytes. In vivo increases in venous return cause corresponding increases in end-diastolic volume and pressure, which in turn change the preload acting on individual cardiomyocytes. Changes in resting sarcomere length alter the overlap between myofilaments and motor proteins that pull against them to generate cardiomyocyte contractions, accounting for the Frank-Starling effect [16, 56]: increasing preload enhances cardiac contractility. However, pathological levels of preload can also inhibit the overlap between myofilaments and motor proteins. In vivo, chronic levels of pathological preload cause eccentric hypertrophy and reduced ejection fraction [8].

Afterload is defined as the pressure the heart works against to eject blood and is related to aortic pressure for the left ventricle and pulmonary artery pressure for the right ventricle. Increases in afterload may cause increases in end-systolic pressure and decreases in stroke volume [57]. In vivo, secondary to the afterload increase, end-diastolic volume (preload) is likely to increase to provide compensatory contractility via the Frank-Starling mechanism [16, 56]. On a cellular level, increases in afterload provoke changes in the sodium-calcium exchanger that result in Ca^{2+} buildup within the sarcoplasmic reticulum, which increases the contractile force generated by cardiac muscle [58]. Chronic afterload elevation can be detrimental for the heart and cause heart disorders such as arrhythmia, heart failure, and maladaptive remodeling [59]. Similar to the preload, afterload triggers cardiac responses/changes from organ-level remodeling to molecular level gene expression. However, conditions that selectively elevate preload versus afterload lead to distinct clinical presentation [57], as well as different phenotypes and molecular signaling in animal models [15]. Knowing the importance of the mechanical cues in heart development and disease and faced with the intrinsic limitations of animal models, it is reasonable to use biophysical tools to recapitulate these cues in vitro.

Studying the Effects of Mechanical Loading on Cardiomyocytes In Vitro

Diverse mechanobiology tools in established iPSC-CM models have been used to show that physical conditioning advances maturation of cardiomyocytes isolated from immature, neonatal rodent hearts [60] and differentiated from iPSC [55•]. Within in vitro models, mechanical loading also affects cardiomyocyte contractility [61••], induces remodeling [62••], activates signaling pathways [63], and regulates gene expression [6, 64].

To study the effects of preload, in vitro models often use tools to manipulate tension within cardiomyocytes, mimicking the effects of volume overload (Fig. 2a). Initial mechanical conditioning/stretch is critical for cardiomyocytes' alignment [35•, 65••]. Aligned, pre-stressed heart cells can be generated in 2D using surface patterning techniques like microcontact printing [66]. Organotypic 3D engineered heart muscles (EHM), typically formed by culturing cardiomyocytes in hydrogels, can also be subjected to passive tension if the engineered tissue is formed under anisotropic boundary conditions. In previous studies, these boundary conditions have been provided by two posts/pillars/bio-wires [55•, 65••, 67–69•], by aligned tissue patches [70], or by anisotropic tissue pinning to a substrate [35•] shown in Fig. 2b.

To dynamically model the changes in preload, active stretch is applied to monolayers and tissues. In 2D, cardiomyocytes can be grown atop stretchable elastomeric membranes which can then be subjected to cyclic stretch with commercially available (e.g., Flexcell) or custom-built devices. Applied substrate cyclic stretch has been used to study how mechanical loading can improve cardiomyocyte gap junction assembly [71]. Other 2D studies have shown that cyclic stretch led to maladaptive remodeling [72] and pathologic conduction in cells that are genetically prone to develop ARVC [73].

In 3D, cell-encapsulating ECM such as collagen type I or fibrin gel is generally required for improved 3D tissue mechanical strength; although in some cases, micro-tissues can been formed without exogenous ECM [35•, 74]. Most dynamic stretching models studying preload effects use a linear motor arm [72, 75••, 76, 77], non-contact electromagnet force [78], or biomimetic volume control chamber [79] to actively apply stretches to the substances which cardiac tissue attached to. Fink et al. [75••] applied cyclic stretch to EHM fabricated from neonatal rodent cardiomyocytes; cardiac hypertrophy and increased contractility were seen. Later on, different dynamic loading approaches demonstrated that cyclic stretch induces cardiac structure remodeling (e.g., cell alignment [76] and eccentric hypertrophy [78]) and affects proliferation rate [76], calcium handling [79], maturation [78, 79], and gene expression [79] of iPSC-CM. In vivo, the peak systolic strain is in the range of 15% to 20% [80]; in the reviewed studies, the stretch conditions range from 5 to 20% strain with 1–3 Hz of the cyclic stretch frequency. Based on one study conducted by Fink et al. [75••]; 4% to 20% stretches all generate significant higher contractile force compared with non-stretched tissue. However, how different cyclic strain amplitudes and frequencies affect cardiomyocytes behavior and maturation has not

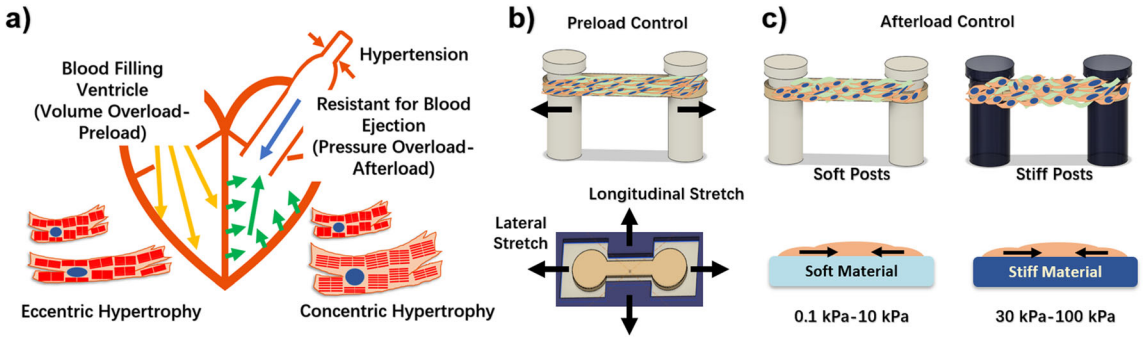


Fig. 2 Modeling cardiac hemodynamic loading in vitro. **a** Two types of hemodynamic loadings heart experiences in vivo: blood filling into ventricles causing stretched cardiomyocytes, which relates to volume overload (preload), and cardiomyocytes contracts against pressure overload (afterload) to eject blood to the body. Mechanical loading causes changes in cell morphology; typically, preload induces cardiac eccentric hypertrophy, and afterload induces cardiac concentric hypertrophy [8]. **b** Control preload in vitro by applying tension/stretch for engineered cardiac muscle. **c** Control afterload in vitro by changing local environment (e.g., materials properties or material geometries) that cardiomyocytes contract against

yet been investigated; whether there is an optimal strain for cardiac tissue maturation is yet to be determined.

In contrast to changes in boundary conditions to model preload, the majority of attempts to model cardiac afterload in vitro use material science tools to manipulate the stiffnesses of the substrates [61••, 81–83], the posts/pillars [58, 62••, 84•, 85], and the fibers [86•] which engineered tissues attach to and contract against. In 2D, substrate stiffness is often controlled by culturing cardiomyocytes on ECM protein-grafted synthetic hydrogels, such as bis-acrylamide [61, 82]. In 3D, cardiac tissues are formed within the posts/pillar configuration (Fig. 2c), the fabrication of the posts/pillars often uses polydimethylsiloxane (PDMS), and the stiffness of which can be precisely controlled by (i) simply changing the material composition to change the material elasticity [84•, 87] or (ii) changing the geometry such as diameter and active height of each posts/pillar [58, 85] to change the moment of inertia. Besides PDMS, photo-curable polymer fibers [86•] can also be used to form cardiac tissue. Similarly, the elasticity of the polymer fibers can be controlled by changing the fiber geometry such as diameter. Despite the versatility of polymers that can be used to control cardiac mechanical environment, one of the challenges for studying afterload effects is that it is hard to dynamically tune afterload in situ. Two studies conducted by Rodriguez et al. [88] and Corbin et al. [89] used magnetic approaches to dynamically and reversibly change cardiac afterload with the presence of cardiomyocytes. Both approaches used PDMS to encapsulate ferromagnetic particles; the PDMS stiffness was then manipulated by changing the magnetic field strength. A similar system based on magnetoactive hydrogel showed that the elasticity of the material can change as rapidly as 30 s upon changing the magnetic flux density [90]. A challenge with magnetic-based actuation is that the ferromagnetic particles themselves may aggregate or have cytotoxicity; this has been addressed by Abdeen et al. by coating the ferromagnetic particles [90].

Table 1 Mechanobiology platforms for mimicking dynamic preload and afterload in vitro

Hemodynamic loading	Methods	Notes
Cyclic stretch for dynamic preload control	Uniaxial stretch pillars [75••], posts [76], and chamber rings [72] that cardiomyocytes are attached to	Linear motor is used to control stretch elongation and frequency; non-direct stretch of cardiac tissues requires strong adhesion
	Gelfoam scaffold [78] with cardiomyocytes is constructed on the apparatus with non-contact electromagnetic force to provide uniaxial stretch	Tissue formed within 3D scaffold constructs, cell adhesion to scaffold is not as critical compared with posts configuration
	3D cardiac tissue in biomimetic chamber [79] that is capable of controlling the filling/stretch of the tissue	Dynamic stretch using volume control, strong cell adhesion, and fluid circulation system is required
Reversible afterload control	PDMS mixed and cured with iron particles [89]; using different magnet spacing to change magnetic field and manipulate PDMS elasticity; 2D cardiomyocytes were formed on PDMS substrate	Iron particles can make substrates opaque; some particles may cause potential toxicity problems for long-term culture
	Encapsulate stainless steel wire in PDMS material [88]; using magnet to control magnetic field in order to change stiffness of the posts. 3D collagen encapsulated cardiac tissue was formed between two posts	Relatively low throughput; technically challenging; tissue deterioration was reported over long-term culture

Table 1 summarizes current mechanobiology tools used to dynamically control preload and afterload in vitro, along with some challenges.

Studying the Effects of Tissue Mechanical Properties on Cardiomyocytes In Vitro

In addition to sensing changes in active mechanical forces (preload and afterload), cardiomyocytes also experience changes in the mechanics of their local tissue environment. In vivo, cardiac ECM contains structural proteins like collagen (approximately 90%) [91], growth factors like bone morphogenetic protein, and other components such as glycosaminoglycans. ECM provides structural support, guides cell behaviors (e.g., adhesion, migration, proliferation, and differentiation) [92], and contributes to mechanical properties of tissue itself (e.g., increased myocardial stiffness from the access production of ECM) [6]. Some changes in cardiac ECM and associated tissue rigidity are linked to preload and afterload and involve responses of cardiac resident fibroblasts [6]. Soluble cues like inflammatory cytokines and changes in ECM composition and rigidity can activate fibroblasts to myofibroblasts. Myofibroblasts are highly contractile cells that secrete marked amounts of ECM to cause rapid wound closure [6]. Despite the immediate need for wound closure during cardiac events like infarction, long-term effects of myofibroblast activation cause cardiac dysfunction [93].

Leveraging polymer technology, natural or synthetic polymer-based hydrogels can be applied to in vitro cardiac models as ECM structures to study the effects that changes in matrix composition and rigidity have on cardiomyocytes. As mechanosensing may be different in 2D compared with 3D cultures [94], 3D cardiac tissue with or without ECM has been established to better recapitulate in vivo-like cell-cell and cell-ECM mechanics and structural configuration in vitro. More than 50 years ago, scientists were able to form 3D mini spherical hearts using rat ventricle cardiomyocytes (without ECM encapsulation); in vivo-like cell-cell orientation and intercalated disk were observed [95]. In 1997, Eschenhagen et al. formed 3D beating EHM using embryonic chick cardiomyocytes encapsulated into collagen gel [65••]. Hydrogels derived from natural ECM are biodegradable and have intrinsic biological recognition [96]. The effects of ECM composition on the formation and function of EHM have been studied by comparing the behavior of EHM formed in collagen type I [65••] vs. in other types of ECM gel, like fibrinogen [84•]. A major drawback of using natural endogenous ECM-forming proteins is that it is challenging to control the rigidity of these gels without affecting other properties like the density of cell-adhesive peptide motifs [94]. Nevertheless, some researchers have used blends of different ECM types to dissect out a role for mechanics in overall EHM function [97, 98]. In addition, the high viscosity and rapid gelation time of these materials present challenges in developing miniaturized tissues for large-scale drug screening. Synthetic hydrogel, or natural hydrogels with synthetic modifications (e.g., gelatin methacryloyl, GelMA) are alternatives to natural ECM. Using synthetic hydrogels enables precise control of substrate elasticity. This allows researchers to mimic the range of elasticity of the human heart in developmental stages (~ 1 kPa), in a healthy adult (10–15 kPa), and during fibrosis (30–90 kPa) [99] within the presence of cells [100, 101]. The synthetic hydrogel enables scientists to investigate cardiomyocyte and fibroblast responses to defined mechanical cues in both 2D [102] and 3D [103]. However, challenges associated with relying on synthetic hydrogels to study cardiac mechanobiology include the following: difficulty in retrieving cells from materials that are not enzymatically degradable (e.g., poly(ethylene glycol) based gels) and in achieving similar EHM function (e.g., contractility) as compared with what is observed with natural ECM materials [104]. Researchers also attempted to solubilize decellularized ECM from animals and process into synthetic modified hydrogel to achieve the native mechanical and biochemical environment of the heart [105, 106]; nevertheless, the limitation of using decellularized ECM is that it is difficult to control the biochemical composition of the ECM. Moreover, the decellularized ECM concentration has to be low for gelation and desired mechanical properties [106]. Overall, various ECM components have opened up many possibilities for consistent 3D cardiac tissue formation in most of the established in vitro cardiac tissue models; in the future, combining natural and synthetic ECM has the potential to mimic in vivo microenvironment and allow researchers to study the effects of cardiac mechanics in vitro.

Combining Mechanical Cues with Functional Readouts in Analyzing Cardiomyocyte Mechanobiology In Vitro

Mechanobiology tools make it possible for in vitro cardiomyocytes to receive the "in vivo-like" mechanical stimulation with different types of mechanical loadings as inputs. An additional challenge is the need to integrate tools used for mechanical conditioning with technology to assess not only how this changes traditional biochemical read-outs (e.g., gene and protein expression) but also how it affects cardiomyocyte physiology. Heart muscle contraction is a complex process driven initially by changes in transmembrane ion channel flux that cause cellular depolarization (action potential), leading to calcium uptake and calcium-induced calcium release from intracellular stores. This calcium next interacts with sarcomere proteins to trigger contraction. Efficient heart pumping and physiological adaptation to hemodynamic changes requires exquisite coordination of all these different processes, both during the initiation of action potential and contraction and

subsequently during electrical repolarization and mechanical relaxation [16].

Techniques for evaluating biochemical readouts such as cell morphology (e.g., sarcomere immunostaining) and gene expression following standard molecular cell biology methods (e.g., qPCR or RNA-seq to analyze gene expression) are relatively similar across different labs. In contrast, differences in the platforms used to control cardiomyocyte loading often necessitate changes in the way physiology is measured. Physiology measurements are challenging and often require specialized equipment such as force transducers, which have relatively lower cost than more sophisticated force measurements such as atomic force microscopy (AFM); both force measures are invasive. Non-invasive methods normally require build-in devices that use biomaterials with defined mechanical properties to measure cardiac contractility, for example, forming cardiomyocytes on PDMS material and using traction force microscopy (TFM) to evaluate contractility; the material cost of the device is relatively low. However, imaging and computational cost of TFM method can be relatively high compared with the above mentioned invasive measures. Nevertheless, refinement of existing technologies and development of new technologies, specifically those that integrate measurements of contractility and electrophysiology in real time, are crucial to understanding how cardiomyocytes respond to different biomechanical inputs.

Understanding cardiomyocyte electrical activity first requires measurement of the action potential, which involves rapid flux of ions across the cell membrane. Technologies to quantify action potential include optical techniques such as voltage sensitive dyes [107] and fluorescence proteins [108], which can be used for both 2D and 3D cardiac systems with relatively low cost; and non-optical techniques including single cell patch clamp (special skills required with relatively higher cost) and microelectrode arrays (MEA) [109]. The action potential happens over a very rapid timescale, and characterizing this event with imaging techniques is challenging and produces large quantities of data, making non-optical approaches especially attractive. Non-optical techniques are often used for 2D cardiac models; however, it is relatively difficult for electrodes to obtain accurate readings of the thicker tissues, although some progress has been made in this area [110]. Action potential activates calcium release from sarcoplasmic reticulum (SR), which is a vital step for subsequent contractility. Optical techniques are often used for visualizing calcium dynamics, for example, chemical molecules that chelate calcium ions such as Fura-2 [111] or a genetically encoded calcium indicator (GCaMP) [112, 113]. Using optical imaging tools for cardiac voltage and calcium measurement have been discussed elsewhere [114].

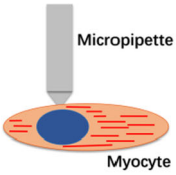
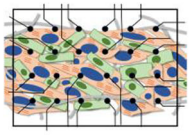
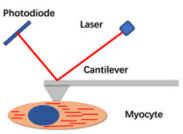
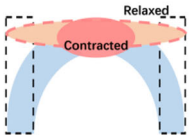
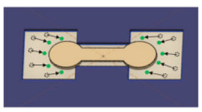
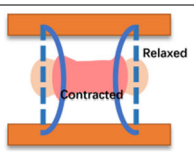
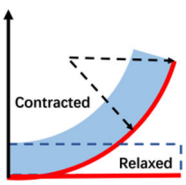
Cardiomyocyte electrical activity and calcium handling ultimately lead to contractility, which is closely related to cardiac output and heart function. Measuring contractility is an important aspect of characterizing cardiac physiology. Like electrophysiology measurements, cardiac contraction measurement can also be implemented using optical and non-optical methods. Direct non-optical measurement of the cardiomyocytes/tissue contraction includes force transducer [35•] and AFM [115, 116]. Contraction force can also be calculated knowing the deformation and material properties of the substances to which cardiomyocytes are attached [117, 118••]; technologies for measuring contractility are shown in Table 2.

Conclusion and Future Perspective

More than a decade ago, the availability of iPSC and the robust production of cardiomyocytes upon iPSC differentiation revolutionized our ability to model inherited diseases in vitro. One of the challenges is the developmental immaturity of the iPSC-CM, which is under intensive investigation and optimization from biochemical [119, 120], electrical [55•], and mechanical [84•] aspects. Another translational obstacle of iPSC-CM is its physiological environment of the simplistic cellular model. With the increasing attention on mechanobiology and the advanced technologies benefit from biomaterials and tissue engineering, versatile tools have been developed and incorporated into the in vitro cardiac model. However, the diversity of the mechanobiology tools sometimes makes it difficult to standardize the cardiac behavior, for example, cyclic stretch induces cardiac alignment, maturity, and contractility [76], but can also cause pathological remodeling of the cardiomyocytes [72]. One challenge is to determine the physiological and pathological level of load conditions for iPSC-CM models. Given different cardiac configurations (e.g., cell sources, cell numbers, ECM, platforms, etc.), it is undoubtedly difficult to set a baseline control value among different models. However, a physiological cardiac model should ideally be capable of simulating a series of loading conditions that reconcile the in vivo physiology from different development stages. More importantly, it will be critical to benchmark the response of cells obtained from in vitro studies to the responses observed in clinical settings and in various animal models of cardiac mechanical loading, to discern whether the same molecular pathways observed and studied in vitro predict the molecular changes that will occur with biophysical stimulation in vivo.

Ultimately, higher throughput, scalable technologies to study cardiomyocyte mechanobiology will be desired, as well as organ-on-a-chip [98] technologies for integrated analysis of organ-level functions and molecular markers. Potential tools equipped with dynamic control of mechanical inputs to simulate complex physiology conditions as well as simple readouts of electrophysiology and contractility are needed. As the field and technologies continue to mature and grow, increased

Table 2 Current tools for evaluating cardiac electrophysiology and contractility

Cardiac Function	Tools	Strengths	Limitations	Schematic
Action Potential	Patch Clamp [45]	Accurate	Invasive and terminal, labor intensive, advanced technical skill required	
	Microelectrode Array (MEA) [109]	Medium throughput, user friendly; non-invasive; capable of mapping conduction velocity	Hard to translate field potential to action potential; may be challenging to obtain readings for 3D tissue	
	Optical methods using voltage sensitive dye [107]	Non-invasive; capable of mapping conduction velocity; chronic imaging	Limited excitation and emission profile; temporal resolution	
Calcium Transient	Genetically encoded calcium indicator (GCaMP) [112,113]	Cell specific calcium mapping; chronic imaging	Requires genetic manipulation	
	Chemical molecule probe [110]	Wide range of excitation/emission spectra and affinities for calcium	Short term imaging; may have cytotoxicity	
Contractility	AFM [115,116]/ Force transducer [35•]	Direct cell/tissue measurement; accurate	Invasive; terminal; no force map	
	Post [55,62••]/pillar [65••] displacement	Relatively simple force calculation	Not continuous adhesion	
	Traction Force Microscopy (TFM) [61••]	Absolute measurement with tunable substrate stiffness; traction map output	High computation cost; optical noise	
	Bio-wire [69•,117]/fiber [86•] deflection	Direct measurement, 2D force map	Fabrication cost and throughput	
	Embedded cantilever for continuous force measurement [118••]	Continuous, real-time force reading	Fabrication cost and throughput	

predictability with accessible fabrication processes will make it possible to transform some preclinical drug testing to in vitro 3D micro-physiological systems.

Acknowledgments We have tried to present a concise review of the current methods of the engineered heart muscle, but we may have missed some significant publications that have contributed to the field. We apologize to any colleagues whose work was not cited in this article. We would like to acknowledge the staff of the Writing Center at Washington University in St. Louis for their feedback on this article.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human Studies/Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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