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REVIEW

Contractility assessment in enzymatically isolated cardiomyocytes

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Abstract The use of enzymatically isolated cardiac myocytes is ubiquitous in modern cardiovascular research. Parallels established between cardiomyocyte shortening responses and those of intact tissue make the cardiomyocyte an invaluable experimental model of cardiac function. Much of our understanding regarding the fundamental processes underlying heart function is owed to our increasing capabilities in single-cell stimulation and direct or indirect observation, as well as quantitative analysis of such cells. Of the many important mechanisms and functions that can be readily assessed in cardiomyocytes at all stages of development, contractility is the most representative and one of the most revealing. The purpose of this review is to provide a survey of various methodological approaches in the literature used to assess adult and neonatal cardiomyocyte contractility. The various methods employed to evaluate the contractile behavior of enzymatically isolated mammalian cardiac myocytes can be conveniently divided into two general categories—those employing optical (image)-based systems and those that use transducer-based technologies. This survey is by no means complete, but we have made an effort to include the most popular methods in terms of reliability and accessibility. These techniques are in constant evolution and hold great promise for the next generation of breakthrough studies in cell biology for the prevention, treatment, and cure of cardiovascular diseases.

Keywords Cardiac myocyte · Cardiomyocyte · Contractility · Sarcomere length · Heart contraction · Cardiovascular research

Celebrating emeritus professor Paul Paolini's 70th birthday. On behalf of the students whose dreams he helped fulfill.

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Introduction

Cardiovascular research based on enzymatically isolated cardiac myocytes (cardiomyocytes) has proven essential to understanding the mechanisms that govern the heart. This experimental model of cardiac function has helped unveil many fundamental processes underlying heart function in health and disease (Maltsev et al. 1998; Ren and Wold 2001). Measurements evaluating the excitation–contraction coupling (ECC), calcium transient signal (movement of the calcium ion, Ca^{2+}), gene and protein expression, and contractility are commonly performed during research studies. These are all important mechanisms and functions which can be readily assessed in cardiomyocytes at all stages of development (Banyasz et al. 2007; Mitcheson et al. 1998; Shevchuk et al. 2001).

The purpose of this review is to provide a survey of the various methodological approaches in the literature used to assess adult and neonatal cardiomyocyte contractility. This survey is by no means exhaustive; however, we have made an effort to include the most popular methods in terms of reliability and accessibility. Approaches used to assess the contractile behavior of cardiomyocytes can be conveniently divided into two general categories—those employing optical (image)-based systems and those using transducer-based technologies. Like most research methods, each approach has its own inherent advantages and limitations. The main

objective is to achieve adequate spatial and temporal resolution in order to unambiguously assess the cardiomyocyte's function (Delbridge and Roos 1997).

Contractility of the enzymatically isolated cardiac myocyte

Contractility—being the most representative of cardiomyocyte functions—has been thoroughly studied both quantitatively and qualitatively. Contractility can be defined as the innate ability of heart muscle to generate force and to shorten. At the molecular level, the contractile response originates from a change in concentrations of Ca^{2+} in the myocardial cytosol. In adult ventricular cardiomyocytes, contractile activity is known to depend primarily upon the release of Ca^{2+} from the sarcoplasmic reticulum (SR) through ryanodine receptor channels (RyR) (ter Keurs 2012; ter Keurs and Boyden 2007). However, in fetal and neonatal cardiomyocytes, SR plays a much smaller role in Ca^{2+} regulation, which decreases the capability of neonatal cardiomyocytes to load Ca^{2+}, as compared to those isolated from mature hearts (Seki et al. 2003).

Fetal and neonatal cardiomyocyte contraction depends largely on trans-sarcolemmal Ca^{2+} influx rather than on Ca^{2+} released by the SR. This difference in the neonatal cardiomyocyte activation process translates into a slower upstroke and decay of Ca^{2+} in comparison to those in adult ventricular cardiomyocytes. This characteristic is confirmed by a major discrepancy between action potentials (AP) in adult and neonatal cardiomyocytes, with a significantly longer repolarization phase in neonatal cardiomyocytes as compared to those in adult cells (Korhonen et al. 2009). Due to its underdeveloped contractile machinery, the neonatal cardiomyocyte is generally unable to retract its cell boundary during contraction, and noticeable changes occur only within the cell perimeter (Bazan et al. 2011). For this reason, it is difficult to perform contractile measurements on this cell type in a manner similar to that of the adult cardiomyocyte, in which changes in the cell boundary can be quantified during contraction. To date, very few methods have been successfully developed to capture the neonatal cardiomyocyte's elusive contractile response. Figure 1 shows an adult (a) and a neonatal (b) cardiomyocytes. The adult and the neonatal cardiomyocytes were enzymatically isolated and cultured as described in (Reuben et al. 1998) and Sprenkle et al. (1995), respectively.

Some of the methods described in this review can also be applied to adult cardiomyocytes undergoing spontaneous (asynchronous) oscillations. These cardiomyocytes have been used to study the spatial distribution of calcium ions during contraction, as well as the function of the different channels involved during the ECC process. Spontaneously contracting cardiomyocytes can be used as an experimental model to study the coordination and communication among sarcomeres in a partially activated state, as well as the interaction between actin and myosin filaments in the absence of Ca^{2+} regulation. If these cells can manifest the functional defects of sarcomeric proteins in the failing myocardium in vitro, they hold the potential to provide an objective and valuable set of parameters to assess the state of human heart failure (Wolfe et al. 2011). Additionally, a large body of work on the physiological properties of myofilaments, sarcoplasmic reticulum, and mitochondria has been done using skinned cardiac cells (disrupted surface membranes) (Best 1983; Fabiato and Fabiato 1976; Saks et al. 1998). This experimental model is not covered in this review.

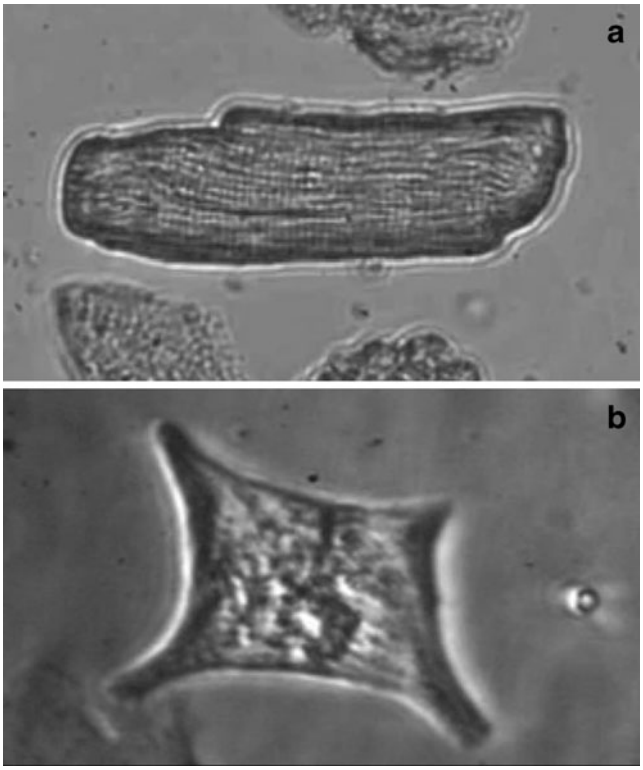

Fig. 1 **a** The adult cardiomyocyte is approximately 25 μm in diameter and about 100 μm in length. It is composed of bundles of myofibrils that contain myofilaments. Myofibrils have distinct, repeating microanatomical units termed sarcomeres, which are the basic contractile elements that make up a developed cardiomyocyte. This conformation gives the adult cardiomyocyte its typical 'rod-shape' ('cigar-shape'). **b** Unlike adult cardiomyocytes, which are highly organized and quite similar in morphology to one another, neonatal cardiomyocytes are in the process of developing their contractile machinery and therefore display a large variety of shapes. The neonatal cardiomyocyte is generally unable to retract its cell boundary during contraction, and noticeable changes occur only within the cell perimeter. For these reasons, it is difficult to perform contractile measurements on this cell type in a manner similar to that of the adult cardiomyocyte, in which changes in the cell boundary can be quantified during contraction

Optical (image)-based systems

Optical (image)-based methods to assess cardiomyocyte contractility have been getting popular over the past decades. This trend is due to both the improvement in imaging techniques and the continuous drop in cost of the necessary equipment. Methods such as the edge detection system, laser diffraction, and Fourier analysis provide the ability to assess the contraction of cardiomyocytes in a non-invasive manner, thereby minimizing the contact with the cells and the introduction of foreign chemicals that might interfere with the natural cardiomyocyte's response.

Edge motion detector

Steadman et al. (1988) introduced the edge detection system to measure contractility in adult cardiomyocytes by exploiting the physiological characteristics of the cell, such as its near-rectangular shape and decrease in length during contraction. The method uses a raster line to track the two longitudinal cell boundaries (left and right) as the adult cardiomyocyte contracts. This tracking is accomplished by detecting sudden changes in pixel intensity at the cell boundaries throughout the duration of the contraction. The user-defined thresholds employed to signal the presence of the cardiomyocyte boundaries are set on a cell-by-cell basis. As the cardiomyocyte contracts and relaxes, the positions of the cell boundaries over the raster line are recorded as a measure of contraction.

The edge detection system has been widely used since its introduction, which is evident by the more than 100 references to its original publication to date. Nonetheless, problems have been identified during the implementation of the method. One of the most common is the difficulty to precisely track the longitudinal edges of the adult cardiomyocyte throughout the contraction. The presence of jagged edges or irregular boundaries can complicate the implementation of this method and introduce errors during the acquisition of the contraction data. Unfortunately, these irregular and jagged edges are very common in isolated adult cardiomyocytes as the cells are often damaged during the isolation process (Brady 1991). Additionally, rotational and translational movements of the adult cardiomyocyte during contraction can introduce errors to the signal (Delbridge and Roos 1997). Figure 2 shows the Myocyte Contractility Recording System commercialized by IonOptix® (Milton, MA, USA).

Micro-machined cantilever beams

Advances in micro-electro-mechanical systems (MEMS) technology have simplified the production of micro-cantilevers, which can be used to measure cardiomyocyte contractions. This approach was introduced by Lin et al. (1995) who attached a heart cell to flexible, hinged polysilicon plates submerged in a nutrient saline solution. The plates bend as the cell contracts and the contractile force is measured based on the spring constant of the plate. Later, Lin et al. (2000) developed a cantilever platform where micro-cantilevers are set up as parallel pairs with the free end attached to the cardiomyocyte. Video recordings are used for measuring the displacement of the bending cantilevers, which, along with the spring constants, allow for the calculation of the forces. Lin et al. (2001) modified this setup by integrating electronic components adhered to a strain gauge whose signal was converted to an amplified electrical output.

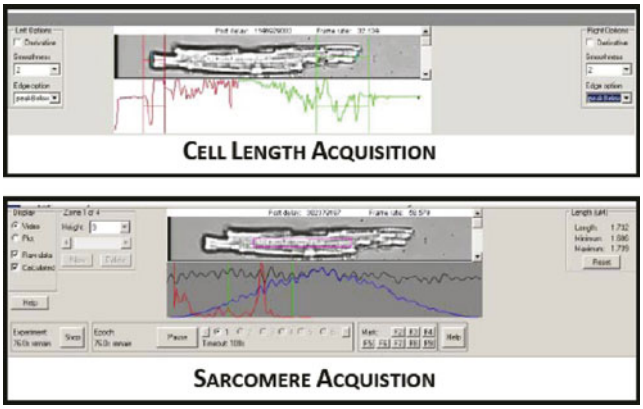

Fig. 2 In the IonOptix® Myocyte Contractility Recording System, the SoftEdge® acquisition module collects cell length at rates up to 250 Hz. The simple user interface enables the precise placement of thresholds for marking cell edges. The output of calibrated length allows real-time cell length collection. Similarly, the sarcormere acquisition module collects real-time sarcomere length at 250 Hz. Line intensity information within a simple region of interest is averaged to generate a well-resolved striation pattern. A fast Fourier transform calculation immediately outputs sarcomere spacing. Along with the fast acquisition MyoCam-S® digital camera, the systems offer precise, real-time calcium and contractility measurements. Reproduced with permission from IonOptix®

The micro-cantilever method has been modified to include vertical cantilevers (see Fig. 3). In this method, PDMS microfabricated post-array-detectors (mPAD) are made with an array of vertical posts on which the cells are cultured. The tips of the posts are fluorescently labeled. The posts are deflected as the cells contract, and their maximum deflections are captured with still images. Forces are derived by combining these images and the post's spring constant (Tan et al. 2003; Zhao et al. 2005, 2006). Zhao et al. (2007) demonstrated the use of this technology to simultaneously orient adult cardiac myocytes in primary culture, and measure the cellular forces in an environment that better mimics in vivo conditions (Kajzar et al. 2008). The most notable contrast between this and prior approaches is that cellular force is derived from the deformation of individual polymer

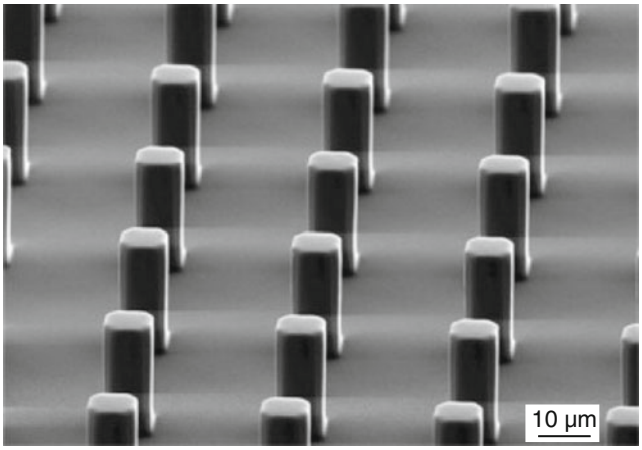

Fig. 3 Scanning electron micrograph of a PDMS elastomer micro-pillar array. In this case, the cross-sections of the pillars were close to square at the base and rounded toward the apex. Additionally, the cross-sectional areas normally increase slightly with height. (Reprinted from Kajzar et al. (2008) with permission from Elsevier)

structures (Zheng and Zhang 2011). Du Roure et al. (2005) were able to manufacture smaller posts in denser arrays using a different process involving projection photolithography and deep reactive ion etching (DRIE). In a somewhat similar approach, Prosser et al. (2011) examined the details of stretch-dependent signal transduction by firmly attaching single cardiomyocytes to stiff glass micro-rods with the biological adhesive MyoTak®. Their work revealed that stretch -activated Ca^{2+} sparks are triggered by a mechano-chemo signaling pathway that regulates local production of reactive oxygen species (ROS) in heart cells.

Flexible sheets with micro-patterned dots or grids

Harris et al. (1980) introduced the elastic substratum method in their search for ways to measure the elastic properties of individual cells, and assess forces exerted during locomotion or motility. The idea involves plating tissue cultures on top of an elastic material thin enough to provide visible and measurable distortions as the cell moves across it. The silicon elastic material was synthesized with cross-linking PDMS by exposing it to heat for a brief period of time (Harris et al. 1980; Schwarz et al. 2002). This proved to be the most effective way to create a thin film that would wrinkle in response to cellular forces. However, the traction forces also proved hard to quantify, since wrinkling would develop slowly and in an extremely chaotic and nonlinear fashion (Dembo and Wang 1999). To overcome these limitations, Lee et al. (1994) modified the assay by embedding two-dimensional beads in the stratum and welding the sides of the film to a rigid vessel (Dembo and Wang 1999). Bead displacement proved to be more accurate in assessing the size and orientation of traction forces. By probing the films with calibrated micro-needles, the force required to displace the bead could be measured (Oliver et al. 1995). Dembo and Wang (1999) have used the elastic substratum method to produce traction maps exerted by single motile cells.

Pelham and Wang (1997) created flexible polyacrylamide substrata. The benefits of these substrata were their ability to adjust the elastic stiffness by controlled variations of the monomer and cross-linker concentrations. Dembo and Wang (1999) embedded fluorescent latex bead markers randomly throughout the polyacrylamide substrata so that deformations could be analyzed more easily. Balaban et al. (2001) developed a novel approach combining micro-patterning of elastomer substrates and fluorescence imaging (see Fig. 4). Their methodology allows for the real-time and direct measurement of forces applied by stationary cells to the underlying matrix at individual focal adhesions. They used this approach to investigate the relationship between local forces at focal adhesions and their assembly. Huang et

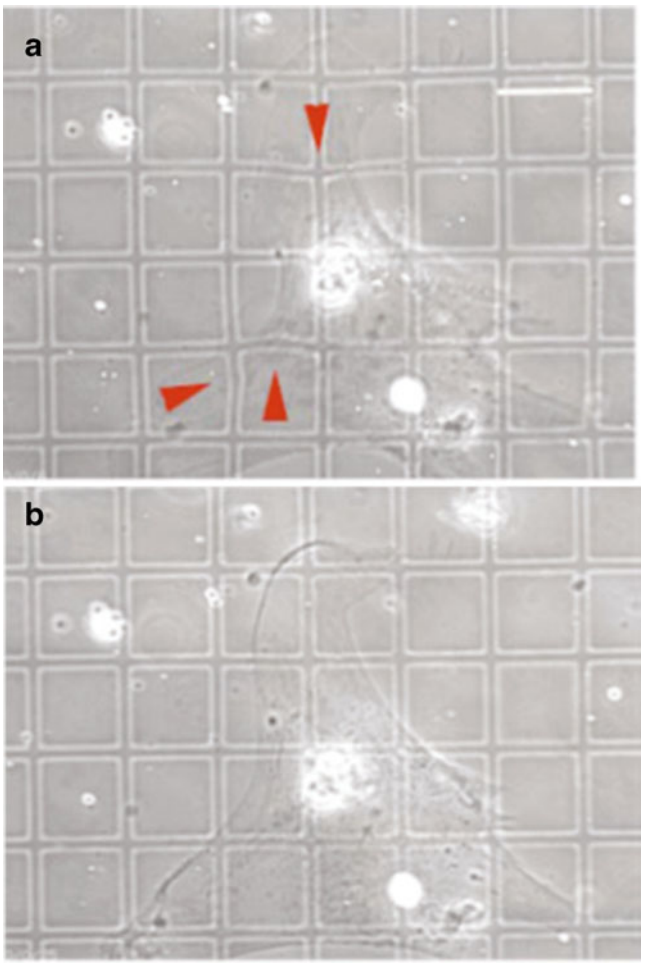

Fig. 4 **a** Fibroblast before relaxation. Phase-contrast image of a rat cardiac fibroblast plated on a large grid pattern. As it contrasts, the cell creates distortions (*arrowheads*) by applying force to the elastomer (Young's modulus=18 kPa). **b** Fibroblast after relaxation. The same cell as in (**a**) 10 min after butanedione monoxime-induced relaxation. We observe the recovery of the regular grid pattern. Grid pitch=30 mm. (Reprinted from Balaban et al. (2001) with permission from Macmillan)

al. (2008) used polyacrylamide substrates embedded with polystyrene fluorescent beads to measure the displacement of single neonatal cardiomyocytes. The deformed pictures of the elastic substrate caused by a single cardiomyocyte provided information for quantitative evaluation of the displacement field by using the Digital Image Correlation (DIC) algorithm. The basic principle of the DIC technique is to track geometric positions before and after deformation, in order to yield displacement as a result of mathematical correlation statistics. The random fluorescence patterns of the bead-embedded substrate, which are captured before and after the distortion induced by cell activity, serve as two consecutive digital images for processing (one as the reference image and the other as the deformed image).

Laser (light) diffraction

Krueger et al. (1980) were among the first to report on the contraction characteristics of unloaded and unattached cells obtained by laser light diffraction. Nonetheless, several variations of laser diffraction techniques have been used for the study of muscle physiology since the early 1970s (Cleworth and Edman 1972; Kawai and Kuntz 1973; Schoenberg et al. 1974; Borejdo and Mason 1976; Paolini et al. 1976; Moss and Halpern 1977; Flitney and Hirst 1978; Rüdel and Zite-Ferenczy 1979; Walcott and Dewey 1980; Magid and Reedy 1980; Edman 1980; Baskin et al. 1981). One of the main advantages of the laser diffraction technique is that the temporal and spatial resolution of the method can be easily calibrated by means of standardized gratings (Niggli 1988). Additionally, this approach permits real-time measurements of the sarcomere length at rest and during contraction. However, there is one principal disadvantage when the method is applied to isolated cardiomyocytes. The small volume of cardiomyocytes reduces the amount of optical grating, which results in a faint diffraction pattern. One way of overcoming this obstacle is by increasing the laser power; however, this results in the reduction of the life of the biological sample to just a few minutes (Krueger et al. 1980).

In order to extend the life of the specimen under investigation beyond a few minutes, Niggli (1988) proposed an improvement of the sensitivity over previously published laser diffraction systems. He developed an opto-electronic device to record the sarcomere length of small muscle cells that presented a well-defined striation pattern (such is the case in isolated cardiomyocytes). This improvement was achieved by a novel approach allowing a reduced susceptibility to optical noise in the detection of laser diffraction patterns. This improvement of the sensitivity, in turn, allowed for a significant reduction of the laser power employed during the experiments. Niggli (1988) also recommended the study of the distribution of the sarcomere lengths in the isolated cardiomyocytes prior to contractility assessment. He argued that this is a sine qua non condition for achieving reliable data concerning the contractile state of cardiomyocytes with the laser diffraction method. Despite the early limitations of the laser diffraction method, several researchers have used it with satisfactory results (Krueger et al. 1980; Lieber et al. 1984; Haworth et al. 1987; Wussling et al. 1987; Ivester et al. 1993).

Fourier transform-based methods

Sarcomere lengths can be used as a measure for contraction, which can be quantified by spectral analysis (Diguet et al. 2011; Delbridge and Roos 1997; Farkasfalvi et al. 2007; Gillis et al. 2005; Iribe et al. 2009). Slawnych et al. (1996) employed the two-dimensional Fourier analysis of rabbit muscle fiber video images to measure sarcomere length. Two separate sarcomere length measurements were assessed: the first used the centroid frequency of the first-order peak, and the second by summing pixels perpendicular to the fiber's axis into a one-dimensional line spectra. Comparison of the two provided a measure of striation skewing. Gannier et al. (1993) developed a non-invasive, easy-to-use method to study sarcomere motion in cardiomyocytes. This was based on analyzing the periodicity of the cell striation pattern using the fast Fourier transform (FFT) algorithm on a video image of the cell during the course of the experiment. This method gives a real-time measurement of the most probable value of sarcomere length in one isolated cell with a temporal resolution of 20 ms.

Bazan et al. (2009) described a computational pipeline for a more comprehensive assessment of contractile responses of adult cardiomyocytes that uses shape representation by Fourier descriptors. In order to use the Fourier descriptors as abstract representation of image features in each frame, they made the Fourier descriptors invariant to translation, rotation, and their starting point. Thus, the Fourier descriptors changed covariantly with the shape of the cell, providing a measure of contraction (see Fig. 5). The method can potentially eliminate the historical concerns and sources of errors caused by cardiomyocyte rotation, bending, or translation during contraction (Delbridge and Roos 1997; Mukherjee et al. 1992, 1993). Similarly, for assessing contractility in neonatal cardiomyocyte, Bazan et al. (2011) proposed a methodology based on image representation by polar Fourier descriptors.

Transducer-based systems

Transducer-based systems to analyze cellular function were originally made available with the introduction of the

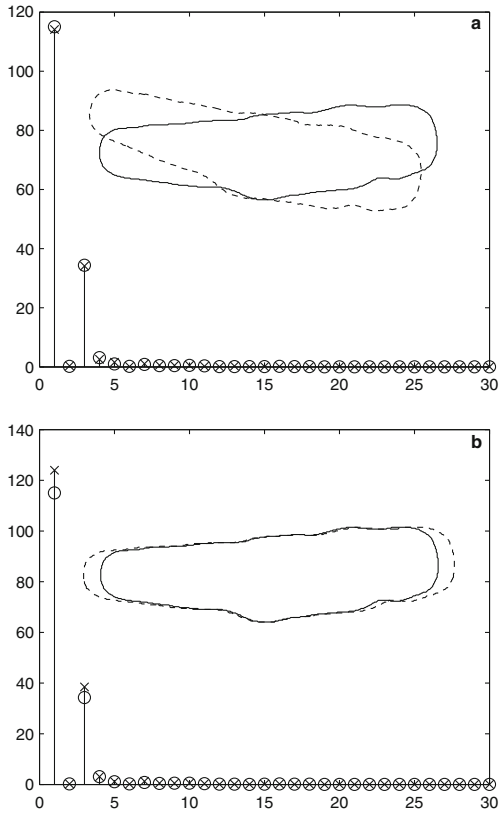

Fig. 5 **a** Two identical cell shapes, one of which has been translated and rotated with respect to the other. First 30 Fourier descriptors of the shapes superimposed for the case of translation, rotation, and starting point invariance. **b** Two cell shapes, one of which is larger than the other, mimicking the ones from a relaxed and contracted cardiomyocyte, respectively. First 30 Fourier descriptors of the shapes superimposed. We observe that the Fourier descriptors are able to capture this change in shape size making the Fourier descriptors variant to scale but invariant to translation, rotation, and starting point. The 'contraction' of the shape is 8.15 %, as measured by the Euclidean distance of the Fourier descriptors. (Reprinted from Bazan et al. (2009) with permission from Hindawi)

scanning tunneling microscope. In general, these methods provide excellent reliability in terms of force tracking and quantification during cardiomyocyte contraction. The accuracy of force measurements with these methods is far superior than that provided by the image-based methods. However, transducer-based systems rely heavily on complex and expensive equipment. Additionally, they are normally invasive and may interfere with the 'natural' function of the cardiomyocyte during the contraction–relaxation process.

Atomic force microscopy

The scanning tunneling microscope (STM) was introduced by Gerd Binnig and Heinrich Rohrer as a mechanism for measuring forces as small as 10^{-18} N (Binnig et al. 1986). This revolutionary idea of using force to image individual surface atoms with unprecedented resolution was recognized with the Nobel Prize in Physics in 1986 (Rugar and Hansma 1990). The prize was divided, with one-half awarded to Ernst Ruska for his fundamental work in electron optics and for the design of the first electron microscope, and the other half going jointly to Gerd Binnig and Heinrich Rohrer for their design of the STM (Nobelprize.org 2012). The atomic force microscope (AFM) is a combination of the principles of the STM and the stylus profilometer (Burke 1999). It takes measurements by lowering a nanometer scale tip onto the surface of the cell. A piezoelectric sensor detects the cantilever deflection and the relative deformation of the cell, and the tip can then be used to calculate the applied force (Bao and Suresh 2003; Mathur et al. 2001). The AFM can also be used to impart force on the cell by applying single or cyclic deflections to the tip (Ting and Sniadecki 2011). Figure 6 shows a schematic of an atomic force microscope setup.

The AFM is now routinely used in cell biology to image cells, quantify single-molecule interactions, and measure cytoskeletal stiffness (Kamm et al. 2010). It has been successfully applied to direct force measurements of properties of whole cells (Yuan and Verma 2006; Radmacher 2002), cell cytoskeletons (Lal et al. 1995; Kim et al. 2009), and subcellular organelles, and biomolecules (Lal and John 1994; Kedrov et al. 2007). Through the use of the AFM, researchers have also been able to study the mechanical pulse of single cardiomyocytes (Domke et al. 1999; Shroff et al. 1995), along with the dynamics and spatial characteristics of sarcomeric structures (Curtis and Russell 2011; Zhu et al. 2009; Yamane et al. 2007), the contractile force of self-

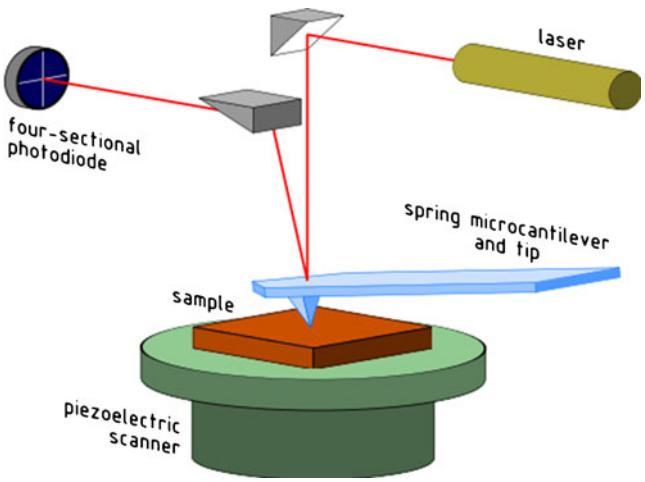

Fig. 6 Schematic of an atomic force microscope setup. The deflection of a micro-fabricated cantilever with a sharp tip (probe) at its end is quantified according to Hooke's law by reflecting a laser beam off the backside of the cantilever into an array of photodiodes while it is scanning over the surface of the specimen. This figure is licensed by Grzegorz Wielgoszewski under the Creative Commons Attribution-Share Alike 3.0 Unported license

organized cardiomyocytes (Park et al. 2005), and how dynamic subcellular mechanical properties of the heart relate to the fundamental underlying process of actin-myosin cross-bridge cycling (Azeloglu and Costa 2010).

The successful implementation of the AFM is contingent to overcoming its main limitations. Weisenhorn et al. (1993) reported that the aforementioned resolution power is very difficult to obtain, as the AFM tip can cause large deformations or indentations in soft samples such as cardiomyocytes. To overcome this issue, they propose correcting the force-versus-indentation curve of the soft sample, with the force-versus-indentation curve measured with the same cantilever on a hard sample. It has been also reported that some experiments conducted with the AFM are difficult to replicate. This is due to the fact that many different tip shapes are used, and the shape of the tip determines the nature of the force-deformation curve (Addae-Mensah and Wikswo 2008).

Scanning ion-conductance microscopy

Scanning Ion-Conductance Microscopy (SICM), patented by Hansma and Drake (1990), produces a topographical map of the surface of nonconductive samples (i.e., living cells) submerged in an electrolyte-rich environment. The SICM works by holding a constant conductance distance over the nonconductive sample bathed in an electrolyte solution. The probe is a micropipette that has been harnessed to detect currents running over the sample, and, in select cases, multiple micropipettes are used to read different ion currents simultaneously. This is achieved by the rapid movement of the micropipette over the surface of the cell without making physical contact. Figure 7 shows the schematic of a scanning ion-conductance microscope setup.

The evolution of this technique has been steady since it first emerged. In order to use the SICM to scan cardiomyocytes, a few alterations in the equipment were made (Miragoli et al. 2011). One of these changes in composition and application of the SICM includes the use of a nanopipette in lieu of a micropipette. This is known as the Hopping Probe Ion-Conductance Microscope (HPICM), and provides a smooth approach of the pipette to the surface of the cell only at selected imaging points, and retraction to a safe distance. This new methodology replaces the previous technique involving a pipette in constant motion above the cell while raster scanning the sample. This new technique allows a fixed distance to be maintained by the pipette during scanning instead of having the distance of the pipette in a broad range in the vertical axis. Korchev et al. (2000) hybridized the SICM by using a piezo-translation stage in conjunction with the SICM and a few other technological advances, helping bring the accuracy and precision of the original SICM to new heights.

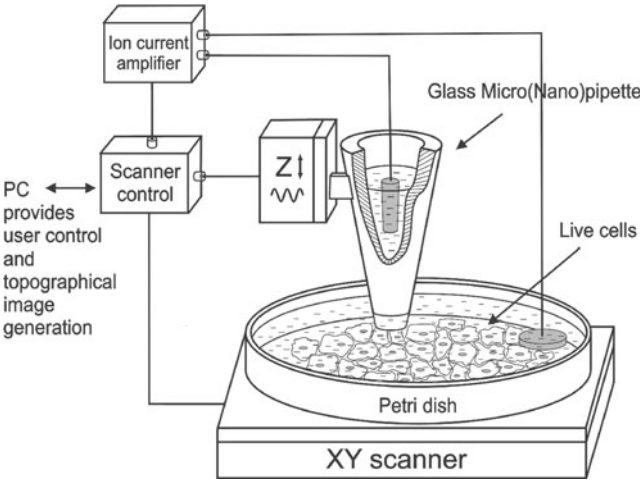

Fig. 7 Schematic of a scanning ion-conductance microscope setup. An electrically-charged glass micropipette (or nanopipette) probe filled with electrolyte is lowered toward the surface of the specimen that is submerged in an oppositely-charged bath of electrolytes. As the micropipette gets closer to the specimen, the ion conductance decreases proportionally to the decrease in the distance between the two elements. These variations in the ion current are amplified and the signal is used to keep a constant distance between the micropipette and the specimen. The surface of the specimen can then be recorded. This figure was released into the public domain by Paul Venter

Magnetic tweezers and magnetic twisting cytometry

Crick and Hughes (1950) first introduced Magnetic Twisting Cytometry (MTC) in an effort to study the in vivo elastic properties of the cytoplasm (Puig de Morales et al. 2001). The technique involves binding specific ligand-coated magnetic beads to cell surface receptors and then stressing the cell by applying an external magnetic twisting field (Fabry et al. 2001). The resulting bead displacement can be recorded and analyzed to study the mechanical properties of the cell. The magnetic twisting device used in this technique consists of several components, including a voltage generator, current sources to twist the particles, a computer, a CCD camera to synchronize images with oscillatory magnetic fields, a temperature controller, and an inverted microscope (Hu et al. 2004) (Fig. 8).

Although MTC has been used successfully in studying cellular properties, it is limited in the range of motions that can be employed to apply force. Another drawback of MTC is its inability to control where on the cell the beads are attaching (Tseng et al. 2002). One advantage of the magnetic tweezers method is that it is a low-cost alternative to other available micromanipulators (Gosse and Croquette 2002). Magnetic tweezers also provide a way to study the mechanical anisotropy of the cell (Hu et al. 2004). Another advantage of magnetic tweezers is that the technique does not require a laser, which may photo-damage the biomaterial (Liu et

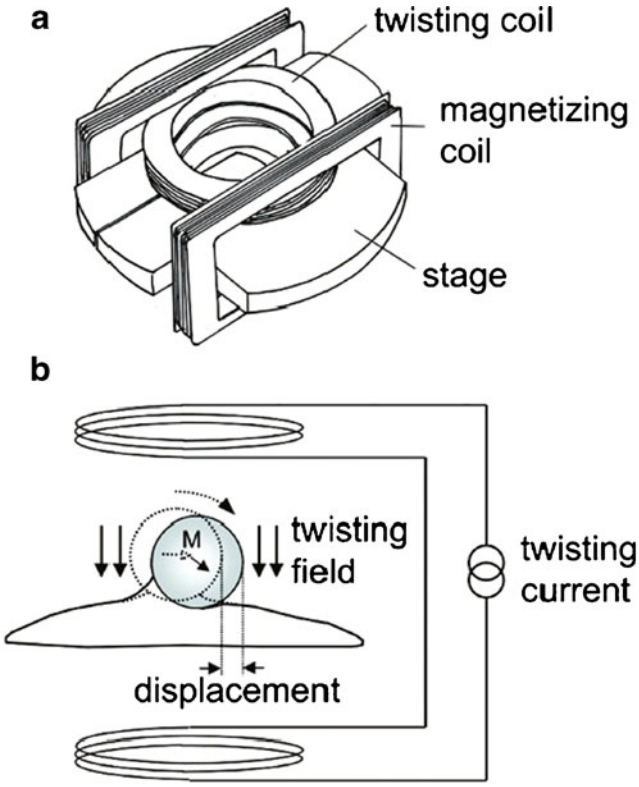

Fig. 8 **a** Schematic of a microscope stage with twisting coils and magnetization coils. **b** A homogeneous magnetic twisting field provokes the bead to rotate and displace. The direction of the bead's magnetic moment is denoted by *M*. (Reprinted from Fabry et al. (2001) with permission from The American Physiological Society)

al. 1996; Neuman et al. 1999) and proteins (Wuite et al. 2000), and, thus, prevent their future use (Gosse and Croquette 2002).

Micropipette aspiration

The micropipette aspiration (MA) method was first proposed by Brady et al. (1979) as an attempt to measure forces of electrically-stimulated rat cardiomyocytes with the use of single-barreled pipettes. The method has since been improved by the use of adhesives and double-barreled pipettes to attain better attachment, and allow a broader scope of force measurements. A schematic diagram of the micropipette aspiration system is shown in Fig. 9. In a more advanced application of this method, as described by Palmer et al. (1996a), custom-made concentric double-barrel micropipettes from glass capillary tubes are used. A monolayer adhesive is applied to each pipette to assist in firm attachment. When a single, chemically-skinned cell of interest is located, the first pipette is lowered for suction. Suction is held for 10–15 min, followed by the lowering of the second pipette for suction. After an additional 20 min for the adhesive to cure, the single cardiomyocyte is attached at either end to a micropipette, thus enabling the measurement of the mechanical properties of the cardiomyocyte.

Variations of the micropipette aspiration method are utilized to obtain reliable measurements of the mechanical properties of single cardiomyocytes. It can measure the elastic and viscous characteristics of both very soft materials like red and white cells, and those of stiffer and more viscous cells, such as endothelial cells and chondrocytes (Hochmuth 2000). Researchers have been able to record length–tension curves of cardiomyocytes by using this method (Copelas et al. 1987). In addition, Sweitzer and Moss (1993) were able to study the force–velocity relationship of shortening cardiomyocytes permeabilized with α-hemolysin. Veksler et al. (1997) have utilized this method for studying the rigor tension yielded by the effects of substrates and products of bound creatine kinase in adenine nucleotide units in myofibrils. The micropipette aspiration method is also widely used in muscular tissue-based experiments. Furthermore, the method has been used in identifying the mechanical properties of cartilage (Buxboim et al. 2010).

Carbon fiber-based analysis

Le Guennec et al. (1990) introduced the etched carbon fiber attachment approach to study the Frank–Starling's law in

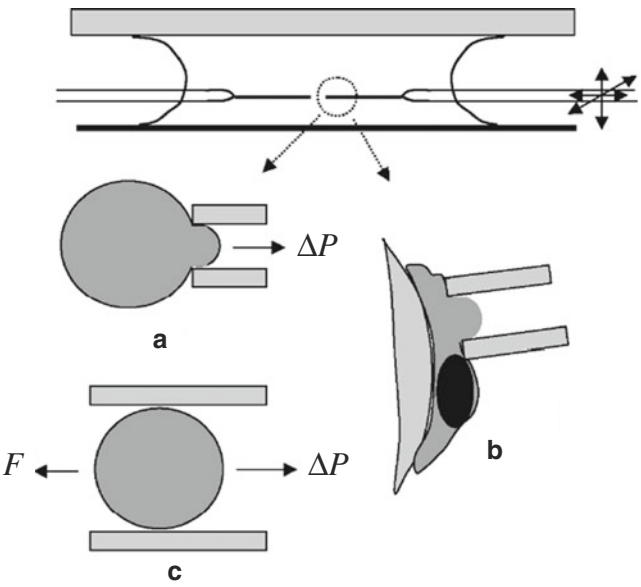

Fig. 9 Two micropipettes in a chamber. A pneumatic micromanipulator controls the movement of a micropipette along three orthogonal axes. **a** A micropipette exerts a suction pressure *P* to aspirate a spherical cell. **b** An attached cell being aspirated into a pipette. **c** A closely-fitting cell or bead moving freely in a pipette like a piston in a cylinder. When static, the suction pressure times the cross-sectional area of the pipette equals the attachment force *F*. (Reprinted from Hochmuth (2000) with permission from Elsevier)

single mammalian cells. They developed a simple method of stretching mammalian ventricular cells using thin carbon fibers, which can be bound electrochemically to the cell's membrane surface. A long, flexible fiber of known compliance is attached to one end of the cell, while a stiff double fiber is adhered to the other end. Yasuda et al. (2001) adapted the aforementioned methodology for the quantitative measurement of contractility in cardiomyocytes. In their implementation, the image of the compliant carbon fiber is projected onto a pair of photodiodes, and their output is fed to a piezoelectric transducer after variable amplifications to alter the effective compliance of the carbon fiber. They were able to quantify contractility under virtually isometric conditions, as well as under auxotonic conditions.

Despite the practical success of carbon fiber-based analysis, early adopters of the methodology reported some concerns regarding the damage of the cardiomyocyte's ends, the difficulty in obtaining uniform sarcomere spacing, and the fact that the system allows full activation of the cells only at short sarcomere lengths, and with a high inherent system compliance (Palmer et al. 1996b). In order to address the fist concern regarding damage to the cell, Sugiura et al. (2006) described a new method for attaching single cardicytes to carbon fibers for mechanical manipulation and measurement. Their technique uses cell-adhesive carbon fibers that attach easily to the cell membrane without causing noticeable damage. Iribe et al. (2007) attached carbon fibers to opposite cell ends to afford dynamic and bidirectional control of the cell's mechanical environment. This system has the ability to independently and dynamically control preload, afterload, and transition between end-diastolic and end-systolic force-length coordinates to study single cardiomyocyte mechanics.

More recently, by combining the efforts of Le Guennec et al. (1990) and Yasuda et al. (2001), as well as the earlier works of Iwazumi (1987), Garcia-Webb et al. (2007) described the design and development of a modular (and inexpensive) instrument for exploring the mechanics of cardiomyocytes using carbon fiber (see Fig. 10). Their goal was to design a system capable of not only quantifying the mechanical properties of individual cardiomyocytes, but that was also appropriate for use in an instrument array setting. This instrument array would allow for high-throughput single-cell muscle physiology. Their innovation utilizes a novel motor design and attachment strategy. The new attachment approach combines a mild clamping force with the inherent attachment of intact cardiomyocytes to borosilicate glass and etched carbon fiber. This attachment permits cardiomyocytes to be stretched farther than before. With this new apparatus, Garcia-Webb et al. (2007) were able to provide the first measure of the passive dynamic stiffness of a single, intact ventricular myocyte at varied sarcomere lengths.

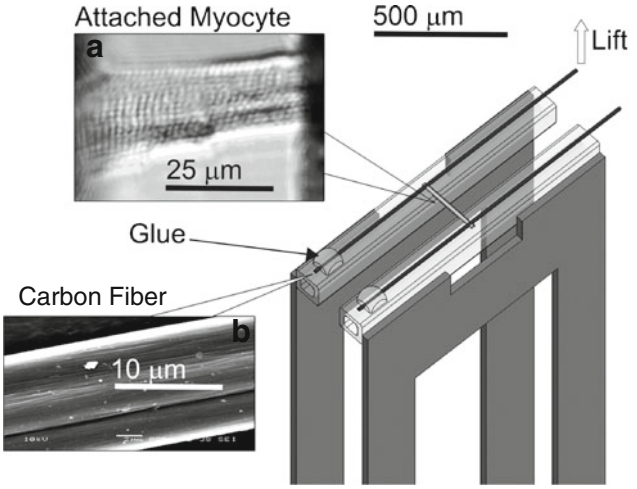

Fig. 10 Novel cell attachment clamp. A cell was loaded and the carbon fiber was lowered onto the cell. **a** A myocyte attached between the cantilevers. **b** Scanning electron microscopy image of the carbon fiber. (Reprinted from Garcia-Webb et al. (2007) with permission from The American Physiological Society)

Conclusions

Over the past few decades, we have seen an unprecedented evolution in our understanding of the mechanics of cells, subcellular components, and essential molecular interactions. This progress was promoted by new capabilities for applying and measuring forces and displacements with pico-Newton and nanometer resolutions, respectively, as well as by advances in live-cell imaging techniques (Addae-Mensah and Wikswo 2008; Kim et al. 2009). This review article provides a survey of the various methodological approaches used to assess cardiomyocyte contractility. We made a concentrated effort to describe the most popular methods in the literature in terms of reliability and accessibility. The key feature required from any methodology is its ability to accurately reproduce and describe the behavior of the cells in the heart (Nishimura et al. 2004). Therefore, measuring contractility in enzymatically isolated cardiac myocytes requires engineering devices that can resemble the microenvironment found in the specific human tissue being utilized (Zheng and Zhang 2011). Nevertheless, if the data are obtained under carefully controlled conditions, the measurement of cell length can provide an adequate index of sarcomere dynamics, and, therefore, of cardiomyocyte contractility (Delbridge and Roos 1997).

For convenience, we divided the different methodologies most commonly used for assessing contractility in cardiomyocytes into two general categories—those employing optical (image)-based systems and those using transducer-based technologies. Transducer-based approaches, through micro-engineering platforms, are leading to new in vitro culture systems in more biomimetic and multi-input/output

(high-throughput) contexts. However, these methodologies rely heavily on expensive equipment and scientific facilities (Zheng and Zhang 2011), and they all share an inherent limitation in that their measurements require direct physical contact with the cell (Curtis and Russell 2011). In cases where the latter is a concern, we must rely on less invasive means to evaluate cardiomyocyte activity. Such concerns can be addressed with the optically-based measurement methods. Among these, the limitations of the one-dimensional assessment methods have fueled a steady transition towards more comprehensive, high-speed, two-dimensional imaging as technological improvements permit.

In summary, the next few years will likely witness the intense development of innovative platforms for cell studies, which will enable us to postulate a multitude of questions which currently remain unasked, while continuing to answer those that have not been addressed due to technological limitations. After all, "Progress in science depends on new techniques, new discoveries and new ideas, probably in that order." (Sydney Brenner, 2002 Nobel Prize laureate in Physiology or Medicine; Brenner 2012).

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Conflict of interest None

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