Dynamic Mechanobiology of Cardiac Cells and Tissues: Current Status and Future Perspective

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Abstract

Mechanical forces impact cardiac cells and tissues over their entire lifespan, from development to growth and eventually to pathophysiology. However, the mechanobiological pathways that drive cell and tissue responses to mechanical forces are only now beginning to be understood, due in part to the challenges in replicating the evolving dynamic microenvironments of cardiac cells and tissues in a laboratory setting. Although many *in vitro* cardiac models have been established to provide specific stiffness, topography, or viscoelasticity to cardiac cells and tissues via biomaterial scaffolds or external stimuli, technologies for presenting time-evolving mechanical microenvironments have only recently been developed. In this review, we summarize the range of *in vitro* platforms that have been used for cardiac mechanobiological studies. We provide a comprehensive review on phenotypic and molecular changes of cardiomyocytes in response to these environments, with a focus on how dynamic mechanical cues are transduced and deciphered. We conclude with our vision of how these findings will help to define the baseline of heart pathology and of how these *in vitro* systems will potentially serve to improve the development of therapies for heart diseases.

Keywords

Dynamic Mechanobiology, Cardiomyocytes, Mechanotransduction, Cardiac Pathophysiology, Cardiovascular Tissue Engineering

Abbreviations

2D:	Two dimensional
3D:	Three dimensional
ACM:	Arrhythmogenic cardiomyopathy
DCM:	Dilated cardiomyopathy
ECM:	Extracellular matrix
EHTs:	Engineered heart tissues
ESCs:	Embryonic stem cells
FA:	Focal adhesion
HCM:	Hypertrophic cardiomyopathy
hiPSCs:	Human induced pluripotent stem cells
hiPSC-CMs:	Human induced pluripotent stem cell derived cardiomyocytes
NO:	Nitric oxide
PDMS:	Polydimethylsiloxane
ROS:	Reactive oxygen species

1. Introduction

Cardiomyocytes, the heart muscle cells responsible for cardiac contraction, experience a dynamic mechanical microenvironment that evolves over diverse timescales associated with heart contraction, heart development and disease progression[1-7]. During early embryonic development, mechanical force enhances expression of the genes *TWIST* and *SNAIL* to drive gastrulation invagination. Nodal signaling guides the formation of a gradient of sonic hedgehog and drives retinoic acid to the left zone of the primitive streak to initiate the transcription of mesodermal programs[5]. In a beating heart, cardiomyocytes are under both rhythmic and static strain fields in the contraction and relaxation cycles[8]. Active contraction of cardiomyocytes raises the ventricle wall stress dramatically to generate the pressure difference versus the aorta that enables efficient blood ejection. During heart diastole, the chamber stress drops rapidly to allow blood filling into the atria. As heart diseases progress, the composition and organization of extracellular matrix (ECM) proteins change due to the activation of cardiac fibroblasts[9-11]. This leads to heart chamber stiffening and maladaptive remodeling of cardiomyocytes, causing the disruption of normal heart functions.

The nature of mechanical load sensed by cardiomyocytes determines their physiological or pathological status. In postnatal life, increase of heart mass is achieved by growth of cell mass, instead of cell proliferation. The continuous exposure of cardiomyocytes to increasing mechanical loads from neighboring cardiomyocytes and ECM, loads that are transmitted through mechanosensory structures, stimulates sarcomere growth and organization. As a result, cardiomyocytes present a hypertrophic morphology with strengthened contractility and establish a mechanical homeostasis with their neighboring environment. In a healthy heart, physiological responses include adaptive remodeling of cardiac tissues under rhythmic beating and ECM modification, which allows the preservation of heart functions in the presence of temporal mechanical overload. The heart could also transit into pathological remodeling if the compensation is interrupted by persistent mechanical overload[12,13]. Pathological remodeling originating from myocardial infarction, hypertension, or inherited defects can initiate maladaptive cardiac remodeling and eventually lead to heart failure. In these cases, cardiomyocytes are able to sense the dramatic ECM changes and develop maladaptive responses such as apoptosis, hypertrophy, and dysfunction[14].

Although cardiomyocyte hypertrophy occurs in both physiological and pathological remodeling, the transcriptional expression, metabolic, and mechanical responses are distinct, leading to different contractile characteristics. For instance, fetal cardiac proteins such as the secretory marker ANP and the transcription factor GATA binding protein 4 (GATA4) are reactivated in diseased cardiomyocytes[15]. One explanation for this observed divergence in hypertrophic signaling under seemingly similar mechanical conditions is that the *timing* of mechanical loading may play a key role in determining cellular responses. For example, short-term activation of protein kinase B (AKT) pathways is considered to be cardioprotective, while prolonged AKT activation may lead to heart dysfunction[16]. Indeed, the effects of many stimuli depend on the context of heart condition and the balance between protective and detrimental factors[17]. More involved signaling cascades and their interactions are still under investigation. Mapping these biomechanical networks related to cardiac behaviors may provide more insight into the phenotypical thresholds of heart physiopathology and help with the identification of reliable therapeutic targets.

To study human cardiomyocyte mechanobiology, *in vitro* model systems not only offer higher throughput than animal models, but also enable specific control over mechanical loading without complex humoral changes *in vivo* that complicate the interpretation of mechanobiology studies[18-21]. Nowadays, creating a dynamic mechanical environment is one of the key considerations of such *in vitro* models, since temporal mechanical changes play an important role in both physiological adaptation and pathophysiological transition. For example, longitudinal stretching (parallel to cell major axis), the most common way used in studies of cardiac mechanobiology, elongates the cardiomyocytes and increases the preload of cardiac contraction[22,23]. In contrast, transverse stretching (perpendicular to cell major axis) was shown to mimic some effects of afterload, such as the increase of sarcomere width[24]. Recently, engineering temporal mechanical property changes of cell culture materials has drawn more attention to induce dynamic mechanical stimuli[25,26]. Cellular mechanotransduction at cell-material interfaces could be controlled by tuning the dynamic properties of the biomaterials used. Active materials, such as smart hydrogels and shape memory polymers, have been explored to mimic the ECM remodeling and the increasing afterload in heart development and disease[22]. These environmental mechanical signals can be transmitted through the integrins,

junctions, ion channels, or tyrosine kinase receptors at cell-material and cell-cell interfaces, which could further modulate biological responses such as cytoskeletal dynamics, protein translocation, and transcriptional activities. Our focus is this concept of dynamic mechanobiology, and we discuss how dynamic mechanical environment could be drivers of physiological or pathological changes in cardiac cells and tissues.

2. Cardiac Mechanobiology: Knowledge from Animal Models

Animal models have been critical to our understanding of cardiac mechanobiology. Using *ex vivo* muscle preparations, classical studies by Starling, Von Anrep and others pointed to distinct changes in muscle function under preload – caused by the stretching of resting muscle – versus afterload – caused by the mechanical resistance during muscle contraction[6,7]. Abnormal increase of preload in pathological conditions is called volume overload, and too much afterload is termed as pressure overload. In animal studies, surgical maneuvers are developed to provide either volume overload through a shunt or pressure overload with transaortic constriction[7,27,28]. While these distinct modes of mechanical loading both lead to cardiac tissue and cellular hypertrophy, the modes of hypertrophy are different: preload leads to eccentric hypertrophy marked by cardiomyocyte elongation, while afterload leads to concentric hypertrophy featured by cardiomyocyte thickening. Preload and afterload lead to unique signaling and transcriptional changes *in vivo*. Previous work found that preload activated AKT signaling, but afterload was more related to calmodulin signaling. However, fibrosis usually occurred in both forms of mechanical overload in 2 – 8 weeks post-surgery[4,8,27,28]. In rodent animal models, cardiac hypertrophy triggered by pressure overload is associated with the upregulation of fetal genes, including those encoding atrial natriuretic factor (ANF) and skeletal α-actin, but decreased expression of peroxisome proliferator-activated receptor α (PPARα) and its regulated genes associated with fatty acid and carbohydrate oxidation[29-32]. These adaptations are induced by the activation of the circulating renin-angiotensin system. Interestingly, activation of angiotensin II is linked to different pathways in different heart chambers: angiotensin converting enzyme was predominant in the atria, but angiotensin II-forming chymase is mostly involved in the ventricles[5].

The motivation for many animal models is observations from human genetics, which has provided a rich dataset linking mechanical cues to cardiomyocyte biology. In young people, especially athletes, sudden cardiac death is often linked to inherited heart diseases. Interestingly, genomic variants associated with arrhythmias are mostly not localized on the ion channels that conduct the electric signal within the heart. Instead, they are either within the sarcomeres, the contractile units of these cells, or in the desmosomes, the intracellular plaque that provides cell-cell mechanical reinforcement[33,34]. Hypertrophic cardiomyopathy (HCM) is an inherited disease associated with sarcomere mutations[35]. This disease is characterized by abnormal thickening of heart muscle, cardiomyocyte disarray, and interstitial fibrosis. These factors were historically thought to be the primary triggers for the arrhythmias associated with this disease. The second most common cause of sudden cardiac death in the young is arrhythmogenic cardiomyopathy (ACM). This inherited disease is associated with genetic variants in the desmosomes, which account for about 50% of all cases[36-38]. One form of the earliest documented cases of ACM is Naxos syndrome caused by the mutation of plakoglobin, a desmosomal protein that links intermediate filaments to cadherins[39,40]. Amongst the desmosomal genes, variants in *PKP2* are the most frequent cause of ACM[41,42].

Genetically defined mouse models were developed to study the cell biology linking genotype to phenotype in these inherited cardiac diseases[6,7,43,44]. For example, heterozygous mutation of MYBPC3 in a mouse model was found to generate contractile dysfunction associated with HCM[45]. In ACM, mouse models and *in vitro* experiments with the cells derived from these models demonstrated the underlying links between desmosome damage and cardiac arrhythmias, which was the physical interactions between desmosomal proteins, gap junction components[46], and/or ion channels (e.g. sodium channel[47]). In addition, desmosome disruption has been linked to arrythmias through transcriptional mechanisms: *Pkp2* deficiency in mice showed a transcriptional dysregulation of molecules involved in intracellular calcium handling/cycling (e.g., calsequestrin 2, ankyrin-B, ryanodine receptor 2)[48].

3. *In Vitro* Model Systems for Cardiac Mechanobiology

Biological differences between humans and model organisms and throughputs limitation are among the motivating factors for studying cell biology in a dish. Early attempts to culture postnatal cardiomyocytes highlighted a

fundamental challenge in studying cardiomyocytes outside the body: cardiomyocytes on glass or on tissue culture plastic do not necessarily die, but they lose their rod-like morphology and cellular identity[49]. Thus, much of the motivation for mechanically actuating cardiomyocytes *in vitro* originates from the experimental need to create "*in vivo*-like" tissue culture platforms to maintain the identity of these cells.

Cells cultured in isolation and confluent two-dimensional (2D) monolayers often fail to exhibit the same biology that is observed *in vivo*, especially regarding long term survival and drug responsiveness[50-53]. In three-dimensional (3D) culture, cells in an intact tissue not only interact with the ECM, but also have a markedly higher incidence of cell-cell contacts that bear mechanical stress. Direct comparison between 2D and 3D cultures based on RNAseq profiling demonstrates that 3D culture can better recapitulate *in vivo* ischemia in response to hypoxic conditions[54]. In 1997, Eschenhagen et al. developed 3D cultures of embryonic cardiomyocytes in collagen matrices as the earliest engineered heart tissues (EHT). The beating cardiac tissue was anchored between tubes that were sutured to Velcro; following designs used earlier for fibroblast-containing tissue constructs[55-57], the Velcro interdigitated with the collagen matrix, and the tubes connected to a mechanical stretching device and a load cell for force measurement[58]. Over the years following this initial publication, EHT systems have been improved by changing the cell sources, cell density, and system apparatus. Higher throughput was enabled by a device developed by Vandenburgh[57,59], in which the load cell was replaced by cantilevered elastic supports. Another version of this functions by indenting EHTs suspended across relatively rigid posts using an indenter connected to a load cell[60]. The Vandenburgh system was subsequently miniaturized to even higher throughput systems that use soft lithography to create polydimethylsiloxane (PDMS) posts and specimens[61]. An additional feature of the Vandenburgh type systems is that their dimensions and material properties can be modulated so as to prescribe the elastic boundary conditions surrounding an EHT specimen. A range of additional geometries and material systems now exist, including elastic membranes, patches, PDMS strips, as well as microwires[62-64]. Recent advances to these systems enable highly flexible generation of devices from 3D prints through hydrogel assisted double molding techniques[65]. By tuning mechanical stimulation[66], *in vitro* tissue constructs can be further used to study genetic cardiac disease phenotypes and drug-induced cardiotoxicity[21,67]. To recapitulate the physical stimulation *in vivo* and study cardiac mechanotransduction, various *in vitro* engineered systems, including 3D EHTs, have been developed to incorporate dynamic mechanical inputs (**Table 1**). Most of these systems are mainly designed to recapitulate either preload or afterload (**Figure 1**). However, it should be noted that strictly speaking, many of these systems in practice induce both preload and afterload changes because *in vivo*, preload, the stretch on heart muscle at rest, is only relevant between beats, whereas afterload, the resistance the heart must work against, is only relevant during systole[68]. Whether or not systems that partially mimic these mechanical inputs – for example, systems that attempt to mimic preload by manipulating the length of engineered heart tissues (**Figure 1**) – can coax cells into recapitulating the molecular behaviors they would exhibit *in vivo* remains an important question for the field.

3.1 Mechanical Interactions between Cardiomyocyte and ECM

2D models that incorporate mechanical loading or physical cues have been widely used to study cardiomyocyte mechanobiology with a focus on cell-ECM interactions. The ECM-costamere-sarcomere linkage a critical structural basis for mechanotransduction, and their deformation triggers myofibril rearrangement. At the early stages of monolayer culture, interactions between cardiomyocyte focal adhesions (FAs) and ECM guide cell spreading, myofibrillogenesis and stabilization of cell-cell junctions into more mature intercalated discs[69,70]. To profile the dynamics of these cell-ECM interactions, Sun et al., established a shape memory polymer platform with a changing surface topography and observed cell remodeling in human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) in response to the formation of topographic wrinkles[71]. Serial structural rearrangements occurred in a sequential order: wrinkle formation in the shape memory polymer platform caused the disassembly and reassembly of FAs at the cell-material interface, which was followed by the reorganization of sarcomere thin filaments and Z discs, while thick filaments did not show significant remodeling during these topographic changes. In a follow-up study, the remodeling of different FA proteins was investigated in response to dynamic surface topographic changes[72]. Paxillin, the closest FA component to the substrate, showed the earliest response to the topographical change. Next, mechanical tension transduced through vinculin and zyxin affected the cytoskeleton. In a similar study, a platform with dynamic topographic cues was fabricated based on a shape memory polymer to

guide the alignment of neonatal rat ventricular cardiomyocytes. Cardiomyocytes aligned along the direction of nano-wrinkles transformed into a circular shape and lost their orientation as they shifted to align orthogonal to the nano-wrinkle direction, which was evidenced by temporal changes of cell polarity and cytoskeleton reorganization[73]. In addition, as cardiomyocytes respond to a topographically curved surface differently than to a flat surface, even if both substrates have the same stiffness. Higher sarcomere shortening and paxillin displacement were observed in cardiomyocytes contracting on soft micropost substrates than on a soft flat substrates, highlighting the importance of surface architecture[74].

Pressure overload forces the heart to contract against a higher mechanical load during each beat and triggers excessive ECM deposition, which can both be modeled *in vitro* by varying substrate stiffness. Ribeiro et al., patterned hiPSC-CMs at physiological aspect ratio on hydrogels with a stiffness mimicking either healthy (10 kPa) or fibrotic (35 kPa) myocardium. Cells on the stiffer substrate showed myofibril discontinuities and produced 90% less contractile force than cells on the soft substrate[75]. Abnormalities in Ca^{2+} handling and action potential were associated with these stiffness-induced cardiac dysfunctions[76,77]. Computer-controlled carbon fibers was able to provide precise and regional mechanical training to single guinea pig cardiomyocytes[78]. The contraction force showed a linear relationship with the length of cardiomyocytes during mechanical stretching to achieve an optimal force-length relationship.

Dynamic stretching of cardiomyocyte monolayers increases electrical signal propagation velocity and action potential upstroke[23]. Compared to static stretching of the same amplitude, dynamic stretching also significantly increases the expression of the gap junction protein connexin 43 (Cx43). Interestingly, dynamic and static stretching activate different signaling pathways. Static stretch causes release of angiotensin II and endothelin, which further activates intracellular signaling via p38 mitogen-activated protein kinase (p38-MAPK) phosphorylation[79 80,81], while pulsatile stretch predominantly mediates activation of extracellular signal-regulated protein kinase 1/2 (ERK1/ERK2) by vascular endothelial growth factor (VEGF) and transforming growth factor beta (TGF-β) signaling[82,83]. In addition to magnitude and frequency the stretch direction could also modulate the behavior of cardiac monolayers. A study comparing the effects of transverse versus longitudinal stretch on aligned neonatal cardiomyocytes grown on a micropatterned elastomer showed increase expression of F-actin, N-cadherin, Cx43, and ANF in response to transverse loading, indicating the development of cardiac hypertrophy[84]. Cyclic uniaxial stretching was found to induce cell reorientation, cytoskeletal reorganization, and accelerated movement of kinesin and dynein towards the cell poles. The study also demonstrated that mechanosensing of stretched cardiac monolayers is mediated by focal adhesion kinase (FAK) Y397-phosphorylation[85].

3.2 Mechanically Enhanced Cardiac Maturation In Vitro

A fundamental challenge in studying cardiomyocyte mechanobiology *in vitro* is that the cells most commonly used for this approach, either neonatal rodent cardiomyocytes or human stem cells-derived cardiomyocytes, do not resemble adult-like cardiomyocytes in terms of structure, functionality, and gene expression[86,87]. Direct *ex vivo* studies of mature, postnatal cardiomyocytes with appropriate biomimetic culture conditions highlighted the involvement of mechanotransduction pathways, such as afterload-induced NO signaling, in the maintenance of mature status of cardiac tissues, although long-term function and survival of adult cardiomyocytes *ex vivo* remains a significant challenge in the field. Additionally, biochemical approaches were also investigated to target specific signaling pathways to maintain the cardiomyocytes in a quiescent state, such as inhibiting mammalian target of rapamycin (mTOR) signaling pathway[88], or force metabolic transition of the cardiac tissues, such as substituting sugars by fatty acids as ATP sources[88-90]

Mechanobiological approaches have been leveraged to achieve structural and functional maturation of cardiomyocytes[91]. Increased substrate stiffness has been shown to promote cardiomyocyte maturation by improving myofibril organization, obtaining adult-mimicking morphology, and reducing cell division[92]. Interestingly, a compliant matrix could reverse the maturation process of neonatal rat and mouse cardiomyocytes and cause cell cycle re-entry, with a loss of sarcomere organization and cardiac markers[92]. Recently, hiPSC-CMs on 3D cylindrical patterns exhibited anisotropic cell morphology and alignment of troponin T structure, and sub-micrometer surface

topography further accelerated the cardiomyocyte maturation[93]. Physiological-relevant mitochondria distribution and enhanced Ca^{2+} transients were also observed from these patterned cells. The benefits of 3D micropatterns could possibly originate from the enforcement of chromatin fibers when mechanical signals were transmitted into the nuclei through the cytoskeleton network.

Compared to static mechanical cues, dynamic cues offer time-specific mechanical modulation to improve the maturation of cardiomyocytes. Temperature-responsive thiolated-hyaluronic acid hydrogel with dynamic stiffness was one of the earliest active biomaterials used for cardiac maturation[94]. During hydrogel stiffening, immature markers in chicken embryonic cardiomyocytes kept decreasing with a simultaneous increase of mature makers. Intracellular myofibrils showed more physiologically relevant structures and better alignment on dynamic hydrogels versus static hydrogels. Unidirectional stretch has been widely used to improve the cell alignment and sarcomere organization of cardiomyocytes[95-97]. Pulsatile hemodynamic force promoted hiPSC-CM maturation, showed as improved cell alignment, enhanced contractility, and formation of mitochondrial network[98].

Although dynamic mechanical loading has a positive effect on cardiac maturation, the stretching protocol needs to be further tuned to maximize the maturation process. On the tissue level, a time-dependent electromechanical training was shown to enhance the maturation of hiPSC-EHT within 4 weeks of culture, with anisotropic gap junctions, formation of t-tubules and a positive force-frequency relationship[99]. When exposed to the cyclic stretch, the sarcomere length and cardiac contractility of hiPSC-CMs plateaued at 15% strain, while the expression of myosin heavy chain continued to increase till 20% strain was applied[100]. Shen et al., investigated the simultaneous effect of shear stress, cyclic strain, and prolonged culture on hESC-CM maturation[101]. A flow rate at 1.48 ml/min, a 5% cyclic strain and 20 days of culture delivered the best outcomes in cardiac gene expressions, sarcomere growth, calcium handling, caffein response, and ion channel formation.

3.3 Mechanically Mediated Cardiac Disease Modeling In Vitro

To mimic the constantly changing mechanical environment of native cardiac tissue, several platforms have been developed to study the impact of dynamic loading on cardiomyocyte biology *in vitro*. For example, Song *et al.* bound magnetic microparticles to the cardiomyocytes to apply torsional forces[102]. These microparticles could be tagged with specific ligands targeting different membrane receptors, thus allowing elucidation of the role of these receptors in cardiomyocyte mechanosensing. This system successfully induced cardiac hypertrophy under hypoxic conditions, shown as the overexpression of hypertrophic markers, including beta myosin heavy chain (β-MHC) and microRNA-21. In aging or diseased myocardium, as tissues become stiffer, maladaptive remodeling was observed with re-formation of FAs adjacent to the cell-cell interface[69,103]. To mimic environmental stiffening *in vitro*, magnetic microparticles were incorporated into bis-acrylamide hydrogels, allowing the elastic modulus of the substrate to be tuned *in situ*. As the substrate was stiffened by a magnetic field, fast nuclear translocation of Yes-associated protein (YAP) was observed, while substrate softening would induce the cytoplasmic translocation of YAP[104]. Corbin *et al.* took a similar approach, using a PDMS-substrate with iron particle inclusions to dynamically tune the stiffness of the substrate[105]. In addition to YAP translocation to the nucleus, two other markers of cardiac myofibroblast activation also increased with increasing substrate stiffness: cell spreading and α-smooth muscle actin expression. Analogous approaches to changing substrate mechanical properties *in situ* using photo-crosslinked materials[106,107] also have great potential for studying cellular mechanosensing.

Force readout has been used extensively for drug screening assays based on 2D traction force microscopy (TFM) and 3D post-based EHTs[108-114]. For example, TFM measurements on single hiPSC-CMs demonstrated the effects of contractile agonists and stimulants on cardiomyocyte force generation[115]. Although these investigations did not examine the mechanical effects on hiPSC-CMs responsiveness to drug toxicity, these experimental systems, such TFM and post arrays, offer both force measurements and mechanical manipulation, allowing assess how cardiomyocytes sense and respond to their environment and cardiotoxic agents simultaneously. As the earliest report on the interplay between mechanical loading and hiPSC-based cardiotoxicity, an in vitro model of three-dimensional (3D) human cardiac tissue was established using synthetic filamentous matrices populated with WT healthy hiPSC-CMs and LQT3 patient-derived hiPSC-CMs. This study showed that LQT3 cardiac tissues exhibited

more contractility abnormality than WT tissues. More importantly, LQT3 cardiac tissues were more susceptible to drug-induced cardiotoxicity when grown on filamentous matrices with low fiber stiffness compared to those with high fiber stiffness and 2D surfaces [116]. More recently, from engineered cardiac microtissues, sunitinib, a cancer chemotherapy drug, showed cardiotoxicity as cardiomyocyte death, a loss of mitochondrial membrane potential, and decreased force generation at clinically relevant doses. The stiffness of the pillar can be used to model the afterload to the cardiac microtissues, which resulted in an increased magnitude of sunitinib-associated caspase activation with stiffer pillars. This finding supports an interaction between sunitinib cardiotoxicity and increased in vitro afterload, indicating that afterload is a key mediator of sunitinib's effects on LV function[117].

To model afterload *in vitro*, adult mouse cardiomyocytes have been encapsulated in a 3D hydrogel, forcing the cells to work against the mechanical rigidity of the hydrogel. This led to the observation that NO synthase plays a role in the dysregulation of Ca^{2+} signaling due to high afterload[118]. In 3D EHT models, afterload can be modeled by stiffening the static supports of the EHT (for example, microcantilevers)[119], applying permanent magnet rods[120], or removing a metal stiffener from the supports[121]. Most of these models show that increasing afterload induces expression of hypertrophic gene programs, with upregulation of *NPPA*, *NPPB*, *MYH7*, *ACTA1* and downregulation of sarcoplasmic reticulum Ca^{2+}-ATPase 2a (SERCA2a) due to ineffective oxidative metabolism[119,120]. The same trend was also observed in a preload-mimicking 3D system applying 5-15% strain to the cardiac tissues[122]. *NPPA*, *MYH7*, and *ACTA1* expression increased in response to a higher strain level, which aggravated a HCM phenotype. To combine preload and afterload, Bliley et al., developed a dynamic platform in which EHT formed against long PDMS strips with a bending stiffness low enough to allow efficient cardiac contraction[63]. The design of the PDMS strips allowed for application of different levels of preload to the cardiac tissues. In addition, the thickness of the strips can be modulated to create different levels of afterload against cardiac contraction. Transcriptional upregulation of genes relevant to desmosomes, sodium channels and gap junctions was observed associated with the change of preload and afterload[63].

In our own previous work, we formed micro-scale EHT on a soft substrate to provide a mild afterload[66]. Interestingly, after 2 weeks of culture, these micro-tissues produced compensatory contractility and decoupled calcium handling, consistent with native responses to mechanical overload. From the same model, EHT with heterozygous mutation of *MYBPC3* exhibited a hypercontractile phenotype with longer upstroke duration on the stiffer fibrotic substrate (65 kPa). In an analogous 3D model based on engineered heart muscle formed on filamentous matrices, EHT with homozygous mutation of *MYBPC3* exhibited a faster decay of Ca^{2+} dynamics, Ca^{2+} desensitization and impaired contraction force under mechanical overload, corresponding to a dilated cardiomyopathy (DCM) phenotype[123].

Modeling volume overload *in vitro* can be achieved by applying exogenous stretches to the cardiomyocytes. High cyclic strain has been shown to provoke pathological responses of cardiomyocytes due to volume overload. Compared to a moderate stretch at 5%, an aggressive stretch at 25% significantly decreased the expression of desmin in hiPSC-CMs[124]. In an *in vitro* ACM model with *PKP2* mutation, a micropatterned substrate with dynamic strain altered transcription program of hiPSC-CMs towards pro-fibrotic dysregulation of the ACM transcriptome, suggesting impaired signaling in response to the mechanical stress[125]. Real-time dynamic sarcomere reorganization in neonatal rat cardiomyocytes under volume overload was observed with second harmonic generation confocal microscopy[126]. A 6% longitudinal stretch caused sarcomere addition within the myofibrils with concurrent damage and repair. Meanwhile, mitochondria accumulated around the sarcomere addition sites, coinciding with the beginning of cell hypertrophy. Nevertheless, it is difficult to determine whether such sarcomere remodeling is physiological or pathological, since the physiological threshold of volume overload is still under debate. Abilez, et al., found that the spacing of anchoring posts affects the consistency of EHT formation and sarcomere alignment and attributed this to strain levels in the EHT[18].

4. Cardiomyocyte Mechanosensitivity and Mechanotransduction

In vitro models, high-throughput animal models (e.g., zebrafish), and *in vivo* mammalian physiology open up the possibility not only to study disease progression in a human or physiological context, but also to unveil molecular mechanisms of disease to identify drug targets. For example, -catenin, a key component of the adherens junction

involved in wingless-related integration site (WNT) signaling, has been noted to exhibit inappropriate localization in the cardiomyocytes of ACM patients[127,128]. SB216763, which stabilizes β-catenin by inhibiting glycogen synthase kinase-3 beta (GSK-3β), was able to prevent and partially reverse the ACM phenotypes[129]. *In vitro* models that phenocopy the beneficial impact of reduction in mechanical loading in the setting of heart diseases may also open the way to discovery of therapeutic approaches that directly target cardiomyocytes in a more targeted manner. For example, volume overload caused by extended endurance training is linked to right-sided heart failure and can exacerbate the chance of arrhythmia in patients with ACM-associated mutations[130]. In mouse models, prolonged volume depletion with diuretics protected mice with ACM-prone plakophilin 2 (PKP2) mutations from developing arrhythmias, even with prolonged exercise[131]. While intriguing, use of such a therapeutic approach may cause stress to other organ systems (e.g., kidneys). Motivated by this goal, researchers have increasingly applied bioengineering tools to explore the mechanisms of cardiomyocyte mechanosensing and mechanotransduction for various applications (**Figure 2**).

4.1 Mechanosensitivity of cardiomyocyte adhesions and junctions

Mechanical signals originating from the extracellular microenvironment can be transduced through FAs, transmembrane ion channels, or intercellular junctions, and can affect the cytoskeleton and nucleus. Connections between cardiac cells and ECM are essential for both mechanical coupling and signal mechanotransduction. ECM ligands bind to the extracellular domains of integrins, and forces transduced at integrins can induce outside-in signaling cascades that upregulate F-actin and α-actinin polymerization and myosin-II activity[132]. Costameres link the outermost Z discs with FA proteins at the cell membrane to facilitate bidirectional mechanotransduction between ECM and cardiomyocytes. Within costameres, dystroglycan and integrins directly bind to the ECM via their transmembrane domains, and their cytoplasmic domains link to myofibrils through different adaptor proteins. The physical linkages among structural proteins result in a rapid transmission of extracellular force to intracellular tension. Force transduction at the adhesion sites activates various signaling kinases, such as FAK, Ras homolog family member A and downstream Rho-associated protein kinase (RhoA-ROCK), and MAPK-dependent pathways, leading to different transcriptional programs that eventually affect the active contraction-relaxation cycles.

Cell-cell junctions are another critical mechanosensitive component of cardiomyocytes[133]. N-cadherins located at the intercalated discs anchor actin filaments and couple actomyosin contractility between neighboring cells[134]. These intercellular junctional proteins are the avenue to transmit mechanical forces between cardiomyocytes and can be reinforced by external forces[135]. For example, α-catenin, a component of adherens junctions, is a direct stress sensor across cell-cell junctions that can transduce mechanical stress and activate YAP signaling to induce cardiomyocyte proliferation[136]. Whereas adherens junctions are ubiquitous across tissues, desmosomes are unique to tissues under high resting tension, most notably the heart and the skin[137]. Interestingly, one of the desmosome components, plakoglobin, was reported to mis-localize to the nucleus in the setting of ACM, where it competitively blocks DNA binding by -catenin[138,139]. It remains uncertain whether this competitive binding would be altered by changes in mechanical loading on the desmosomes.

4.2 Mechanosensitivity of the cardiomyocyte cytoskeleton

Sarcomeres are the major cytoskeletal structures and contractile units in cardiomyocytes. Titin and its associated protein complex, spanning from Z discs to M lines, are the most widely acknowledged mechanosensors within sarcomeres. Titin serves as a scaffolding protein in sarcomere formation and interacts with many proteins participating in sarcomere assembly. The elastic domain of titin defines the physiological range of sarcomere slack length. Titin can link to muscle LIM protein (MLP) and stabilize its interaction with T-cap, which enables efficient ECM-titin force transmission and load-induced signaling events[140]. Both titin and MLP mutations have been observed in DCM and heart failure, which further confirms their indispensable role in normal cardiac functions[141,142]. Myosin is the major component of thick filaments. The availability of myosin heads and their interactions with actin filaments ensure efficient systolic function[143]. Disruption of myosin tension leads to a decrease of sarcomere content and inefficient cardiac contraction[3,144]. Cardiomyocytes with deficiency in other sarcomere proteins, such as myosin binding protein C and troponins, also showed different levels of myofibril damage responding to the variation of mechanical load[123,145], but mechanistic insights associated with their mechanotransduction roles are not well-

elucidated yet.

In addition to myofibril filaments, microtubule reorganization is also involved in hypertrophic remodeling. As post-translational modification, detyrosination of α-tubulin affects X-ROS signaling and cytoskeleton stiffness[146,147]. The mechanism behind detyrosinase activity in response to mechanical stress is still unclear. However, it was shown that tubulin mRNA upregulation can be transcriptionally induced by signal transducer and activator of transcription 3, Janus tyrosine kinase and adrenergic stress[148]. Another study also showed that mechanical stress activated AMP activated protein kinase (AMPK) signaling at the intercalated disc and further induced the phosphorylation of cytoplasmic linker protein-170 (CLIP170), which is essential for microtubule dynamics[149]. Deleting AMPK or blocking CLIP170 caused microtubule accumulation under mechanical stress, which led to impaired cardiac functions and heart chamber dilation.

4.3 Mechanosensitivity of cardiomyocyte ion channels

Extracellular mechanical stress can activate transmembrane ion channels and receptors and modulate ion transportation and secondary messengers. For example, the mechano-gated channel, potassium channel subfamily member 2 (TREK-1), contributes to potassium conductance in cardiomyocytes for normal repolarization and stable diastole. The increase of TREK-1 current due to mechanical stretching could shorten action potential duration and promote arrythmia[150]. Sarcolemmal channels, like L-type Ca^{2+} channels, can be indirectly modulated by the mechanical microenvironment. Polycystin-1 is a recently discovered mechanosensitive protein that governs the stability of L-type Ca^{2+} channels. Its blockage decreased the abundance of L-type Ca^{2+} channels and induced hypertrophic growth of cardiomyocytes[151]. During contraction, Ca^{2+} influx triggers intracellular release of Ca^{2+} from the sarcoplasmic reticulum that is regulated by the ryanodine receptor[152]. Acute stretch to rat ventricular cardiomyocytes could cause a rapid increase of Ca^{2+} spark rate, which was very sensitive to the spatial location of mechanical stimulation[153]. The spark rate was independent of the availability of sarcolemmal ion channels or extracellular Ca^{2+} concentrations, but affected by the integrity of microtubules, which are major intracellular force-bearing components. Piezo1, a force-activated Ca^{2+} channel, was recently found to be the cardiomyocyte mechanosensors directly association with overload-induced cardiomyopathy[154]. Piezo1 deletion could prevent the upregulation of the sodium–calcium exchanger and changes in other Ca^{2+} handling proteins after pressure overload.

4.4 Mechanosensitivity of cardiomyocyte nuclei

Cell nuclei can sense dynamic mechanical cues through several direct and indirect pathways. Nuclei their linker connections between nucleoskeleton and cytoskeleton complexes, which are the primary units to support nuclei structure and tether intranuclear components. This allows a rapid force transmission from peripheral domains to the nuclei for the regulation of transcription factors and genome integrity. Lamin, a core protein in the nucleus sensing complex, is responsible to set the nuclear stiffness according to the external mechanical microenvironment. Lamin-A depletion drove the nuclear rupture and caused cytoplasmic mis-localization of DNA repair factors, which led to DNA damage and cell-cycle arrest[155]. *LMNA*-S143P mutant could cause Lamin A less bound to the Lamin network, which eventually led to familial DCM in patients[156]. Mechanosensitive transient receptor potential cation channel subfamily V member 4 (TRPV4) were found to localize only in the nuclei of neonatal rat ventricular cardiomyocytes, although their functionality in nucleus mechanotransduction is still under investigation[157]. Moreover, cell nuclei were capable of sensing the stimulation of tensile force, evidenced by the increase of nuclei volume and the modulation of YAP, Lamin A/C, and desmin[158].

4.5 Mechanisms of Cardiomyocyte Mechanotransduction

Mechanical-induced cardiomyocyte remodeling is dependent on intracellular signaling pathways and transcriptional activities. The complexity of mechanotransduction induced by dynamic mechanical cues gives rise to distinct responses of cardiomyocytes. RhoA and Ras-related C3 botulinum toxin substrate 1 from Rho family can be activated by mechanical stretching. Downstream signaling pathways involve nuclear translocation of myocardin-related transcription factor-A (MRTF-A), a co-activator with serum response factor (SRF) that induces brain natriuretic peptide (BNP) gene expression and hypertrophic responses[159]. MRTF-A and SRF were the critical transcription factors that maintained sarcomere organization and regulated cardiomyocyte maturation.[159,160]

Mechanical transduction through cardiomyocyte cell-cell junctions involves actin remodeling that is stabilized by RhoA-ROCK signaling and the MRTF-SRF transcriptional program[161]. This mechanotransduction cascade helps maintain cardiomyocyte identity, and its perturbation can induce conversion from the cardiomyocyte lineage to the adipocyte lineage.

A key signaling pathway for cardiac development is WNT signaling, activated through cytoplasmic accumulation of β-catenin, which can then translocate into cell nuclei to initiate transcription of cardiac-related genes[5]. Mechanosensitive cadherins at cell-cell junctions can grasp β-catenin to blunt its nuclear translocation via mechanical regulation of WNT/β-catenin[162]. Cyclic strain increases expression and translocation of β-catenin, and thereby facilitates cardiac differentiation of mouse ESCs[163]. GSK3β and phosphoinositide 3-kinase (PI3K) /AKT play opposite roles in controlling β-catenin degradation. Time-dependent stiffening of hydrogels upregulates PI3K/AKT in chicken embryonic cardiomyocytes, and downregulates GSK3β[164]. GSK3β inhibition in cardiomyocytes on static matrices promotes contraction and myofibril organization, while AKT inhibition in cardiomyocytes on dynamic matrices interferes with cardiomyocyte maturation.

The TGF-β superfamily is composed of three branches: TGF-β, bone morphogenetic proteins (BMPs) and Nodal. Nodal signaling primarily mediates early mesoderm patterning, while BMPs are more important in late cardiac specification[5]. TGF-β plays a positive role in early cardiac differentiation; TGF-β repression is necessary for blocking the commitment of cardiac progenitors into smooth muscle or endothelial cells[165]. Congenital heart defects like hypoplastic left heart syndrome might be attributed to mis-regulation of TGF-β signaling[166]. Mechanical factors influence TGF-β, as seen in mechanical-induced changes in downstream suppressor of mothers against decapentaplegic (SMAD)-mediated cardiac gene transcription, TGF-β receptor endocytosis, and ligand sequestration[167]. Multiple members of the TGF-β family are suppressed by physiological stretch of embryonic mouse cardiomyocytes and are thereby related to stretch-induced cell proliferation, cardiac gene expression and cardiomyocyte contractility. Inhibition of TGF-β2 significantly abrogates these positive effects on cardiomyocyte maturation while increasing the size of cardiomyocytes. Upregulation of BMP2 and transient downregulation of BMP4 observed in neonatal rat cardiomyocytes under dynamic stretch is positively correlated with the expression of cardiac marker GATA4[168].

Hippo pathway and its downstream effectors, YAP and WW domain-containing transcription regulator protein 1 (TAZ), are key regulators of cardiomyocyte proliferation and myocardial growth during heart development[169]. Hippo activation leads to cytoplasmic retention and degradation of YAP/TAZ, which reduces their nuclear translocation and thus downregulates their transcriptional activity. YAP translocation is mechanosensitive due for reasons that are not fully clear, but that may relate to its interactions with dystrophin-glycoprotein complex at cell-ECM interface and the cellular cytoskeletons[170,171]. During the process of attachment-detachment-reattachment of human cardiac progenitor cells on a dynamic substrate, YAP/TAZ shows a temporal nuclei-cytoplasm-nuclei translocation profile, suggesting sensitivity to cell adhesion and cytoskeletal assembly[172]. In contrast, YAP silencing correlates to increased cell proliferation and decreased cardiac differentiation[173].

MAPK pathways are composed of four major subfamilies, including ERK1/2, c-Jun N-terminal kinase (JNK), p38, and ERK5, which play diverse and overlapping roles in heart development and pathology. MAPK could be activated by physical force through mechanical and biochemical factors, such as integrin transformation, ROS and FAK[174]. Mechanical strain elevates ROS concentration by stimulating membrane oxidase of mouse ESCs, which triggers the ERK1/2, JNK and p38 pathways to orchestrate cardiac differentiation[175,176]. Mechanical training of 15% strain at 60 cycles/min for 24 h causes hypertrophy of neonatal rat cardiomyocytes due to the activation of ERK1/2 and JNK[177]. Low-density lipoprotein receptor-related protein 6 (LRP6)/β-catenin and JNK are both activated by mechanical stretch in adult human ventricular cells, mediated by the passivation of mechanosensitive Piezo1 transmembrane channels[178]. However, Piezo1 inhibition affects these pathways only under aggressive stretch, suggesting that mechanical cues of different magnitude may activate distinct intracellular cascades.

5. Conclusions and Future Perspectives

In the past decades, cardiac tissue engineering has advanced through biomaterial design and device fabrication that enable dynamic *in vitro* models of cardiac physiology and physiopathology. Studies using these have demonstrated that mechanical stimuli regulate the biological responses of single cardiomyocytes, 2D cardiac monolayers, as well as 3D EHTs. While many signaling pathways have been revealed to play critical roles in the mechanotransduction for cell-ECM and cell-cell interactions, many others remain elusive. Thus, there is a need for new dynamic systems that replicate development, physiology, and disease.

Key open challenges relate to mechanical loading, mechanical environment, and population heterogeneity, as well as to the time evolution of these factors. The local environment of cells provides mechanical loads as well as compliance and damping to cardiomyocytes, and system that can effectively integrate both preload and afterload to mimic natural physiology of the heart are needed. Anisotropic mechanical cues, critical for maintaining normal cardiac physiology, are difficult to prescribe in a hydrogel system, and the mechanisms underlying cellular responses to anisotropy still need to be further explored. Recent cardiac models that achieve organ-level organization[179-182] opens new possibilities to understand how mechanical stimuli drive functional maturation, and to induce disease-specific conditions.

Transmembrane proteins activated dynamic changes to the extracellular microenvironment transmit mechanical signals via intracellular pathways to affect cardiac behavior. Current mechanistic studies focus on characterization and validation of mechanoresponsive signaling from YAP/TAZ translocations and MAPK pathways, but additional pathways certainly exist, and much work is left to identify how all of these pathways interact. Activation of mechanosensors and mechanosensitive pathways varies with the magnitude and temporal history of force[183]. Rapid inactivation of mechanosensitive Piezo channels occurs after their force-induced opening, and its functionality thus varies with frequency of mechanical stimulation[184]. Thus, differential responses of cardiomyocytes to static or dynamic mechanical cues exist, but the roles of these and the uniqueness of the underlying pathways are not clear.

The study of cardiomyocyte mechanobiology will continue to benefit from interdisciplinary approaches. Molecular biology and analytical chemistry toolboxes for manipulating and studying cell biology increasingly enable the probing of cardiomyocyte mechanosensing. Study of dynamic mechanobiology, requires real-time tracking of mechanoresponsive molecules following mechanical stimulation. This can be achieved by transforming the activity of these molecules into quantitative fluorescent or electrical signals. For example, fluorescence resonance energy transfer (FRET) tagged integrin proteins and fluorescence-tagged DNA sensors enable measurement of molecular tension during cell-ECM, the latter via irreversible rupture of DNA structure triggered by the mechanical stress[185]. PDMS-based elastic scaffolds coated with conductive gold nanotubes can detect NO produced by cells into measurable voltage changes in response to cell stretch[186]. However, length scales for FRET are small (~10 nm), not all proteins are amenable to addition of fluorophores, and electrical measurement can interfere with physiology. Only a handful of mechanobiological phenomena lend themselves to these sorts of measurements, and much need exists for future innovation.

Computational simulation and analysis have been evolving in mechanobiology, enabling the testing of hypotheses, the analysis of differential and combinational effects, and the interpretation of experimental data. Integrating micro-devices and computational modeling has been effective in understanding cell and ECM mechanobiology of mesenchymal stromal cells[187] and in guiding loading regimes for promoting ECM production. Electrophysiological models of cardiomyocytes are extremely well-developed[188]. However, models of mechanical-electrical coupling of cardiomyocytes are still emerging, especially over the course of development, and understanding electrical and mechanical coupling between cardiomyocytes and other cardiac tissues is still an emerging science[189]. Models of how cytoskeletal and cytoplasmic protein systems evolve in response to mechanical loads are developing for smooth muscle cells and fibroblasts but are still needed for cardiomyocyte signaling and development.

Progress to date has arisen from collaboration between bioengineers, bioinformaticians, biologists and biochemists. Going forward, cross-disciplinary collaboration may introduce new opportunities to design bioengineered tissues and systems to facilitate downstream analysis. For example, in recent years, the biomedical research enterprise has

seen explosive growth of molecular techniques for deep-phenotyping and "omics,[190,191]" both on the level of bulk averages over many cells[192] in a sample as well as on the single cell level. Especially given powerful tools for deep phenotyping of individual cells in complex environments, ranging from single cell RNA sequencing (scRNAseq[193,194]) to multiplexed approaches to immunostaining[195,196] and flow cytometry[197], we have the opportunity to link the mechanical inputs cells receive to their molecular phenotype with unprecedented power. However, these approaches demand a shift from shallow molecular profiling of many samples to deep profiling of a few.

A fundamental challenge facing groups developing and applying *in vitro* models, especially complex models involving setups required to control forces on cells like cardiomyocytes, is that this shift requires the experimentalist to have a robust and quantitative understanding of the range of normal sample-to-sample variability. In a similar vein, application of bioengineered tools to high-throughput screening may facilitate multidisciplinary collaboration but will require a similarly deep understanding of tissue-to-tissue and/or device-to-device variability. In some cases, pursuing high throughput screens or deep molecular phenotyping may require researchers to sacrifice complexity of the experimental setup in order to achieve lower experimental variability within individual groups so that the variability across groups can be better assessed. For example, bioengineering teams devising engineered tissue platforms may need to achieve a lower degree of variability in morphology and electrophysiology amongst engineered tissues made from cells with a single genotype so that the effects of genotype and/or genotype combined with mechanical cues may be better appreciated.

Finally, given a growing need in the field to improve throughput and robustness, efforts to reduce costs and simplify approaches to deriving and maintaining cardiomyocyte and supporting cell populations are essential, especially given the high cost and complexity of developing these from stem cells or maintaining postnatal cardiomyocytes *ex vivo.* Inexpensive, flexible, quantifiable, and precision systems for EHT represent important and continuing needs in the field, and may achievable through a convergence of machine learning[198], additive manufacturing[199] and synthetic biology[200] approaches.

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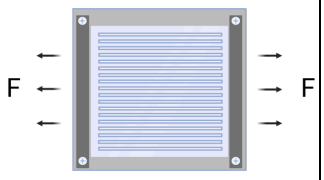
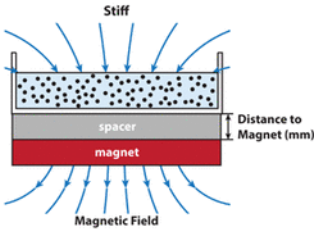
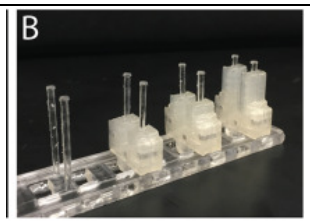
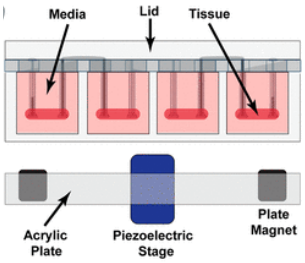
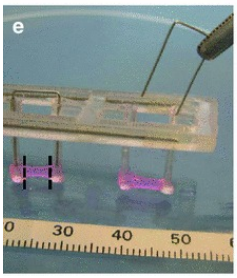
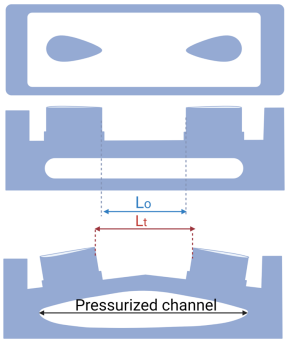
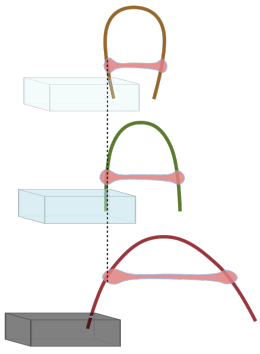
Conflict of Interest
The authors declare no competing financial interest.

Author Contributions
C.W. and G.R. contributed equally to this work.

Data Availability
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Table 1. Current dynamic system for evaluating cardiac mechanobiology and pathophysiology.

REF	Level (2D/3D)	Schematic of the system	Source of mechanical signal	Findings: Biology	Findings: Physiology
[125]	3D isotropic on PDMS micro-groove membrane		After 5 days: 10% cyclic stretching of the membrane for 60 mins	Significant arrhythmogenic cardiomyocyte response to acute stretch: cell–cell and cell–substrate adhesion (PKP2, Protein kinase G, and sodium channel protein type 5); strongly pro-fibrotic genetic program represents an early marker of ACM.	n/a
[105]	2D layer: magnetic inclusions into a soft PDMS elastomer	 Reproduced with permission from ACS Appl. Mater. Inter. 11, 20603 (2019) Copyright 2019 American Chemical Society	Ramp-up and down using spacer & magnet every 3 hours	In the stage of ramp-up, significant enhancement shown by *AURKA*, followed by *CNNB1*, *CTGF*, and *FN1* expression. During the ramp down, the genes *AURKA*, *CNN B1*, *CTGF*, and *FN1* all exhibit significant decreases in expression. During all process, *MYH7* shows an increase in the expression.	With magnet (stiffer): higher myofibroblast activation, higher *MYH7*, lower sarcomere organization, fast translocation of the YAP into the nucleus as the substrate is stiffened.
[119]	3D micropillar	 Peproduced with permission from J. Mol. Cell. Cardiol. 118, 147 (2018). Copyright 2018 Elsevier.	Stiffer pillar by adding support (static); and 1.75 Hz biphasic field stimulation	Higher stiffness leads to upregulation of *MYH6*, *MYH7*, *MYL2*, *ACTC1*, and *TNNI3* (promote maturation), *CASQ2*, *CACNA1C*, and *SCN5A* (calcium handling related genes), and *NPPA*, *NPPB*, *ACTA1*. Decreased expression of the endoplasmic reticulum calcium pump SERCA2a (coding by *ATP2A2*) and showed ineffective oxidative metabolism.	Increasing stiffness improved cell size and sarcomere structure, as well as calcium handling in function of time.

120	3D micropillar with magnet & piezoelectric mount	 Reproduced with permission from ACS Biomater. Sci. Eng. 5, 3663 (2019) Copyright 2019 American Chemical Society	After 20 days: N40 neodymium permanent magnets into silicone posts as afterload enhancement (stepwise increase)	No significant upregulation of fetal cardiac genes observed. The magnet tuning was able to initiate a physiological force response to increased afterload.	n/a
121	3D micropillar with metal brace	 Reproduced with permission from Basic. Res. Cardiol. 107, 307 (2012) Copyright 2012 Elsevier.	After 2weeks: afterload enhancement using metal braces	Afterload enhancement induced hypertrophic programs: ANP, BNP, α-skeletal-actin, and β-MHC & downregulation of SERCA2a. Higher glucose consumption per beat, increased glucose consumption, and increased mRNA levels and extracellular deposition of Collagen-1.	Cardiomyocytes in EHTs enlarged by 28.4 % under afterload enhancement and to a similar extent by endothelin-1- or phenylephrine-stimulation (40.6 or 23.6 %).
122	3D micropillars on top of fluidic channel		After 3 days: pressurizing the fluidic channel à 5 to 15% strain from the displaced pillars	Upregulation of the fetal protein program, ANP, βMHC, and skeletal α-actin, in dose-dependent manner with increasing strains, similar to dose-response of HCM.	n/a
63	Dynamic-EHT with an elastic PDMS strip		On day 14 and until day 28, tissues are exposed to constrained (EHTs) or dynamic (dyn-EHTs) loading. Dynamic loading enabled to model preload conditions	Dynamic EHT leads to up-regulation in sarcomere-related genes: *TTN* and *TNNI3*, *DSP*, *PKP2*, and *DSC2* and the intermediate filament gene *DES*. Metabolic markers upregulated: *CPT1B* and *PDK4*; sodium channel gene *SCN5A* and gap junction protein Cx43.	The applied force leads to improved alignment, conduction velocity, and contractility of EHT. With desmosome mutation, dyn-EHT showed contractile shortening in the dynamic, which further led to faster contraction and relaxation times.

FIGURES

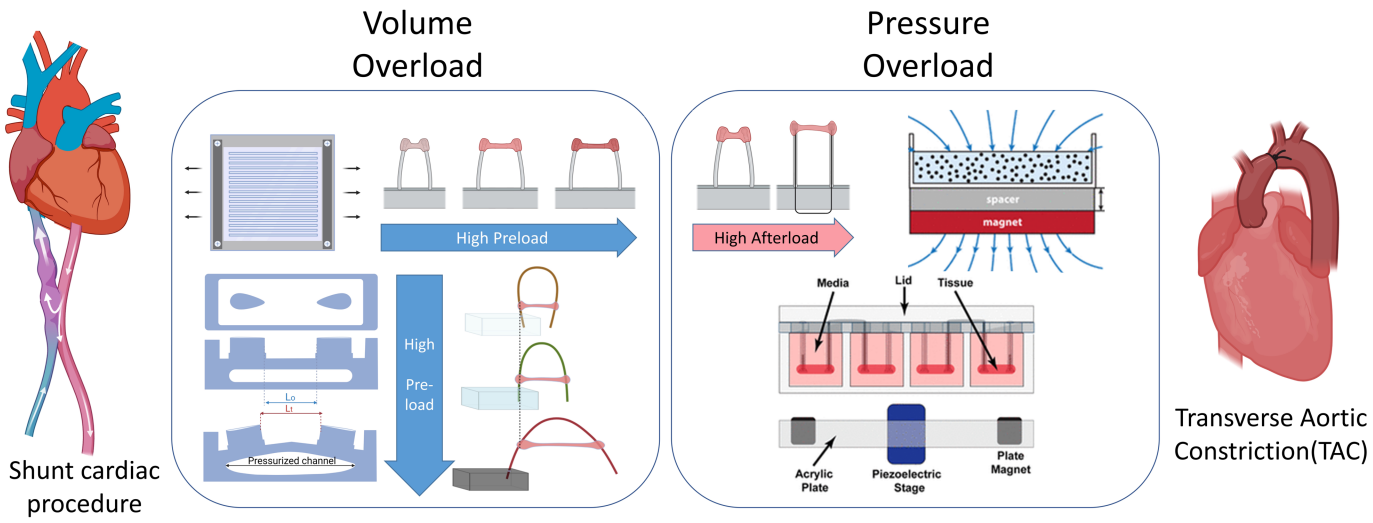


Figure 1. Existing studies to model dynamic cardiac overload *in vitro* and surgical procedures in animal study to mimic volume and pressure overload. Image created with BioRender. Two images are reproduced with permission from Corbin et al., *ACS Appl Mater Inter* 11, 20603 (2019). Copyright 2019 American Chemical Society and permission from Rodriguez et al., *ACS Biomater Sci Eng* 5, 3663 (2019). Copyright 2019 American Chemical Society.

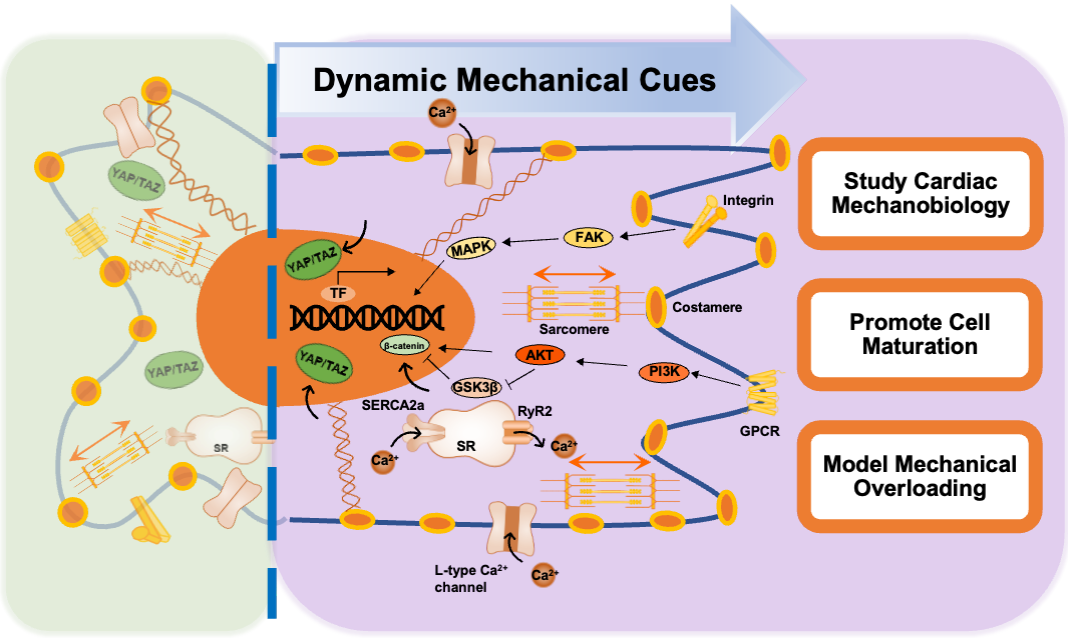


Figure 2. Cardiomyocyte mechanosensitivity to dynamic mechanical microenvironment.

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