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STATE-OF-THE-ART REVIEW

Assessing Cardiac Contractility From Single Molecules to Whole Hearts

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HIGHLIGHTS

- **Heart failure therapies do not address dysregulated contraction.**
- **New therapies increasing contractility come from multiscale models of contraction.**
- **Multiscale models of contraction study disease mechanism from molecules to animals.**
- **Utilizing multiscale models may create new therapeutic paradigms.**
- **The authors review how the technologies for these models measure contractility.**

SUMMARY

Fundamentally, the heart needs to generate sufficient force and power output to dynamically meet the needs of the body. Cardiomyocytes contain specialized structures referred to as sarcomeres that power and regulate contraction. Disruption of sarcomeric function or regulation impairs contractility and leads to cardiomyopathies and heart failure. Basic, translational, and clinical studies have adapted numerous methods to assess cardiac contraction in a variety of pathophysiological contexts. These tools measure aspects of cardiac contraction at different scales ranging from single molecules to whole organisms. Moreover, these studies have revealed new pathogenic mechanisms of heart disease leading to the development of novel therapies targeting contractility. In this review, the authors explore the breadth of tools available for studying cardiac contractile function across scales, discuss their strengths and limitations, highlight new insights into cardiac physiology and pathophysiology, and describe how these insights can be harnessed for therapeutic candidate development and translational. (J Am Coll Cardiol Basic Trans Science 2024;9:414–439)

A key hallmark of both cardiomyopathies and heart failure (HF) is altered contractility, which is commonly demonstrated by changes in systolic or diastolic function on echocardiography, cardiac magnetic resonance imaging, and/or invasive hemodynamic assessment directly measuring cardiac output.[1] Per current American College of Cardiology/American Heart Association guidelines, HF is defined as “a complex clinical syndrome with symptoms and signs that result from any

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structural or functional impairment of ventricular filling or ejection of blood."[1] HF is subdivided on the basis of left ventricular ejection fraction into heart failure with reduced ejection fraction (HFrEF) (left ventricular ejection fraction <40%), HF with midrange or mildly reduced ejection fraction (left ventricular ejection fraction 41%-49%), and heart failure with preserved ejection fraction (HFpEF) (left ventricular ejection fraction >50%).[2] HFrEF, characterized by reduced systolic function, is broadly divided into ischemic and nonischemic forms, but standard guideline-directed medical therapy (GDMT) for HF are agnostic to the etiology of HFrEF. HF with midrange or mildly reduced ejection fraction is a more recently defined category that shares similarities with HFrEF and has some positive clinical benefit from the same GDMT, but research is ongoing to better define this category.[2] HFpEF, which is generally characterized by impaired diastolic function, is less well defined clinically and mechanistically, but it represents a growing percentage of HF cases. A subset of nonischemic cardiomyopathies associated with altered contractility are inherited genetic cardiomyopathies, including hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and restrictive cardiomyopathy. Mutations in sarcomeric and contractile proteins are major causes of inherited cardiomyopathies, with worse outcomes for patients with mutations in sarcomeric genes,[3-7] and therefore, it is unsurprising that many of these mutations affect cardiac contraction. As the burden of HF continues to strain limited resources such as donor hearts, there is an outstanding need to better understand the mechanistic basis of contractile dysfunction and design new therapies.

Although HF and cardiomyopathies are both characterized by altered contractility, current treatments for these diseases do not target cardiac contraction. Current GDMT for HFrEF addresses preventing adverse remodeling of the heart. Although this has improved the prognosis for patients with HFrEF, many patients still progress to end-stage HF, requiring mechanical ventricular assist devices and/or cardiac transplantation.[8] In the case of HFpEF, there were no treatments available until recently, with the development of sodium-glucose cotransporter-2 inhibitors.[9] With genetic cardiomyopathies, mutation-induced changes in contraction can be observed in genotype-positive patients before the onset of pathologic ventricular remodeling; however, current GDMT focuses on treating patients after the onset of symptomatic disease rather than treating the contractile defects driving the disease pathogenesis before adverse remodeling.[10-13]

Given the central role of altered contractility in both HF and cardiomyopathies, there has been a long-standing interest to develop molecules that directly modulate contractility. Inotropes, such as dobutamine and milrinone, increase contractility along with intracellular calcium levels, increasing the risk for malignant arrhythmias and mortality.[14] As such, inotropes are typically used in a palliative and critical care setting or as a temporary bridging therapy to ventricular assist devices or cardiac transplantation.[1] Recently, there has been a push to develop compounds known as myotropes, which directly target sarcomeric machinery to modulate contractility without affecting intracellular calcium.[3,5,7,15-17] For example, the phase 3 randomized control trial GALACTIC-HF (Registrational Study With Omecamtiv Mecarbil [AMG 423] to Treat Chronic Heart Failure With Reduced Ejection Fraction) demonstrated that omecamtiv mecarbil (OM), a small molecule targeting myosin, increases contractility in patients with HFrEF and improves clinical outcomes.[18] Another myosin targeting myotrope, mavacamten, was shown to reduce contractility, and it recently became the first U.S. Food and Drug Administration-approved drug for treating patients with hypertrophic obstructive cardiomyopathy.[19] The development of these myotropes was driven by the ability to study and model contractility across scales in preclinical models that span from single molecules to whole organisms. Moreover, these multiscale models have revealed new insights into the pathogenesis of cardiac diseases, and they have been applied to investigate the pathogenicity of variants of unknown significance. It is clear that a multiscale approach to defining contractility has the potential to create new treatment paradigms.

In this review, we describe several multiscale approaches to investigating contractility and provide specific examples of clinically informative insights gleaned from these studies (**Central Illustration**). A multiscale approach starts with considering the most fundamental interactions between myosin and actin, the principal components of the thick and thin filaments of the sarcomere, respectively. We describe the use of biochemical techniques to reconstitute aspects of contraction at the molecular scale. We follow this with a discussion on contractile studies in skinned

ABBREVIATIONS AND ACRONYMS

3D = 3-dimensional

DCM = dilated cardiomyopathy

EHT = engineered heart tissue

GDMT = guideline-directed medical therapy

HCM = hypertrophic cardiomyopathy

HF = heart failure

HFpEF = heart failure with preserved ejection fraction

HFrEF = heart failure with reduced ejection fraction

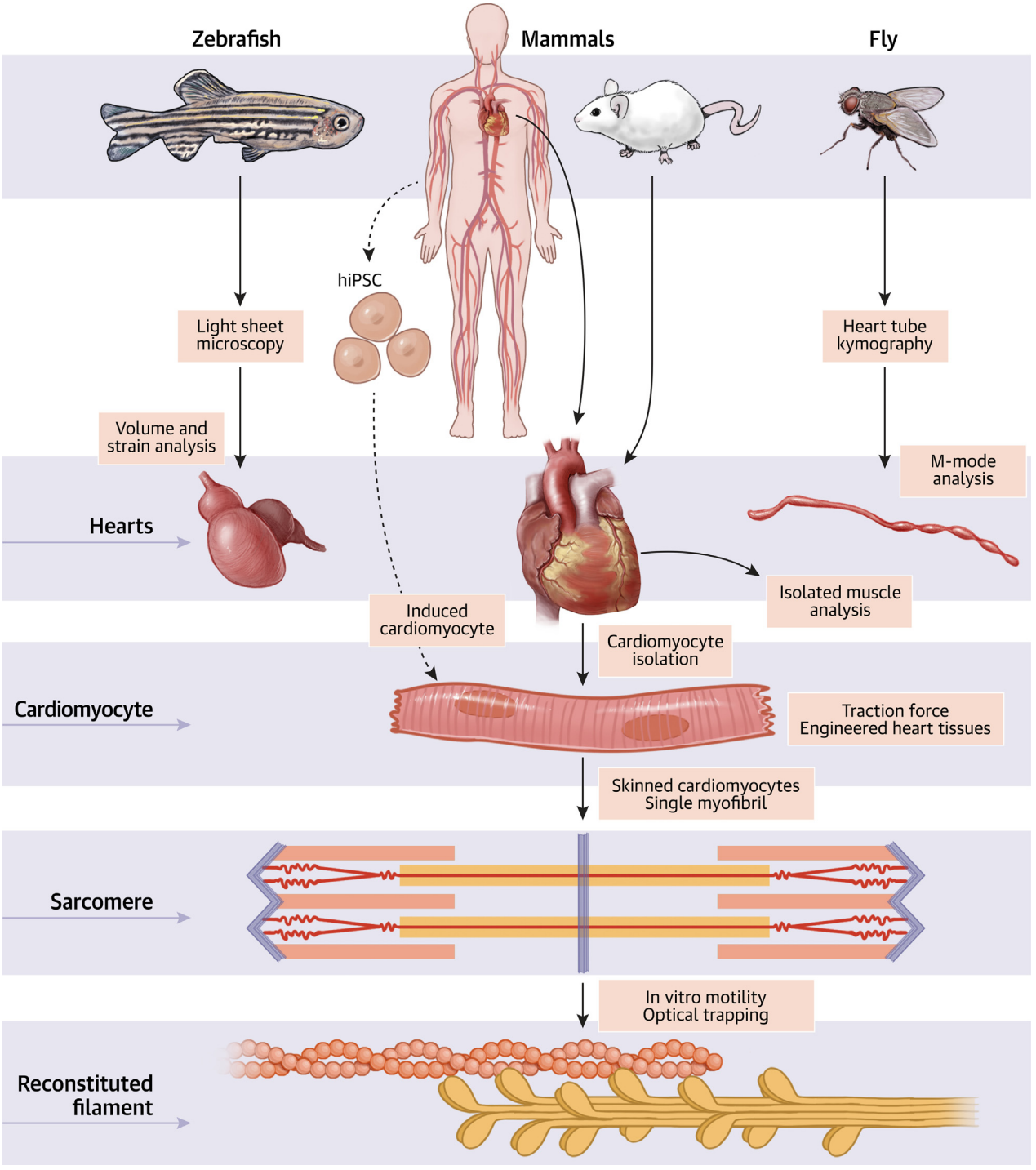
iCM = induced cardiomyocyte

LSFM = light-sheet fluorescence microscopy

OM = omecamtiv mecarbil

SRX = super-relaxed state

CENTRAL ILLUSTRATION **Multiscale Modeling of Cardiac Contractility**



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iPSC = induced pluripotent stem cell.

cardiomyocytes and single myofibrils that allow biochemical studies while retaining native sarcomeric structures. Then, we explore studies of contractility in human induced pluripotent stem cell-derived cardiomyocytes (iCMs), cardiomyocytes isolated from patients, and engineered heart tissues (EHTs). We close with an overview of selected techniques used to study contractility in model organisms including isolated mouse papillary muscles, *Drosophila* heart tubes using kymography, and zebrafish hearts using light-sheet microscopy. We discuss the inherent strengths and limitations of each approach and how these tools can complement each other. Finally, we discuss the prospects of these techniques to drive innovative and integrative approaches to studying and treating HF and cardiomyopathies.

SINGLE-MOLECULAR AND MACROMOLECULAR MEASUREMENTS OF CONTRACTILITY

At the molecular scale, force generation occurs in the sarcomere, a macromolecular complex. Force generation in the sarcomere is driven by myosin motors in the thick filament that pull on actin containing thin filaments. This motion draws the Z-discs toward the center of the sarcomere (demarcated by the M-line), resulting in cell shortening and contraction (**Figure 1A**). Troponin and tropomyosin on the thin filament regulate the calcium-dependent interactions between myosin and actin by sterically blocking myosin binding to the thin filament during diastole (ie, at low calcium levels) (**Figure 1B**) and enabling these interactions during systole (ie, at high calcium concentrations) (**Figure 1C**).[20,21] In this section, we focus on how in vitro reconstitution techniques can be applied to study the sarcomeric machinery that generates and regulates contraction.

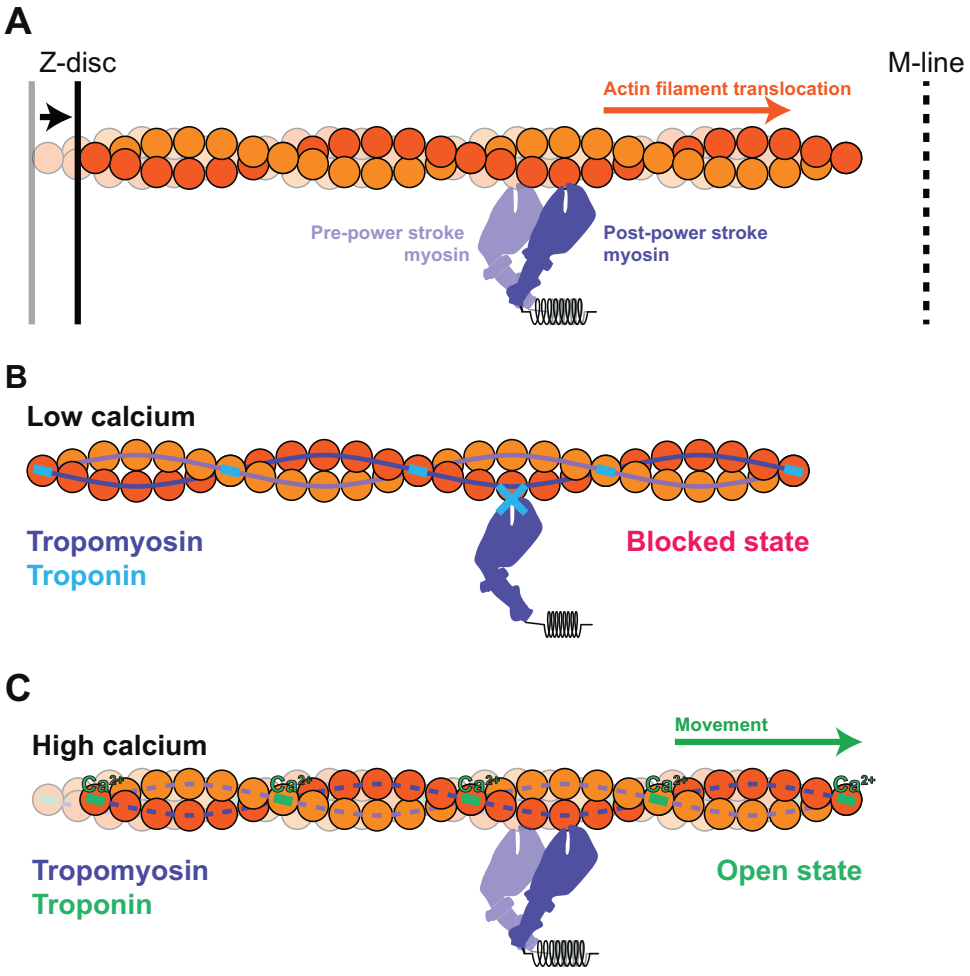
MEASURING SINGLE MYOSIN FORCE GENERATION WITH OPTICAL TRAPPING. A reductionist approach to studying the molecular mechanism of cardiac contractility is the use of reconstituted systems with sarcomere proteins. The simplest contractile unit is a single myosin motor pulling on an actin filament.[22] Although several techniques have been developed to study this simplified system, a commonly used tool for studying cardiac myosin is optical trapping.[23] Optical trapping uses a tightly focused laser beam to measure nanometer movements and piconewton forces, which are within the range of forces generated by single myosin motors as they interact with actin.[24] Importantly, as will be discussed later, optical trapping experiments can directly measure fundamental properties of single myosins related to shortening velocity (eg, myosin's step size and kinetics), force generation (eg, myosin's stiffness and unitary force), and power output (eg, load-dependent kinetics). For further information on myosin's single-molecule properties and their relationships to physiology, the reader is referred to reviews.[22,23,25]

The most common setup for studying single cardiac myosin molecules is a 3-bead arrangement in which a fluorescent actin filament is tethered to 2 optically active beads and then guided to a third surface-bound bead that is sparsely coated with myosin (**Figure 2**).[23,24,26-30] When the myosin binds to the actin, it displaces the beads, and one can measure both the mechanics (ie, the size of myosin's displacement) and kinetics (ie, how long myosin remains bound to actin) of this interaction.[23] As such, it is an ideal system for studying the coupling between biochemistry and mechanical movements. Optical trapping systems have been used to measure displacements generated by both single[27,29-32] and small ensembles of cardiac myosin motors.[33-35]

The optical trapping systems can be modified to measure how external mechanical forces affect myosin's stepping rate.[29,31,36-38] This is important as myosin in the heart must generate power against external mechanical loads such as peripheral resistance or outflow obstruction seen with aortic valve narrowing or hypertrophic obstructive cardiomyopathy. External force slows the rate of crossbridge cycling, and this gives rise to the force-velocity relationship for muscle.[29,31] Importantly, slowing with load influences power production, enabling the myosin to fine-tune its power output in response to physiological and pathologic loads experienced during the cardiac cycle.[29-31] Changes in the force-velocity or force-power relationship are important for contractile function, and they are often observed in failing hearts and hearts with mutations in sarcomeric proteins.[39,40]

Optical trapping techniques have been applied to study pathologic mutations in β-cardiac myosin, the major human adult ventricular myosin isoform (MYH7, interchangeably referred to as "myosin" in this review).[31,37,38,41,42] Some HCM mutations in myosin, such as D239N and H251N, generate greater power output, while some DCM-associated mutations, such as A223T and R237W, decrease power output.[43] These findings have contributed to the hypothesis that the HCM and DCM phenotypes due to myosin mutations are caused by hypercontractility and hypocontractility, respectively, at the molecular scale.[25] It should be noted that multiple parameters contribute to power output that are not completely captured with the optical trapping assay alone.[22,44]

FIGURE 1 Calcium-Dependent Actin-Myosin Interactions

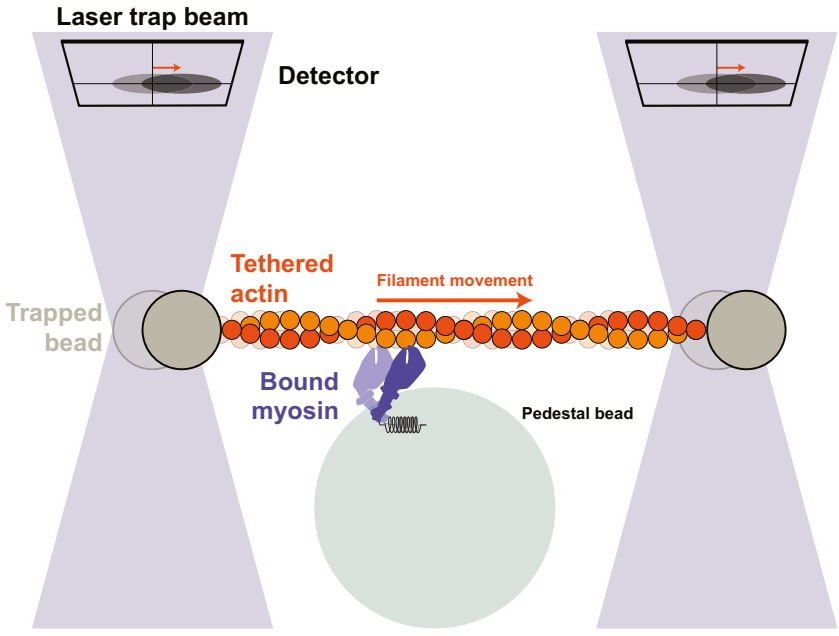


(A) Single myosin motor (purple) bound to an actin filament (orange/yellow) in pre- and post-power stroke states that results in movement of the Z-disc (left, solid black line) toward the M-line (right, dashed black line). (B) In the absence of calcium, tropomyosin (dark/light purple line) and troponin (green box) block binding of myosin to the thin filament. This is referred to as the "blocked state." (C) In the presence of high calcium, calcium (Ca^{2+}) binds to troponin (green), resulting in movement of tropomyosin (dashed light/dark purple lines), allowing myosin binding and thin filament translocation.

For example, the widely studied myosin HCM mutation R403Q, the first mutation associated with HCM,[45,46] showed reduced power output when examined at the single-motor level[42] but increased power output when examined in the context of more complex systems such as full-length myosin,[47] skinned cardiac myocytes and myofibrils,[48-51] intact muscle fibers,[52] and targeted mouse models.[48,49,51] Thus, the hypercontractility and hypocontractility hypothesis for some MYH7 mutations requires testing at multiple scales.

Optical trapping has also been used to study drugs that bind directly to contractile proteins. Such studies have revealed fundamental insights into the mechanism of OM, a myosin-binding drug that has completed phase 3 clinical trials and has shown some benefits in treating patients with HFrEF.[18] Initially, OM was identified in a high-throughput screen for molecules that increase the cardiac myosin adenosine triphosphatase activity, which in isolation would be expected to increase contractile force.[3] Consistent with this notion, OM increases the force of contraction in cardiomyocytes and patients at low doses. However, at higher doses, the force of contraction is reduced, and relaxation becomes longer, impairing diastolic function,[16,53] suggesting a narrow

FIGURE 2 3-Bead Optical Trap



Myosin motors are sparsely coated on a fixed pedestal bead (large bead). A single myosin can then bind to and displace an actin filament held between to 2 optically trapped beads (smaller gray beads). When the myosin motor moves the actin filament, a detector senses the displacement of the beads.

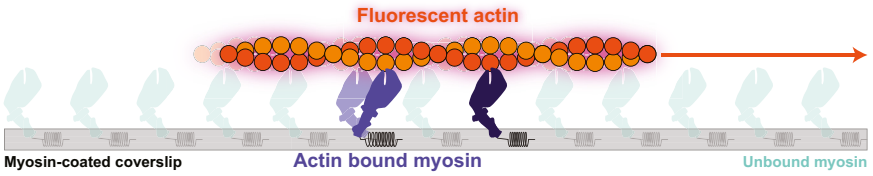
therapeutic window that may explain the modest benefit in clinical trials. Using optical trapping, it was found that OM-bound myosin heads do not generate force but instead prolong the binding of myosin to the thin filament.[31,32] At low concentrations of OM, prolonged binding of myosin to the thin filament leads to cooperative opening of additional myosin binding sites on the thin filament (ie, increased thin filament activation) and enhanced force production.[31,32] However, at high OM concentrations, an excessive number of OM-bound myosin heads ineffectually produce force and decrease systolic function. Furthermore, the prolongation of myosin binding to the thin filament keeps the thin filament in an active state as calcium levels drop, consistent with the prolonged time to relaxation and diastolic dysfunction observed in vitro and in animal models.[54] In summary, optical trapping can directly measure both the mechanics and kinetics of the actomyosin interaction, revealing mechanistic insights into physiology, pathologic mutations, and drug mechanisms.

MULTIMOLECULAR SYSTEMS TO UNDERSTAND CONTRACTILITY. The sarcomere is a complex macromolecular environment consisting of many molecules beyond single myosin molecules interacting with a single actin filament. Importantly, the sarcomere displays additional biophysical properties that emerge as complexity is built into the system.

A popular multimolecular assay that can broadly measure actin-myosin-driven contraction is the in vitro actin motility assay.[55] In this assay, fluorescently labeled actin filaments are propelled over a bed of myosin, generating movement (**Figure 3**). Using a standard epifluorescence microscope, one can observe the speed of many filaments moving, which directly correlates with muscle shortening velocity. This assay has the advantages of solution biochemistry (eg, one can completely define the system, including solution conditions that match physiological or pathologic states) and the ability to directly observe changes in contractility. Furthermore, compared with optical trapping, in vitro motility has increased throughput and does not require engineering expertise to setup the system.

The ability to completely define the system for the in vitro motility assay has enabled studies of actomyosin contractility in the presence of thin-filament regulatory proteins, mechanical load, and intact thick filaments. The thin-filament regulatory proteins troponin and tropomyosin can be added to the assay

FIGURE 3 In Vitro Motility Assay



Cartoon showing fluorescently labeled actin gliding on top of a bed of nonfluorescent myosin adsorbed to a glass coverslip. This movement can be measured using a standard fluorescence microscope.

to recapitulate calcium-dependent regulation of actomyosin interactions.[55-58] With regulated thin filaments, the speed of myosin-driven actin translocation depends on the concentration of calcium, enabling the measurement of thin filament activation (ie, the availability of myosin binding sites on actin).[35] In vitro motility assays can also be modified to exert mechanical loads on myosin, enabling the measurement of how load affects the speed of translocation.[59-61] Unlike optical traps that require complex engineering to apply loads to myosin, actin filaments in the in vitro motility assay can be loaded by adding actin-binding proteins (eg, α-actinin or utrophin) that apply a frictional force opposing movement by myosin.[60] In contrast to optical trapping which directly quantifies the force produced by single motors, loaded motility assays provide qualitative measurements of force production by many motors referred to as the "ensemble" force. Load can be increased by increasing the concentration of the actin-binding proteins on the surface, which allows generation of qualitative force-velocity curves. Finally, these assays can be conducted using either reconstituted thick filaments from isolated proteins, native thick filaments isolated from tissue, or engineered thick filaments created on DNA scaffolds.[62-64] For example, native thick filaments were used in a motility assay to investigate how myosin-binding protein C affects the speed of thin filament translocation over the thick filament.[62,65]

The in vitro motility assay has been widely used to characterize the effects of pathogenic mutations in myosin, actin, tropomyosin, troponin, and myosin-binding protein C. Some recent examples include characterizing HCM mutations in the lever arm of MYH7, actin mutations (DCM-associated R312H and HCM-associated R312C), and HCM-associated mutations in tropomyosin (D219V).[66-68] We have used in vitro motility to characterize mutations in troponin T associated with both HCM and DCM.[57,58,69] The in vitro motility assay has also been applied to study the effects of small molecules. A recent study showed that OM caused a seemingly paradoxical decrease in motility with increasing concentrations.[70] The decreased motility is inconsistent with the initially proposed mechanism of OM as a myosin activator. This key result spurred the optical trapping studies described earlier, which found that OM suppressed myosin's ability to generate motility.[32]

LIMITATIONS. In vitro reconstitution contractility assays are restricted to a subset of contractile proteins that can be readily purified or expressed in a functional state such as actin, myosin, tropomyosin, the troponin complex, myosin-binding protein C, and small molecules that directly interact with these proteins. Importantly, these specific assays exclude proteins such as titin, which is frequently mutated in inherited DCM. The massive protein titin has an important role in regulating diastolic and systolic function through complex mechanisms.[71,72] However, studying the role of integral sarcomere proteins, including titin, can be accomplished in higher order systems such as skinned cardiomyocytes, as discussed in subsequent sections. Although the majority of HCM mutations are found in sarcomeric proteins, which are amenable to these reconstituted contractility assays, the genetic landscape of DCM is much broader, and it includes many proteins not directly involved in sarcomeric contraction.[73,74] For example, BAG3 and LMNA mutations are prominent causes of DCM, but these proteins are not directly involved in cardiac contraction, and contractile dysfunction is a secondary, indirect consequence of mutation-induced changes in protein function.[75-79] That being said, it is still possible to study contractile aspects of these diseases in vitro. Native thick and thin filaments can be extracted from cells, tissue from animal models, and human samples to study secondary effects of these mutations on myofilament contractility.

Native thick and thin filaments retain post-translational modifications such as phosphorylation and alternative protein isoforms expressed in different pathologic conditions, both of which can regulate myofilament function.[65] An alternative approach to studying these mutations is using the higher order systems detailed later. Finally, these reconstituted systems do not recapitulate the complex sarcomere architecture including the spacing between thin and thick filaments.

SKINNED CARDIOMYOCYTES AND SINGLE MYOFIBRILS

Skinned myocytes or single myofibrils extracted from muscle can be used to study sarcomeric proteins in their native architecture, including full complements of accessory proteins. A skinned cardiomyocyte is a cell that has had its sarcolemma removed by mechanical or chemical means, and their permeabilization allows biochemical manipulation. Permeabilization allows unique simultaneous measurements of force and myosin adenosine triphosphatase rate[80-82] and definition of fine details of muscle physiology using chemically caged compounds that can be activated using light, which is referred to as flash photolysis.[83-85] Permeabilization also has the advantage that it enables studying the direct effects of calcium, drugs, and mutations on contractile function without secondary effects related to altered electrophysiology and calcium handling that are often perturbed in disease.[86,87] Moreover, because of their permeabilization, it is possible to exchange certain mutant proteins, as has been done extensively with the troponin complex,[88-122] tropomyosin,[113,123-131] myosin light chains,[132,133] and even actin.[134-138] Direct protein exchange circumvents the need for patient tissue harboring mutant protein or generation of genetic model organisms. Finally, skinned cardiomyocytes and myofibrils can be processed from unfixed patient biopsy samples and tissue.[139,140]

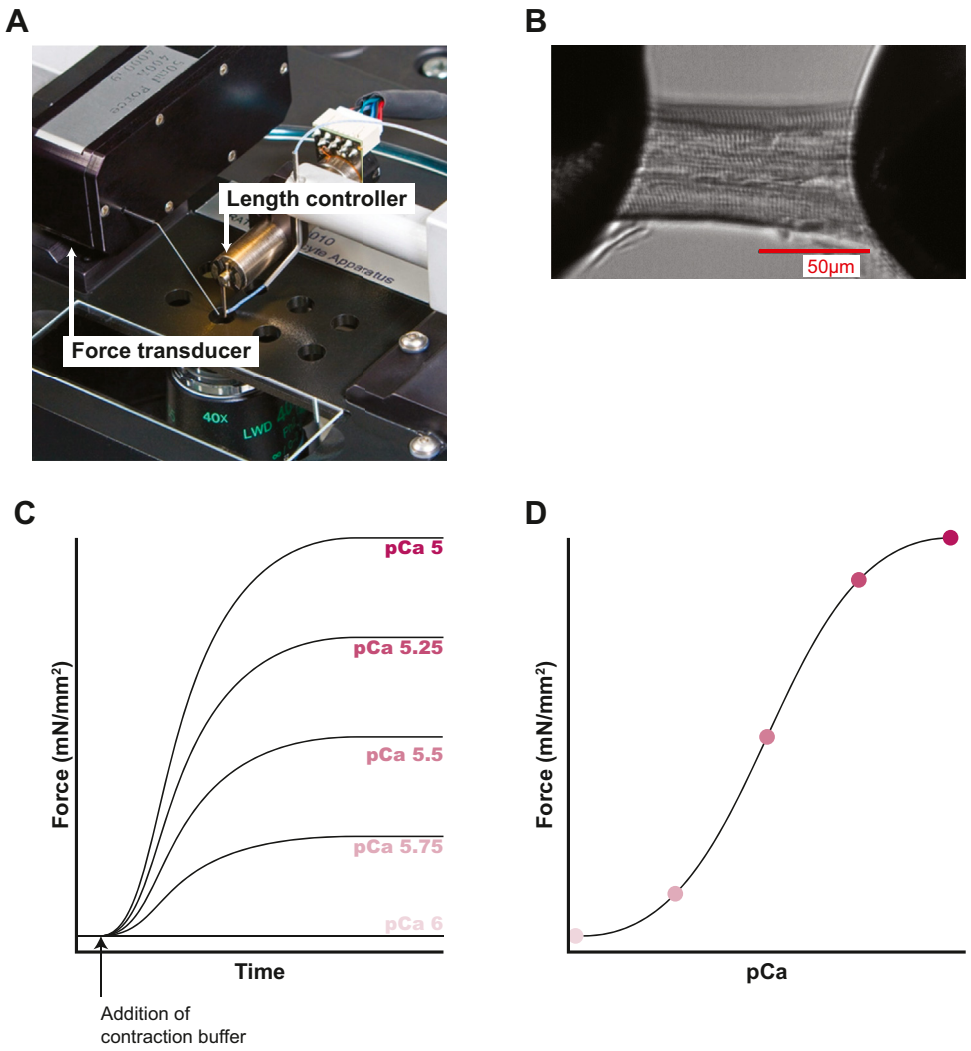
SKINNED CARDIOMYOCYTES. Direct measurement of contractile force in skinned myocytes was first described in skeletal muscle[141] and soon after applied to cardiomyocytes.[142] In a typical setup for examining skinned myocytes, the myocyte is mounted between a force transducer and a length controller (**Figure 4**).[143] The solution conditions can be controlled by exchanging the bath surrounding the myocyte, and systems have been designed to move samples between solutions (eg, different calcium concentrations). For measurements of length-dependent activation,[144] the sarcomere length can be altered by stretching and measured using laser diffraction. More complicated systems have been designed with feedback control of sarcomere length or tension, enabling measurements of additional important parameters such as the force-velocity relationship that can be altered in disease.[145,146]

The force exerted by the muscle on the force transducer is the sum of active and passive forces. The active force is the force of muscle contraction generated by myosin pulling on thin filaments, and it is related to systolic function. The passive force that resists stretch comes from several sources, most notably the protein titin, though this protein has important roles in both systolic and diastolic function.[147-149] A common measurement in skinned myocyte is the force-calcium relationship, which provides useful information about thin filament activation.[150] In this assay, the cardiomyocyte is bathed in solutions containing different amounts of calcium, and the active force generated is measured (**Figure 4**). This setup has been used to examine multiple pathogenic cardiomyopathy mutations. Studies of mutations in the thin-filament regulatory proteins troponin and tropomyosin have demonstrated that these mutations often affect the calcium sensitivity of activation, where HCM increases calcium sensitivity (which is thought to increase contractility) while DCM decreases calcium sensitivity (which is thought to decrease contractility).[88,90,91,94-98,100,102,104-111,116-122,124,126-130,134-138] These changes are most consistently seen with mutations in thin-filament regulatory proteins, but this correlation is less consistent for mutations in other protein such as actin and thick-filament proteins.[151,152]

Similar to the optical trapping and in vitro motility assays, skinned cardiomyocytes can also undergo force-velocity and force-power testing. In these experiments, the skinned muscle is pulled to isometric tension, and then a feedback loop is used to maintain either a constant velocity or constant load as the muscle is allowed to shorten. These techniques were recently applied to study decompensation in a hypertensive animal model.[153] Using skinned cardiomyocytes, the investigators found that as hypertensive animals aged, there was a dramatic loss of power output independent of calcium handling. The onset of this power loss was correlated with the onset of HF, reinforcing the importance of looking beyond simple force production and demonstrating the need to consider power production in HF.

Beyond mechanical relationships, skinned cardiomyocytes can also elucidate actin-myosin cross-bridge kinetics both in the absence and presence of

FIGURE 4 Skinned Cardiomyocyte Contractility



(A) A commercially available system that can be used to measure the force of single myocytes (Aurora Scientific 1600A). The myocyte is mounted between a length mover and a force transducer. Image courtesy of Chris Rand and Aurora Scientific. (B) Magnified view of the attached skinned cardiomyocyte. Reproduced from Greenman et al[288] and licensed under a Creative Commons CC BY license, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. Scale bar in red. (C) Cartoon showing force development in buffers containing differing calcium concentrations. Lower calcium concentrations result in less force generated, with no force generated in the low-calcium pCa 6 (10^{-6} M calcium) buffer. (D) Cartoon showing the sigmoidal relationship between the maximum force generated and the concentration of calcium (pCa). Colored dots correspond to respectively colored pCa concentrations displayed in C.

load. A common technique that is used to measure crossbridge kinetics is the slack-restretch maneuver, in which a myocyte is held at isometric tension, and then the tension is rapidly released and then re-established.[154] This causes myosin crossbridges to release from the thin filaments and then reattach, giving a measurement of the rate of tension redevelopment (k_{tr}) which is proportional to the crossbridge cycling rate. This rate has been shown to be altered in some inherited and diabetic cardiomyopathies (discussed later).[155-157] More advanced techniques and modeling are available to measure the rates of key steps of the crossbridge cycle in skinned myocytes, unloaded shortening velocity, and sinusoidal stiffness, all of which can guide downstream biochemical experiments.[158-166]

In addition to probing actomyosin interactions, skinned fibers have been instrumental in deciphering the autoregulation of myosin and its perturbation in diseases including cardiomyopathies.[22] It has been shown that muscle myosin can form an autoinhibited state known as the "super-relaxed state" (SRX). The presence of the SRX was originally shown in skeletal muscle fibers and later demonstrated in cardiac muscle.[167-170] This state was first observed using skinned fibers, in which the rate of adenosine triphosphate release from relaxed fibers was measured in a pulse-chase experiment using a fluorescently labeled adenosine triphosphate. Autoinhibited myosins in the SRX showed very slow adenosine triphosphate turnover kinetics, while uninhibited myosin in the "disordered relaxed state" had a much faster adenosine triphosphate turnover rate.[171,172] Myosins in the disordered relaxed state can generate force in the presence of calcium, whereas myosins in the SRX cannot. Thus, the fraction of myosins in the SRX is a critical determinant of force generation, and this can be dynamically modulated.[170] Recent studies of muscle fibers from HCM model organisms have demonstrated that the SRX can be destabilized by many pathogenic mutations, contributing to the hypercontractility seen in patients.[133,173-176] Similarly, skinned cardiomyocytes from a DCM mouse model with a mutation in MYL2 (myosin regulatory light chain), D94A, demonstrated an increase in the population of the SRX, which contributes to hypocontractility.[175]

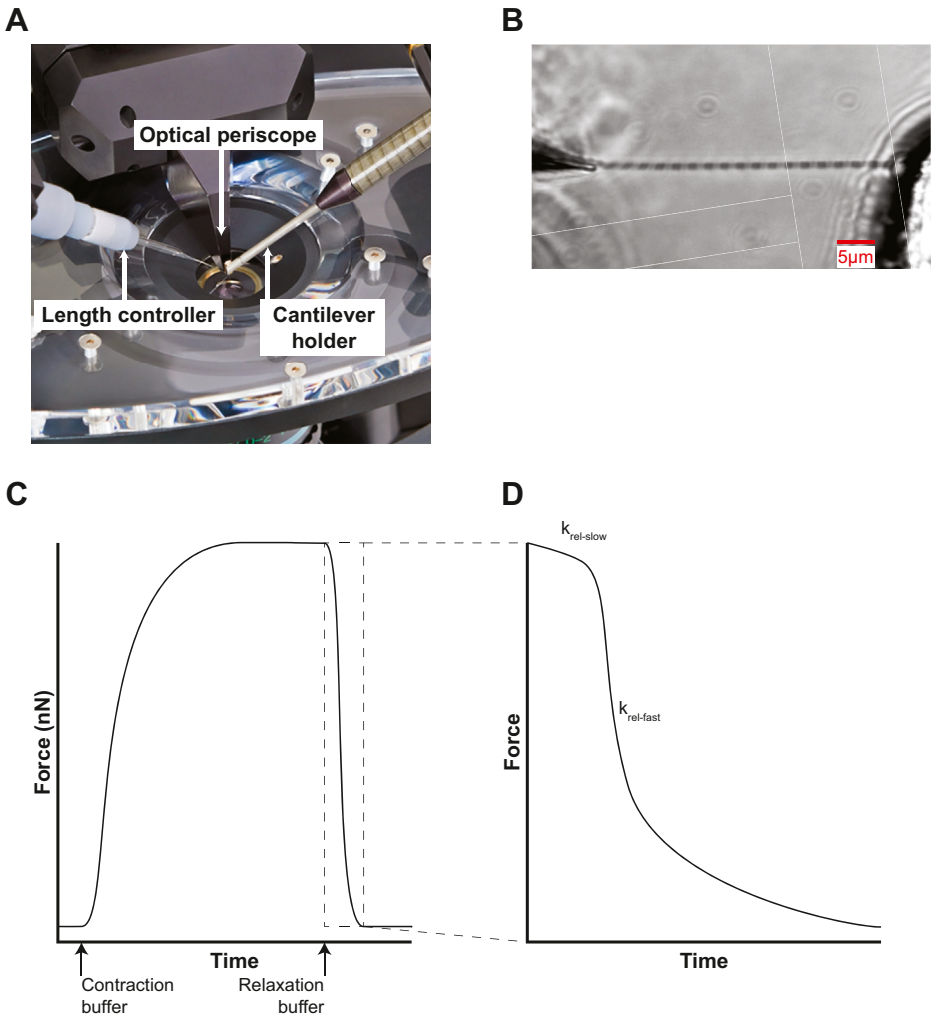
Along with genetic cardiomyopathies, skinned cardiomyocytes can be used to study pathologies with complex effects on contractility, which was done in a study of patients with diabetic cardiomyopathy.[157] Chronic exposure to elevated glucose causes glycation of key sarcomeric proteins; however, the functional consequences of this modification and its relationship to diabetic cardiomyopathy were not well understood.[177] Skinned cardiac muscle fibers were treated with a compound to glycate sarcomeric proteins, and it was shown that glycation reduces the peak tension generated and slows the rate of tension redevelopment. These effects likely contribute the systolic dysfunction in diabetic cardiomyopathy.

Another complex phenotype that has been successfully studied with skinned cardiomyocytes is HFpEF, and this study demonstrated the potential for direct bedside-to-bench studies using samples from patients. As stated previously, HFpEF remains both poorly defined clinically and mechanistically. A recent study used skinned cardiomyocytes from patients with HFpEF to elucidate the contractile effects of this disease.[178] In that study, the investigators obtained right ventricular septal biopsies from patients with HFpEF undergoing right heart catheterization, which is a standard diagnostic procedure for cardiologists. Active and passive forces were measured for individual skinned cardiomyocytes from flash-frozen biopsy tissues. The investigators found increased passive force, consistent with diastolic dysfunction but also a striking decrease in calcium-dependent active force generation in myocytes from patients with HFpEF. The defect in active force generation at the skinned myocyte level seemingly differs from the clinical observation that hearts from patients with HFpEF show intact contractility with impaired relaxation. On the basis of their observation, the investigators speculated that applying pro-contractility agents may present a new avenue of treatment for HFpEF; however, further studies are required to test this hypothesis.

Finally, skinned cardiomyocytes can be used to study the effects of direct sarcomeric modulators such as OM and mavacamten, furthering the translational power for this system.[53,179-186] For example, skinned cardiomyocytes from donor and failing hearts were used to explore the effects of OM on force generation, the relaxation rate, and the rate of tension redevelopment.[182] Interestingly, although OM increased force generation in the myocytes from failing hearts, relaxation time was increased as was the time to redevelop force and reach maximum force compared with nonfailing donor myocytes. The investigators speculated that high concentrations of OM may compromise diastolic filling, which may blunt the overall inotropic effect of OM, consistent with molecular studies; however, titrating the dose of OM in patients with HF could improve its therapeutic benefit while minimizing the adverse effects and will need to be explored more in the future.

SINGLE-MYOFIBRIL CONTRACTILITY. In contrast to an array of many parallel sarcomeres, single myofibrils consisting of several sarcomeres linked in series can be isolated from skinned myocytes for mechanical studies.[187,188] A single myofibril can give similar information about contractile function as a skinned cardiomyocyte but without contributions of other cellular elements that may confound these measurements such as nonsarcomeric cytoskeletal components. Moreover, the amount of starting material needed for a single myofibril assay is less than that of a skinned cardiomyocyte, which may be beneficial for maximizing replicates when tissue amount can be limiting, such as the case with patient biopsies. Finally, the small size of a single myofibril enables

FIGURE 5 Single-Myofibril Contractility



(A) Commercially available system for single myofibril measurements (Aurora Scientific 1700A). The myofibril is mounted between a length mover (left) and cantilever holder (right), which is visualized by an optical periscope (middle). Image courtesy of Chris Rand and Aurora Scientific. (B) Magnified view of attached single myofibril held between a length mover and the stationary force transducer. Approximate scale bar displayed in red. Image used with permission from Aurora Scientific. (C) Cartoon showing the force generated by a single myofibril. The myofibril is initially bathed in relaxing buffer, then bathed in activating buffer, and then relaxing buffer again. Note the difference in the magnitude of forces between a single myofibril shown here and a skinned cardiomyocyte in Figure 4 (nanonewtons vs millinewtons). (D) Cartoon showing the time expanded view of region of interest from C showing transient linear relaxation phase ($k_{rel-slow}$) and the longer exponential relaxation phase ($k_{rel-fast}$).

more rapid switching of buffer conditions compared with skinned myocytes.

The evolution of single-myofibril techniques was extensively reviewed recently.[189] In a typical single myofibril experiment, a myofibril is dissected from a skinned myocyte and then attached between a length mover and a force transducer (eg, pulled glass pipette or cantilever) (Figure 5). A dual-chamber pipette is then brought near the myofibril and different buffers (e.g., high and low calcium buffers) are flowed through each chamber. The dual-chamber pipette can be rapidly moved for quickly switching of buffer conditions. This rapid switching without the need to move the myofibril enables the observation of fast contractile changes that cannot be seen with skinned myocytes.

When a myofibril is rapidly switched from a high-calcium activating buffer to a low-calcium relaxing buffer, the tension relaxes in 2 distinct phases.[86] The first phase is short lived, but the rate of relaxation is slow and linear ($k_{rel\text{-}slow}$). The rate of this phase is proportional to the rates of thin filament deactivation and crossbridge detachment. This linear phase is not observed in skinned myocytes, as it is not possible to switch buffers fast enough with full myocytes. The linear phase is followed by a fast exponential decay ($k_{rel\text{-}fast}$) that relates to the active and passive relaxation forces[190] (**Figure 5C**). This technique was recently used to study the effects of the small molecule danicamtiv that is currently being investigated to increase contractility.[191] The investigators found that danicamtiv changes the linear phase of relaxation when calcium levels drop. This suggests that danicamtiv slows the rate of crossbridge release from the thin filament, which would slow the rate of myofibril relaxation and could induce changes in diastolic function. Notably, slowing the rate of myofibril relaxation is thought to attenuate the therapeutic benefit of OM. It is possible that danicamtiv may have similar issues[54]; however, a recent study showed a more mild effect on diastolic function with danicamtiv compared with OM.[16] This mechanistic insight would not have been gleaned from skinned myocytes which do not show a linear phase.

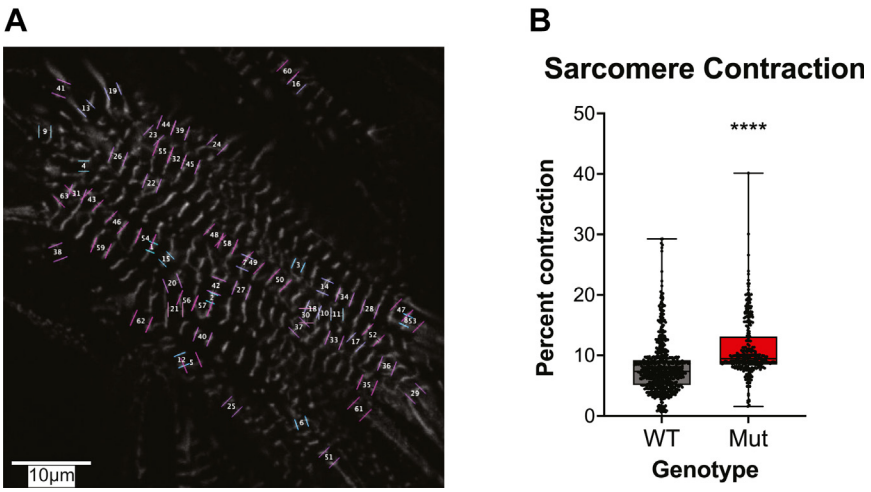
Numerous studies have used single myofibrils to study changes in contractility and kinetics under physiological conditions, in the context of genetic cardiomyopathies, and in the presence of proteins with specific post-translational modifications.[189] In one study using a transgenic mouse model of DCM-associated cardiac actin E361G, the investigators showed that single cardiac myofibrils had increased calcium sensitivity but nearly one-half the rate of relaxation for both $k_{rel\text{-}slow}$ and $k_{rel\text{-}fast}$.[192] By testing other parameters that change calcium sensitivity, such as the dephosphorylation of troponin I or a small-molecule activator of troponin C, the investigators found that changes in calcium sensitivity correlate with changes in $k_{rel\text{-}slow}$ and $k_{rel\text{-}fast}$. This study and others demonstrate the ability to glean multiple biochemical parameters from a single myofibril experiment.

LIMITATIONS. Removal of the sarcolemma influences myofilament organization, passive mechanical properties, calcium responsiveness, and force-length relationships of myofibers compared with intact myofibers.[193] Furthermore, probing changes in contractility that rely on electrochemical coupling or cell metabolism are not feasible in demembranated cells. Additionally, though the skinned cardiomyocyte is freely permeable with its bathing solution, measurements are limited by diffusion of buffer into the cells, though this issue is greatly mitigated in single myofibrils. In comparison with biochemical reconstitution systems, contractile changes seen in cardiomyocytes and myofibrils are not as readily relatable to a single molecular defect given the increased molecular complexity of these systems. For example, it is difficult to decipher whether changes in force observed in skinned fibers come from changes in the force generated by individual myosins, the number of active crossbridges, the kinetics of individual crossbridges, and/or the stiffnesses of individual crossbridges. Finally, although the skinned cardiomyocyte and single myofibril experiments are conceptually very similar, they require very different equipment to execute (**Figures 4 and 5**), with single myofibrils presenting a greater technical challenge.

MEASURING CONTRACTILITY IN LIVING CELLS

CARDIAC CONTRACTILITY IN INDUCED CARDIOMYOCYTES. There have been several technical advances that have enabled the study of human cardiomyocytes. One advance is the ability to generate stem cell-derived iCMs.[194] These cells can be paired with gene-editing techniques such as CRISPR/Cas9 to generate isogenic cell lines containing mutations or isogenic control cell lines from patients from whom the mutation has been removed.[195-200] The process for creating iCMs from human induced pluripotent stem cells has become substantially more streamlined with multiple protocols and commercially available reagents.[201,202] A long-standing challenge with this model is the immaturity of these cells compared with adult cardiomyocytes[203,204]; however, there are various approaches being used to improve this maturity, which is discussed in a recent review.[205] Although these cells cannot recapitulate end-stage HF, they can give powerful insights into the early disease pathogenesis before the onset of maladaptive remodeling.[206] Currently, mature cardiomyocytes can be derived only from heart tissue, and protocols have been optimized to obtain these cells from rodents.[207] There have been efforts to improve the isolation of human primary cardiomyocytes, with one protocol successfully carrying cells through a cryopreservation and replating cycle.[208] However, even with these improvements, experiments taking more than 1 week to complete remain challenging, and there are still major hurdles with obtaining sufficient tissue from

FIGURE 6 Fluorescent Sarcomere Contractility Tracking in Live iCMs



(A) Stem cell–derived cardiomyocytes expressing ACTN2-mEGFP, which demarcates Z-discs. Sarcomeres were identified and tracked using the SarcTrack software package.[213] Note that not all sarcomeres are tracked, on the basis of a filtering algorithm in SarcTrack, which removes sarcomeres that are not reliably tracked for the entirety of the recorded video. (B) Representative data generated using SarcTrack showing a mutant (Mut) cell line that has greater contractility compared with the wild-type (WT) line.

appropriately matched patients and with the inherent variability of human samples. Here, we focus on measuring contractility from single cells and microtissues derived from human induced pluripotent stem cells.

MEASURING SARCOMERIC SHORTENING IN LIVING CELLS. Two important contractile parameters of cardiac muscle are fractional shortening (ie, the percentage length change during shortening) and shortening velocity, both of which can be directly measured for individual cardiomyocytes.[209] It has recently become possible to measure these parameters for individual sarcomeres within live cardiomyocytes using fluorescently labeled probes. The sarcomere is particularly amenable to these approaches because of its regular, repeating structure. Several approaches have been applied to introduce fluorescent proteins into live sarcomeres, including using small molecules (eg, LifeAct), viral transduction of plasmids for the overexpression of tagged sarcomeric proteins, and gene editing of endogenous proteins to fuse them to fluorescent tags (eg, alpha actinin, myomesin, titin, myosin regulatory light chain).[210-212] A large selection of validated human stem cell lines with genomic expression of tagged sarcomere proteins are available from the Coriell Institute for Medical Research.

Fluorescently labeled sarcomeric proteins enable the measurement of several important parameters, including sarcomeric length, fractional shortening, and shortening velocity (Figure 6). There are several excellent open-source software packages for the analysis of sarcomeric contraction in living cells, and the reader is referred to one of these packages.[213] Sarcomeric tracking was used to study myosin-binding protein C haploinsufficient cells, and that study demonstrated that it is possible to improve hypercontractility in these cells using mavacamten.[214] Similarly, this technique has been used to prospectively study several other mutations in *TNNI3* (cardiac troponin I), *TNNT2* (cardiac troponin T), and *MYH7*.[176,215]

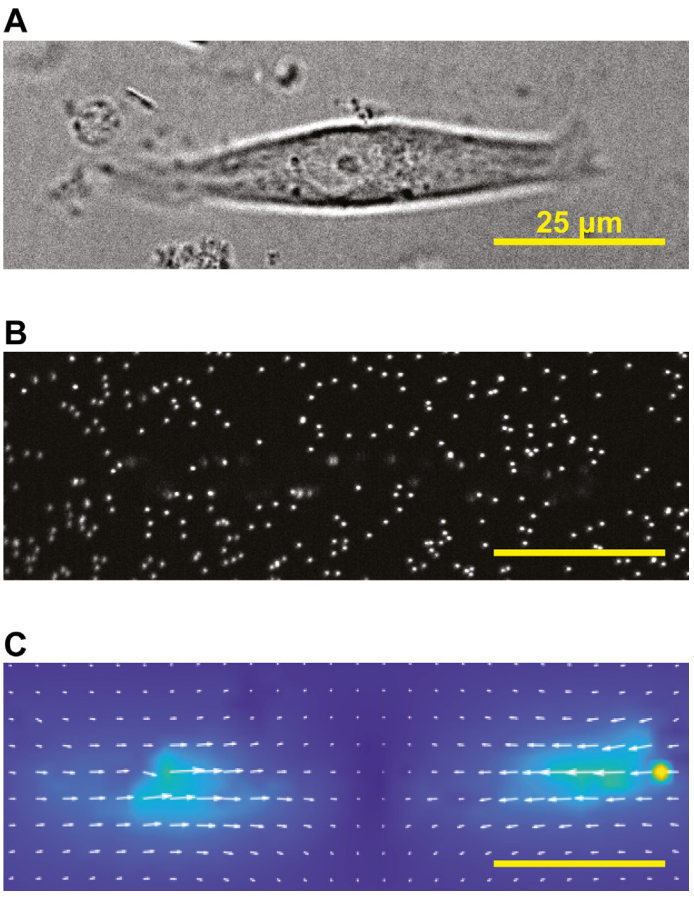
One limitation of this approach is the need for fluorescent proteins to track sarcomeric contractions, which requires either genomic modification or lentiviral overexpression. When generating new lines with fluorescently tagged sarcomeric proteins, it is important to conduct controls to ensure that the fluorescent protein does not affect sarcomeric function.[212] Gene editing of isolated cardiomyocytes from patients is currently not possible, because of challenges with long-term culture; however, these cells could be treated with viral overexpression of tagged proteins or peptides. Another limitation is that this

technique does not directly measure force. Force-tuned fluorescent tensions sensors have been developed for other cytoskeletal proteins, but these have not yet been applied to measuring forces in the sarcomere.[216] Finally, a general theme of single-cell techniques is that it is challenging to apply force to cells to analyze the force-length relationship, although studying force-frequency responses is technically possible.

MEASURING CARDIOMYOCYTE FORCE GENERATION. Several techniques have been developed for measuring the contractility of single intact cells, but the most frequently used technique is traction force microscopy,[217] in which a cardiomyocyte is placed on a deformable hydrogel of known stiffness that contains small, embedded fluorescent beads (Figure 7). When the cardiomyocyte contracts, this force deforms the substrate, displacing the beads. From the known stiffness of the substrate and the displacement of the beads, it is possible to calculate the active force of contraction.[211] There are several open-source packages for analyzing traction force experiments, and these programs can calculate important parameters, including peak force, power, and contraction and relaxation rates.[211,218,219] Moreover, the stiffness of the substrate can be tuned to several different stiffnesses to mimic physiological and pathologic conditions.[211,220,221] Very few cells are needed to perform analysis, which can considerably lessen resource commitment.

A key advantage of a live cell system is that it enables the study of physiology, including signaling pathways, protein isoform switching, and genetic and epigenetic mechanisms. At the molecular scale, point mutations directly affect the structure, function, and/or abundance of the mutated protein, and this molecular dysfunction can lead to the activation of adaptive and maladaptive downstream signaling.[44,57,58,69] It is often not possible to examine these changes outside of the context of a live cell, necessitating the pairing of molecular and cellular techniques. For example, we showed that the HCM R92Q mutation in troponin T causes molecular hypercontractility, but this leads to downstream changes in calcium handling, gene expression, and electrophysiology.[58] Intact cellular systems have been applied to study nonsarcomeric DCM mutations, including ones in the chaperone protein BAG3, the intermediate filament protein LMNA, and the calcium handling protein PLN.[75,76,78,222,223] These studies all revealed reduced contractility, consistent with the known DCM phenotype, but they have very different molecular mechanisms, and the observed changes in contractility are secondary to the direct mutation-induced changes in protein function.[44]

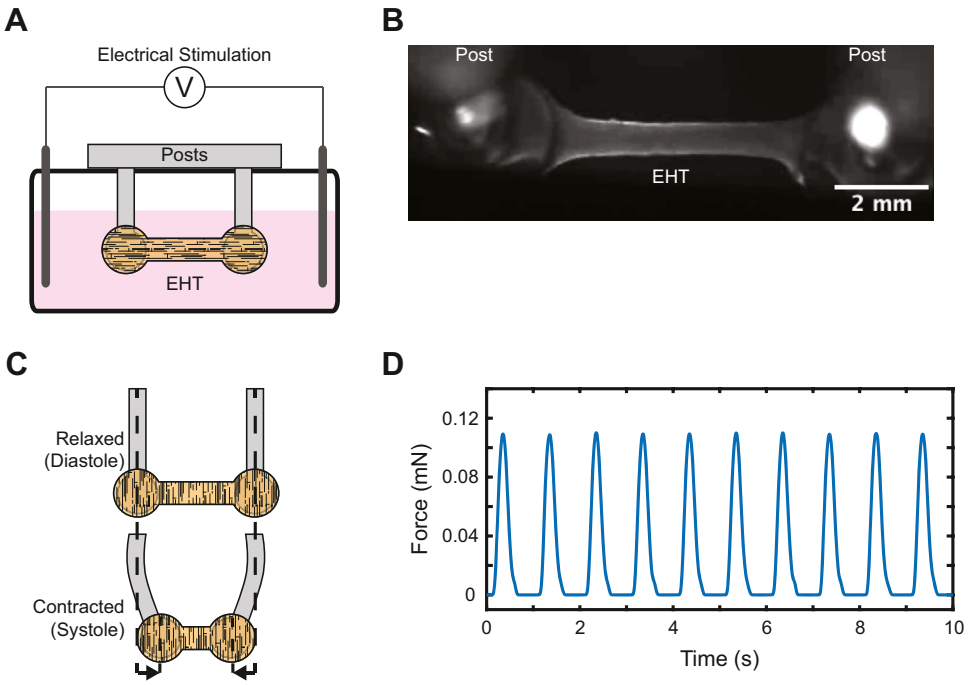
FIGURE 7 Traction Force Microscopy of Cardiomyocytes



(A) Bright-field image of a patterned stem cell–derived cardiomyocyte on a hydrogel. (B) Fluorescent beads embedded within the hydrogel. (C) Resultant strain map showing forces generated by contraction. This was calculated using the software package Contrax. Note that the strain points toward the center of the cell with maximal strain at opposite poles of the cell.[289]

LIMITATIONS. As stated previously, immaturity remains an ongoing issue for studies with iCMs, especially when it comes to electrochemical coupling, metabolism and expressing mature sarcomere protein isoforms.[205,224] Mechanistically, deciphering the direct connection between the initial molecular insult and pathogenesis of disease in living cells is more difficult given the inherent complexity of a cell compared with a biochemically reconstituted system or skinned cardiomyocytes. However, this system lacks the complexity of myocardial tissue, which

FIGURE 8 Force Measurement With Passive Linear EHT System



(A) Side-view schematic of a single engineered heart tissue (EHT) (orange) adhered around posts of known stiffness and immersed in media with electric stimulation. (B) Image of a linear EHT courtesy of Lina Greenberg (Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine). (C) Schematic showing post deformation with contraction. The force can be calculated from the stiffness of the posts and the magnitude of the displacement. (D) Sample force trace showing EHT contraction with electric stimulation.

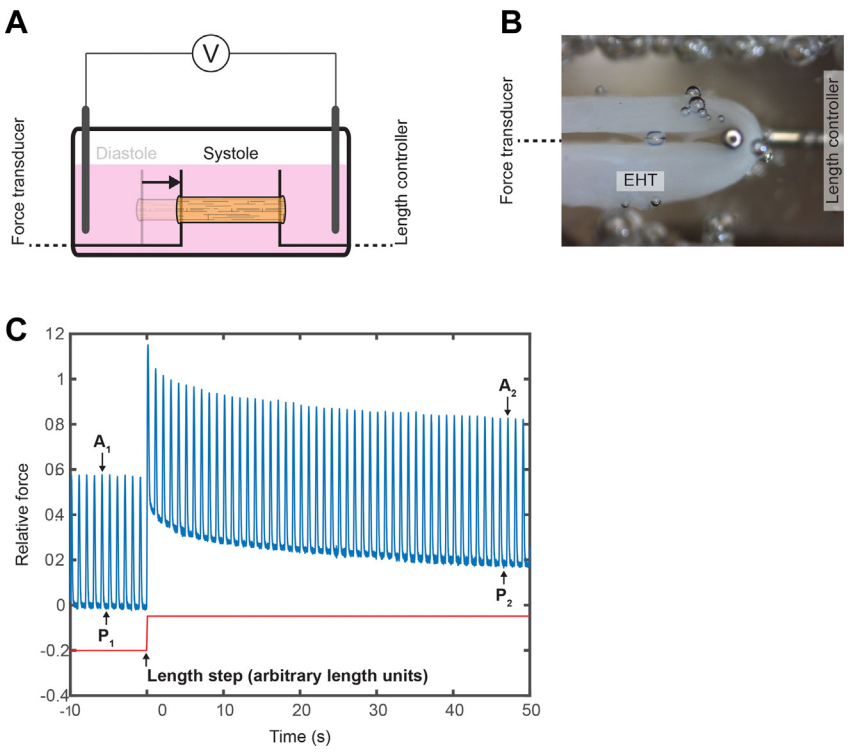
contains additional cell types and structures that may influence the observed contractile phenotype.[225,226]

MEASURING CARDIAC TISSUE CONTRACTILITY IN VITRO AND EX VIVO

ENGINEERED HEART TISSUES. Multiple platforms have been developed to generate 3-dimensional (3D) human EHTs.[219,227-236] EHTs can recapitulate critical cues experienced in the heart that are missing from most single-cell assays, such as mechanical and electric stimulation, 3D arrangement, and the presence of noncardiomyocyte cell populations.[237-240] EHTs are typically designed in specialized microfabricated or microelectromechanical systems to investigate key parameters of contractile function while limiting the required amount of input material relative to macroscale tissues.[228,230,233,234] Recently, it has become possible to generate EHTs on 3D printed scaffolds that geometrically resemble a heart and can recapitulate key aspects of whole-heart contractility.[241] The reader is referred to recent reviews on these technologies.[239] Currently, it is not possible to make tissues from adult cardiomyocytes, and systems have focused primarily on iCMs or neonatal rat ventricular cardiomyocytes, which readily form tissues.

The most common EHTs used to study contractility consist of iCMs and cardiac fibroblasts embedded in an extracellular matrix (usually collagen or fibrin). Broadly speaking, there are 2 types of EHT systems for measuring contractility: passive systems, which measure forces without allowing the user to actively manipulate the force on the tissue, and active systems, with which the user can manipulate the force.[237] One type of passive system is a system in which a linear tissue strip is formed between 2 deformable posts of known stiffness, and then the force of contraction during beating can be calculated from the displacement of the posts (**Figure 8**).[238,242] This system has been used to study several conditions, including cardiomyopathy mutations, doxorubicin-induced cardiotoxicity, and the effects of drugs on tissue function.[229,231,243] An advantage of this system is the ability to add in additional cell

FIGURE 9 **Force Measurement With Active Ring Shaped EHT System**



(A) Side-view schematic of a ring tissue (orange) attached between a force transducer (left) and a length controller (right). The tissue is immersed in media and electrically stimulated. When the tissue contracts, this results in displacement of the force transducer. (B) Partial overhead image of ring tissue attached to force transducer (left, off screen) and length controller (right). (C) Representative force tracing (blue) showing tissue beating before and after a length step (red). The length step results in an increase from the initial passive force (P1) to a new passive force (P2). Active force modestly increases after stretch (A1 vs A2) because of the Frank-Starling mechanism. The tissue shows evidence of viscoelasticity from the increase and subsequent relaxation in total force following the length step. EHT = engineered heart tissue.

types, and we recently used this system to investigate how SARS-CoV-2 affects the contractile properties of human heart tissue.[244]

One example of an active system is a ring-shaped tissue[245,246] that can be attached between a force transducer and a length mover, allowing one to measure the active and passive forces of the tissue and establish force-length relationships (ie, Frank-Starling) (**Figure 9**).[247] This ring-tissue configuration was recently applied to study a female patient with severe HF carrying an X-linked Δ45-58 *DMD* mutation and a heterozygous *PLOD3* stop variant.[248] iCMs generated with both the Δ45-58 *DMD* mutation and the *PLOD3* mutation showed a decrease in active force and stiffness. Upon correction of the *PLOD3* mutation, stiffness, but not active force generation, was restored. Analysis of the EHT showed decreases in collagen synthesis offering a possible explanation to the decrease in tissue stiffness.

LIMITATIONS. Although EHTs improve the maturation of iCMs, the iCMs do not reach the same level of maturity as adult cardiomyocytes.[239] It should be noted that the required degree of maturity needed to model a disease will vary depending on the disease process being studied. An additional limitation to EHTs is that they require more cells than single-cell techniques. Moreover, although these tissues have a 3D architecture, they lack the organization of cells and extracellular matrix as well as the diversity of cells found in the human heart. Recently, it was demonstrated that it is possible to generate cardiac organoids that contain many of the cell types in the heart, but this technology is still under development.[249] There have been advances in 3D printing of

heart tissues and the use of decellularized heart scaffolds, but these tools still cannot completely recapitulate this organization.[250,251]

INTACT MUSCLE TISSUE ISOLATED FROM MODEL ORGANISMS. It is possible to directly study the contractility of isolated intact papillary or trabecular muscles from transgenic animals.[252] This technique can be applied to tissue from human patients, but there are major logistical challenges with these studies, as human samples are usually obtained from explanted hearts and require immediate use, unlike skinned cardiomyocytes and myofibrils.[253] With that said, there have been successful contractility studies of intact trabeculae muscle obtained from human hearts.[254-256]

In contrast to skinned cardiomyocytes and single myofibrils, isolated papillary muscles retain electrochemical coupling with their intact sarcolemma, allowing cardiac conduction. In papillary muscle studies, the muscle tissue is mounted between a length mover and a force transducer. By retaining cell viability and electric coupling, the contractile effects of changes to ion channel conduction, calcium signaling, adrenergic signaling, and additional pathways can be studied. Techniques have also been developed to simultaneously measure force and calcium transients.[81,82,257,258] Furthermore, these fibers can be manipulated to measure force-length, force-velocity or force-power, and force-frequency relationships.[252,259,260] These tissues retain the native architecture of cells and matrices, enabling the study of complex interactions between cells and the extracellular matrix.

Isolated murine papillary muscle fibers were used to test whether overexpression of SERCA2a could mitigate the impact of diabetic cardiomyopathy.[261] In the absence of overexpression, isolated papillary muscles from a diabetic animal model showed a >60% reduction in systolic force and diastolic relaxation; however, these effects were completely reversed with SERCA2a overexpression. In another example the effects of mechanical unloading by heterotopic heart transplantation (ie, connecting a second donor heart to a recipient while maintaining the original heart) were investigated using papillary muscles isolated from a rat DCM model.[262] The investigators found that mechanical unloading improved contractility and β-adrenergic sensitivity, which are important clinical targets for preserving overall cardiac function.

LIMITATIONS. Unlike skinned cardiomyocytes and single myofibrils, isolated muscles must be used immediately after harvesting. Furthermore, proteins cannot be exchanged into the intact muscle fiber as they can be for skinned cardiomyocytes and single myofibrils. Thus, mutant proteins must already be present in the muscle fiber prior to harvest. Despite these limitations, measuring the contractility of intact muscle remains an important technique given the ability to retain a viable muscle fiber that is capable of being electrically stimulated while retaining other important cell types and matrix organization.

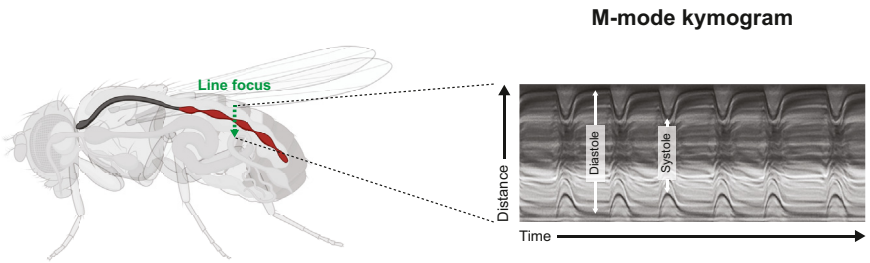
MEASURING CONTRACTILITY IN VIVO

In vivo models of cardiac disease provide the most complete model of disease, as they do not require simplifications and they contain the diverse cell types found in the myocardium such as fibroblasts, immune cells, and endothelial cells.[240,263] In vivo systems can be used to investigate signaling between organs and organ systems that are critical to normal physiology and disease processes. This is important for understanding complex diseases such as HFpEF, whose pathophysiology is believed to be significantly influenced by cardiac remodeling resulting from endothelial inflammation and oxidative stress.[264,265] The mouse is the most popular model organism to use to study cardiac disease, and many tools used to study cardiac function in humans, such as echocardiogram, cardiac magnetic resonance imaging, and invasive catheterization, are also available in mice and have been extensively discussed previously (for a recent review, see Lindsey et al[265]). Here, we focus on the application of cutting-edge optical imaging techniques to study cardiac function in vivo using fly and zebrafish models.

***DROSOPHILA* CARDIAC KYMOGRAPHY.** *Drosophila melanogaster* has been an important genetic platform for uncovering the molecular biology underlying development.[266,267] Powerful tools have been developed to study *Drosophila* enabling cost-effective and high-throughput study of genetic perturbations. The *Drosophila* heart is a linear contractile tube that creates an open circulatory network that transports a mix of blood and interstitial fluids called hemolymph throughout the organism (**Figure 10**).[268] Despite the anatomical differences between *Drosophila* and humans, *Drosophila* cardiomyocytes possess a similar sarcomeric architecture with many conserved cardiac regulatory proteins and networks.[269]

The *Drosophila* cardiac tube is routinely visualized by dissection. High-frame rate optical imaging of the cardiac tube enables the determination of end-systolic and end-diastolic diameter, fractional shortening, and shortening and relaxation times and

FIGURE 10 ***Drosophila*** **Heart Tube Kymography**



Adult heart tube (red) with a line of focus drawn through the walls of the heart (green arrow) is recorded at high frame rate to produce a kymograph (right). Diastole and systole can be observed on the basis of the positioning of the anterior and posterior walls, similar to M-line echocardiography. Fly image created using BioRender.com (left). Kymogram (right) courtesy of Anthony Cammarato (Division of Cardiology, Department of Medicine, Johns Hopkins University School of Medicine).

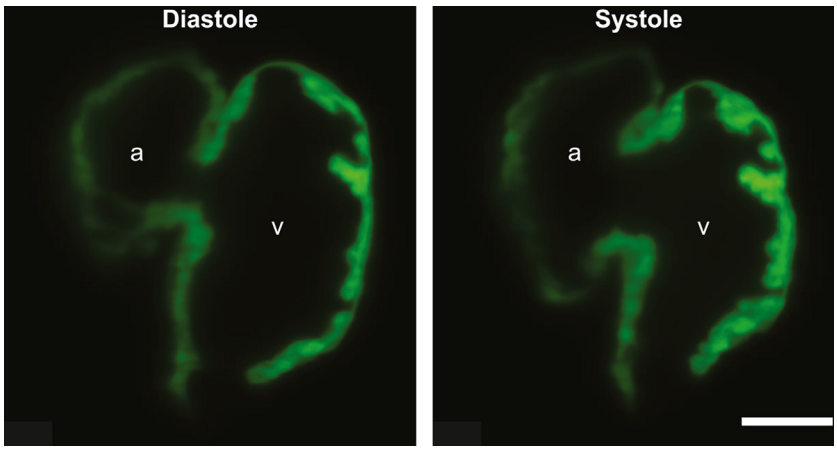
velocities (Figure 10).[270] Similar to M-mode echocardiography, a line of focus can be drawn transversely through the cardiac tube, allowing the direct calculation of systolic and diastolic diameters with subsequent derivation of fractional shortening. This method was applied to study the HCM-associated mutation M305L in cardiac actin.[271] Mutant hearts showed decreased fractional shortening, prolonged systolic contraction, and prolonged diastolic relaxation. This study demonstrated clear defects in diastolic function with mutation, consistent with what is observed in patients. Similarly, the DCM-associated S532P MYH7 mutation in was studied using this technique.[272] The mutant hearts recapitulated a DCM phenotype of reduced systolic function. Taken together, these studies demonstrate the utility of heart tube kymography for studying the in vivo effects of cardiomyopathy-associated mutations.

ZEBRAFISH AND LIGHT-SHEET FLUORESCENCE MICROSCOPY. Zebrafish are another important vertebrate model for studying development. Advances in optical technology have made it possible to view zebrafish heart contractility with greater dynamic volumetric resolution similar to human cardiac magnetic resonance imaging. The developing zebrafish heart is visible to optical techniques without dissection. The zebrafish heart is also physiologically different from the human heart, with only a single atrium and ventricle; however, zebrafish models can faithfully recapitulate key aspects of human diseases such as DCM[273,274] (Central Illustration).

Several methods have been developed to measure contractility in zebrafish hearts, and recent advances in imaging technologies have enabled studies with dramatically improved spatial and temporal resolution. One such technology is light-sheet fluorescence microscopy (LSFM). LSFM excites a thin section of the sample using a thin sheet of light that is orthogonally projected to the detection pathway. This method requires lower light intensity than other optical methods which reduces phototoxicity. LSFM also has a greater sample penetration than light confocal microscopy.[275] Moreover, this technique illuminates only a single sample plane, reducing the background signal. Using this technique, it is possible to generate time synchronized, 3D reconstructions of the heart, allowing accurate volumetric measurements of both diastole and systole (Figure 11). Furthermore, this technique also enables the estimation of global longitudinal strain, which is a common method in clinical echocardiography to ascertain subtle changes in cardiac function that are not captured by 2-dimensional or 3D echocardiography.[276] An interesting future application of LSFM may be studying cardiac contractility in *Xenopus laevis* tadpoles, which are similarly transparent like zebrafish embryos, for laboratories that prefer this model organism. Mutations in sarcomeric proteins such as cardiac troponin that cause DCM in patients have been shown to recapitulate the DCM morphology in genomically engineered *X. laevis* tadpoles.[277] However, the compatibility of LSFM for these applications has yet to be demonstrated.

LIMITATIONS. Care needs to be taken when extrapolating from results seen in *Drosophila* and zebrafish to human studies because of physiological differences in anatomy and protein expression. Moreover, despite the extensive methodological development and validation of LSFM for measuring cardiac contractility, it

FIGURE 11 Light-Sheet Microscopy of Zebrafish Heart



Single-frame captures of light-sheet fluorescence microscopic videos of fluorescently labeled zebrafish hearts that are 96 hours post-fertilization. Scale bar represents 40 μm. Image courtesy of Jamison Leid (Center for Cardiovascular Research, Washington University School of Medicine). a = atrium; v = ventricle.

has yet to be applied to modeling cardiomyopathy. Finally, these techniques require technical expertise, and techniques such as LSFM require sophisticated equipment (reviewed by Reynaud et al[278]).

CONCLUSIONS AND PROSPECTS FOR THE FUTURE

The development of multiscale tools for studying cardiac contractility has enabled deeper phenotyping of cardiomyopathies and HF. These studies have revealed new mechanistic categories that lead to contractile defects which have helped drive the development of modulators of cardiac contraction that may eventually complement GDMT. Therapeutics targeting contraction are still new; however, we anticipate that these tools will help fuel the development of the next generation of therapeutics targeting contractility and that these deep phenotyping tools can be used to optimize the characteristics of these compounds.

One outstanding challenge to the field is the need to integrate data across spatial and temporal scales. New, integrative computational models are being developed that can model aspects of the emergent complexity of these systems.[35,279-287] However, modeling remains difficult, and additional studies that use multiscale techniques are required to establish correlations between results generated from different techniques. This will be best accomplished by collaborative efforts using techniques that span scales of organization. The eventual goal will be to use data from a single experimental system and predict functional outcomes at another scale. For example, one might be able to use biochemical results to predict effects on cardiomyocyte contractility and identify ideal treatment options in silico. With such a platform, we hope it will be eventually possible to rapidly assess functional consequences of genetic variants of unknown significance, identify additional processes for drug targeting, and provide better care for patients overall.

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