Stephen P. Ma

Department of Biomedical Engineering,
Columbia University,
622 West 168th Street,
VC12-234,
New York, NY 10032
e-mail: spm2145@columbia.edu

Gordana Vunjak-Novakovic¹

Department of Biomedical Engineering
and Department of Medicine,
Columbia University,
622 West 168th Street,
VC12-234,
New York, NY 10032
e-mail: gv2131@columbia.edu

Tissue-Engineering for the Study of Cardiac Biomechanics

The notion that both adaptive and maladaptive cardiac remodeling occurs in response to mechanical loading has informed recent progress in cardiac tissue engineering. Today, human cardiac tissues engineered in vitro offer complementary knowledge to that currently provided by animal models, with profound implications to personalized medicine. We review here recent advances in the understanding of the roles of mechanical signals in normal and pathological cardiac function, and their application in clinical translation of tissue engineering strategies to regenerative medicine and in vitro study of disease. [DOI: 10.1115/1.4032355]

1 Introduction

Tissue engineering was officially established at an NSF meeting in 1987 by Y. C. Fung, followed by the first tissue engineering workshop at Lake Tahoe in 1988 [1]. The 1993 Science review by Robert Langer and Joseph Vacanti helped to establish tissue engineering as its own discipline [2]. One of the common unifying themes since the inception of the field has been the importance of biomechanical cues, which can act on cells through a number of different pathways. Examples include changes in gene expression secondary to forces transmitted to the nucleus [1], kinase phosphorylation [3,4], conformational changes in the cytoskeleton [1], localization of proteins [4], and stretch-activated ion channels [1,5–7].

In tissue engineering, biomechanical signals are being harnessed in two primary ways. First, knowledge of the effects of different mechanical stimuli is being applied to engineer functional tissues in vitro [8]. Even a simple change in substrate stiffness has been shown to differentiate stem cells toward different lineages [9,10]. Cyclic compression has been shown to beneficially regulate cartilage tissue development [11,12], while cyclic tension has greatly increased the tensile strength of engineered arteries [13,14]. Perfusion bioreactors providing fluid shear stresses have been used to enhance osteoblast differentiation and mineralization [15]. Second, biomimetic in vitro systems incorporating controllable mechanical stimuli are being used as models for better understanding the complex relationships between mechanical cues and biology. We review here recent progress in cardiac biomechanics and cardiac tissue engineering, and discuss the outlooks for future work.

2 Cardiac Biomechanics

The intrinsically mechanical nature of the heart makes cardiac tissue engineering an obvious field for the study and application of biomechanics. In particular, the observation that both adaptive and maladaptive cardiac remodeling occurs in response to altered mechanical loads is a foundational concept for clinical cardiology [16–18] and has also informed recent progress in cardiac tissue engineering. Here, we discuss cardiac physiology and pathophysiology, with an emphasis on their relationship with mechanical loading.

The heart receives blood from the systemic and pulmonary circulations into the right and left atria, and pumps blood into the

pulmonary and systemic circulations from the right and left ventricles, respectively. The flow of blood is controlled by four valves: (i) the tricuspid between the right chambers, (ii) the mitral between the left chambers, (iii) the pulmonary between the right ventricle and pulmonary trunk, and (iv) the aortic between the left ventricle and the aorta (Fig. 1(a)). Of primary clinical interest is the behavior of the left ventricle. The basic contractile cycle of the left ventricle is commonly presented as a pressure–volume loop with four distinct phases controlled by the state of the mitral and aortic valves (Fig. 1(b)): (1) ventricular filling through the open mitral valve (isotonic relaxation), (2) ventricular contraction against a closed aortic valve (isometric contraction), (3) ejection of blood through the open aortic valve (isotonic contraction), and (4) ventricular relaxation with a closed mitral valve (isometric relaxation) [19,20].

The contours of the PV loop are partially governed by the intrinsic biomechanical properties of the heart itself, commonly depicted as the end-diastolic pressure–volume relationship (EDPVR) and the end-systolic pressure–volume relationship (ESPVR) (Fig. 1(b)). Changes in the stiffness/compliance of the relaxed ventricles alter the filling properties of the heart, contributing to changes in the EDPVR (Fig. 1(c)). Changes in the contractility and ionotropy of the ventricles alter the ejection properties of the heart, contributing to changes in the ESPVR (Fig. 1(d)). Both of these relationships have major consequences for the stroke volume and cardiac output of the heart.

The major external mechanical stimuli of interest to the heart are the preload/volume load (determined by the extent of ventricular filling) (Fig. 2(a)) and the afterload/pressure load (determined by the pressure against which the heart pumps) (Fig. 2(b)). The preload in vivo can be altered by a number of phenomena including mitral valve regurgitation, arteriovenous shunts, and pregnancy [21]. Frank–Starling’s law for the heart governs the instantaneous interaction between ventricular filling and stroke volume/contraction force [6]. Mechanical stretch induces an immediate increase in force within a beat [6] that is followed by a secondary increase in force over the course of several minutes, possibly related to stretch-dependent changes in the action potential [6]. Regardless of mechanism, the presence of a positive force–length relationship is considered a hallmark of healthy ventricular tissue and is absent in many patients with chronic heart failure.

Chronically, increased preload can cause serial addition of sarcomeres, lengthening of myocytes and dilation of the left ventricular wall, resulting in eccentric hypertrophy [16,22,23] and reduced ejection fraction (EF), where the ventricle is eventually unable to contract with enough force to maintain circulatory

¹Corresponding author.

Manuscript received November 11, 2015; final manuscript received December 15, 2015; published online January 27, 2016. Editor: Victor H. Barocas.

Fig. 1 Mechanical function of the heart. (a) The heart consists of four chambers that circulate blood through the systemic and venous circulations. (b) Blood flow through the heart is controlled by the four valves as depicted pictorially in the diagrams. The opening and closing of the valves is controlled by the relative pressures between the various compartments. The contours of the left ventricular PV loop for each contractile cycle are partially determined by the intrinsic properties of the heart (EDPVR and ESPVR). (c) Changes in mechanical stiffness change the EDPVR. (d) Changes in ionotropy change the ESPVR. (Images in (a) and (b) were modified from work done by Eric Pierce, available under a GNU Free Documentation License or a Creative Commons Attribution-ShareAlike License.)

output. The molecular phenotype is generally distinct from that associated with increased afterload: upregulation of Akt [22], no upregulation of B-type natriuretic peptide (BNP) [22], no upregulation of α -skeletal actin [21], and impaired focal adhesion kinase (FAK) signaling [24]. A number of mechanisms have been proposed to explain mechanotransduction in the heart, including stretch-sensing complexes in titin [25], stretch-sensing proteins localized to the Z-disk [23,26,27], stretch-activated ion transporters [7,28], stretch-activated receptors [29], and integrin signaling [30]. However, the field is still fairly fragmented about the exact roles of the various downstream signaling pathways acted on by

these transducers [31]. One of the best understood pathways activated by volume loads involves TNF- α , which is upregulated with stretch [32], downregulated following surgical correction of mitral regurgitation [33], and interacts with other signaling pathways to promote eccentric hypertrophy [16]. Other biomechanical and neurohumoral mediators associated with eccentric hypertrophy include CT-1 [21], LIF [21,34], and IGF-1 [21], and downregulation of FAK [21] and RhoA [21,34].

Increased afterload is commonly caused by systemic hypertension [35], but can also be caused by localized conditions such as aortic stenosis. Chronic increases in afterload lead to parallel

Fig. 2 Normal and pathological conditions of preload and afterload in the heart. The contours of the left ventricular PV loop are further modified by mechanical loading, which depend on (a) the volume of blood in the ventricle prior to the stroke and (b) the pressure against which the ventricle contracts. (c) Chronic increases in these loads can lead to pathological changes in the heart. (Images in C were reproduced from Servier Medical Art library of images.)

addition of sarcomeres and thickening of individual myocytes and the left ventricular wall, resulting in concentric hypertrophy of the heart and impaired diastolic filling [16,22,23]. The canonical hypertrophic fetal gene program involves upregulation of atrial natriuretic factor (ANF) [36], BNP [22,36], α -skeletal actin [36] (as opposed to α -cardiac actin [37]), a shift in myosin heavy chain (MHC) expression (downregulation of the α isoform in humans [37], shift from α - to β in mice [36,37]), and downregulation of sarcoplasmic endoplasmic reticulum calcium ATPase2a (SERCA2a) [36,38,39].

The pathway most commonly associated with ventricular remodeling in response to increased afterload is the release of angiotensin II in response to increased systolic wall stress that binds to its G_q protein receptor and activates the ϵ isoform of protein kinase C, which eventually leads to activation of mitogen-activated protein kinases (MAPKs) [16,40]. These kinases upregulate both the prosurvival and pro-apoptotic pathways via extracellular-signal-regulated kinases (ERKs) and Jun N-terminal kinase (JNK), respectively, reflecting the competition between adaptive and maladaptive responses to mechanical stress [16,41]. Angiotensin II is generally considered a maladaptive signal, upregulating fibrosis via separate pathways [16], and drugs that block the formation of angiotensin II are front-line treatments for heart

failure.[42] Other pressure-induced pathways include adrenergic activation [16,21], the formation of reactive oxygen species [16], and endothelin-1 [21]. In addition to their direct effects on myocardium, many of these signals can also cause an imbalance in matrix metalloproteinases and their inhibitors [16,43], which can lead to collagen degeneration and ventricular dilation [16,43].

Of note, the classical binary division described here (Fig. 2(c)) is overly simplistic. Many patients exhibit characteristics of both impaired EF and impaired ventricular filling, particularly as remodeling proceeds in response to the initial insult [16,20,44]. As such, the historical division of heart failure into systolic and diastolic heart failure [45] has been superseded by a division into heart failure with reduced EF (HFrEF) and heart failure with preserved EF (HFpEF), each with distinct risk profiles and therapeutic outcomes [46]. The change in terminology also reflects our increased understanding of the importance of previously overlooked factors, such as increased collagen deposition in HFpEF [47]. However, despite complicated clinical picture, the basic concepts described here still provide a useful framework for thinking about mechanical loading of the heart.

There are two important points to keep in mind. First, TNF- α , angiotensin II, and the neurohumoral system at-large are important contributions to the progression of ventricular dysfunction to

clinical heart failure, but are systemically regulated [48], making it difficult to separate them from mechanical loading. Second, the sheer number of downstream signaling pathways that respond to mechanical stimuli have wide-ranging effects that can be harnessed for medical and tissue engineering purposes.

3 Cardiac Tissue Engineering

3.1 Knowledge Application: Mechanical Maturation of Tissue Engineered Constructs. The heart is the first functional organ in the human body, and it starts to beat only three weeks into gestation. Therefore, most of the heart's development and all adult function occur in the presence of mechanical contractions induced by electrical signals. Observations of congenital malformations in response to overloading or unloading in the embryo suggest that mechanical loading is a strong developmental cue [49], and that abnormal ventricular morphologies correspond to load imbalances between the right and left ventricles [19].

These observations suggest that mechanical loading could also be an important factor in engineering cardiac tissue constructs. The simplest strategy for biomechanical stimulation of cultured heart muscle is the use of static holders for isometric loading (Fig. 3(a)), which can be adjusted to manipulate the preload and were first used in 1997 [50]. This approach has been improved upon since through the use of auxotonic loading (Fig. 3(b)) [51], which is defined as contraction against an increasing load followed by extension under a defined force [19]. This is commonly achieved through contraction against flexible holders such as elastic pillars made of polydimethylsiloxane that are easily molded in a variety of shapes to facilitate optimal loading, and where contractions can be imaged for real-time readouts of force generation [52,53]. Studies using these systems demonstrate the importance of contraction against anisotropic loading for the alignment of cells and matrix in engineered cardiac tissue [51,54].

Slightly more complex is the use of cyclic mechanical stress (Fig. 3(c)), which requires adaptation of the cycle length with respect to the endogenous beating frequency of the engineered tissue [19]. This method was first used in 2002 to create engineered heart tissues that had better ultrastructural organization and contractile properties [55] than the unstimulated tissues [50]. Cyclic mechanical stress of tissue engineered constructs shifted the expression of cardiac MHC from the α - to β -isoform [56,57], and also upregulated ANP [56–58], BNP [56], cardiac troponin T(cTnT) [56], α -actinin [58], and connexin-43 [3]. These changes correspond to activation of the fetal/hypertrophic gene program and/or maturation of cardiomyocytes to a more adultlike phenotype. Structurally, cyclic stretch also leads to longitudinally oriented cells [56,58] and matrix [56], increased matrix deposition [59], increased cell-to-nucleus ratio [58], increased cell size [56,58], localization of connexin-43 [4], closer association of mitochondria with myofilaments [58], and enhanced myotube formation through phosphorylation of FAK and RhoA [60]. RhoA is an important regulator of cardiac hypertrophy, is downregulated in eccentric hypertrophy [34], and has been shown to be critical for cardioprotective hypertrophy without dilation in response to chronic afterload [61].

Functionally, the application of cyclic stretch leads to increased force of contraction [57,58], greater acute response to β -adrenergic stimulation [57], and increased twitch tension as a function of calcium concentration [51], possibly mediated through greater expression of L-type Ca^{2+} channels [56], the ryanodine receptor [56], and SERCA2a [56]. Mechanically stimulated constructs derived from human pluripotent stem cells have reproduced the classic force-length Frank–Starling relationship characteristic of native cardiac muscle [56].

While significant advances have been made with engineering cardiac tissues using mechanical stimulation, the results have been inconsistent, with some groups not achieving improved contractile function with cyclic stretch [62], and others demonstrating

immature electrophysiology in cells subjected to passive mechanical loading [54]. An alternative strategy for cardiac maturation is electrical stimulation, which was first used on in vitro tissues in 2004 to induce synchronous tissue contractions [63] and has since seen widespread use [64,65]. Recently, it was shown that electrical stimulation can improve calcium handling in cardiomyocytes, resulting in more mature electrophysiology, in addition to eliciting a hypertrophic response [66]. However, while promising, electrical stimulation alone was not able to achieve terminal differentiation of human pluripotent stem cell-derived cardiomyocytes at an ultrastructural level, as shown by the absence of T-tubules and M-lines [66].

The next generation of maturation regimens seeks to combine the use of mechanical stimulation with electrical stimulation [67–70]. These range from simple passive tension in combination with electrical stimulation (Fig. 3(d)) [67] to more complicated patterns, such as active mechanical stretch followed by delayed electrical stimulation, that seek to mimic the in vivo cardiac cycle described previously [69,70]: (1) mechanical stretch (isometric stretch), (2) electrically stimulated contraction against the stretcher (isometric contraction), (3) release of the mechanical stretcher (isotonic contraction), and (4) relaxation (isometric relaxation) [6]. These studies have added to our knowledge of the different signaling pathways upregulated in response to preload and afterload [70]. In particular, in vitro studies have shown that SERCA2a is upregulated by increased preload (isotonic contraction vs. slack) [38,69] but not by increased afterload (isometric contraction vs. slack) [38,69], possibly through BNP antagonization [38,39], making the relative timings of mechanical and electrical stimulation critical for maturing this aspect of calcium handling [69].

Continued progress must still be made to achieve truly adultlike human engineered heart tissue. In addition to the lack of T-tubules and M-lines, human pluripotent stem cell-derived cardiomyocytes have yet to demonstrate a positive force–frequency relationship (Bowditch phenomenon), another hallmark of adult ventricular myocardium that was only recently demonstrated in engineered tissue created using neonatal rat cardiomyocytes [71]. Further optimization of maturation protocols, based on new biological insights, will be needed to achieve these goals. In particular, the effects of cyclic mechanical loading have been speculated to be frequency-dependent, with higher frequencies corresponding to increased kinase phosphorylation [72] and greater gene expression changes [73]. Similar suggestions have been made with respect to electrical stimulation [66,71], and the frequency-dependence of maturation stimuli is an active area of research. Additionally, long-term β -adrenergic stimulation was shown to further increase contraction forces in engineered tissues on top of gains already achieved through mechanical stretch alone [57], suggesting that the use of chemical stimulation will be important as well.

3.2 Knowledge Generation: Tissue Response to Controlled Mechanical Stimuli. Historically, in vitro models for the study of cardiac biomechanics have included stimulation using chemicals, such as α -adrenergic agonists or endothelin-1 [36,74], or stretching of cardiomyocyte monolayers on flexible membranes [74–76]. These approaches revealed that while both chemical stimulation and mechanical stretching can both lead to cardiac hypertrophy, they each result in different gene expression programs [74,76]. Mechanical stretch is a frequent tool for modeling conditions of increased preload (Fig. 4(a)), with in vitro models providing insight into the underlying mechanisms such as the role of the ϵ isoform of protein kinase C in regulating sarcomere length following longitudinal stretch [77].

However, 2D monolayer systems are not particularly biomimetic, as they lack the 3D cell-matrix environment. The tissue engineered models discussed in Sec. 3.1 address this fundamental limitation through the creation of 3D tissue constructs [36]. A recent study modeled the increased afterload by creating 3D tissue

Fig. 3 Mechanical and electrical stimulation strategies for the maturation of cardiac tissue constructs. (a) Brightfield and α -actinin staining depict cardiac response to static, isometric stretch in a biaxial arrangement (reproduced with permission from [167]). **(b)** Auxotonic stretch is more biomimetic, and allows for the tuning of tissue properties by adjusting the spring constant of the resisting material [168]. The sequence of brightfield images shows shrinkage of the gel and alignment of the tissue over seven days. The bar graphs depict changes in cross-sectional area and force generation as a function of the pillar spring constant and collagen concentration (reproduced with permission from [168]). **(c)** Cyclic stretch substitutes active dynamic loading [169] for the passive loads described in (a) and (b) (reproduced with permission from [169]). **(d)** Electrical stimulation of tissue constructs subjected to auxotonic stretch (spring device on the left) is commonly achieved through the use of bioreactors with carbon rod electrodes (black rectangular blocks on the right), and have produced aligned tissues with electrophysiological maturity (reproduced with permission from [71]).

organoids around silicone tubes with a low spring constant [36]. The baseline resistance could be increased 12-fold through the addition of metal rods into the tubing, mimicking a sudden increase in afterload (Fig. 4(b)) [36]. Afterload resulted in myocyte hypertrophy, activation of the hypertrophic genetic program, and increased glycolysis/fibrosis in engineered tissues created from neonatal rat ventricular cells [36]. These changes correlated with decreased functional outputs including the contractile forces and relaxation velocities [36].

Particularly intriguing were the similarities between gene expression associated with afterload enhancement and that

associated with application of endothelin-1. Such correlation was not observed with phenylephrine (an α -adrenergic agonist), indicating that endothelin-1 might be a more physiologic stimulus for hypertrophy [36]. Indeed, the use of endothelin receptor antagonists blunted many of the effects of afterload, including the activation of the hypertrophic gene program, fibrosis, and the changes in relaxation times [36]. It remains to be better understood why these benefits appeared in the tissue engineered systems, while the use of endothelin receptor antagonists has largely failed in clinical trials [78]. One likely explanation is the use of neonatal rat ventricular cells for this study, as endothelin receptor antagonists

Fig. 4 In vitro methods for studying preload and afterload. (a) Increased preload is commonly modeled by stretching cardiomyocytes grown on 2D membranes (reproduced with permission from [75]). (b) Increased afterload can be modeled by actively changing the spring constant of the resisting material after tissues have been formed (reproduced with permission from [36]).

were found to be beneficial in animal models for postMI therapy [79].

This particular study was significant for a number of reasons. First, it showed that isolated afterload enhancement in tissue engineered models is a sufficient stimulus for pathological hypertrophy, independent of the accompanying systemic neurohumoral activity and blood vessel-myocyte mismatch found in small animal models [36]. Second, this is the first tissue-engineered model of isolated afterload enhancement, as opposed to the much more common preload enhancement or neurohumoral stimulus as discussed above. The Framingham Heart Study has indicated that afterload enhancement is more important than preload enhancement in the pathophysiology of heart failure [35], an observation that has been supported in comparison of TAC mice and shunt mice [22]. Moreover, the observed upregulation of both the glycolytic and fibrotic pathways in vitro demonstrated the presence of native plasticity in cardiac tissue without the need for systemic regulation. Finally, the protective effects of endothelin receptor blockade without any alteration in endothelin expression and no interference from external stimuli suggests a possible mechanism: mechanically induced activation of endothelin receptors (similar to that proposed for angiotensin II-receptors) [29]. Recapitulating this study with human cells would potentially shed further light on this suggested mechanism as well as the discrepancy between animal models and clinical trials.

3.3 Knowledge Interpretation: Comparison of Current In Vitro Models to the Clinical Setting. The differences between engineered cardiac tissues and native heart tissue must be kept in mind when translating results from in vitro models to the clinical setting. Native heart tissue contains high concentration of cells (on the order of 10^8 cells/cm³) [80] of multiple types: cardiomyocytes, endothelial cells, smooth muscle cells, and fibroblasts [81]. It has been estimated that cardiomyocytes occupy ~75% of the volume of the heart but comprise <40% of the total number of cells [82]. Functionally, native adult human heart tissue has been reported to generate maximum stresses of 18–44 mN/mm² [83] and support ventricular conduction velocities of 46 cm/s [83]. It should be noted that conduction velocities in the heart are highly heterogeneous, with major differences between the atria, the AV node, the bundle of His/Purkinje fibers and the ventricles.

Engineered heart tissue differs from the adult native heart tissue in a number of regards. The cellular mixture is simplified, with

many constructs formed from pure cardiomyocytes [66] and only sometimes supplemented with either supporting cells [83]. In particular, the closely integrated microvasculature of the heart where every cardiomyocyte is adjacent to a capillary has yet to be replicated in vitro, which means that the parenchymal–vascular relationship is not faithfully reproduced in these models [82]. Moreover, the limits on tissue oxygenation set constraints on the cell density and thickness of tissue constructs, with only a few published engineered tissues approaching the cell densities reported for native myocardium [66]. Functionally, the largest stress generated by engineered human heart tissues has been reported as 11.8 mN/mm² [83], which is two-to-three-fold lower than that of native tissue. The conduction velocity of these same tissues was 21.2 cm/s [83], or about half that reported for native tissue.

Building relationships between parameters measured in in vitro systems with those measured in the clinical setting is difficult. As mentioned previously, we are interested in the contractility and compliance of the heart, illustrated by the ESPVR and the EDPVR, respectively. The in vitro proxy for contractility is the maximum stress generated during the contractile cycle, which changes the slope of the ESPVR. However, while it is easy to directly measure the stresses generated by cardiac tissue in the in vitro setting, this is not the case clinically. The gold standard for measuring these relationships clinically is the pressure-conductance catheter combined with load-altering interventions such as balloon occlusion of the inferior vena cava [84]. This method is generally restricted to research settings as it is quite involved. The most common proxy for contractility in the clinical setting is the EF, which is defined as ratio of the stroke volume to the end diastolic volume. While this is easily determined from echocardiography, a brief inspection of the previously depicted graphs (Figs. 1 and 2) is enough to see that contractility is not the only factor that affects the EF. Research into the use of novel methods for explicitly measuring the ESPVR and the EDPVR is ongoing [85].

The two extrinsic mechanical factors are similarly problematic. The most meaningful measure of muscle preload is the sarcomere length, which can be measured in vitro. However, this is impractical in the clinic, where the end-diastolic pressure is the preferred proxy. This is usually determined as the pulmonary capillary wedge pressure measured through the use of a Swan-Ganz catheter. Similarly, the afterload is directly measurable in many in vitro

systems where it is simply the resistance that must be overcome by the tissue during contraction. On the other hand, the clinical conditions are complex, with several potential proxies ranging from simple measures such as the aortic pressure, to the total peripheral resistance (defined as the ratio between the mean pressure drop across the vasculature and the cardiac output), and the complex frequency-dependent arterial impedance [86].

In summary, a number of issues arise should we wish to translate the results of in vitro measurements to the clinical setting. In addition to the shortfalls in current in vitro models, oftentimes completely different measurements are used to represent the variables of interest. We believe that the relationship between in vitro and in vivo measurements needs to be assessed on a platform-by-platform basis. Moreover, in many cases it will likely be more useful to directly relate in vitro measurements to clinical endpoints of interest such as heart failure or arrhythmogenic potential through the use of machine learning algorithms rather than passing through an intermediate in vivo measurement. Until these platforms are systematically characterized, their utility will remain dependent on the target application.

4 Future outlook

4.1 Future Outlook: Comparison With Animal Models for Preclinical Testing. A number of animal models have been created for the study of cardiac biomechanics. As an example, the rat coronary ligation model for the study of MI and heart failure was developed in 1979 [87]. Using this model, the authors observed a proportional relationship between infarct size and left ventricular dilation and function [88], and subsequently used this model to study the effects of angiotensin converting enzyme (ACE) inhibitors for postmyocardial infarction (postMI) therapy. ACE inhibitors were the first vasodilators to be explored for cardiac therapy through a decrease in afterload. The results from small animal studies showed reduced left ventricular dilation, improved systolic function and increased survival in the treated animals [17,89], eventually leading to the human Survival And Ventricular Enlargement (SAVE) trial comparing captopril against placebo with a 19% reduction in all-cause mortality and 22% reduction in heart failure hospitalizations during the mean follow-up period of 42 months [90]. These results were strongly correlated to quantitative changes in left ventricular end-diastolic and end-systolic areas on 2D transthoracic echocardiograms [91]. This series of studies provides an example of the link between mechanical stimuli and cardiac health, and is an elegant application of a controllable experimental model system for the study of mechanical interventions.

However, while some studies in animal models have succeeded, others have failed. The same rat MI model also indicated that postMI administration of endothelin receptor antagonists increased survival and reduced left ventricular dilation [79]. These drugs subsequently failed in human trials [92,93], demonstrating some of the shortcomings of animal models. The classic murine model for increased afterload is transverse aortic constriction [94], which begins with compensated hypertrophy and temporary enhancement of cardiac contractility followed by cardiac dilatation and heart failure [95]. However, only a subset develop heart failure and the functional phenotypes are highly variable [96], and dependent on the mouse strain [97]. Similar caveats apply to the corresponding rat model, ascending aortic banding [98], though the larger animal sizes result in relaxed technical requirements [99]. The rat coronary artery ligation model discussed above is one of the most popular heart failure models, but it does not mimic the gradual progression of pressure overload through compensated hypertrophy to decompensated heart failure, which is the predominant sequence of events in hypertension-induced heart failure in humans [99].

In this context, tissue engineered in vitro models provide several clear benefits when coupled with animal models. Animal

studies are resource-intensive, requiring comparatively large investments of technical expertise, money, and time. Tissue engineering provides easily accessible platforms for pilot studies that are nevertheless more biomimetic than simple cell culture systems, and can be used to study human cells and tissues rather than those from animals. Moreover, in vitro platforms offer the ability to isolate mechanical stimuli from systemic effects. Indeed, combined studies using both in vitro and animal models are increasingly common and leverage the relative strengths of each system. For instance, a recent study looking at the different effects of increased preload and afterload on the endoplasmic reticulum (ER) of cardiomyocytes used in vitro and in vivo models to dissect out the effects of mechanical loading from those of purely neurohumoral conditioning, showing that afterload, but not preload, induces myocardial ER stress independently of angiotensin II signaling [100].

4.2 Future Outlook: In Vitro Integration With Stem Cell Research and Precision Medicine. The fields of tissue engineering, stem cell research, and gene therapy have matured together over the past decade, creating a confluence of methods ripe for integration. In the field of stem cells, the ability to create induced pluripotent stem cells (iPS cells) from human somatic cells was first reported in 2007 [101,102] and was rapidly applied to the creation of patient-specific stem cell lines recapitulating disease phenotypes in 2008 [103,104]. Generation of cardiomyocytes from iPS cells (iPS-CMs) soon followed in 2009 [105,106] and was recently optimized for consistency and efficiency across hundreds of cell lines using chemically defined conditions in 2014 [107,108]. The first patient-specific iPS-CM models were created in 2010 [109,110] and have been steadily expanded upon since [111,112] with 39 publications as of January 2015 [113].

The use of patient-specific iPS-CMs provides a mechanism for clinicians to link genetic mutations with clinical phenotypes and establish causality, particularly when targeted gene-editing is used to generate isogenic control lines [114]. The recent application of the CRISPR/cas9 system to human cells has greatly improved the efficiency and precision with which we are able to genetically engineer human cell lines [115–118]. Combined with the tissue engineering platforms discussed previously, we are for the first time able to reliably generate human genetic disease models in vitro, and subsequently subject them to the mechanical, electrical, and biological stresses that they might encounter in vivo. These systems hold enormous promise, both for better understanding clinically relevant diseases, and for developing patient-specific therapeutic regimens.

As a prominent recent example, the most common genetic causes of dilated cardiomyopathy are truncating mutations in the sarcomere protein titin, accounting for 25% of familial cases of idiopathic dilated cardiomyopathy and 18% of sporadic cases [119]. While single-cell assays showed no significant difference between iPS-CMs derived from patients carrying these mutations and wild-type controls, the same type of assay carried out on 3D microtissues showed that the mutated line generated 50% of the contractile force compared to wild-type controls (Fig. 5(a)) [120]. These results were confirmed through the use of CRISPR/Cas9 to create corresponding mutations in independent cell lines to account for possible interpatient background genetic differences [120]. The tissue engineering platform was further used to test the response of the tissues to differential mechanical loads, demonstrating that the mutations prevented adaptation to increased mechanical loads [120]. Further analysis identified alternative exon splicing as a possible mitigating factor for the observed differences in pathogenicity of I-band mutations in comparison with A-band mutations [120]. This study demonstrates the many possibilities provided by controlled in vitro systems and provides a road map forward for future studies.

On the therapeutic side, there is great interest in the use of genetically engineered systems for drug development. The biggest

Fig. 5 The use of engineered human cardiac tissues to model cardiac disease. (a) Titin mutations are a common cause of dilated cardiomyopathy. The structure of the cardiac sarcomere is depicted on the left, with TTN, thick filaments (rods with globular heads), and thin filaments (coiled ovals). TTN protein segments (Z disk, I band, A band, M band) are shown below, along with the locations of patient-derived (p) and CRISPR-induced (c) mutations. hiPS-CMs carrying these mutations were used to create microtissues (center, brightfield and immunofluorescence of phalloidin staining). These in vitro models recapitulated a number of relevant parameters including the change in contractile force [120] in mutated lines as shown on the right (reproduced with permission from [120]). (b) The development of organ-on-a-chip models using hiPS-derived cells has advanced to the point where multi-organ integration is a possibility. One avenue of exploration is to combine heart, liver, and vasculature for the purpose of drug testing [126]. The CAD drawings on the left depict a modular platform with different compartments specifically designed for the culture of heart and liver tissues. The bottom photos on the left show cardiac microtissues. The middle column of images from top to bottom depict (1) the scale of the individual modules, (2) dissolvable sugar lattices for the introduction of vasculature, (3) a cardiac microtissue, and (4) the even propagation of electrical signals through cardiac tissue. The images on the right depict angiogenic sprouting from the initial channels created through the use of sacrificial sugar filaments coated by human endothelial stem cells (reproduced with permission from [126]).

immediate impact of these systems may be with regards to cardiovascular safety, as cardiovascular toxicity accounts for the greatest percentage of drug withdrawals and black box warning additions [121,122]. As of 2015, the guidelines for nonclinical cardiotoxicity assessments recommend repeated-dose toxicological studies in one rodent and one nonrodent species, with endpoints based on histopathological examination and

electrocardiographic recordings. These findings are to be aggregated with in vitro ion channel studies to paint a complete picture of cardiotoxicity [123,124]. Patch clamp assays capture single-cell ion channel dynamics but the data is often insufficient to predict the behavior of aggregates of cells [125]. Conversely, small animal models capture the geometric complexity of the heart, but the differences between human cells and animal cells prevent

Fig. 6 The application of optogenetics in cardiology. (a) Light-induced multisite pacing of a Langendorff-perfused heart was demonstrated through the use of AAV-9 as a vector for ChR2 delivery, recapitulating the benefits of multisite electrode pacing (reproduced with permission from [158]). (b) Optogenetics is also being used as a basic science tool in vivo to probe the mechanisms that underlie spiral wave arrhythmias and their termination, such as an increased first half winding distance (1/2 W.D.) (reproduced with permission from [159]).

successful prediction [121]. In vitro tissues created using hiPS-CMs have the potential to capture the best of both worlds—human biological fidelity and geometric complexity—and increase the predictive power of preclinical studies. The most ambitious research in this area aims to recapitulate the physiology of the liver and vasculature in addition to the heart, to account for the effects of absorption, metabolism, and excretion of the drug (Fig. 5(b)) [126]. However, systematic characterization of the response of engineered heart tissues to drugs, and the predictive value of these measurements, has yet to be attempted.

Clinically, this line of research meshes nicely with the narrative of personalized medicine, in which every patient is approached in a targeted way. Patient-specific iPS-CMs have already been used to identify potential therapies for a number of genetic cardiac diseases. While largely focused on channelopathies, primarily biomechanical diseases including familial hypertrophic [127,128] and dilated [128,129] cardiomyopathies have also been studied. The mutations associated with hypertrophic cardiomyopathy (β -MHC, cardiac troponin T, α -tropomyosin, myosin-binding protein C) [130] appear to cause failures in force generation, prompting secondary hypertrophy [131], while those resulting in dilated

cardiomyopathy seem to be associated with problems in force sensing and transmission [132]. These studies have identified potential novel therapeutic options [127,129] as well as variable adverse reactions to existing drugs [128]. The phenotype of the derived hiPS-CMs, which have been seen to vary between patients with similar mutations [113], have also been proposed as an assay output in and of itself for differentiating between patients. However, further research is still necessary to validate the results of these systems, especially with respect to possible interactions between the disease and the culture conditions that may not be indicative of the in vivo phenotype [113].

4.3 Future Outlook: In Vivo Integration With Mechanical, Electrical, and Optical Devices. Finally, we briefly discuss the opportunities for clinical application of regenerative medicine based on tissue engineering. In particular, advances in biocompatible materials, the miniaturization of electronics, and the development of novel forms of electromechanical and optomechanical actuation have greatly increased the possibilities of interfacing with biological tissues in the in vivo setting, enabling the clinical

translation of methods currently used for tissue engineering in vitro.

Passive mechanical constraint for the prevention of ventricular dilation is one of the simplest interventions from a conceptual standpoint, and was studied in the Acorn Clinical Trial with modest benefits [133,134], possibly through the reduction of shear strain abnormalities and left ventricular dilatation [135] mediated by both cellular and extracellular mechanisms [136]. While the efficacy of this particular device is debatable and has yet to be approved by the FDA, more sophisticated applications would leverage implantation of a patch for the delivery of growth factors or cells [51,137–139].

Another primarily mechanical intervention is the use of left-ventricular assist devices in patients with intractable heart failure either as a bridge to transplant or as a destination therapy. These devices directly pump some of the blood from the left ventricle to the aorta, which in some patients can even lead to long-term reverse remodeling in addition to acutely unloading the ventricle [140].

Electrical interventions include cardiac pacemakers/implantable cardioverter-defibrillators and cardiac resynchronization therapy [141], the latter of which can often also lead to reverse remodeling in patients with mechanical dyssynchrony. However, due to limitations in battery lifespan, replacement surgery must be made every 7–10 yr [142]. The first conceptual demonstration of battery-free pacemakers was a piezoelectric device that generated sufficient to power a small pacemaker by coupling to the natural movement of the heart [143]. This was improved upon by two separate groups in 2014: a Korean group used an advanced piezoelectric material for in vivo pacing in a rat, while a Swiss group used the technology behind automatic wristwatches for pacing in a pig [142,144]. One can imagine applying this type of power source to applications such as in vivo maturation of the previously mentioned cardiac tissue patches through electromechanical actuation, particularly in conjunction with novel electronics printed on flexible substrates and unique geometries [145], or through the use of organic electronics [146]. Actuation could be combined with the use of electrical sensing systems for feedback control, which would additionally ensure proper entrainment of the tissue patch with the underlying syncytium.

Perhaps the most exciting avenue ahead of us involves the use of optical actuation. A major breakthrough occurred in 2005 with the seminal paper describing the transduction of channelrhodopsin-2 from the algae *Chlamydomonas reinhardtii* into cultured rat neurons via a lentiviral vector [147]. First characterized and cloned by in 2003 [148], channelrhodopsin-2 is a seven-transmembrane helix protein with covalently linked retinal [148]. It acts as a nonspecific, light-gated cation channel that opens when its component retinal molecule changes from its all-trans conformation to its 13-cis confirmation [149] upon exposure to 470 nm light. Boyden et al. took advantage of its fast gating kinetics (inward currents evoked within 50 μ s of exposure to blue light [148]), substantial photocurrent, and known stability to demonstrate continuous control of mammalian neurons with millisecond temporal resolution during sustained experiments lasting over an hour [147]. Further exploration led to the demonstration of inhibitory action via the use of halorhodopsin [150] and a host of other modified rhodopsins engineered for various purposes. Gene fusion strategies have also been developed for the precise co-localized 1:1 expression of any two rhodopsins for increased quantitative accuracy [151]. Since 2005, there have been over 1,000 publications and more than 23,000 citations, leading to the recognition of optogenetics by Nature Methods as the “Method of the Year” in 2010 [152].

Despite its popularity in neuroscience, only a small number of publications have utilized cardiac optogenetics [153–158]. Two studies demonstrated pacemaker ability in vivo, one in a transgenic mouse [155] and one through the use of intramyocardial adeno-associated virus 9 (AAV-9) delivery to the apex of a rat heart [158]. The latter also demonstrated multisite pacing through

the use of multiple injections, mimicking the increased synchrony achieved through cardiac resynchronization therapy (Fig. 6(a)) [158]. Switching to optical pacing and defibrillation has the potential to decrease pain and incidental current-based myocardial damage [159]. This technology is already being used in vitro to garner novel insights into biological mechanisms (Fig. 6(b)) [159], but major barriers remain for in vivo translation, particularly the safety concerns associated with the introduction of exogenous genes using viral vectors [160,161]. However, the potential is enormous with a number of additional refinements, including the use of cell-type specific promoters targeting structures such as the SA and AV nodes [162], the use of fiber optics for light delivery or the use of red-shifted opsins to obviate the need for close apposition of light sources [163], and the use of light-induced proteins with a wider range of targets such as adrenergic signaling [164], G-protein signaling [165], or transcriptional regulation [166].

5 Conclusion

In the two decades since Langer’s seminal review paper, the positive feedback cycle of knowledge application and generation has brought us to the precipice of the ability to engineer in vitro high-fidelity human tissues. These models offer complementary knowledge to that currently provided by animal models, with profound implications for clinical applications in the age of personalized medicine. Moreover, advances in materials science, electrical engineering, and mechanical engineering have made increasingly feasible the clinical translation of tissue engineering strategies for regenerative medicine. Tissue engineering stands at the crossroads of these varied disciplines at an exciting time, and the next two decades should see the investment of the previous two come to fruition.

Acknowledgment

The authors gratefully acknowledge funding support of their cardiac tissue engineering research by the National Heart, Lung and Blood Institute, NHLBI (Grant No. HL076485), National Institute for Biomedical Imaging and Bioengineering, NIBIB (Grant Nos. EB17103 and EB002520) and the New York State Department of Health (Grant No. C028119).

References

- [1] Butler, D. L., Goldstein, S. A., Guldberg, R. E., Guo, X. E., Kamm, R., Laurencin, C. T., McIntire, L. V., Mow, V. C., Nerem, R. M., Sah, R. L., Soslowsky, L. J., Spilker, R. L., and Tranquillo, R. T., 2009, “The Impact of Biomechanics in Tissue Engineering and Regenerative Medicine,” *Tissue Eng. Part B: Rev.*, **15**(4), pp. 477–484.
- [2] Langer, R., and Vacanti, J. P., 1993, “Tissue Engineering,” *Science*, **260**(5110), pp. 920–926.
- [3] Salameh, A., Karl, S., Djilali, H., Dhein, S., Janousek, J., and Daehnert, I., 2010, “Opposing and Synergistic Effects of Cyclic Mechanical Stretch and α - or β -Adrenergic Stimulation on the Cardiac Gap Junction Protein Cx43,” *Pharmacol. Res.*, **62**(6), pp. 506–513.
- [4] Salameh, A., Wustmann, A., Karl, S., Blanke, K., Apel, D., Rojas-Gomez, D., Franke, H., Mohr, F. W., Janousek, J., and Dhein, S., 2010, “Cyclic Mechanical Stretch Induces Cardiomyocyte Orientation and Polarization of the Gap Junction Protein Connexin43,” *Circ. Res.*, **106**(10), pp. 1592–1602.
- [5] Jalife, J., and Zipes, D. P., 2013, *Cardiac Electrophysiology: From Cell to Bedside*, Saunders, Philadelphia, PA.
- [6] Calaghan, S. C., Belus, A., and White, E., 2003, “Do Stretch-Induced Changes in Intracellular Calcium Modify the Electrical Activity of Cardiac Muscle?,” *Prog. Biophys. Mol. Biol.*, **82**(1–3), pp. 81–95.
- [7] Von Lewinski, D., 2004, “Functional Relevance of the Stretch-Dependent Slow Force Response in Failing Human Myocardium,” *Circ. Res.*, **94**(10), pp. 1392–1398.
- [8] Radisic, M., Park, H., Gerecht, S., Cannizzaro, C., Langer, R., and Vunjak-Novakovic, G., 2007, “Biomimetic Approach to Cardiac Tissue Engineering,” *Philos. Trans. R. Soc. B: Biol. Sci.*, **362**(1484), pp. 1357–1368.
- [9] Engler, A. J., Rehfeldt, F., Sen, S., and Discher, D. E., 2007, “Microtissue Elasticity: Measurements by Atomic Force Microscopy and its Influence on Cell Differentiation,” *Methods Cell Biol.*, **83**, pp. 521–545.
- [10] Engler, A. J., Sen, S., Sweeney, H. L., and Discher, D. E., 2006, “Matrix Elasticity Directs Stem Cell Lineage Specification,” *Cell*, **126**(4), pp. 677–689.

- [11] Kim, Y. J., Sah, R. L. Y., Grodzinsky, A. J., Plaas, A. H. K., and Sandy, J. D., 1994, "Mechanical Regulation of Cartilage Biosynthetic Behavior: Physical Stimuli," *Arch. Biochem. Biophys.*, **311**(1), pp. 1–12.
- [12] Mauck, R. L., Soltz, M. A., Wang, C. C., Wong, D. D., Chao, P. H., Valhmu, W. B., Hung, C. T., and Ateshian, G. A., 2000, "Functional Tissue Engineering of Articular Cartilage Through Dynamic Loading of Chondrocyte-Seeded Agarose Gels," *ASME J. Biomech. Eng.*, **122**(3), pp. 252–260.
- [13] Niklason, L. E., Gao, J., Abbott, W. M., Hirschi, K. K., Houser, S., Marini, R., and Langer, R., 1999, "Functional Arteries Grown In Vitro," *Science*, **284**(5413), pp. 489–493.
- [14] Syedain, Z. H., Weinberg, J. S., and Tranquillo, R. T., 2008, "Cyclic Distension of Fibrin-Based Tissue Constructs: Evidence of Adaptation During Growth of Engineered Connective Tissue," *Proc. Natl. Acad. Sci. U.S.A.*, **105**(18), pp. 6537–6542.
- [15] Datta, N., Pham, Q. P., Sharma, U., Sikavitsas, V. I., Jansen, J. A., and Mikos, A. G., 2006, "In Vitro Generated Extracellular Matrix and Fluid Shear Stress Synergistically Enhance 3D Osteoblastic Differentiation," *Proc. Natl. Acad. Sci. U.S.A.*, **103**(8), pp. 2488–2493.
- [16] Opie, L. H., Commerford, P. J., Gersh, B. J., and Pfeffer, M. A., 2006, "Controversies in Ventricular Remodelling," *Lancet (London, England)*, **367**(9507), pp. 356–367.
- [17] Pfeffer, J. M., Pfeffer, M. A., and Braunwald, E., 1985, "Influence of Chronic Captopril Therapy on the Infarcted Left Ventricle of the Rat," *Circ. Res.*, **57**(1), pp. 84–95.
- [18] Grossman, W., Jones, D., and McLaurin, L. P., 1975, "Wall Stress and Patterns of Hypertrophy in the Human Left Ventricle," *J. Clin. Invest.*, **56**(1), pp. 56–64.
- [19] Zimmermann, W.-H., 2013, "Biomechanical Regulation of In Vitro Cardiogenesis for Tissue-Engineered Heart Repair," *Stem Cell Res. Ther.*, **4**(6), p. 137.
- [20] Lilly, L. S., and School, H. M., 2011, *Pathophysiology of Heart Disease: A Collaborative Project of Medical Students and Faculty*, Wolters Kluwer/Lippincott Williams and Wilkins, Baltimore, MD.
- [21] Sciarretta, S., and Sadoshima, J., 2010, "New Insights Into the Molecular Phenotype of Eccentric Hypertrophy," *J. Mol. Cell. Cardiol.*, **49**(2), pp. 153–156.
- [22] Toischer, K., Rokita, A. G., Unsold, B., Zhu, W., Kararigas, G., Sossalla, S., Reuter, S. P., Becker, A., Teucher, N., Seidler, T., Grebe, C., Preuss, L., Gupta, S. N., Schmidt, K., Lehnart, S. E., Kruger, M., Linke, W. A., Backs, J., Regitz-Zagrosek, V., Schafer, K., Field, L. J., Maier, L. S., and Hasenfuss, G., 2010, "Differential Cardiac Remodeling in Preload Versus Afterload," *Circulation*, **122**(10), pp. 993–1003.
- [23] Russell, B., Curtis, M. W., Koshman, Y. E., and Samarel, A. M., 2010, "Mechanical Stress-Induced Sarcomere Assembly for Cardiac Muscle Growth in Length and Width," *J. Mol. Cell. Cardiol.*, **48**(5), pp. 817–823.
- [24] Sabri, A., Rafiq, K., Seqqat, R., Kolpakov, M. A., Dillon, R., and Dell'Italia, L. J., 2008, "Sympathetic Activation Causes Focal Adhesion Signaling Alteration in Early Compensated Volume Overload Attributable to Isolated Mitral Regurgitation in the Dog," *Circ. Res.*, **102**(9), pp. 1127–1136.
- [25] Linke, W. A., 2008, "Sense and Stretchability: The Role of Titin and Titin-Associated Proteins in Myocardial Stress-Sensing and Mechanical Dysfunction," *Cardiovasc. Res.*, **77**(4), pp. 637–648.
- [26] Frank, D., Kuhn, C., Katus, H. A., and Frey, N., 2006, "The Sarcomeric Z-Disc: A Nodal Point in Signalling and Disease," *J. Mol. Med.*, **84**(6), pp. 446–468.
- [27] Knöll, R., Hoshijima, M., Hoffman, H. M., Person, V., Lorenzen-Schmidt, I., Bang, M.-L., Hayashi, T., Shiga, N., Yasukawa, H., Schaper, W., McKenna, W., Yokoyama, M., Schork, N. J., Omens, J. H., McCulloch, A. D., Kimura, A., Gregorio, C. C., Poller, W., Schaper, J., Schultheiss, H. P., and Chien, K. R., 2002, "The Cardiac Mechanical Stretch Sensor Machinery Involves a Z Disc Complex That is Defective in a Subset of Human Dilated Cardiomyopathy," *Cell*, **111**(7), pp. 943–955.
- [28] Sugden, P. H., 2001, "Mechanotransduction in Cardiomyocyte Hypertrophy," *Circulation*, **103**(10), pp. 1375–1377.
- [29] Zou, Y., Akazawa, H., Qin, Y., Sano, M., Takano, H., Minamino, T., Makita, N., Iwanaga, K., Zhu, W., Kudoh, S., Toko, H., Tamura, K., Kihara, M., Nagai, T., Fukamizu, A., Umemura, S., Iiri, T., Fujita, T., and Komuro, I., 2004, "Mechanical Stress Activates Angiotensin II Type 1 Receptor Without the Involvement of Angiotensin II," *Nat. Cell Biol.*, **6**(6), pp. 499–506.
- [30] Sadoshima, J., and Izumo, S., 1997, "The Cellular and Molecular Response of Cardiac Myocytes to Mechanical Stress," *Annu. Rev. Physiol.*, **59**(1), pp. 551–71.
- [31] Baines, C. P., and Molkentin, J. D., 2005, "Stress Signaling Pathways That Modulate Cardiac Myocyte Apoptosis," *J. Mol. Cell. Cardiol.*, **38**(1), pp. 47–62.
- [32] Palmieri, E. A., Benincasa, G., Di Rella, F., Casaburi, C., Monti, M. G., De Simone, G., Chiariotti, L., Palombini, L., Bruni, C. B., Saccà, L., and Cittadini, A., 2002, "Differential Expression of TNF-Alpha, IL-6, and IGF-1 by Graded Mechanical Stress in Normal Rat Myocardium," *Am. J. Physiol. Heart Circ. Physiol.*, **282**(3), pp. H926–H934.
- [33] Oral, H., Sivasubramanian, N., Dyke, D. B., Mehta, R. H., Grossman, P. M., Briesmiester, K., Fay, W. P., Pagani, F. D., Bolling, S. F., Mann, D. L., and Starling, M. R., 2003, "Myocardial Proinflammatory Cytokine Expression and Left Ventricular Remodeling in Patients With Chronic Mitral Regurgitation," *Circulation*, **107**(6), pp. 831–837.
- [34] Nakaoka, Y., Shioyama, W., Kunimoto, S., Arita, Y., Higuchi, K., Yamamoto, K., Fujio, Y., Nishida, K., Kuroda, T., Hirota, H., Yamauchi-Takahara, K., Hirano, T., Komuro, I., and Mochizuki, N., 2010, "SHP2 Mediates gp130-Dependent Cardiomyocyte Hypertrophy Via Negative Regulation of Skeletal Alpha-Actin Gene," *J. Mol. Cell. Cardiol.*, **49**(2), pp. 157–164.
- [35] Levy, D., 1996, "The Progression From Hypertension to Congestive Heart Failure," *JAMA J. Am. Med. Assoc.*, **275**(20), pp. 1557–1562.
- [36] Hirt, M. N., Sörensen, N. A., Barthold, L. M., Boeddinghaus, J., Schaaf, S., Eder, A., Vollert, I., Stöhr, A., Schulze, T., Witten, A., Stoll, M., Hansen, A., and Eschenhagen, T., 2012, "Increased Afterload Induces Pathological Cardiac Hypertrophy: A New In Vitro Model," *Basic Res. Cardiol.*, **107**(6), p. 307.
- [37] Taegtmeier, H., Sen, S., and Vela, D., 2010, "Return to the Fetal Gene Program," *Ann. N. Y. Acad. Sci.*, **1188**(1), pp. 191–198.
- [38] Toischer, K., Kogler, H., Tenderich, G., Grebe, C., Seidler, T., Van, P. N., Jung, K., Knoll, R., Korfer, R., and Hasenfuss, G., 2008, "Elevated Afterload, Neuroendocrine Stimulation, and Human Heart Failure Increase BNP Levels and Inhibit Preload-Dependent SERCA Upregulation," *Circ. Heart Fail.*, **1**(4), pp. 265–271.
- [39] Kögler, H., Schott, P., Toischer, K., Milting, H., Van, P. N., Kohlhaas, M., Grebe, C., Kassner, A., Domeier, E., Teucher, N., Seidler, T., Knöll, R., Maier, L. S., El-Banayosy, A., Körfer, R., and Hasenfuss, G., 2006, "Relevance of Brain Natriuretic Peptide in Preload-Dependent Regulation of Cardiac Sarcolemmal Reticulum Ca²⁺ ATPase Expression," *Circulation*, **113**(23), pp. 2724–2732.
- [40] Sugden, P. H., and Clerk, A., 1998, "Cellular Mechanisms of Cardiac Hypertrophy," *J. Mol. Med. (Berl.)*, **76**(11), pp. 725–746.
- [41] Sopontammarak, S., Aliharoo, A., Ocampo, C., Arcilla, R. A., Gupta, M. P., and Gupta, M., 2005, "Mitogen-Activated Protein Kinases (p38 and c-Jun NH2-Terminal kinase) are Differentially Regulated During Cardiac Volume and Pressure Overload Hypertrophy," *Cell Biochem. Biophys.*, **43**(1), pp. 61–76.
- [42] Mitchell, G. F., Lamas, G. A., Vaughan, D. E., and Pfeffer, M. A., 1992, "Left Ventricular Remodeling in the Year After First Anterior Myocardial Infarction: A Quantitative Analysis of Contractile Segment Lengths and Ventricular Shape," *J. Am. Coll. Cardiol.*, **19**(6), pp. 1136–1144.
- [43] Bradham, W. S., Bozkurt, B., Gunasinghe, H., Mann, D., and Spinale, F. G., 2002, "Tumor Necrosis Factor-Alpha and Myocardial Remodeling in Progression of Heart Failure: A Current Perspective," *Cardiovasc. Res.*, **53**(4), pp. 822–830.
- [44] Antonini-Canterin, F., Huang, G., Cervesato, E., Faggiano, P., Pavan, D., Piazza, R., and Nicolosi, G. L., 2003, "Symptomatic Aortic Stenosis: Does Systemic Hypertension Play an Additional Role?," *Hypertension*, **41**(6), pp. 1268–1272.
- [45] Van Heerebeek, L., 2006, "Myocardial Structure and Function Differ in Systolic and Diastolic Heart Failure," *Circulation*, **113**(16), pp. 1966–1973.
- [46] Borlaug, B. A., 2013, "Heart Failure With Preserved and Reduced Ejection Fraction: Different Risk Profiles for Different Diseases," *Eur. Heart J.*, **34**(19), pp. 1393–1395.
- [47] Udelson, J. E., 2011, "Heart Failure With Preserved Ejection Fraction," *Circulation*, **124**(21), pp. e540–e543.
- [48] Middlekauff, H. R., and Mark, A. L., 1998, "The Treatment of Heart Failure: The Role of Neurohumoral Activation," *Intern. Med.*, **37**(2), pp. 112–122.
- [49] Trevett, T. N., and Cotton, J., 2004, "Idiopathic Constriction of the Fetal Ductus Arteriosus," *Ultrasound Obstet. Gynecol.*, **23**(5), pp. 517–519.
- [50] Eschenhagen, T., Fink, C., Remmers, U., Scholz, H., Wattchow, J., Weil, J., Zimmermann, W., Dohmen, H. H., Schäfer, H., Bishopric, N., Wakatsuki, T., and Elson, E. L., 1997, "Three-Dimensional Reconstitution of Embryonic Cardiomyocytes in a Collagen Matrix: A New Heart Muscle Model System," *FASEB J.*, **11**(8), pp. 683–694.
- [51] Zimmermann, W.-H., Melnychenko, I., Wasmeier, G., Didié, M., Naito, H., Nixdorff, U., Hess, A., Budinsky, L., Brune, K., Michaelis, B., Dhein, S., Schwoerer, A., Ehmke, H., and Eschenhagen, T., 2006, "Engineered Heart Tissue Grafts Improve Systolic and Diastolic Function in Infarcted Rat Hearts," *Nat. Med.*, **12**(4), pp. 452–458.
- [52] Boudou, T., Legant, W. R., Mu, A., Borochin, M. A., Thavandiran, N., Radisic, M., Zandstra, P. W., Epstein, J. A., Margulies, K. B., and Chen, C. S., 2012, "A Microfabricated Platform to Measure and Manipulate the Mechanics of Engineered Cardiac Microtissues," *Tissue Eng. Part A*, **18**(9–10), pp. 910–919.
- [53] Legant, W. R., Pathak, A., Yang, M. T., Deshpande, V. S., McMeeking, R. M., and Chen, C. S., 2009, "Microfabricated Tissue Gauges to Measure and Manipulate Forces From 3D Microtissues," *Proc. Natl. Acad. Sci. U.S.A.*, **106**(25), pp. 10097–10102.
- [54] Schaaf, S., Shibamiya, A., Mewe, M., Eder, A., Stöhr, A., Hirt, M. N., Rau, T., Zimmermann, W.-H., Conradi, L., Eschenhagen, T., and Hansen, A., 2011, "Human Engineered Heart Tissue as a Versatile Tool in Basic Research and Preclinical Toxicology," *PLoS One*, **6**(10), p. e26397.
- [55] Zimmermann, W.-H., Schneiderbanger, K., Schubert, P., Didié, M., Münzel, F., Heubach, J. F., Kostin, S., Neuhuber, W. L., and Eschenhagen, T., 2002, "Tissue Engineering of a Differentiated Cardiac Muscle Construct," *Circ. Res.*, **90**(2), pp. 223–230.
- [56] Tulloch, N. L., Muskheili, V., Razumova, M. V., Korte, F. S., Regnier, M., Hauch, K. D., Pabon, L., Reinecke, H., and Murry, C. E., 2011, "Growth of Engineered Human Myocardium With Mechanical Loading and Vascular Coculture," *Circ. Res.*, **109**(1), pp. 47–59.
- [57] Kensah, G., Gruh, I., Vierung, J., Schumann, H., Dahlmann, J., Meyer, H., Skvorc, D., Bär, A., Akhyari, P., Heisterkamp, A., Haverich, A., and Martin, U., 2011, "A Novel Miniaturized Multimodal Bioreactor for Continuous In

- Situ Assessment of Bioartificial Cardiac Tissue During Stimulation and Maturation," *Tissue Eng. Part C: Methods*, 17(4), pp. 463–473.
- [58] Fink, C., Ergun, S., Kralisch, D., Remmers, U., Weil, J., and Eschenhagen, T., 2000, "Chronic Stretch of Engineered Heart Tissue Induces Hypertrophy and Functional Improvement," *FASEB J.*, 14(5), pp. 669–679.
 - [59] Akhyari, P., Fedak, P. W. M., Weisel, R. D., Lee, T.-Y. J., Verma, S., Mickle, D. A. G., and Li, R.-K., 2002, "Mechanical Stretch Regimen Enhances the Formation of Bioengineered Autologous Cardiac Muscle Grafts," *Circulation*, 106(12 Suppl. 1), pp. I137–I142.
 - [60] Zhang, S. J., Truskey, G. A., and Kraus, W. E., 2007, "Effect of Cyclic Stretch on Beta1D-Integrin Expression and Activation of FAK and RhoA," *Am. J. Physiol. Cell Physiol.*, 292(6), pp. C2057–C2069.
 - [61] Lauriol, J., Keith, K., Jaffre, F., Couvillon, A., Saci, A., Goonasekera, S. A., McCarthy, J. R., Kessinger, C. W., Wang, J., Ke, Q., Kang, P. M., Molkenkin, J. D., Carpenter, C., and Kontaridis, M. I., 2014, "RhoA Signaling in Cardiomyocytes Protects Against Stress-Induced Heart Failure But Facilitates Cardiac Fibrosis," *Sci. Signal.*, 7(348), p. ra100.
 - [62] Kensah, G., Roa Lara, A., Dahmann, J., Zweigert, R., Schwanke, K., Hegemann, J., Skvorc, D., Gawol, A., Azizian, A., Wagner, S., Maier, L. S., Krause, A., Dräger, G., Ochs, M., Haverich, A., Gruh, I., and Martin, U., 2013, "Murine and Human Pluripotent Stem Cell-Derived Cardiac Bodies Form Contractile Myocardial Tissue In Vitro," *Eur. Heart J.*, 34(15), pp. 1134–1146.
 - [63] Radisic, M., Park, H., Shing, H., Consi, T., Schoen, F. J., Langer, R., Freed, L. E., and Vunjak-Novakovic, G., 2004, "Functional Assembly of Engineered Myocardium by Electrical Stimulation of Cardiac Myocytes Cultured on Scaffolds," *Proc. Natl. Acad. Sci. U.S.A.*, 101(52), pp. 18129–18134.
 - [64] Lasher, R. A., Pahnke, A. Q., Johnson, J. M., Sachse, F. B., and Hitchcock, R. W., 2012, "Electrical Stimulation Directs Engineered Cardiac Tissue to an Age-Matched Native Phenotype," *J. Tissue Eng.*, 3(1), p. 2041731412455354.
 - [65] Lucía-Valldeperas, A., Sanchez, B., Soler-Botija, C., Gálvez-Montón, C., Prat-Vidal, C., Roura, S., Rosell-Ferrer, J., Bragos, R., and Bayes-Genis, A., 2013, "Electrical Stimulation of Cardiac Adipose Tissue-Derived Progenitor Cells Modulates Cell Phenotype and Genetic Machinery," *J. Tissue Eng. Regen. Med.*, 9(11), pp. E76–E83.
 - [66] Nunes, S. S., Miklas, J. W., Liu, J., Aschar-Sobbi, R., Xiao, Y., Zhang, B., Jiang, J., Massé, S., Gagliardi, M., Hsieh, A., Thavandiran, N., Laflamme, M. A., Nanthakumar, K., Gross, G. J., Backs, P. H., Keller, G., and Radisic, M., 2013, "Biowire: A Platform for Maturation of Human Pluripotent Stem Cell-Derived Cardiomyocytes," *Nat. Methods*, 10(8), pp. 781–787.
 - [67] Miklas, J. W., Nunes, S. S., Sofla, A., Reis, L. A., Pahnke, A., Xiao, Y., Laschinger, C., and Radisic, M., 2014, "Bioreactor for Modulation of Cardiac Microtissue Phenotype by Combined Static Stretch and Electrical Stimulation," *Biofabrication*, 6(2), p. 024113.
 - [68] Wang, B., Wang, G., To, F., Butler, J. R., Claude, A., McLaughlin, R. M., Williams, L. N., de Jongh Curry, A. L., and Liao, J., 2013, "Myocardial Scaffold-Based Cardiac Tissue Engineering: Application of Coordinated Mechanical and Electrical Stimulations," *Langmuir*, 29(35), pp. 11109–11117.
 - [69] Morgan, K. Y., and Black, L. D., 2014, "Mimicking Isovolumic Contraction With Combined Electromechanical Stimulation Improves the Development of Engineered Cardiac Constructs," *Tissue Eng. Part A*, 20(11–12), pp. 1654–1667.
 - [70] Yamamoto, K., Dang, Q. N., Maeda, Y., Huang, H., Kelly, R. A., and Lee, R. T., 2001, "Regulation of Cardiomyocyte Mechanotransduction by the Cardiac Cycle," *Circulation*, 103(10), pp. 1459–1464.
 - [71] Godier-Furnémont, A. F. G., Tiburcy, M., Wagner, E., Dewenter, M., Lämmle, S., El-Armouche, A., Lehnart, S. E., Vunjak-Novakovic, G., and Zimmermann, W.-H., 2015, "Physiologic Force-Frequency Response in Engineered Heart Muscle by Electromechanical Stimulation," *Biomaterials*, 60, pp. 82–91.
 - [72] Senyo, S. E., Koshman, Y. E., and Russell, B., 2007, "Stimulus Interval, Rate and Direction Differentially Regulate Phosphorylation for Mechanotransduction in Neonatal Cardiac Myocytes," *FEBS Lett.*, 581(22), pp. 4241–4247.
 - [73] Shimko, V. F., and Claycomb, W. C., 2008, "Effect of Mechanical Loading on Three-Dimensional Cultures of Embryonic Stem Cell-Derived Cardiomyocytes," *Tissue Eng. Part A*, 14(1), pp. 49–58.
 - [74] Frank, D., Kuhn, C., Brors, B., Hanselmann, C., Lüdde, M., Katus, H. A., and Frey, N., 2008, "Gene Expression Pattern in Biomechanically Stretched Cardiomyocytes: Evidence for a Stretch-Specific Gene Program," *Hypertension*, 51(2), pp. 309–318.
 - [75] Lee, A. A., Delhaas, T., Waldman, L. K., MacKenna, D. A., Villarreal, F. J., and McCulloch, A. D., 1996, "An Equibiaxial Strain System for Cultured Cells," *Am. J. Physiol.: Cell Physiol.*, 271(4 Pt. 1), pp. C1400–C1408.
 - [76] Malhotra, R., Sadoshima, J., Brosius, F. C., and Izumo, S., 1999, "Mechanical Stretch and Angiotensin II Differentially Upregulate the Renin-Angiotensin System in Cardiac Myocytes In Vitro," *Circ. Res.*, 85(2), pp. 137–146.
 - [77] Mansour, H., de Tombe, P. P., Samarel, A. M., and Russell, B., 2004, "Restoration of Resting Sarcomere Length After Uniaxial Static Strain Is Regulated by Protein Kinase Cepsilon and Focal Adhesion Kinase," *Circ. Res.*, 94(5), pp. 642–649.
 - [78] Ertl, G., and Bauersachs, J., 2004, "Endothelin Receptor Antagonists in Heart Failure: Current Status and Future Directions," *Drugs*, 64(10), pp. 1029–1040.
 - [79] Sakai, S., Miyauchi, T., Kobayashi, M., Yamaguchi, I., Goto, K., and Sugishita, Y., 1996, "Inhibition of Myocardial Endothelin Pathway Improves Long-Term Survival in Heart Failure," *Nature*, 384(6607), pp. 353–355.
 - [80] Guo, Y., Zhang, X., Wei, Y., Guo, C., Li, R., Zeng, Q., and Zhang, Y., 2009, "Culturing of Ventricle Cells at High Density and Construction of Engineered Cardiac Cell Sheets Without Scaffold," *Int. Heart J.*, 50(5), pp. 653–662.
 - [81] Xin, M., Olson, E. N., and Bassel-Duby, R., 2013, "Mending Broken Hearts: Cardiac Development as a Basis for Adult Heart Regeneration and Repair," *Nat. Rev. Mol. Cell Biol.*, 14(8), pp. 529–541.
 - [82] Brutsaert, D. L., 2003, "Cardiac Endothelial-Myocardial Signaling: Its Role in Cardiac Growth, Contractile Performance, and Rhythmicity," *Physiol. Rev.*, 83(1), pp. 59–115.
 - [83] Zhang, D., Shadrin, I. Y., Lam, J., Xian, H.-Q., Snodgrass, H. R., and Bursac, N., 2013, "Tissue-Engineered Cardiac Patch for Advanced Functional Maturation of Human ESC-Derived Cardiomyocytes," *Biomaterials*, 34(23), pp. 5813–5820.
 - [84] Steendijk, P., Tulner, S. A. F., Wiemer, M., Bleasdale, R. A., Bax, J. J., van der Wall, E. e., Vogt, J., and Schalij, M. J., 2004, "Pressure-Volume Measurements by Conductance Catheter During Cardiac Resynchronization Therapy," *Eur. Heart J. Suppl.*, 6(Suppl. D), pp. D35–D42.
 - [85] Schmitt, B., Steendijk, P., Lunze, K., Ovrutski, S., Falkenberg, J., Rahmanzadeh, P., Maarouf, N., Ewert, P., Berger, F., and Kuehne, T., 2009, "Integrated Assessment of Diastolic and Systolic Ventricular Function Using Diagnostic Cardiac Magnetic Resonance Catheterization: Validation in Pigs and Application in a Clinical Pilot Study," *JACC Cardiovasc. Imaging*, 2(11), pp. 1271–1281.
 - [86] Brands, P. J., Hoeks, A. P. G., Rutten, M. C. M., and Reneman, R. S., 1996, "A Noninvasive Method to Estimate Arterial Impedance by Means of Assessment of Local Diameter Change and the Local Center-Line Blood Flow Velocity Using Ultrasound," *Ultrasound Med. Biol.*, 22(7), pp. 895–905.
 - [87] Pfeffer, M. A., Pfeffer, J. M., Fishbein, M. C., Fletcher, P. J., Spadaro, J., Kloner, R. A., and Braunwald, E., 1979, "Myocardial Infarct Size and Ventricular Function in Rats," *Circ. Res.*, 44(4), pp. 503–512.
 - [88] Fletcher, P. J., Pfeffer, J. M., Pfeffer, M. A., and Braunwald, E., 1981, "Left Ventricular Diastolic Pressure-Volume Relations in Rats With Healed Myocardial Infarction. Effects on Systolic Function," *Circ. Res.*, 49(3), pp. 618–626.
 - [89] Pfeffer, M. A., Pfeffer, J. M., Steinberg, C., and Finn, P., 1985, "Survival After an Experimental Myocardial Infarction: Beneficial Effects of Long-Term Therapy With Captopril," *Circulation*, 72(2), pp. 406–412.
 - [90] Pfeffer, M. A., Braunwald, E., Moyé, L. A., Basta, L., Brown, E. J., Cuddy, T. E., Davis, B. R., Geltman, E. M., Goldman, S., and Flaker, G. C., 1992, "Effect of Captopril on Mortality and Morbidity in Patients With Left Ventricular Dysfunction After Myocardial Infarction. Results of the Survival and Ventricular Enlargement Trial. The SAVE Investigators," *N. Engl. J. Med.*, 327(10), pp. 669–677.
 - [91] St. John Sutton, M., Pfeffer, M. A., Plappert, T., Rouleau, J. L., Moyé, L. A., Dagenais, G. R., Lamas, G. A., Klein, M., Sussex, B., and Goldman, S., 1994, "Quantitative Two-Dimensional Echocardiographic Measurements are Major Predictors of Adverse Cardiovascular Events After Acute Myocardial Infarction. The Protective Effects of Captopril," *Circulation*, 89(1), pp. 68–75.
 - [92] Packer, M., McMurray, J., Massie, B. M., Caspi, A., Charlon, V., Cohen-Solal, A., Kiowski, W., Kostuk, W., Krum, H., Levine, B., Rizzon, P., Soler, J., Swedberg, K., Anderson, S., and Demets, D. L., 2005, "Clinical Effects of Endothelin Receptor Antagonism With Bosentan in Patients With Severe Chronic Heart Failure: Results of a Pilot Study," *J. Cardiol. Fail.*, 11(1), pp. 12–20.
 - [93] Handoko, M. L., de Man, F. S., and Vonk-Noordegraaf, A., 2011, "The Rise and Fall of Endothelin Receptor Antagonists in Congestive Heart Failure," *Eur. Respir. J.*, 37(3), pp. 484–485.
 - [94] Rockman, H. A., Ross, R. S., Harris, A. N., Knowlton, K. U., Steinhilber, M. E., Field, L. J., Ross, J., and Chien, K. R., 1991, "Segregation of Atrial-Specific and Inducible Expression of an Atrial Natriuretic Factor Transgene in an In Vivo Murine Model of Cardiac Hypertrophy," *Proc. Natl. Acad. Sci. U.S.A.*, 88(18), pp. 8277–8281.
 - [95] deAlmeida, A. C., van Oort, R. J., and Wehrens, X. H. T., 2010, "Transverse Aortic Constriction in Mice," *J. Visualized Exp.*, 38, epub.
 - [96] Mohammed, S. F., Storlie, J. R., Oehler, E. A., Bowen, L. A., Korinek, J., Lam, C. S. P., Simari, R. D., Burnett, J. C., and Redfield, M. M., "Variable Phenotype in Murine Transverse Aortic Constriction," *Cardiovasc. Pathol.*, 21(3), pp. 188–198.
 - [97] Barrick, C. J., Rojas, M., Schoonhoven, R., Smyth, S. S., and Threadgill, D. W., 2007, "Cardiac Response to Pressure Overload in 129S1/SvImJ and C57BL/6J Mice: Temporal- and Background-Dependent Development of Concentric Left Ventricular Hypertrophy," *Am. J. Physiol. Heart Circ. Physiol.*, 292(5), pp. H2119–H2130.
 - [98] Weinberg, E. O., Schoen, F. J., George, D., Kagaya, Y., Douglas, P. S., Litwin, S. E., Schunkert, H., Benedict, C. R., and Lorell, B. H., 1994, "Angiotensin-Converting Enzyme Inhibition Prolongs Survival and Modifies the Transition to Heart Failure in Rats With Pressure Overload Hypertrophy Due to Ascending Aortic Stenosis," *Circulation*, 90(3), pp. 1410–1422.
 - [99] Patten, R. D., and Hall-Porter, M. R., 2009, "Small Animal Models of Heart Failure: Development of Novel Therapies, Past and Present," *Circ. Heart Fail.*, 2(2), pp. 138–144.
 - [100] Toischer, K., Kochhäuser, S., Nguyen van, P., Leineweber, K., Hasenfuss, G., and Kögler, H., 2012, "Mechanical Load-Dependent Cardiac ER Stress In Vitro and In Vivo: Effects of Preload and Afterload," *FEBS Lett.*, 586(9), pp. 1363–1369.

- [101] Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S., 2007, "Induction of Pluripotent Stem Cells From Adult Human Fibroblasts by Defined Factors," *Cell*, **131**(5), pp. 861–872.
- [102] Yu, J., Vodyanik, M. A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J. L., Tian, S., Nie, J., Jonsdottir, G. A., Ruotti, V., Stewart, R., Slukvin, I. I., and Thomson, J. A., 2007, "Induced Pluripotent Stem Cell Lines Derived From Human Somatic Cells," *Science*, **318**(5858), pp. 1917–1920.
- [103] Dimos, J. T., Rodolfa, K. T., Niakan, K. K., Weisenthal, L. M., Mitsumoto, H., Chung, W., Croft, G. F., Saphier, G., Leibel, R., Goland, R., Wichterle, H., Henderson, C. E., and Eggan, K., 2008, "Induced Pluripotent Stem Cells Generated From Patients With ALS Can be Differentiated Into Motor Neurons," *Science*, **321**(5893), pp. 1218–1221.
- [104] Park, I.-H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., Lensch, M. W., Cowan, C., Hochedlinger, K., and Daley, G. Q., 2008, "Disease-Specific Induced Pluripotent Stem Cells," *Cell*, **134**(5), pp. 877–886.
- [105] Zhang, J., Wilson, G. F., Soerens, A. G., Koonce, C. H., Yu, J., Palecek, S. P., Thomson, J. A., and Kamp, T. J., 2009, "Functional Cardiomyocytes Derived From Human Induced Pluripotent Stem Cells," *Circ. Res.*, **104**(4), pp. e30–e41.
- [106] Zwi, L., Caspi, O., Arbel, G., Huber, I., Gepstein, A., Park, I.-H., and Gepstein, L., 2009, "Cardiomyocyte Differentiation of Human Induced Pluripotent Stem Cells," *Circulation*, **120**(15), pp. 1513–1523.
- [107] Burridge, P. W., Matsa, E., Shukla, P., Lin, Z. C., Churko, J. M., Ebert, A. D., Lan, F., Diecke, S., Huber, B., Mordwinkin, N. M., Plews, J. R., Abilez, O. J., Cui, B., Gold, J. D., and Wu, J. C., 2014, "Chemically Defined Generation of Human Cardiomyocytes," *Nat. Methods*, **11**(8), pp. 855–860.
- [108] Burridge, P. W., Holmström, A., and Wu, J. C., 2015, "Chemically Defined Culture and Cardiomyocyte Differentiation of Human Pluripotent Stem Cells," *Curr. Protoc. Hum. Genet.*, **87**, pp. 21.3.1–21.3.15.
- [109] Moretti, A., Bellin, M., Welling, A., Jung, C. B., Lam, J. T., Bott-Flügel, L., Dorn, T., Goedel, A., Höhnke, C., Hofmann, F., Seyfarth, M., Sinnecker, D., Schömg, A., and Laugwitz, K.-L., 2010, "Patient-Specific Induced Pluripotent Stem-Cell Models for Long-QT Syndrome," *N. Engl. J. Med.*, **363**(15), pp. 1397–1409.
- [110] Carvajal-Vergara, X., Sevilla, A., D'Souza, S. L., Ang, Y.-S., Schaniel, C., Lee, D.-F., Yang, L., Kaplan, A. D., Adler, E. D., Rozov, R., Ge, Y., Cohen, N., Edelmann, L. J., Chang, B., Waghay, A., Su, J., Pardo, S., Lichtenbelt, K. D., Tartaglia, M., Gelb, B. D., and Lemischka, I. R., 2010, "Patient-Specific Induced Pluripotent Stem-Cell-Derived Models of LEOPARD Syndrome," *Nature*, **465**(7299), pp. 808–812.
- [111] Itzhaki, I., Maizels, L., Huber, I., Zwi-Dantsis, L., Caspi, O., Winterstern, A., Feldman, O., Gepstein, A., Arbel, G., Hammerman, H., Boulos, M., and Gepstein, L., 2011, "Modelling the Long QT Syndrome With Induced Pluripotent Stem Cells," *Nature*, **471**(7337), pp. 225–229.
- [112] Yazawa, M., Hsueh, B., Jia, X., Pasca, A. M., Bernstein, J. A., Hallmayer, J., and Dolmetsch, R. E., 2011, "Using Induced Pluripotent Stem Cells to Investigate Cardiac Phenotypes in Timothy Syndrome," *Nature*, **471**(7337), pp. 230–234.
- [113] Eschenhagen, T., Mummery, C., and Knollmann, B. C., 2015, "Modelling Sarcomeric Cardiomyopathies in the Dish: From Human Heart Samples to iPSC Cardiomyocytes," *Cardiovasc. Res.*, **105**(4), pp. 424–438.
- [114] Xue, H., Wu, J., Li, S., Rao, M. S., and Liu, Y., 2016, "Genetic Modification in Human Pluripotent Stem Cells by Homologous Recombination and CRISPR/Cas9 System," *Methods Mol. Biol.*, **1307**, pp. 173–190.
- [115] Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., Norville, J. E., and Church, G. M., 2013, "RNA-Guided Human Genome Engineering Via Cas9," *Science*, **339**(6121), pp. 823–826.
- [116] Cong, L., Ran, F. A., Cox, D., Lin, S., Barretto, R., Habib, N., Hsu, P. D., Wu, X., Jiang, W., Marraffini, L. A., and Zhang, F., 2013, "Multiplex Genome Engineering Using CRISPR/Cas Systems," *Science*, **339**(6121), pp. 819–823.
- [117] Jinek, M., East, A., Cheng, A., Lin, S., Ma, E., and Doudna, J., 2013, "RNA-Programmed Genome Editing in Human Cells," *Elife*, **2**, p. e00471.
- [118] Cho, S. W., Kim, S., Kim, J. M., and Kim, J.-S., 2013, "Targeted Genome Engineering in Human Cells With the Cas9 RNA-Guided Endonuclease," *Nat. Biotechnol.*, **31**(3), pp. 230–232.
- [119] Herman, D. S., Lam, L., Taylor, M. R. G., Wang, L., Teekakirikul, P., Christodoulou, D., Conner, L., DePalma, S. R., McDonough, B., Sparks, E., Teodorescu, D. L., Cirio, A. L., Banner, N. R., Pennell, D. J., Graw, S., Merlo, M., Di Lenarda, A., Sinagra, G., Bos, J. M., Ackerman, M. J., Mitchell, R. N., Murry, C. E., Lakdawala, N. K., Ho, C. Y., Barton, P. J. R., Cook, S. A., Mestroni, L., Seidman, J. G., and Seidman, C. E., 2012, "Truncations of Titin Causing Dilated Cardiomyopathy," *N. Engl. J. Med.*, **366**(7), pp. 619–628.
- [120] Hinson, J. T., Chopra, A., Nafissi, N., Polacheck, W. J., Benson, C. C., Swist, S., Gorham, J., Yang, L., Schafer, S., Sheng, C. C., Haghighi, A., Homys, J., Hubner, N., Church, G., Cook, S. A., Linke, W. A., Chen, C. S., Seidman, J. G., and Seidman, C. E., 2015, "Titin Mutations in iPSC Cells Define Sarcomere Insufficiency as a Cause of Dilated Cardiomyopathy," *Science*, **349**(6251), pp. 982–986.
- [121] Lasser, K. E., 2002, "Timing of New Black Box Warnings and Withdrawals for Prescription Medications," *JAMA*, **287**(17), pp. 2215–2220.
- [122] Fung, M., Thornton, A., Mybeck, K., Wu, J. H.-H., Hornbuckle, K., and Muniz, E., 2001, "Evaluation of the Characteristics of Safety Withdrawal of Prescription Drugs From Worldwide Pharmaceutical Markets-1960 to 1999," *Ther. Innov. Regul. Sci.*, **35**(1), pp. 293–317.
- [123] EMA, 2008, "Guideline on Repeated Dose Toxicity," European Medicines Agency, London.
- [124] ICH, 2005, "The Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals," International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Safety Guideline S7B.
- [125] Mandenius, C.-F., Steel, D., Noor, F., Meyer, T., Heinze, E., Asp, J., Arain, S., Kraushaar, U., Bremer, S., Class, R., and Sartipy, P., 2011, "Cardiotoxicity Testing Using Pluripotent Stem Cell-Derived Human Cardiomyocytes and State-of-the-Art Bioanalytics: A Review," *J. Appl. Toxicol.*, **31**(3), pp. 191–205.
- [126] Vunjak-Novakovic, G., Bhatia, S., Chen, C., and Hirschi, K., 2013, "HeLiVa Platform: Integrated Heart-Liver-Vascular Systems for Drug Testing in Human Health and Disease," *Stem Cell Res. Ther.*, **4**(Suppl. 1), p. S8.
- [127] Lan, F., Lee, A. S., Liang, P., Sanchez-Freire, V., Nguyen, P. K., Wang, L., Han, L., Yen, M., Wang, Y., Sun, N., Abilez, O. J., Hu, S., Ebert, A. D., Navarrete, E. G., Simmons, C. S., Wheeler, M., Pruitt, B., Lewis, R., Yamaguchi, Y., Ashley, E. A., Bers, D. M., Robbins, R. C., Longaker, M. T., and Wu, J. C., 2013, "Abnormal Calcium Handling Properties Underlie Familial Hypertrophic Cardiomyopathy Pathology in Patient-Specific Induced Pluripotent Stem Cells," *Cell Stem Cell*, **12**(1), pp. 101–113.
- [128] Liang, P., Lan, F., Lee, A. S., Gong, T., Sanchez-Freire, V., Wang, Y., Diecke, S., Sallam, K., Knowles, J. W., Wang, P. J., Nguyen, P. K., Bers, D. M., Robbins, R. C., and Wu, J. C., 2013, "Drug Screening Using a Library of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes Reveals Disease-Specific Patterns of Cardiotoxicity," *Circulation*, **127**(16), pp. 1677–1691.
- [129] Sun, N., Yazawa, M., Liu, J., Han, L., Sanchez-Freire, V., Abilez, O. J., Navarrete, E. G., Hu, S., Wang, L., Lee, A., Pavlovic, A., Lin, S., Chen, R., Hajjar, R. J., Snyder, M. P., Dolmetsch, R. E., Butte, M. J., Ashley, E. A., Longaker, M. T., Robbins, R. C., and Wu, J. C., 2012, "Patient-Specific Induced Pluripotent Stem Cells as a Model for Familial Dilated Cardiomyopathy," *Sci. Transl. Med.*, **4**(130), p. 130ra47.
- [130] Spirito, P., Seidman, C. E., McKenna, W. J., and Maron, B. J., 1997, "The Management of Hypertrophic Cardiomyopathy," *N. Engl. J. Med.*, **336**(11), pp. 775–785.
- [131] Watkins, H., Seidman, C. E., Seidman, J. G., Feng, H. S., and Sweeney, H. L., 1996, "Expression and Functional Assessment of a Truncated Cardiac Troponin T That Causes Hypertrophic Cardiomyopathy. Evidence for a Dominant Negative Action," *J. Clin. Invest.*, **98**(11), pp. 2456–2461.
- [132] Olson, T. M., Michels, V. V., Thibodeau, S. N., Tai, Y. S., and Keating, M. T., 1998, "Actin Mutations in Dilated Cardiomyopathy, A Heritable Form of Heart Failure," *Science*, **280**(5364), pp. 750–752.
- [133] Acker, M. A., Bolling, S., Shemin, R., Kirklin, J., Oh, J. K., Mann, D. L., Jessup, M., Sabbah, H. N., Starling, R. C., and Kubo, S. H., 2006, "Mitral Valve Surgery in Heart Failure: Insights From the Acorn Clinical Trial," *J. Thorac. Cardiovasc. Surg.*, **132**(3), pp. 568–577.
- [134] Mann, D. L., Kubo, S. H., Sabbah, H. N., Starling, R. C., Jessup, M., Oh, J. K., and Acker, M. A., 2012, "Beneficial Effects of the CorCap Cardiac Support Device: Five-Year Results From the Acorn Trial," *J. Thorac. Cardiovasc. Surg.*, **143**(5), pp. 1036–1042.
- [135] Cheng, A., Nguyen, T. C., Malinowski, M., Langer, F., Liang, D., Daughters, G. T., Ingels, N. B., and Miller, D. C., 2006, "Passive Ventricular Constraint Prevents Transmural Shear Strain Progression in Left Ventricle Remodeling," *Circulation*, **114**(1 Suppl.), pp. 179–186.
- [136] Blom, A. S., Mukherjee, R., Pilla, J. J., Lowry, A. S., Yarbrough, W. M., Mingoia, J. T., Hendrick, J. W., Stroud, R. E., McLean, J. E., Affuso, J. G., Gorman, R. C., Gorman, J. H., Acker, M. A., and Spiale, F. G., 2005, "Cardiac Support Device Modifies Left Ventricular Geometry and Myocardial Structure After Myocardial Infarction," *Circulation*, **112**(9), pp. 1274–1283.
- [137] Chang, Y., Lai, P.-H., Wei, H.-J., Lin, W.-W., Chen, C.-H., Hwang, S.-M., Chen, S.-C., and Sung, H.-W., 2007, "Tissue Regeneration Observed in a Basic Fibroblast Growth Factor-Loaded Porous Acellular Bovine Pericardium Populated With Mesenchymal Stem Cells," *J. Thorac. Cardiovasc. Surg.*, **134**(1), pp. 65–73.
- [138] Freytes, D. O., Santambrogio, L., and Vunjak-Novakovic, G., 2012, "Optimizing Dynamic Interactions Between a Cardiac Patch and Inflammatory Host Cells," *Cells Tissues Organs*, **195**(1–2), pp. 171–182.
- [139] Godier-Furnémont, A. F. G., Tekabe, Y., Kollars, M., Eng, G., Morales, A., Vunjak-Novakovic, G., and Johnson, L. L., 2013, "Noninvasive Imaging of Myocyte Apoptosis Following Application of a Stem Cell-Engineered Delivery Platform to Acutely Infarcted Myocardium," *J. Nucl. Med.*, **54**(6), pp. 977–983.
- [140] Dipla, K., Mattiello, J. A., Jeevanandam, V., Houser, S. R., and Margulies, K. B., 1998, "Myocyte Recovery After Mechanical Circulatory Support in Humans With End-Stage Heart Failure," *Circulation*, **97**(23), pp. 2316–2322.
- [141] Yu, C.-M., Fung, W.-H., Lin, H., Zhang, Q., Sanderson, J. E., and Lau, C.-P., 2003, "Predictors of Left Ventricular Reverse Remodeling After Cardiac Resynchronization Therapy for Heart Failure Secondary to Idiopathic Dilated or Ischemic Cardiomyopathy," *Am. J. Cardiol.*, **91**(6), pp. 684–688.
- [142] Hwang, G.-T., Park, H., Lee, J.-H., Oh, S., Park, K.-I., Byun, M., Park, H., Ahn, G., Jeong, C. K., No, K., Kwon, H., Lee, S.-G., Joung, B., and Lee, K. J., 2014, "Self-Powered Cardiac Pacemaker Enabled by Flexible Single Crystalline PMN-PT Piezoelectric Energy Harvester," *Adv. Mater.*, **26**(28), pp. 4880–4887.
- [143] Dagdeviren, C., Yang, B. D., Su, Y., Tran, P. L., Joe, P., Anderson, E., Xia, J., Doraiswamy, V., Dehdashti, B., Feng, X., Lu, B., Poston, R., Khalpey, Z.,

- Ghaffari, R., Huang, Y., Slepian, M. J., and Rogers, J. A., 2014, "Conformal Piezoelectric Energy Harvesting and Storage From Motions of the Heart, Lung, and Diaphragm," *Proc. Natl. Acad. Sci. U.S.A.*, **111**(5), pp. 1927–1932.
- [144] Zurbuchenn, A., Haeblerlin, A., Schaerer, J., Wagner, J., Pfenninger, A., Vandenbergh, S., Huber, C. H., Fuhrer, J., and Vogel, R., 2014, "A Batteryless Cardiac Pacemaker Powered by Cardiac Motion," *Eur. Heart J.*, **35**, pp. 173–512.
- [145] Dagdeviren, C., Shi, Y., Joe, P., Ghaffari, R., Balooch, G., Usgaonkar, K., Gur, O., Tran, P. L., Crosby, J. R., Meyer, M., Su, Y., Chad Webb, R., Tedesco, A. S., Slepian, M. J., Huang, Y., and Rogers, J. A., 2015, "Conformal Piezoelectric Systems for Clinical and Experimental Characterization of Soft Tissue Biomechanics," *Nat. Mater.*, **14**(7), pp. 728–736.
- [146] Martiradonna, L., 2014, "Bioresorbable Organic Electronics," *Nat. Mater.*, **13**(5), pp. 428–428.
- [147] Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K., 2005, "Millisecond-Timescale, Genetically Targeted Optical Control of Neural Activity," *Nat. Neurosci.*, **8**(9), pp. 1263–1268.
- [148] Nagel, G., Szellas, T., Huhn, W., Kateriya, S., Adeishvili, N., Berthold, P., Ollig, D., Hegemann, P., and Bamberg, E., 2003, "Channelrhodopsin-2, A Directly Light-Gated Cation-Selective Membrane Channel," *Proc. Natl. Acad. Sci. U.S.A.*, **100**(24), pp. 13940–13945.
- [149] Gerwert, K., and Siebert, F., 1986, "Evidence for Light-Induced 13-cis, 14-s-cis Isomerization in Bacteriorhodopsin Obtained by FTIR Difference Spectroscopy Using Isotopically Labelled Retinals," *EMBO J.*, **5**(4), pp. 805–811.
- [150] Gradinaru, V., Thompson, K. R., and Deisseroth, K., 2008, "eNpHR: A Natronomonas Halorhodopsin Enhanced for Optogenetic Applications," *Brain Cell Biol.*, **36**(1–4), pp. 129–139.
- [151] Kleinlogel, S., Terpitz, U., Legrum, B., Göckbuget, D., Boyden, E. S., Bamann, C., Wood, P. G., and Bamberg, E., 2011, "A Gene-Fusion Strategy for Stoichiometric and Co-Localized Expression of Light-Gated Membrane Proteins," *Nat. Methods*, **8**(12), pp. 1083–1088.
- [152] Entcheva, E., 2013, "Cardiac Optogenetics," *Am. J. Physiol. Heart Circ. Physiol.*, **304**(9), pp. H1179–H1191.
- [153] Ambrosi, C. M., and Entcheva, E., 2014, "Optogenetic Control of Cardiomyocytes via Viral Delivery," *Methods Mol. Biol.*, **1181**, pp. 215–228.
- [154] Arrenberg, A. B., Stainier, D. Y. R., Baier, H., and Huisken, J., 2010, "Optogenetic Control of Cardiac Function," *Science*, **330**(6006), pp. 971–974.
- [155] Bruegmann, T., Malan, D., Hesse, M., Beiert, T., Fuegemann, C. J., Fleischmann, B. K., and Sasse, P., 2010, "Optogenetic Control of Heart Muscle In Vitro and In Vivo," *Nat. Methods*, **7**(11), pp. 897–900.
- [156] Jia, Z., Valiunas, V., Lu, Z., Bien, H., Liu, H., Wang, H.-Z., Rosati, B., Brink, P. R., Cohen, I. S., and Entcheva, E., 2011, "Stimulating Cardiac Muscle by Light: Cardiac Optogenetics by Cell Delivery," *Circ. Arrhythm. Electrophysiol.*, **4**(5), pp. 753–760.
- [157] Park, S. A., Lee, S.-R., Tung, L., and Yue, D. T., 2014, "Optical Mapping of Optogenetically Shaped Cardiac Action Potentials," *Sci. Rep.*, **4**, p. 6125.
- [158] Nussinovitch, U., and Gepstein, L., 2015, "Optogenetics for In Vivo Cardiac Pacing and Resynchronization Therapies," *Nat. Biotechnol.*, **33**(7), pp. 750–754.
- [159] Bingen, B. O., Engels, M. C., Schali, M. J., Jangsangthong, W., Neshati, Z., Feola, I., Ypey, D. L., Askar, S. F. A., Panfilov, A. V., Pijnappels, D. A., and de Vries, A. A. F., 2014, "Light-Induced Termination of Spiral Wave Arrhythmias by Optogenetic Engineering of Atrial Cardiomyocytes," *Cardiovasc. Res.*, **104**(1), pp. 194–205.
- [160] Kaplitt, M. G., Feigin, A., Tang, C., Fitzsimons, H. L., Mattis, P., Lawlor, P. A., Bland, R. J., Young, D., Strybing, K., Eidelberg, D., and During, M. J., 2007, "Safety and Tolerability of Gene Therapy With an Adeno-Associated Virus (AAV) Borne GAD Gene for Parkinson's Disease: An Open Label, Phase I Trial," *Lancet*, **369**(9579), pp. 2097–2105.
- [161] Zsebo, K., Yaroshinsky, A., Rudy, J. J., Wagner, K., Greenberg, B., Jessup, M., and Hajjar, R. J., 2014, "Long-Term Effects of AAV1/SERCA2a Gene Transfer in Patients With Severe Heart Failure: Analysis of Recurrent Cardiovascular Events and Mortality," *Circ. Res.*, **114**(1), pp. 101–108.
- [162] Doetschman, T., and Azhar, M., 2012, "Cardiac-Specific Inducible and Conditional Gene Targeting in Mice," *Circ. Res.*, **110**(11), pp. 1498–1512.
- [163] Lin, J. Y., Knutsen, P. M., Muller, A., Kleinfeld, D., and Tsien, R. Y., 2013, "ReaChR: a Red-Shifted Variant of Channelrhodopsin Enables Deep Transcranial Optogenetic Excitation," *Nat. Neurosci.*, **16**(10), pp. 1499–1508.
- [164] Siuda, E. R., McCall, J. G., Al-Hasani, R., Shin, G., Il Park, S., Schmidt, M. J., Anderson, S. L., Planer, W. J., Rogers, J. A., and Bruchas, M. R., 2015, "Optodynamic Simulation of β -Adrenergic Receptor Signalling," *Nat. Commun.*, **6**, p. 8480.
- [165] Maseck, O. A., Rubelowski, J. M., Spoida, K., and Herlitz, S., 2011, "Light- and Drug-Activated G-Protein-Coupled Receptors to Control Intracellular Signalling," *Exp. Physiol.*, **96**(1), pp. 51–56.
- [166] Hisatomi, O., Takeuchi, K., Zikihara, K., Ookubo, Y., Nakatani, Y., Takahashi, F., Tokutomi, S., and Kataoka, H., 2012, "Blue Light-Induced Conformational Changes in a Light-Regulated Transcription Factor, Aureochrome-1," *Plant Cell Physiol.*, **54**(1), pp. 93–106.
- [167] Thavandiran, N., Dubois, N., Mikryukov, A., Massé, S., Beca, B., Simmons, C. A., Deshpande, V. S., McGarry, J. P., Chen, C. S., Nanthakumar, K., Keller, G. M., Radisic, M., and Zandstra, P. W., 2013, "Design and Formulation of Functional Pluripotent Stem Cell-Derived Cardiac Microtissues," *Proc. Natl. Acad. Sci. U.S.A.*, **110**(49), pp. E4698–E4707.
- [168] Wang, H., Svoronos, A. A., Boudou, T., Sakar, M. S., Schell, J. Y., Morgan, J. R., Chen, C. S., and Shenoy, V. B., 2013, "Necking and Failure of Constrained 3D Microtissues Induced by Cellular Tension," *Proc. Natl. Acad. Sci. U.S.A.*, **110**(52), pp. 20923–20928.
- [169] Eschenhagen, T., Didié, M., Heubach, J., Ravens, U., and Zimmermann, W.-H., 2002, "Cardiac Tissue Engineering," *Transpl. Immunol.*, **9**(2–4), pp. 315–321.