Load dependency in force-length relations in isolated single cardiomyocytes

Gentaro Iribe, Toshiyuki Kaneko*, Yohei Yamaguchi, and Keiji Naruse

Department of Cardiovascular Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1, Shikata-cho, Okayama 700-8558, Japan

* Toshiyuki Kaneko contributed as a co-first author

Address correspondence to: Gentaro Iribe, MD, PhD
Cardiovascular Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
Tel.: +81-86-235-7115
Fax: +81-86-235-7430
E-mail: iribe@okayama-u.ac.jp
Abstract

The previously reported pressure-volume (PV) relationship in frog hearts shows that end-systolic PV relation (ESPVR) is load dependent, whereas ESPVR in canine hearts is load independent. To study intrinsic cardiac mechanics in detail, it is desirable to study mechanics in a single isolated cardiomyocyte that is free from interstitial connective tissue. Previous single cell mechanics studies used a pair of carbon fibers (CF) attached to the upper surface of opposite cell ends to stretch cells. These studies showed that end-systolic force-length (FL) relation (ESFLR) is load independent. However, the range of applicable mechanical load using the conventional technique is limited because of weak cell-CF attachment. Therefore, the behavior of ESFLR in single cells under physiologically possible conditions of greater load is not yet well known. To cover wider loading range, we contrived a new method to hold cell-ends more firmly using two pairs of CF attached to both upper and bottom surfaces of cells. The new method allowed stretching cells to 2.2 μm or more in end-diastolic sarcomere length. ESFLR virtually behaves in a load independent manner only with end-diastolic sarcomere length less than 1.95 μm. It exhibited clear load dependency with higher preload, especially with low afterload conditions. Instantaneous cellular elastance curves showed that decreasing afterload enhanced relaxation and slowed time to peak elastance, as previously reported. A simulation study of a mathematical model with detailed description of thin filament activation suggested that velocity dependent thin filament inactivation is crucial for the observed load dependent behaviors and previously reported afterload dependent change in Ca^{2+} transient shape.

Keywords
mechano-electric coupling, cell mechanics, shortening deactivation, modeling
1. Introduction

1.1. General background

The heart is a mechanically functioning blood pump whose mechanical properties can be described by left ventricular (LV) pressure (LVP) and LV volume (LVV). Therefore, many cardiac mechanics studies focusing on pressure-volume (PV) relation have been conducted. In the late 19th century, Otto Frank studied PV relations in isolated frog hearts and obtained PV diagrams such as that shown in Figure 1A (Frank, 1990). The shallow concave curve at the bottom shows the end-diastolic PV relation (EDPVR), and the steep convex curves illustrate end-systolic PV relations (ESPVR). Interestingly, the steepness of ESPVR is greatly affected by afterload conditions. ESPVR in isovolumic contraction (highest afterload; top curve) is the steepest, and that in isobaric condition (lowest afterload, middle solid curve) is more moderate. The ESPVR of normal “working” contractions (dashed line) depends on the end-diastolic state of the heart and can be described by a line between the corresponding points on isovolumic and isobaric ESPVR. Although there is an afterload dependency of ESPVR, one can generalize that the higher the preload (higher end-diastolic LVV), the greater the mechanical work, within a physiologically possible range of preloads.

Suga and Sagawa investigated mammalian cardiac LV pump behavior by analyzing the PV diagram in detail (Sagawa, 1981; Suga and Sagawa, 1974; Suga et al., 1973). Suga et al. reported that, in the absence of changes in contractility, ESPVR is independent of variations in pre- and afterload in excised cross-circulated canine hearts: ESPVR maxima follow a single, linear relation. Suga found that all isochronal points of the PV relation are also located on a single line, which is located between the ESPVR and EDPVR curves (Figure 1B). During each heartbeat, the slope of the isochronal connection line initially becomes steeper (during contraction), and after reaching a maximum at the end-systolic point, returns to end-diastolic levels (during relaxation). The slope of the isochronal connection line describes the instantaneous PV ratio, that is, the ventricular elastance. This concept is called the “time-varying elastance model,” which describes a ventricle as an elastic pouch whose instantaneous elastance can be determined using the instantaneous PV ratio. In this context, the shallow EDPVR indicates that the end-diastolic heart behaves as a soft “balloon,” whereas it behaves as a more rigid balloon at end-systole, as shown by the far steeper ESPVR. The slope of ESPVR indicates the maximum elastance of the ventricle, called $E_{\text{max}}$ (Suga and Sagawa, 1974; Suga et al., 1973).
1.2. Specific background

As mentioned above, the unique concept of the time-varying elastance model is that the ESPVR is independent of preload and afterload within a physiologically possible range of load conditions. Strictly speaking, however, instantaneous ventricular pressure can be more or less reduced from isovolumic pressure, when the velocity of ejection is increased (Baan and Van der Velde, 1988; Leach et al., 1980; Suga et al., 1980). In papillary muscle preparation, similar force reduction has been observed in force-length (FL) relations (Hisano and Cooper, 1987). One of the factors that may explain the pressure/force reduction during shortening is viscoelastic resistance of the extracellular matrix like connective tissue. Other factors are shortening-dependent change in Ca\(^{2+}\) and crossbridge dynamics (shortening deactivation) (Backx and Ter Keurs, 1993; Janssen and de Tombe, 1997; Kentish and Wrzosek, 1998; Lab et al., 1984; Yasuda et al., 2003).

To study intrinsic properties of cardiac mechanics in detail, it is desirable to study mechanics in single isolated cardiomyocyte that is free from viscoelastic resistance of the extracellular matrix. Interestingly, previous studies on FL relations in single cells demonstrated that end-systolic FL relation (ESFLR) is load independent in guinea pig and even frog cardiomyocytes (Iribe et al., 2007; Parikh et al., 1993). Given these findings, one might conclude that the intrinsic myocardial mechanical property is load independent. However, the contractile profile of individual cells, characterized by their elastance, shows clear load dependency (slower time-to-peak elastance, faster decay in low afterload conditions compared to high afterload) (Iribe et al., 2007). Previous studies also showed that the shape of Ca\(^{2+}\) transient is load dependent (Backx and Ter Keurs, 1993; Janssen and de Tombe, 1997; Kentish and Wrzosek, 1998; Lab et al., 1984; Yasuda et al., 2003). Regarding findings in single cell preparations, Yasuda et al. reported that the isotonic Ca\(^{2+}\) transient is higher than that of isometric contractions initially, and then the two transients cross each other in the early decay period, so that the isometric Ca\(^{2+}\) transient is higher than that of isotonic contractions for the rest of the decay period (Yasuda et al., 2003).

Why did load dependent Ca\(^{2+}\) transient and elastance profiles yield load independent ESFLR (maximum elastance) in previous single cell studies? One possibility we need to consider is that the applied preload range was not wide enough to cover loading conditions in which load dependent behavior of ESFLR can be observed, if it indeed does exist. Even in the PV relation for a frog’s heart, ESPVR of isotonic and isometric contraction are close with small LV volume (Figure 1A) (Frank, 1990). In a ferret papillary muscle study, ESFLR in shortening contraction
from $L_{\text{max}}$ does not reach the ESFLR in isometric contraction, whereas ESFLR in shortening contraction from lower preload does reach that in isometric contraction (Hisano and Cooper, 1987). In our previous single cell study, diastolic sarcomere length (SL) was elongated up to approximately 2.0 $\mu$m using a carbon fiber technique (Iribe et al., 2007), whereas the diastolic SL with $L_{\text{max}}$ in most papillary muscle studies is approximately 2.2–2.3 $\mu$m (Page, 1974). To confirm the inherent mechanical properties of single cardiomyocytes, cells must be stretched to this range of length.

To study single cell mechanics, we have been using a pair of piezo translator (PZT)-positioned carbon fibers (CF) attached to the upper surface of opposite cell ends to stretch isolated cardiomyocytes (Iribe et al., 2007). However, the CF attachment force in this conventional method is limited; therefore, it is difficult to stretch cardiomyocytes beyond 2.0 $\mu$m in SL. In the present study, to overcome this limitation, we develop a novel cell gripping technique that allows attaching two CFs to both upper and lower cell surfaces to improve cell holding and apply more stretching force. Using this new technique, we investigate load dependency/independency in single cell mechanics under a wider range of mechanical loading conditions than was previously possible. Additionally, mathematical model simulations are performed to examine the possible mechanisms underlying observed load dependency/independency.

2. Materials and methods
2.1. Myocyte preparation

We conducted all experiments in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan, and the study protocol was approved by the Animal Subjects Committee of Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences. Ventricular myocytes were enzymatically isolated from hearts excised from C57BL/6 mice (aged 9–11 weeks), which were killed by cervical dislocation. The detailed cell isolation method is described elsewhere (Iribe et al., 2013). Isolated cells were stored in normal tyrode solution (140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl$_2$, 1 mM MgCl$_2$, 5 mM HEPES, 11 mM glucose, pH of 7.4 adjusted using NaOH).

2.2. Experimental setup

All CFs used in the present study (10 $\mu$m in diameter) were provided by Tsukuba Materials Information Laboratory, Ltd., Tsukuba, Japan. The single myocyte stretch system used in the
present study is based on our conventional system (Figure 2A). In the conventional system, each CF is in a glass capillary with a protrusion (1.2 mm in length) and is mounted to a 3D hydraulic manipulator for CF positioning to attach a cell end (Iribe et al., 2007). In the new system, each cell end is held by two CFs attached to the top and bottom surfaces of the cell (Figure 2B). One cell end is fixed using a CF directly mounted on a coverslip (CF1 in Figure 2B) attached to the bottom surface of the cell and another short CF (CF2 in Figure 2B) attached to the top surface. The other cell end is the side to be stretched, and it is held using two 1.8 mm CFs attached to the bottom and top surfaces (CF3 and CF4 in Figure 2B, respectively). Each CF on the stretch side is mounted on a computer-controlled PZT with capacitive sensors of which positioning resolution is under 1 nm (P-621.1CL; Physik Instrumente, Karlsruhe/Palmbach, Germany). Both CFs receive the same control command to achieve identical CF position control in stretching the cell end in one direction. To avoid friction between the cell and the surface of the coverslip, the bottom CF1 was mounted on lumps of glue to keep the CF1 above the coverslip. The height of CF3 was confirmed to be lifted up from the coverslip by keeping optical focus at the same level as for CF1. All single cell experiments were performed at 4 Hz stimulation at 37°C. Temperature was controlled with an MPRE8 in-line heater (Cell Microcontrols, Norfolk, VA).

2.3. Cell attachment procedure

For cell attachment, first, the right end of the cell was grabbed using CF2, and the cell was lifted up to a sufficiently safe height. Then, the microscope stage was moved to bring CF1 into view, followed by lowering CF3 to the same level as CF1. Thereafter, the cell held by CF2 was lowered to hold the right cell end between CF1 and CF2. Finally, CF4 was lowered to hold the left cell end between CF3 and CF4. After all CFs were set, the cells were paced at 4 Hz for 3 min to enhance cell-CF adhesion (Iribe et al., 2007).

2.4. Length and force measurements

The detailed method for performing length and force measurements is described elsewhere (Iribe et al., 2007). Briefly, cell length signal (CF tip distance) and SL changes were recorded using the IonOptix equipment and software (IonOptix Corporation, Milton MA, USA). Active and passive forces ($F$) were calculated using the following equation:

$$F = K (\Delta L_F - \Delta L_P)$$

Here, $\Delta L_F$ is the change in distance between CF2 and CF4, and $\Delta L_P$ is the change in PZT
position. The parenthetical term on the right side of the equation indicates total carbon fiber bending.  \( K \) is the combined stiffness of CF2 and CF4. Each CF stiffness was measured directly using a force transducer system (406A; Aurora Scientific, Aurora, ON, Canada).

2.5. Afterload control

The detailed method for adaptive feed-forward control to vary afterload to achieve isometric and isotonic contraction is described elsewhere (Iribe et al., 2007). Briefly, length signal in steady state contraction was recorded and averaged for 10 beats, and was used as source signal for a PZT command. For isometric contractions (highest afterload), an appropriately amplified source signal was inverted and applied to make outward PZT movement compensate for the cell shortening. For isotonic contractions (lowest afterload), an appropriately amplified source signal was applied to make inward PZT movement prevent changes in CF bending. This process was repeated once the preload was changed.

2.6. Statistics

All the values are presented as mean ± standard error of means (SEM). One-way or two-way analysis of variance followed by a post-hoc Dunnett’s Multiple Comparison Test was used as appropriate for statistical analyses, and a \( p \) value of <0.05 was considered to indicate a significant difference between means.

2.7. Mathematical modeling

To simulate experimental findings, we modified our previously reported single cardiac cell model (2006 Iribe-Kohl-Noble model) (Iribe et al., 2006), whose electrophysiology component was based on that of the Noble-91 guinea pig cell model (Noble et al., 1991), by updating the electrophysiology part to the Noble-98 guinea pig model (Noble et al., 1998) and by introducing detailed description of thin filament activation, crossbridge cycling, and \( \text{Ca}^{2+} \) kinetics from the Schneider model (Schneider et al., 2006).

The Schneider model describes the process of thin filament activation by \( \text{Ca}^{2+} \), including conformational changes and crossbridge cycling. Figure 3 shows a state diagram of the Schneider model. First, \( \text{Ca}^{2+} \) binds to the troponin (Tn) C (TnC) of a non-activated regulatory unit (RUNA), which consists of seven actin molecules, a Tn complex (TnC, TnI, TnT), and tropomyosin (Tm). RUTCaoff is the state in which \( \text{Ca}^{2+} \) is bound to TnC but with TnI still connected to actin. A conformational change releases TnI from its inhibitory site on actin
The subsequent conformational change of Tm creates fully activated RUs (RUTMon) with a free myosin-binding site. Myosin with ATP (ADP-Pi) bound (MADPPi) can bind to RUTMon to form a force-generating crossbridge (RUA_MADPPi), cycling via a non-force-generating crossbridge state (RUAMADPPi). When Pi is released, another force-generating crossbridge state is formed (RUA_MADP). Finally, ADP is released, and the crossbridge detaches (RUTMon).

To simulate the effects of known velocity dependent response to cellular elastance and Ca\textsuperscript{2+} transient, a number of small modifications were introduced to the Schneider model. For the shortening mechanism, we combined the sliding crossbridge from Negroni and Lascano's model (Negroni and Lascano, 1996). Shortening deactivation was implemented by adding velocity dependency to \( k_{off} \), which is the inactivation rate for thin filament shown in Figure 3 (Eq. A86 in Appendix). Afterload was changed by varying external elastance (\( E_{ex} \)), which corresponds to CF elastance. We paced the model at 1 Hz with varying end-diastolic SL either in isometric or isotonic contraction modes.

3. Results

3.1. Cell-CF attachment

We compared the maximum SL at which CFs start sliding or detach from the cell in the 4-Hz stimulation between the novel method and the conventional method. The new gripping system allowed 16.5 ± 2.4 % (n = 12) stretch in SL before CF started to slide relative to the cell surface or detach, while the conventional method allowed 4.0 ± 0.4 % (n = 16) stretch in SL (Figure 4).

3.2. ESFLR

Figure 5A shows superimposed FL relation curves under various pre- and afterload conditions. Afterloads were varied from isometric to isotonic contraction, and preloads ranged up to 2.3 \( \mu \)m in end-diastolic SL. ESFLR of all isometric contractions (isometric run: black solid line) was reasonably fitted by a linear regression line as well as ESFLR of contractions with differing afterloads from a certain preload point (afterload run: black dotted lines). The slopes of ESFLR of isometric contractions and afterload run were similar with lower preload. However, as the preload increased, the latter gradually became steeper because the amount of shortening at lower afterload gradually decreased. The higher the preload or the lower the afterload, the larger the decrease in the shortening. Figure 5B shows the correlation between end-diastolic SL and the ratio of ESFLR slope of afterload run against isometric runs for five
animals. There was a significant correlation ($p < 0.0001$). The slope of afterload run is comparable to that of isometric run (ratio of the slope is around 1.0) only for SL less than approximately $1.95 \mu m$. In other words, ESFLR behaves in a load independent manner only at low preload condition.

3.3. Instantaneous elastance curve

Figure 6 shows experimentally obtained profiles of instantaneous elastance curves with different preloads and afterloads. Instantaneous elastance $E(t)$ is the ratio of instantaneous force $F(t)$ to instantaneous length $L(t)$, defined as $E(t) = [F(t) - F_0]/[L(t) - L_0]$, where $L_0$ and $F_0$ are the length and force coordinates, respectively, at the intersection of ESFLR and EDFLR (Iribe et al., 2007). In the present study, owing to the load dependency of ESFLR and non-linearity of EDFLR, the coordinates $(L_0, F_0)$ were obtained as the intersection point of EDFLR line for end-diastolic SL less than $1.94 \mu m$, where EDFLR is virtually linear, with ESFLR for same SL range, where ESFLR is virtually load independent (Figure 5A, gray dashed lines). Decreasing afterload significantly decreased the relaxation time constant ($\tau$) and increased the time to peak elastance ($T_{max}$). Increasing preload showed tendency to increase $T_{max}$ (not statistically significant). Both of these findings are consistent with many previous findings for papillary muscles and single cells (Allen and Kurihara, 1982; Iribe et al., 2007; Kentish and Wrzosek, 1998; Lab et al., 1984; White et al., 1995).

3.4 Model simulation

Figures 7A and 7B illustrate simulated FL relations in isometric and isotonic contractions and normalized instantaneous elastance curves of isometric and isotonic contractions with low and high preload (1.8 and 2.2 $\mu m$, respectively, in end-diastolic SL) of the model with velocity dependency. The time course of simulated contractions in Figures 7 is longer than the wet experimental data in Figure 6 because our electrophysiology model is based on a guinea pig model that has a longer action potential duration than mouse. Here, velocity dependency was introduced by setting $v_{off}$ (velocity dependent factor for $k_{off}$, see Eq. A86 in Appendix) to 0.15, and visco-elastic resistance ($V_R$) was set to virtually zero ($V_R = 10^{-5}$). End-systolic FL points of isometric and isotonic contractions with low preload (SL < 1.825 $\mu m$) show a nearly linear relation (Figure 7A), rendering ESFLR load independent within the load range. The elastance profile shows larger $T_{max}$ and faster relaxation in isotonic contraction compared to isometric contraction, especially with the higher preload (Figure 7B, upper right panel). The higher
preload shows increase in $T_{\text{max}}$ and relaxation in isometric contractions (Figure 7B, lower left panel). These behaviors are consistent with the experimental results presented in Figures 5 and 6.

Even without velocity dependency ($v_{\text{off}} = 0$), one can reproduce the same load dependent ESFLR as shown in Figure 7A by increasing the visco-elastic resistance during shortening. Figure 7C illustrates this, showing results with increased visco-elastic resistance ($V_R = 3.8$). As a result of this modification, ESFLR is almost similar to that in Figure 7A. However, the modification failed to reproduce the rapid relaxation observed in isotonic contractions (Figure 7D).

Figure 8 shows simulated instantaneous elastance curves and Ca$^{2+}$ transients of isometric and isotonic contractions in the model for 2.2-$\mu$m end-diastolic SL with velocity dependency in two different parameters: $k_{12}$ and $k_{\text{off}}$. As shown in Figure 3, $k_{12}$ indicates the crossbridge detachment rate. Velocity dependency was introduced to $k_{12}$ by adjusting $v_{12}$ (velocity dependent factor for $k_{12}$; see Eq. A90 in Appendix). The elastance profile of isotonic contraction with velocity dependency in $k_{12}$ ($v_{12} = 0.5$) shows almost identical shape to that in $k_{\text{off}}$ (Figure 8A and 8B, top). A velocity dependent increase in $k_{12}$ during shortening in isotonic contraction accelerates crossbridge detachment, which may increase the Ca$^{2+}$ transient by affecting thin filament inactivation. However, the Ca$^{2+}$ transient shows almost no change from that in isometric contractions (Figure 8B, middle) even if its velocity dependency is increased by six times ($v_{12} = 3$, Figure 8B, bottom). On the other hand, a velocity dependent increase in $k_{\text{off}}$ ($v_{\text{off}} = 0.15$, Figure 8A, middle), which primarily accelerates thin filament inactivation, gives rise to a “crossover” in the Ca$^{2+}$ transient during isotonic contraction (from higher to lower transient at lower afterload), which is consistent with previously reported changes (Yasuda et al., 2003). This change in the Ca$^{2+}$ transient is enhanced as the velocity dependency in $k_{\text{off}}$ is increased ($v_{\text{off}} = 0.9$, Figure 8A, bottom).

4. Discussion
4.1. New gripping system

In our previous report, the conventional stretch method allowed 5 to 8% stretch, approximately up to 2.0 $\mu$m in SL (Iribe et al., 2013, 2009, 2007). On the other hand, the present improved cell gripping method allowed more than 15% stretch, approximately up to $L_{\text{max}}$. Therefore, this new technique reasonably covers the range of mechanical load encountered in mechano-electric coupling studies. Although it is possible to improve cell attachment with the
conventional two CF method by combining with gluing agents, another important advantage of the new method is that it involves cell holding from both top and bottom surfaces of the cell. This may avoid potentially inhomogeneous mechanical load in the conventional method due to the cell holding only on the top surface of the cell, especially under intensive loading conditions.

4.2. Load dependency in ESFLR

Our previous experiments on single isolated cardiac myocytes showed that ESFLR is load independent within the range of loads that we could apply using the conventional method (Iribe et al., 2007). Even in the present study, ESFLR of any contractions with end-diastolic SL shorter than 1.95 µm (Figure 5) was shown to be virtually load independent. However, applying more mechanical load using our new method revealed that ESFLR of a single cell is inherently load dependent.

Although ESFLR of contractions with end-diastolic SL shorter than 1.94 µm in Figure 5A is virtually load independent, this does not mean that all contractions (especially low-afterload contractions) in which “end-systolic” SL is shorter than 1.94 µm fall on the same ESFLR curve. For instance, the end-systolic point of isotonic contraction at which SL is near 1.94 µm in Figure 5A is located below the virtually load independent ESFLR. Therefore, strictly speaking, the load independency of ESFLR is only applicable for SL far shorter than 1.94 µm. Simulated ESFLR curves demonstrate this well. As shown in Figure 9, although simulated ESFLR curves of two sets of afterload runs, for which end-systolic SL values are shorter than 2.1 µm, virtually fall on one line (Figure 9, left), simulated ESFLR curves of isometric and isotonic contractions of the same model show that virtual load independency can be observed only for end-systolic SL around 1.8 µm (Figure 9, right).

Under physiological in situ conditions, extremely high and low loading conditions, especially for afterload, are unlikely. Therefore, preload and afterload of the heart do not necessarily vary over the full range that we examined. Although we concluded that ESFLR of a single cardiomyocyte is inherently load dependent, Suga and Sagawa’s approximation, linearity, and load independency of ESFLR and time-varying elastance model are still reasonable for a physiologically possible range of loads.

Our mathematical modeling study suggested that velocity dependent thin filament inactivation may be one of the important contributors to the reduced shortening in low afterload contractions shown in Figure 5. We excluded viscoelastic resistance as a main contributor
because, as in Figure 7D, our modeling study indicated that viscoelastic resistance does not reproduce rapid relaxation in isotonic contractions. It also failed to reproduce shortening-induced change in Ca\(^{2+}\) transient shape (data are not shown). From the viewpoint of myocardial energetics, if the reduced shortening is attributable to the internal viscoelastic resistance, part of the mechanical energy should be dissipated during ejection. However, it has been reported that the myocardial resistance is related to the contractile process, which is not energy dissipative (Kawaguchi et al., 1993; Shroff et al., 1990). Furthermore, the resistive extracellular matrix does not affect cellular mechanics in the present single-cell preparations, in contrast with multicellular preparations. Therefore, we concluded that velocity dependent thin filament inactivation may be more crucial than visco-elastic resistance in shaping the observed load independency of ESFLR.

4.3. Load dependency in Ca\(^{2+}\) transient

In single cell preparations, Yasuda et al. reported a crossover in Ca\(^{2+}\) transients between isotonic and isometric contractions, probably due to the shortening-induced decrease in Ca\(^{2+}\) binding to the thin filament in isotonic contractions and the higher Ca\(^{2+}\) binding affinity of TnC in isometric contractions (Yasuda et al., 2003).

We assessed possible effects at the level of cross-bridge dynamics and Ca\(^{2+}\) kinetics in the present model. Indeed, this model succeeded in reproducing Ca\(^{2+}\) transient crossover by adding velocity dependency to thin filament inactivation (\(k_{offI}\); see Figure 8A). However, the primary velocity dependency in crossbridge detachment (\(k_{-12}\)), followed by secondary inactivation of thin filament conformational change, did not have a prominent effect on the Ca\(^{2+}\) transient (Figure 8, right). PKA phosphorylation of cardiac TnI has been reported to desensitize myofilament MgATPase activity to calcium, shifting the force-pCa relationship rightward and enhancing relaxation (Solaro et al., 2008; Zhang et al., 1995). Takimoto et al. used transgenic mice of which serine residues of cardiac TnI normally targeted by PKA are mutated to aspartic acid to mimic native phosphorylation, and found that delay in LV relaxation in high afterload condition was significantly smaller compared to non-transgenic mice. PKA activation by isoproterenol also had same effects (Takimoto et al., 2004). These previous findings suggest that PKA activation process seems to be velocity dependent. Therefore, we comprehensively concluded that velocity dependent TnI inactivation may be responsible for the afterload dependency of single cell mechanics and Ca\(^{2+}\) transient, however, underlying molecular mechanisms for the behavior remain unclear.
5. Conclusion

The present study introduces a new cell-gripping technique using CFs that enables the application of a wide range of mechanical load that covers the physiologically possible range of load. By investigating single cell mechanics in conditions with higher load than previously possible, we find that single cell ESFLR behaves in a load dependent manner in the same manner as Otto Frank’s whole heart ESPVR. The combination of experimental findings and simulation results suggests that the load dependent behavior of ESFLR, instantaneous elastance curve, and Ca^{2+} transient can be explained by velocity dependent thin filament inactivation.

Acknowledgments

This study was supported by the Japan Society for the Promotion of Science (JSPS KAKENHI: 23300167, 22136008, 26282121).
References


Figure legends

Figure 1.
Schematic diagram of pressure-volume loop in different species. A: frog heart. End-systolic pressure-volume (PV) relations (ESPVR) are affected by afterload conditions. B: canine heart. ESPVR is a load independent single linear line. Different markers (open and solid circles and squares) indicate different isochronal points. The slope of the isochronal connection line increases in the systole and decreases in the diastole (time-varying elastance model).

Figure 2.
Overview of carbon fiber (CF) technique in cell stretch system. A: conventional method. Each cell end is held by one CF. B: new method. Each cell end is held by two CFs from the top and bottom surfaces of the cell.

Figure 3.
State diagram for Schneider’s cardiac muscle contraction model. Velocity dependency was implemented in $k_{off}$, which is an off-rate parameter of conformational change of troponin I (TnI). See text for a detailed explanation about each state.

Figure 4.
Change in end-diastolic sarcomere length during maximum stretch application with conventional (n = 16) and new (n = 12) methods. * $p < 0.05$ vs. before stretch, † $p < 0.05$ vs. conventional method.

Figure 5.
Load dependency in end-systolic force-length (FL) relation curves (ESFLR). A: superimposed FL relation curves at various pre- and afterload conditions. Black dashed lines indicate ESFLR of contractions with varied afterload (afterload run) at each preload point. Black solid line indicates ESFLR of isometric contractions (isometric run). B: correlation between end-diastolic sarcomere length (EDSL) and the ratio of the slope of ESFLR of afterload run to that of isometric run. There is significant correlation between them. $(L_0, F_0)$: intersection coordinates of the ESFLR and end-diastolic FL relation curves (EDFLR) with low preloads (gray dashed lines).
Figure 6.
Load dependent morphological changes in instantaneous elastance curve. A: superimposed elastance curves of isometric (thin solid line) and isotonic (thin dashed line) contractions with low preload (SL 1.84 µm). B: superimposed elastance curves of isometric (thick solid line) and isotonic (thick dashed line) contractions with high preload (SL 2.02 µm). C: superimposed elastance curves of isometric contractions with low preload (thin solid line) and high preload (thick solid line). D: superimposed elastance curves of isotonic contractions with low preload (thin dashed line) and high preload (thick dashed line). E: load dependent changes in time to peak elastance ($T_{\text{max}}$) (n = 7). F: load dependent changes in relaxation time constant ($\tau$) (n = 7). $(L_{0}, F_{0})$: intersection coordinates of the EDFLR and ESFLR. * $p < 0.05$ vs. isometric contractions.

Figure 7.
Simulated FL relations and instantaneous elastance curves at various pre- and afterload conditions with either velocity dependency in thin filament inactivation (A and B) or viscoelastic resistance (C and D). Although both settings resulted in similar FL relations, viscoelastic resistance does not reproduce rapid relaxation in isotonic contractions, especially at high preload (D top right). Line style code in B and D is identical to that in Figure 6. $v_{\text{offI}}$: velocity dependent factor in thin filament inactivation. $V_{R}$: visco-elastic resistance.

Figure 8.
Simulated instantaneous elastance curves and $Ca^{2+}$ transients of isometric and isotonic contractions for end-diastolic sarcomere length of 2.2 µm with velocity dependency in different parameters. Velocity dependency in thin filament inactivation ($v_{\text{offI}} > 0$) well reproduced known afterload dependent morphological changes in instantaneous elastance and $Ca^{2+}$ transient (A), while velocity dependency in cross-bridge detachment ($v_{-12} > 0$) failed to reproduce the crossover change in $Ca^{2+}$ transient (B). $v_{\text{offI}}$: velocity dependent factor in thin filament inactivation. $v_{-12}$: velocity dependent factor in cross-bridge detachment.

Figure 9.
Simulated ESFLR of afterload runs (A) and isometric and isotonic contractions (B). The model setting is identical to that in Figure 7A.
Appendix

In the present version of the model, the fraction of the dyadic space ($f_{vd}$) was reduced to 0.2% from the original value (10%) in Noble-98. The transfer rate from dyadic to bulk cytosolic space was increased to achieve comparable dyadic space Ca$^{2+}$ concentration ($Ca_{ds}$) values during systole (Eq. A77 and 78). The $f_{2ds}$ gating in the L-type Ca$^{2+}$ channel (Eq. A41) was adjusted to fit the modifications of the dyadic space parameters mentioned above.

All the mathematical model equations are listed in this section. All the model parameters are listed in Table A1. Except for the modifications mentioned, parameters were identical to the original published model (2006 Iribe-Kohl-Noble model, Noble-98 and the Schneider model). The model is not temperature-corrected. A full CellML version of the whole model is available as an online supplement file and run using suitable modelling environments (such as COR; http://cor.physiol.ox.ac.uk/).

Cell volume

$$v_{Cell} = \frac{3.14 \left( \frac{r_{Cell}}{1000} \right)^2 l_{Cell}}{1000}$$  \hspace{1cm} \text{Eq. A1}

$$v_j = f_{vd} v_{Cell}$$  \hspace{1cm} \text{Eq. A2}

Reversal potentials

$$E_{Ca} = \frac{RT}{2F} \ln \left( \frac{Ca_o}{Ca_i} \right)$$  \hspace{1cm} \text{Eq. A3}

$$E_{Na} = \frac{RT}{F} \ln \left( \frac{Na_o}{Na_i} \right)$$  \hspace{1cm} \text{Eq. A4}

$$E_K = \frac{RT}{F} \ln \left( \frac{K_o}{K_i} \right)$$  \hspace{1cm} \text{Eq. A5}

Fast Na$^+$ current ($I_{Na}$)

$$I_{Na} = G_{Na} m^3 h (V - E_{mh})$$  \hspace{1cm} \text{Eq. A6}
\[ E_{mb} = \frac{RT}{F} \ln \left( \frac{Na_o + 0.12K_o}{Na_t + 0.12K_t} \right) \]  
Eq. A7

\[ \frac{dm}{dt} = \alpha_m (1 - m) - \beta_m m \]  
Eq. A8

\[ \frac{dh}{dt} = \alpha_h (1 - h) - \beta_h h \]  
Eq. A9

\[ \alpha_m = \frac{200(V + 41)}{1 - e^{-0.3(V+41)}} \]  
Eq. A10

\[ \beta_m = 8000e^{-0.056(V+66)} \]  
Eq. A11

\[ \alpha_h = 20e^{-0.125(V+75)} \]  
Eq. A12

\[ \beta_h = \frac{2000}{1 + 320e^{-0.1(V+75)}} \]  
Eq. A13

Persistent Na\(^+\) current \((I_{pNa})\)

\[ I_{pNa} = \frac{G_{pNa}(V - E_{Na})}{1 + e^{-(V+52)/8}} \]  
Eq. A14

Background Na\(^+\) current \((I_{bNa})\)

\[ I_{bNa} = G_{bNa}(V - E_{Na}) \]  
Eq. A15

Time-dependent rectifier K\(^+\) current \((I_K)\)

\[ I_K = I_{Kr} + I_{Ks} \]  
Eq. A16

\[ I_{Kr} = \frac{(G_{Kr1} \cdot x_{r1} + G_{Kr2} \cdot x_{r2})(V - E_K)}{1 + e^{(V+9)/22.4}} \]  
Eq. A17
\[
\frac{dx_{r1}}{dt} = \alpha_{s_{r1}} (1-x_{r1}) - \beta_{s_{r1}} x_{r1}
\]
Eq. A18

\[
\frac{dx_{r2}}{dt} = \alpha_{s_{r2}} (1-x_{r2}) - \beta_{s_{r2}} x_{r2}
\]
Eq. A19

\[
\alpha_{s_{r1}} = \frac{50}{1 + e^{-(V-5)/9}}
\]
Eq. A20

\[
\beta_{s_{r1}} = 0.05 e^{-(V-20)/15}
\]
Eq. A21

\[
\alpha_{s_{r2}} = \frac{50}{1 + e^{-(V-5)/9}}
\]
Eq. A22

\[
\beta_{s_{r2}} = 0.4 e^{-(V+30)/15}
\]
Eq. A23

\[
I_{Ks} = G_{Ks} x_s^2 (V - E_{Ks})
\]
Eq. A24

\[
E_{Ks} = \frac{RT}{F} \ln \left( \frac{0.03 Na_o + K_o}{0.03 Na_i + K_i} \right)
\]
Eq. A25

\[
\frac{dx_s}{dt} = \alpha_{s_s} (1-x_s) - \beta_{s_s} x_s
\]
Eq. A26

\[
\alpha_{s_s} = \frac{14}{1 + e^{-(V-405)/9}}
\]
Eq. A27

\[
\beta_{s_s} = 0.05 e^{-V/45}
\]
Eq. A28

Time-independent K⁺ rectifier current (\(I_{Ks}\))
\[ I_{K1} = \frac{G_{K1}(V - E_K) + \frac{K_o}{K_o + K_{mK1}}}{1 + e^{1.4F(V - E_K - 10)/RT}} \]  

Eq. A29

Transient outward K\(^+\) current \((I_{to})\)

\[ I_{to} = G_{to}r_s(V - E_K) \]  

Eq. A30

\[
\frac{dr}{dt} = 333\left(\frac{1}{1 + e^{-0.2(V + 4)}} - r\right) \]  

Eq. A31

\[
\frac{ds}{dt} = \alpha_s(1 - s) - \beta_s s \]  

Eq. A32

\[ \alpha_s = 0.033e^{-V/17} \]  

Eq. A33

\[ \beta_s = \frac{33}{1 + e^{-0.125(V + 10)}} \]  

Eq. A34

L-type Ca\(^{2+}\) current \((I_{CaL})\)

\[ I_{CaL} = I_{CaLCa} + I_{CaLK} + I_{CaLNc} \]  

Eq. A35

\[ I_{CaLCa} = 4dfff \frac{P_{CaL}(V - 50)F/RT}{1 - e^{-2(V - 50)/F/RT}} \left( Ca_0 e^{100F/RT} - Ca_0 e^{-2F(V - 50)/RT} \right) \]  

Eq. A36

\[ I_{CaLK} = 0.002dfff \frac{P_{CaL}(V - 50)F/RT}{1 - e^{-4(V - 50)/F/RT}} \left( K_0 e^{50F/RT} - K_0 e^{-F(V - 50)/RT} \right) \]  

Eq. A37

\[ I_{CaLNc} = 0.0 dfff \frac{P_{CaL}(V - 50)F/RT}{1 - e^{-50F/RT}} \left( Na_0 e^{50F/RT} - Na_0 e^{-F(V - 50)/RT} \right) \]  

Eq. A38

\[
\frac{dd}{dt} = 3\alpha_d(1 - d) - 3\beta_d d \]  

Eq. A39

\[
\frac{df}{dt} = 0.3\alpha_f(1 - f) - 0.3\beta_f f \]  

Eq. A40

\[
\frac{df_{2ds}}{dt} = 25 \left( 1 - \frac{Ca_{ds}}{0.0003 + Ca_{ds}} - f_{2ds} \right) \]  

Eq. A41
\[
\alpha_d = \frac{30(V + 19)}{1 - e^{-0.25(V + 19)}} \quad \text{Eq. A42}
\]
\[
\beta_d = \frac{-12(V + 19)}{1 - e^{0.1(V + 19)}} \quad \text{Eq. A43}
\]
\[
\alpha_f = \frac{6.25(V + 34)}{-1 + e^{0.25(V + 34)}} \quad \text{Eq. A44}
\]
\[
\beta_f = \frac{12}{1 + e^{-0.25(V + 34)}} \quad \text{Eq. A45}
\]

Background Ca\(^{2+}\) current \((I_{bCa})\)

\[
I_{b_{Na}} = G_{b_{Na}}(V - E_{Na}) \quad \text{Eq. A46}
\]

Stretch activated current \((I_{str})\)

\[
I_{str} = I_{strNa} + I_{strCa} + I_{strK} + I_{strAn} \quad \text{Eq. A47}
\]

\[
I_{strNa} = G_{strNa} f_{str}(V - E_{Na}) \quad \text{Eq. A48}
\]

\[
I_{strCa} = G_{strCa} f_{str}(V - E_{Ca}) \quad \text{Eq. A49}
\]

\[
I_{strK} = G_{strK} f_{str}(V - E_{K}) \quad \text{Eq. A50}
\]

\[
I_{strAn} = G_{strAn} f_{str}(V + 20) \quad \text{Eq. A51}
\]

\[
f_{str} = \frac{1}{1 + e^{-\frac{\text{force}}{1000}}} \quad \text{Eq. A52}
\]

Na\(^+\)/K\(^+\) pump current \((I_{NaK})\)

\[
I_{NaK} = \frac{I_{NaK_{max}} \left( \frac{K_o}{K_o + 1} \left( \frac{Na_i}{Na_i + 21.7} \right) \right)}{1 + 0.1245 e^{-0.1VF/RT} + 0.0353 e^{-VF/RT}} \quad \text{Eq. A53}
\]
Na\(^+\)/Ca\(^{2+}\) exchanger current \((I_{NaCa})\)

\[ I_{NaCa} = I_{cytNaCa} + I_{dsNaCa} \]  
Eq. A54

\[ I_{cytNaCa} = I_{NaC max} \frac{0.999(e^{0.557F/RT Na_i^3Ca_o - e^{-0.557F/RT Na_o^3Ca_i}})}{1 + \frac{Ca_i}{0.0069}} \]  
Eq. A55

\[ I_{dsNaCa} = I_{NaC max} \frac{0.001(e^{0.557F/RT Na_i^3Ca_o - e^{-0.557F/RT Na_o^3Ca_d}})}{1 + \frac{Ca_d}{0.0069}} \]  
Eq. A56

Membrane potential \((V)\)

\[ \frac{dV}{dt} = -\frac{1}{C_m}(I_{Na} + I_{hNa} + I_{pNa} + I_{K1} + I_K + I_{lo} + I_{CaL} + I_{hCa} + I_{str} + I_{NaCa} + I_{NaK}) \]  
Eq. A57

SR Ca\(^{2+}\) handling

The RyR model was modified to take into account dyadic Ca\(^{2+}\) handling by adjusting Ca\(^{2+}\)-induced RyR activation rate \((ActRate)\).

RyR channel Ca\(^{2+}\) release flux \((J_{rel})\)

\[ J_{rel} = \left( K_{relmax} \frac{F_{SRCaRyR}}{F_{SRCaRyR} + 0.2} \right) \left( \frac{ActFrac}{ActFrac + 0.25} \right)^2 (Ca_{rel} - Ca_{ds}) \]  
Eq. A58

\[ \frac{dF_{SRCaRyR}}{dt} = \frac{Ca_{rel} - F_{SRCaRyR}}{0.05} \]  
Eq. A59

\[ \frac{dActFrac}{dt} = ActRate \cdot PrecFrac - InactRate \cdot ActFrac \]  
Eq. A60

\[ \frac{dProdFrac}{dt} = InactRate \cdot ActFrac - RecoverRate \cdot ProdFrac + 1.8PrecFrac \]  
Eq. A61

\[ PrecFrac = 1 - (ActFrac + ProdFrac) \]  
Eq. A62
\[ CaiReg = \left( \frac{Ca_i}{Ca_i + 0.0005} \right) \]  
Eq. A63

\[ CadsReg = \left( \frac{Ca_{ds}}{Ca_{ds} + 0.01} \right) \]  
Eq. A64

\[ RegBind = CaiReg + CadsReg (1.0 - CaiReg) \]  
Eq. A65

\[ \text{ActRate} = 125 \times \text{RegBind} \]  
Eq. A66

\[ \text{InactRate} = \frac{450}{1 + 0.36/Ca_{rel}} \]  
Eq. A67

\[ \text{RecoverRate} = 1.885 \left( \frac{F_{SRCaR}R}{0.22} \right)^{N_{CaMK}} \]  
Eq. A68

\[ N_{CaMK} = \left( \frac{F_{CaMK}}{0.7} \right)^2 \]  
Eq. A69

\[ \frac{dF_{CaMK}}{dt} = \frac{F_{CaMK} - F_{CaMK_{\infty}}}{0.8} \]  
Eq. A70

\[ F_{CaMK_{\infty}} = \frac{Ca_{Calmod}}{0.00005} \]  
Eq. A71

SERCA pump Ca\(^{2+}\) uptake flux (\(J_{up}\))

\[ J_{up} = \frac{F_{CaMK_{\infty}}Ca_i - 0.00024 \beta_{up} Ca_{up}}{Ca_i + 0.00024Ca_{up} + 0.00042} \]  
Eq. A72

Ca\(^{2+}\) translocation flux in SR (\(J_{trans}\))

\[ J_{trans} = 50(Ca_{up} - Ca_{rel}) \]  
Eq. A74

Ionic concentration
\[
\frac{dNa}{dt} = -\frac{I_{Na} + I_{pNa} + I_{hNa} + I_{arNa} + I_{CaNa} + 3I_{NaCa} + 3I_{NaK}}{v_{f}F} 
\]
Eq. A75

\[
\frac{dK_i}{dt} = -\frac{I_{Kl} + I_{Ko} + I_{hK} + I_{CaK} - 2I_{NaK}}{v_{f}F} 
\]
Eq. A76

\[
\frac{dCa_{ds}}{dt} = -\frac{I_{CaCa} - 2I_{dsNaCa}}{2f_{vd}v_{f}F} - 1000Ca_{ds} 
\]
Eq. A77

\[
\frac{dCa_i}{dt} = -\frac{I_{CaCa} + I_{arCa} - 2I_{NaCa}}{2v_{f}F} + 1000f_{vd}Ca_{ds} - J_{up} + J_{rel} \frac{f_{r}}{f_{i}} - \left( \frac{dCalmod_{ca}}{dt} + QCaB \right) 
\]
Eq. A78

where \( QCaB \) is the net rate of \( Ca^{2+} \) binding to troponin, which is defined in the modified Schneider model section below (Eq. A84).

\[
\frac{dCa_{up}}{dt} = J_{up} \frac{f_{r}}{f_{i}} - J_{trans} 
\]
Eq. A79

\[
\frac{dCa_{rel}}{dt} = J_{trans} \frac{f_{i}}{f_{r}} - J_{trans} 
\]
Eq. A80

\[
\frac{dCalmod_{ca}}{dt} = 10000 Ca_{i} (Calmod - Calmod_{ca}) - 500 Calmod_{ca} 
\]
Eq. A81

Contraction model (modified Schneider model)

All states and transition rates are defined in Figure 3.

\[
[RU_{total}] = [RUN_{A}] + [RUTCa_{off}] + [RUTCa_{on}] + [RUTMo] + [RUAMADP] + [RUA_{_MADPP}] + [RUA_{_MADP}] 
\]
Eq. A82

\[
[RUA] = [RUTCa_{off}] + [RUTMo] + [RUAMADP] + [RUA_{_MADPP}] + [RUA_{_MADP}] 
\]
Eq. A83

\[
Q_{CaB} = k_{on}[Ca_{i}][RUN_{A}] - k_{off}[RUTCa_{off}] 
\]
Eq. A84
\[ Q_{Ca} = k_{out}[RUTCao] - k_{off}[RUTCao] \]  

Eq. A85

\[ k_{off} = 75 \left[ 1 + v_{off} \left( \frac{dX}{dt} \right) \right] \left( 1 - 0.32 \left[ \frac{RUA}{RU_{total}} \right] \right)^2 \times \left( 1 - 1.37 \left[ \frac{RUA \_ MADPPi + RUA \_ MADP}{RU_{total}} \right] \right)^{4.4} \]  

Eq. A86

\[ Q_{TMA} = k_{mon}[RUTCao] - k_{mon}[RUTMoi] \]  

Eq. A87

\[ k_{mon} = 14 \left[ \frac{RUTCao}{RU_{total}} \right] \left( 1 + 10 \left[ \frac{RUTMoi}{RU_{total}} \right] \right)^2 \times \left( 1 - 1.86 \left[ \frac{RUA \_ MADPPi + RUA \_ MADP}{RU_{total}} \right] \right)^2 \]  

Eq. A88

\[ Q_{MB} = k_{MB} K_{Titin \_ MADP} [RUTMoi] \_ [RUTMoi] - k_{MB} K_{AMADP}^{\_ [RUTMoi]} \_ [RUTMoi] \]  

Eq. A89

\[ k_{MB} = 600 \left( 1 + v_{MB} \left( \frac{dX}{dt} \right) \right) \]  

Eq. A90

if \( L < 1 \mu m \),

\[ \alpha = 1.5L - 0.5 \]

if \( 1 \leq L \leq 1.1 \mu m \)

\[ \alpha = 1 \]

if \( L > 1.1 \mu m \)

\[ \alpha = -1.6L + 2.76 \]  

Eq. A91

if \( L \leq 1.1 \mu m \)

\[ K_{Titin} = 0.2 \times \frac{9.9663}{1.0639 - L} \times 1 + e^{-0.0696} \]  

if \( L > 1.1 \mu m \)

\[ K_{Titin} = 0.2 \times \left( 40 - \frac{3L}{0.97} \right) \]  

Eq. A92
\[ Q_{\text{gen}} = k_3[RUAMADP] + k_3[RUA\_MADP] \]  

Eq. A93

\[ k_3 = 25 \left[ 1 + 50 \left( \alpha[RUTCaon] + \alpha[RUTMon] + [RUAMADP] \right) \right]^{1/2} \times \left( 1 - 1.23 \left[ RUA\_MADPPi \right] + \left[ RUA\_MADP \right] \right)^2 \]  

Eq. A94

\[ Q_{\text{PR}} = k_4[RUAMADP] + k_4[RUA\_MADP] \]  

Eq. A95

\[ Q_{\text{ADPR}} = k_5[RUA\_MADP] \]  

Eq. A96

\[ \frac{d[RUTCaoff]}{dt} = Q_{Ca} - Q_{TCaA} \]  

Eq. A97

\[ \frac{d[RUTCaon]}{dt} = Q_{TCaA} - Q_{TM4} \]  

Eq. A98

\[ \frac{d[RUTMon]}{dt} = Q_{TM4} - Q_{MB} + Q_{\text{ADPR}} \]  

Eq. A99

\[ \frac{d[RUAMADP]}{dt} = Q_{MB} - Q_{\text{gen}} \]  

Eq. A100

\[ \frac{d[RUA\_MADPPi]}{dt} = Q_{\text{gen}} - Q_{\text{PR}} \]  

Eq. A101

\[ \frac{d[RUA\_MADP]}{dt} = Q_{\text{PR}} - Q_{\text{ADPR}} \]  

Eq. A102

Force calculation:

\[ \text{Force} = F_b + F_p \]  

Eq. A103

\[ F_b = 2(L - X)[RUA\_MADP] + [RUA\_MADP] \times 10^7 \]  

Eq. A104

\[ F_p = 2000(L - 0.97)^3 + 40(L - 0.97) \]  

Eq. A105

Length calculation:
\[
\frac{dL}{dt} = \frac{E_{ex}\left(L_{ed} + \frac{2000(L_{ed} - 0.97)^3 + 40(L_{ed} - 0.97)}{E_{ex}}\right)}{V_R} - \text{Force}
\]

Eq. A106

where \(L_{ed}\) is end-diastolic half-SL, \(E_{ex}\) is the external elastance that corresponds to carbon fiber stiffness in our experimental settings, and \(V_R\) is the visco-elastic resistance.

Crossbridge sliding calculation:

\[
\frac{dX}{dt} = 500(L - 0.005 - X)
\]

Eq. A107

Table A1.  Model Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(R)</td>
<td>Gas constant</td>
<td>8314 mJ·K(^{-1})·mol(^{-1})</td>
</tr>
<tr>
<td>(T)</td>
<td>Temperature</td>
<td>310 K</td>
</tr>
<tr>
<td>(F)</td>
<td>Faraday’s constant</td>
<td>96485 C/mol</td>
</tr>
<tr>
<td>(C_m)</td>
<td>Membrane capacitance</td>
<td>9.5(\times)10(^{-5}) (\mu)F</td>
</tr>
<tr>
<td>(r_{Cell})</td>
<td>Cell radius</td>
<td>12 (\mu)m</td>
</tr>
<tr>
<td>(l_{Cell})</td>
<td>Cell length</td>
<td>74 (\mu)m</td>
</tr>
<tr>
<td>(f_{ei})</td>
<td>Volume fraction of cytosolic space</td>
<td>0.49</td>
</tr>
<tr>
<td>(f_{eds})</td>
<td>Volume ratio of dyadic space</td>
<td>0.002</td>
</tr>
<tr>
<td>(f_{vrel})</td>
<td>Volume ratio of SR release site</td>
<td>0.1</td>
</tr>
<tr>
<td>(f_{vup})</td>
<td>Volume ratio of SR uptake site</td>
<td>0.01</td>
</tr>
<tr>
<td>(G_{Na})</td>
<td>Maximum (I_{Na}) conductance</td>
<td>2.5 (\mu)S</td>
</tr>
<tr>
<td>(G_{Na}^{\beta Na})</td>
<td>Maximum (I_{Na}^{\beta Na}) conductance</td>
<td>4.0(\times)10(^{-3}) (\mu)S</td>
</tr>
<tr>
<td>(G_{Kr1})</td>
<td>Maximum conductance of (x_{r1}) gate</td>
<td>2.1(\times)10(^{-3}) (\mu)S</td>
</tr>
<tr>
<td>(G_{Kr2})</td>
<td>Maximum conductance of (x_{r2}) gate</td>
<td>1.3(\times)10(^{-3}) (\mu)S</td>
</tr>
<tr>
<td>(G_{Ks})</td>
<td>Maximum (I_{Ks}) conductance</td>
<td>2.6(\times)10(^{-3}) (\mu)S</td>
</tr>
<tr>
<td>(G_{Ki})</td>
<td>Maximum (I_{Ki}) conductance</td>
<td>1.0 (\mu)S</td>
</tr>
<tr>
<td>(G_{to})</td>
<td>Maximum (I_{to}) conductance</td>
<td>5.0(\times)10(^{-3}) (\mu)S</td>
</tr>
<tr>
<td>(P_{CaL})</td>
<td>L-type Ca(^{2+}) channel permeability</td>
<td>0.1</td>
</tr>
<tr>
<td>(G_{bNa})</td>
<td>Maximum (I_{bNa}) conductance</td>
<td>6.0(\times)10(^{-4}) (\mu)S</td>
</tr>
<tr>
<td>(G_{bCa})</td>
<td>Maximum (I_{bCa}) conductance</td>
<td>2.5(\times)10(^{-4}) (\mu)S</td>
</tr>
</tbody>
</table>
\( G_{\text{strNa}} \) Maximum \( I_{\text{strNa}} \) conductance 0.01 \( \mu \text{S} \)
\( G_{\text{strCa}} \) Maximum \( I_{\text{strCa}} \) conductance 0.01 \( \mu \text{S} \)
\( G_{\text{strK}} \) Maximum \( I_{\text{strK}} \) conductance 0.01 \( \mu \text{S} \)
\( G_{\text{strAm}} \) Maximum \( I_{\text{strAm}} \) conductance 0.01 \( \mu \text{S} \)
\( I_{\text{NaKmax}} \) Maximum \( I_{\text{NaK}} \) 1.36 nA
\( I_{\text{NaCamax}} \) Maximum \( I_{\text{NaCa}} \) current \( 5.0 \times 10^{-4} \) nA
\( K_{\text{relnmax}} \) RyR \( \text{Ca}^{2+} \) release flux constant 750 s\(^{-1}\)
\( \alpha_{\text{up}} \) SERCA \( \text{Ca}^{2+} \) uptake flux rate 0.4 s\(^{-1}\)
\( \beta_{\text{up}} \) SERCA \( \text{Ca}^{2+} \) reverse flux rate 0.03 s\(^{-1}\)
\( k_{\text{on}} \) Rate parameter 17300 mM\(^{-1}\cdot\text{s}^{-1}\)
\( k_{\text{off}} \) Rate parameter 200 s\(^{-1}\)
\( k_{\text{ond}} \) Rate parameter 200 s\(^{-1}\)
\( k_{\text{onoff}} \) Rate parameter 67 s\(^{-1}\)
\( k_{12} \) Rate parameter 2000 mM\(^{-1}\cdot\text{s}^{-1}\)
\( k_{3} \) Rate parameter 8 s\(^{-1}\)
\( k_{4} \) Rate parameter 77 s\(^{-1}\)
\( k_{4} \) Rate parameter 1 s\(^{-1}\)
\( k_{5} \) Rate parameter 37.23 s\(^{-1}\)
\( E_{\text{ex}} \) External elastance \( 10^5 \) mN-mm\(^2\cdot\mu\text{m}^{-1} \) (isometric)
\( V_{\text{R}} \) Visco-elastic resistance \( 10^{-5} \) mN-mm\(^2\cdot\mu\text{m}^{-1}\cdot\text{s} \)
Figure 1

A

ESPVR
(physiological contraction)

ESPVR
(isovolumic contraction)

ESPVR
(isobaric contraction)

EDPVR

LV Pressure

LV Volume

B

ESPVR

EDPVR

LV Pressure

LV Volume

V₀
Figure 2
Figure 3

- **Ca^2+ binding to TnC**
  - RUNA
    - \[ \text{Ca}^{2+} \] on \( k_{\text{on}} \) \( \text{Ca}^{2+} \) off \( k_{\text{off}} \)
  - RUTCaoff
    - \( k_{\text{onI}} \) \( \text{Ca}^{2+} \) on \( k_{\text{offI}} \)
  - RUTCaon
    - \( k_{\text{tmon}} \) \( \text{Ca}^{2+} \) off \( k_{\text{tmooff}} \)

- **Conformational change of TnI**
  - Conformational change of Tm

- **Conformational change of Tm**
  - Myosin binding
    - MADPPI
      - \( k_{12} \) \( \text{MADPPI} \) on \( k_{-12} \) \( \text{MADPPI} \) off
    - RUAMADPPI
      - \( k_{3} \) \( \text{RUAMADPPI} \) off \( k_{-3} \)

- **Force generation**
  - RUTMon
    - \( \text{RUA\_MADP} \)
      - \( k_{5} \) \( \text{ADP} \) on \( k_{4} \) \( \text{Pi} \) off
    - RUAMADPPI
      - \( k_{4} \) \( \text{Pi} \) on
Before stretch Stretch Before stretch Stretch
Conventional method New method

End-diastolic sarcomere length (μm)

Figure 4
Figure 5

A

B

(Left) Graph showing the relationship between cell length and force, with EDSL values indicated at 1.94 μm, 2.01 μm, and 2.30 μm. The graph plots force (μN) against cell length (μm).

(Right) Scatter plot showing the ratio of ESFLR slope against EDSL (μm).
Figure 6

(A) EDSL = 1.84 μm

(B) EDSL = 2.02 μm

(C) Isometric

(D) Isotonic

(E) $T_{max}$ (ms)

(F) Relaxation $\tau$ (ms)

* Indicates significant difference

Low preload  High preload
Figure 7

(A) $v_{off} = 0.15$

$V_R = 10^{-5}$

(B) EDSL = 1.8 μm

(C) $v_{off} = 0$

$V_R = 3.8$

(D) EDSL = 2.2 μm

Isometric

Isotonic
Figure 8
Figure 9