Force-length relations in isolated intact cardiomyocytes subjected to dynamic changes in mechanical load

Gentaro Iribe,1 Michiel Helmes,1,2 and Peter Kohl1
1Oxford University, Oxford, United Kingdom; and 2IonOptix Europe, Wageningen, The Netherlands
Submitted 23 August 2006; accepted in final form 9 November 2006

Iribe G, Helmes M, Kohl P. Force-length relations in isolated intact cardiomyocytes subjected to dynamic changes in mechanical load. Am J Physiol Heart Circ Physiol 292: H1487–H1497, 2007. First published November 10, 2006; doi:10.1152/ajpheart.00909.2006.—We developed a dynamic force-length (FL) control system for single intact cardiomyocytes that uses a pair of compliant, computer-controlled, and piezo translator (PZT)-positioned carbon fibers (CF). CF are attached to opposite cell ends to afford dynamic and bidirectional control of the cell’s mechanical environment. PZT and CF tip positions, as well as sarcomere length (SL), are simultaneously monitored in real time, and passive/active forces are calculated from CF bending. Cell force and length were dynamically adjusted by corresponding changes in PZT position, to achieve isometric, isotonic, or work-loop style contractions. Functionality of the technique was assessed by studying FL behavior of guinea pig intact cardiomyocytes. End-diastolic and end-systolic FL relations, obtained with varying preload and/or afterloads, were near linear, independent of the mode of contraction, and overlapping for the range of end-diastolic SLs tested (1.85–2.05 μm). Instantaneous elastance curves, obtained from FL relation curves, showed an afterload-dependent decrease in time to peak elastance and slowed relaxation with both increased preload and afterload. The ability of the present system to independently and dynamically control preload, afterload, and transition between end-diastolic and end-systolic FL coordinates provides a valuable extension to the range of tools available for the study of single cardiomyocyte mechanics, to foster its interrelation with whole heart pathophysiology.

single cell mechanics; preload; afterload; time-varying elastance

CLASSICALLY, LOAD-DEPENDENT characterization of cardiac mechanics has been conducted in whole heart preparations (25, 27). The externally homogeneous mechanical activity at this level (i.e., the whole organ) arises from spatiotemporal integration of the ordered, but intrinsically heterogeneous, activity of cardiac muscle elements (14). Currently, the tools to study mechanical interaction of heterogeneous contractile elements in near-physiological conditions are limited (23), and the vast majority of research on single intact cardiomyocytes is conducted in mechanically unloaded cells (15).

Isolated cardiomyocytes are an important preparation for studying cardiac mechanics, because they are free from connective tissue and endothelium, which cause significant modification of tissue viscoelastic and contractile properties. In addition, subcellular mechanisms can be assessed in greater detail in single cardiomyocytes compared with intact tissue preparations. However, controlling the mechanical environment of single intact cells has been a challenging task fraught with intrinsic difficulties of related techniques (8).

In the late 1980s, Le Guennec et al. (19) developed a technique that uses carbon fibers (CF), attached to opposite cell ends, to manipulate preload. CF-based stretching gives rise to a relatively homogeneous increase in sarcomere length (SL) and allows calculation of passive and active forces from optically monitored changes in CF bending. This CF technique has been used successfully for the characterization of cardiomyocyte electromechanical properties during auxotonic contractions (3, 7, 34). More recently, Yasuda et al. (37), using a modified version of the CF technique, introduced an adaptive force-length (FL) control system. This technique has been used to subject individual cardiomyocytes to near-isometric, near-isotonic, and work-loop style contractions by anchoring one cell end with a stiff CF and dynamically controlling the attachment position of the other, compliant CF (21). A limitation of that technique is the absence of overall positional control (single-sided displacements), which contributed to sarcomere blurring, even during isotropic contractions.

In this report, we present a bidirectional CF-based FL control system, which uses cells that are suspended from two compliant CF (fully removed from the coverslip). Each CF is mounted to a piezo translator (PZT) supported by an optical-grade railing system that is coaligned with the bath plane. With bidirectional positional control, the cell center remains stationary relative to the optical axis (thereby improving x/y resolution for optical imaging), while coalignment with the bath plane avoids sarcomere blurring and improves resolution of optical detail. Functionality of this system is illustrated by investigating the effects of independently varying preload and afterload, as well as mode of contraction, on end-systolic FL relation (ESFLR) and elastance of guinea pig single ventricular cardiomyocytes.

MATERIALS AND METHODS

Myocyte Preparation

All experiments were carried out in accordance with the UK Home Office guidance on the Operation of Animals (Scientific Procedures) Act of 1986. Ventricular myocytes were enzymatically isolated from hearts, excised from female guinea pigs (350–400 g) after death by cervical dislocation. Hearts were swiftly (within 70–90 s after death) cannulated via the aorta and Langendorff perfused at a rate of 8 ml/min. Perfusion was conducted at 37°C, and all solutions were checked for osmolality (Semi-Micro osmometer; Knauer, Berlin, Germany); for composition of individual solutions, see Table 1.

After being washed by heparinized (10,000 IU/l) Tyrode solution (5 min), the heart was perfused with Ca2+-free solution (5 min), followed by high-K+ solution to arrest the heart in preparation for enzymatic digestion (5 min). The perfusate was then changed to

Address for reprint requests and other correspondence: G. Iribe, Cardiac Mechano-Electric Feedback Group, Dept. of Physiology, Anatomy and Genetics, Univ. of Oxford, Sherrington Bldg., Parks Road, Oxford, UK OX1 3PT (e-mail: gentaro.ribe@dpag.ox.ac.uk).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Table 1. Composition of solutions used in the isolation procedure

<table>
<thead>
<tr>
<th>Composition</th>
<th>Tyrode Solution</th>
<th>Ca²⁺-Free Solution</th>
<th>High-K⁺ Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl, mM</td>
<td>140.0</td>
<td>140.0</td>
<td>4.0</td>
</tr>
<tr>
<td>KCl, mM</td>
<td>5.4</td>
<td>5.4</td>
<td>10.0</td>
</tr>
<tr>
<td>K-glutamate, mM</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>CaCl₂, mM</td>
<td>1.8</td>
<td></td>
<td>0.025</td>
</tr>
<tr>
<td>EGTA, mM</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>HEPES, mM</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Glucose, mM</td>
<td>11.0</td>
<td>11.0</td>
<td>11.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 (with NaOH)</td>
<td>7.4 (with NaOH)</td>
<td>7.2 (with KOH)</td>
</tr>
</tbody>
</table>

high-K⁺ solution containing collagenase (Sigma Blend Type L: 1 mg/ml, 10 min) for digestion (note: a small but controlled content of calcium is beneficial for ensuring steady and reproducible enzyme activity; see composition in Table 1).

Next, ventricles were harvested by cutting along the atrioventricular border, chopped into small cubes (~1–2 mm³), and gently agitated in oxygenated high-K⁺ solution (free of enzyme). The supernatant containing ventricular cells was collected after tissue fragments were allowed to settle, and the remaining tissue was resuspended in fresh high-K⁺ solution. This procedure was repeated six times until little or no visible tissue remained.

The supernatant, containing cells, was passed through a 100-μm nylon mesh (Cadish Precision Meshes, London, UK) and centrifuged for 1 min at 16,000 g (PK121R; ALC, Cologno Monzese, Italy) using a soft-start/soft-stop protocol. After centrifugation, the supernatant was discarded and the cell pellet was carefully resuspended in storage solution (50 ml of Tyrode solution, with 50 mg of bovine serum albumin and 0.83 mg of trypsin inhibitor to terminate further enzyme activity).

Experimental Setup

Carbon fibers. CF (12–14 μm in diameter), kindly provided by Prof. J.-Y. Le Guennec (University of Tours) were mounted in glass capillaries pulled from 1.16-mm inner diameter/2.0-mm outer diameter glass tubes (GC200F-10; Harvard Apparatus, Holliston, MA). The final section (1.2–1.4 mm) of the glass capillary, containing the CF, was bent by 30° to allow near-parallel alignment of the CF with the bottom of the perfusion chamber (Fig. 1, right). The CF protruding from the glass capillary was trimmed to 1.20 mm and fixed in position using cyanoacrylate adhesive.

**CF position control.** Each CF is mounted to a position control system with six regularly exploited degrees of freedom (explained below). Before use, CF are aligned near-horizontal (a minor slant is required to ensure that the CF tip is slightly lower than the rim of the glass capillary that holds the CF) and near-parallel to each other (again, a slight angle is needed to allow CF tip proximity without interference of the two glass capillaries holding them; Fig. 1). This is largely achieved by fitting the glass tube (with the CF at its pulled end) in a modified patch-clamp pipette holder (rubber seal compression fixing), which provides rotational control along the glass tube (1st degree of freedom). This, in conjunction with connecting the two pipette holders at a slight angle to each other, ensures that the CF tips can be aligned near-parallel to the bath and to each other.

For use, CF are lowered into the bath with a rotational coupling (roughly aligned with the x-axis of the system’s coordinate space; 2nd degree of freedom) between the pipette holder and the hydraulic manipulators that are used for CF fine positioning. The correct combination of height of the attachment point, length of the glass capillary, angle of rotation of pipette holder, and angle of capillary tip ensures seamless alignment and positioning of CF in the bath near the optical path.

For cell attachment, CF are manipulated using one Narishige miniature hydraulic manipulator on each side (SM-28; Narishige, Tokyo, Japan) with 5-mm travel in x/y/z directions (3rd to 5th degrees of freedom). Once CF positioning and cell attachment have been completed, cells are lifted off the glass coverslip (see below for further detail).

From this point in time, bidirectional position control along the x-axis is implemented via a pair of high-accuracy PZT (P-621.1CL; Physik Instrumente, Karlsruhe/Palmbach, Germany; 6th degree of freedom). Each PZT, carrying one CF assembly, is fixed (with opposite intrinsic directionality) on sleighs, mounted on an optical-path.
grade railing system. The sleighs provide for easy coarse alignment (and a park-position for access to the bath when not in use), whereas the railing system ensures precise alignment of the cell manipulation axis with the x-axis of the bath and microscope table and strict bidirectionality of the movement of both CF assembles along a single axis (to avoid undesired shearing or bending of the cell).

The PZT used are capacitance-encoded and combine a 100-μm working range with 0.4-nm motion resolution, at motion linearity accuracy better than 0.01%. Custom-written LabView software (available from the authors) and a fast analog-to-digital/digital-to-analog interface (NI DAQCard-6062E; National Instruments, Austin, TX) were used to drive the PZT.

Cell chamber and perfusion. A custom stage was designed to fit on an inverted microscope (Diaphot-TMD; Nikon, Tokyo, Japan) with a 330° rotatable chamber (16). Cells were placed on a poly-HEMA (2-hydroxyethyl methacrylate; Sigma, Poole, UK)-coated coverslip to interface (NI DAQCard-6062E; National Instruments, Austin, TX) working range with 0.4-nm motion resolution, at motion linearity accuracy better than 0.01%. Custom-written LabView software (available from the authors) and a fast analog-to-digital/digital-to-analog interface (NI DAQCard-6062E; National Instruments, Austin, TX) were used to drive the PZT.

Cell Attachment Procedure

Cell inclusion criteria were as follow: a rod-shaped cardiomyocyte with clear striation and well-delineated (‘bleb’ free) membrane, resting SL > 1.8 μm, and active shortening by 5% or more in response to electrical field stimulation. Suitable cells were positioned under the CF and aligned with their main axis in parallel to the direction of stretch (i.e., perpendicular to CF) by rotation/translocation of the bath. For attachment, CF were lowered, one at a time, to opposite cell ends and gently pushed onto the cell surface (as identified by minor lateral deformation of the cell at the attachment points, see Fig. 1, “top view”). Initial cell attachment was confirmed by briefly lifting each CF, and the attached cell end, off the coverslip. Subsequently, both CF were again gently lowered and, to enhance cell-CF adhesion, cells were paced at 2–4 Hz for 3 min. This procedure significantly improved cell attachment, to the point that all experimental protocols could be performed with the cell completely detached from the bottom of the chamber and suspended only from the two CF. Slippage (sliding of CF relative to the cell surface) was an exclusion criterion.

At the end of experiments, CF were briefly lifted out of the solution, which stripped them of any cell debris by the perfusate surface tension at passage through the liquid-air interface. CF do not need to be otherwise cleaned before the next cell attachment and normally can be reused during more than 10 experimental days.

Measurements

Length measurements. The myocyte and CF tips were monitored through an inverted microscope (Nikon Diaphot TMD) using a ×40 long working distance objective (NA 0.75). Both cardiomyocyte SL and CF tip distances were recorded at 240 Hz and analyzed in real time, using IonOptix equipment and software (IonOptix, Milton, MA).

Effective cell cross-sectional area was calculated from measured cell width, assuming an elliptical shape of the cross section with a 3:1 ratio of long (measured cell width, y-direction) and short axis (estimated cell height, z-direction), according to Ref. 21.

Force measurement. Active and passive forces (F) were calculated from CF bending, assessed by monitoring CF tip and PZT positions (Fig. 2), using Eq. 1:

\[ F = K(ΔL_e - ΔL_p) \]  

where \( ΔL_e \) is the change in distance between the two CF tips that are attached to the cell, and \( ΔL_p \) is the change in distance between the two PZT to which the CF holders are mounted (compared with “force-free” attachment positions). The parenthetic term on the right side of the equation yields total CF bending. K is the stiffness of the CF (0.15–0.25 μN·μm−1).

F was measured directly, using a force transducer system (406A; Aurora Scientific, Aurora, ON, Canada) factory calibrated for compression measurement, and mounted under a microscope. The tip of experiment-ready CF (held in a glass capillary) was carefully positioned just in front of the force transducer probe. By using the PZT, the CF was then moved toward the transducer, past the contact point, applying forces of up to 5.0 μN, while force and PZT position were continuously recorded. Because the stiffness of the force transducer (100 μN·μm−1) is about three orders of magnitude higher than that of CF, displacement of the transducer is negligible. Calibration runs were repeated five to six times, and the slope of the linear regression curve through the recorded data (force vs. movement) was taken as the stiffness of the CF.

Force and Length Control

An adaptive feed-forward control process (Fig. 2) was implemented as follows. After firm attachment of CF tips to opposite ends of a cardiomyocyte, the cell was stimulated at 2 Hz. Contractions cause force development and cell shortening (auxotonic contractions; Fig. 3A). After 2–3 min, and on the background of steady-state contractions, the length signal (changes in CF tip distance, \( ΔL_p \)) was recorded for 10 beats and averaged. This was used as the source signal for PZT command signal design to achieve any of the following loading states.

Isometric and isotonic contraction. For isometric contractions, the source signal was 1) inverted (to induce an outwardly directed countermovement of PZT); 2) its amplitude was divided by two (one-half of the movement applied to each PZT to cause symmetric effects on both CF to retain the cell center quasi-stationary); and 3) the gain was adjusted until CF tip position remained near constant (Fig. 3B). For isotonic contractions, step 1 was omitted (to induce inwardly directed PZT movement) and step 2 was tuned to minimize changes in CF bending (Fig. 3C; see also isometric_isometric.mp4 in the supplementary data contained in the online version of this article).

Physiological work-loop style contraction. Physiological work-loop style contractions at the whole organ level involve four phases: isovolumetric contraction, ejection, isovolumetric relaxation, and filling. Similar phasic behavior can be mimicked at the single-cell level via a sequence consisting of isometric contraction (phase 1 in Fig. 4, left), quasi-isotonic shortening (phase 2), isotropic relaxation (phase 3), and quasi-isotonic lengthening (phase 4). The PZT command algorithm for work-loop style contractions is explained below (see also Fig. 4), including a pseudo-code listing (which is simplified in...
isometric relaxation, until the time work-loop style contractions (D) is referred to throughout as the “source signal.”

The length change during auxotonic contraction (bottom left). The length change during auxotonic contraction (bottom left) is referred to throughout as the “source signal.”

[Image: Fig. 3. PZT command (top; ∆Lρ) and related force (middle) and length (bottom; ∆Lρ) curves for auxotonic (A), isometric (B), isotonic (C), and physiological work-loop style contractions (D). The length change during auxotonic contraction (bottom left) is referred to throughout as the “source signal.”]

For phase 1 (isometric contraction), both PZT are moved outward to keep cell length and position constant. The PZT command is identical to roughly the first quarter of the curve labeled A\([−\text{SourceSignal}]\) (t) in Fig. 4, right (i.e., an inverted and tuned version of the source signal) until the time \(T_{1-2}\), at which active force reaches a user-defined afterload. Up to this point, the PZT command signal (heavy solid line in Fig. 4, right) \([\text{Command}]\) can be described as follows:

\[
\text{if } 0 \leq t \leq T_{1-2} \\
\text{\quad } [\text{Command}]\!(t) = A \cdot [−\text{SourceSignal}]\!(t)
\]

where \([−\text{SourceSignal}]\!(t)\) is the inverted source signal at each point in time \(t\) (obtained during auxotonic contraction, as described above) and \(A\) is the command signal gain that yields isometric contraction.

For phase 2 (isotonic shortening), both PZT are moved inward to keep CF bending constant, following the shape of the source signal with a gain \(B\) that yields isotonic behavior, until the time point at which the source signal reaches peak contraction \((T_{\text{max}})\), so that:

\[
\text{if } T_{1-2} < t \leq T_{\text{max}} \\
\text{\quad } [\text{Command}]\!(t) = [\text{Command}]\!(T_{1-2}) - B \cdot [\text{SourceSignal}]\!(t)
\]

For phase 3 (isometric relaxation), both PZT are moved further inward to avoid distension of the relaxing cell by the force stored in the CF, bent during the previous contraction. This is done by following the shape of the inverted source signal with a gain \(C\) that supports isometric relaxation, until the time \(T_{3-4}\), at which the command signal meets a value corresponding to an isotonic contraction initiated from the original end-diastolic point:

\[
\text{if } T_{\text{max}} < t \leq T_{3-4} \\
\text{\quad } [\text{Command}]\!(t) = [\text{Command}]\!(T_{\text{max}}) - C \cdot [−\text{SourceSignal}]\!(t)
\]

where \(C\) is the gain that supports an isotonic state.

For phase 4 (isotonic lengthening), both PZT are moved outward again to lengthen the cell while keeping CF bending (i.e., force) constant. The PZT command in this phase is identical to roughly the last quarter of the active control sequence labeled D\([\text{SourceSignal}]\) in Fig. 3C.

\[
\text{if } T_{3-4} < t \\
\text{\quad } [\text{Command}]\!(t) = D \cdot [\text{SourceSignal}]\!(t)
\]

where \(D\) is the gain that yields relengthening of the cell at constant force.

Statistics

All values are presented as means ± SE. Two-way analysis of variance was used for statistical analyses, and \(P < 0.05\) was considered to indicate a significant difference between means.

RESULTS

Cell-CF Attachment

CF attach glue-free to cardiac cells (19). The actual mechanism of CF-cell adhesion is not currently understood in detail, but cell adhesion is usually strong enough to support passive diastolic distension and auxotonic contraction studies (3). Prior research involving isometric contractions (where more significant forces occur between cell and CF) was limited to working at minute preloads (end-diastolic SL near slack values; Ref.
Preventing premature cell separation tended to require clamping of cells between CF and the bottom of the perfusion chamber, impeding force detection. A major improvement in cell-CF attachment was achieved in this study by pacing cells after initial adhesion, for 3 min at 2–4 Hz, before starting any protocol involving mechanical cell manipulation. Cell adhesion was assessed by applying stretch to resting cardiomyocytes suspended from two carbon fibers. As diastolic SL approached 2.4 μm, most myocytes started to display spontaneous contractions (mechanical activation). The average peak force where detachment occurred during isometric contractions was 2.56 ± 0.18 N (16.2 ± 1.7 mN/mm²) (n = 11).

Pacing-induced amplification of cell-CF attachment allowed us to conduct all investigations with cells lifted completely off the glass surface of the chamber and to extend the range of diastolic stretch (preload) from which isometric contractions could reliably be supported to SL of up to 2.05 μm.

**Force and Length Control**

Figure 3 shows representative recordings of PZT commands, force, and length, for auxotonic, isometric, isotonic, and work-loop style contractions. Isometric and isotonic modes differed in the polarity (directionality) of PZT control commands, as described in MATERIALS AND METHODS. Best results usually required an additional offset in PZT command timing (up to a 10-ms delay in isotonic mode compared with matched isometric points). This was mainly required to correct for the delay in time to peak isometric contraction compared with isotonic mode (Table 2). In work-loop style contractions, FL control is sufficiently precise to yield the required isometric and isotonic phases (see work-loops in Figs. 6 and 9), illustrating the suitability of the control algorithm.

**Internal Sarcomere Shortening**

Although CF distance is fixed in isometric contractions, there is limited internal sarcomere shortening. The level of

---

**Table 2. Load-dependent morphological changes in elastance curve**

<table>
<thead>
<tr>
<th>Preload Condition:</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>End-Diastolic SL, μm:</td>
<td>1.939±0.008</td>
<td>2.050±0.025</td>
</tr>
<tr>
<td>Afterload Condition:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (Isotonic)</td>
<td>High (Isometric)</td>
<td>Low (Isotonic)</td>
</tr>
<tr>
<td>Peak elastance time, ms</td>
<td>60.26±2.9</td>
<td>53.5±1.8*</td>
</tr>
<tr>
<td>Relaxation time constant, ms</td>
<td>9.74±0.89</td>
<td>15.83±1.28*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 13). *P < 0.05 vs. isotonic contraction. †P < 0.05 vs. low preload condition.
internal shortening was variable; contractions that were also isometric at the SL level could sometimes be measured, but usually internal shortening took place. Sarcomere shortening was reproducible when switching back and forth between varying preloads and afterloads, so although there was internal sarcomere shortening, there appeared to be no slippage of CF-myocyte attachment points. In addition, internal sarcomere shortening is fairly homogeneous in the area between CF.

**FL Relationship**

We traced FL relation curves to identify ESFLR, independently varying preload and/or afterload. Between interventions, cells were routinely released to record auxotonic contractions from a range of preloads (see Fig. 5A). These were compared with preintervention reference ESFLR to assess maintained cell mechanical performance (instability of which was an exclusion criterion for data analysis). The observed consistency of ESFLR throughout experiments with dynamic FL control suggests that effects of slow force responses to stretch (1) are negligible in this setting. For all results presented in this communication, ESFLR was steady over experimental periods of up to 20 min.

Figure 5A shows superimposed FL relation curves of auxotonic contractions (reference ESFLR) and of isometric contractions from a range of preloads (end-diastolic SL: 1.85 to 1.97 μm). Figure 5B shows FL relation curves with afterloads

![Graph](http://example.com/graph1.png)

Fig. 5. FL relation curves of auxotonic, isometric, and isotonic contractions with varied preloads and afterloads and their end-systolic FL relation (ESFLR). A: superimposed FL curves of auxotonic contractions (no PZT command; shaded lines) and of isometric contraction (solid lines), obtained with varied preloads ranging from 1.85- to 1.97-μm end-diastolic sarcomere length (EDSL). The ESFLR was near linear for all contractions, regardless of mode of contraction (dashed line). B: superimposed FL contraction curves with varying afterload (from isometric to isotonic), obtained at low (SL 1.90 μm; shaded lines) and high preloads (SL 1.97 μm; solid lines). ESFLR was near linear and independent of preload and afterload (dashed line).

![Graph](http://example.com/graph2.png)

Fig. 6. FL relation curves of auxotonic and work-loop style contractions with varied preload and afterload. A: superimposed FL curves of auxotonic contractions (shaded lines) and work-loops (solid lines), initiated from varying preloads (SL 1.85 to 2.04 μm). ESFLR was load and contraction mode independent and near linear (dashed line). B: superimposed FL curves of auxotonic contractions (shaded lines) with varied preload (SL 1.82 to 1.97 μm) and high preload (SL 1.97 μm; solid lines). ESFLR remains independent of contraction mode and near linear (dashed line).
Contractile Profiles

To further investigate the time-course profiles of contraction, we analyzed the time-varying ratio of instantaneous force $F(t)$ to instantaneous length $L(t)$ under different preloads and afterloads. Instantaneous FL ratio is defined as $E(t) = F(t)/[L(t) - L_0]$, where $L_0$ is the cell length at the intersection of ESFLR and EDFLR (Fig. 8A). This concept is an analog of instantaneous pressure-volume ratio in whole heart (28, 30, 31). Its numerical value corresponds to the slope of a line that connects the intersection of ESFLR and EDFLR with any

![Fig. 7. Superimposed force-sarcomere length (FLS) relation curves of auxotonic contractions (shaded lines) and of externally isometric contractions (solid lines) of the same case as Fig. 5A. The ESFL$\alpha$R was near linear, similarly to ESFLR in Fig. 5A, for all contractions, regardless of mode of contraction (dashed line).

varied from isometric to isotonic contraction, initiated from two different preloads (end-diastolic SL: 1.90 and 1.97 $\mu$m).

As explained in MATERIALS AND METHODS, isometric and isotonic phases can be combined to create work-loop style contractions. Examples are shown in Fig. 6. A and B. Figure 6A shows superimposed FL relation curves of auxotonic contractions (as reference) and work-loop style contractions with varied preloads (end-diastolic SL: 1.85 to 2.04 $\mu$m). Figure 6B shows superimposed FL relation curves of auxotonic contractions (reference) and a set of work-loop style contractions initiated from a constant preload (SL: 1.91 $\mu$m) and working against different afterloads. A cellular equivalent of the classic Frank-Starling effect could be observed in all cases: an increase in preload (diastolic length) leads to an increased ability to perform work (see increased area of work-loops in Fig. 6A).

In the case of isometric contractions, increased preload raised peak force production (Fig. 5A), whereas in isotonic mode, an increase in active shortening was observed (Fig. 5B).

In all experiments, the slope of ESFLR was constant and independent of mode of contraction, preload, or afterload (Figs. 5 and 6) over the length range studied. In the range of SL from 1.85 to 2.05 $\mu$m, ESFLR could be fitted by a linear regression ($R^2 = 0.9905 \pm 0.0014$; $n = 16$). The slope of ESFLR (whole cell force production over cell length change) was $0.20 \pm 0.03$ N/um$^1$ ($n = 16$). End-diastolic FL relations (EDFLR) also could be well fitted by linear regression for SL up to $\sim 2.0 \mu$m ($R^2 = 0.9066 \pm 0.0185$; $n = 9$), with a slope of $0.022 \pm 0.003$ N/um$^1$ (for larger SL, EDFLR increased more steeply; the limited extent of data in that length range did not allow for qualified curve fitting)

Figure 7 presents force-SL (FLS) relation curves of the same case as in Fig. 5A. Although the result shows that there is internal sarcomere shortening during isometric contractions, as mentioned above, the data do confirm that the ESFL$\alpha$R is linear and load independent as well. The slope of the ESFL$\alpha$R was $10.9 \pm 2.2$ N$\cdot$um SL$^{-1}$ (linear fit), and the slope against the force normalized to the cross-sectional area was $76.1 \pm 13.9$ mN$\cdot$mm$^{-2}$ $\mu$m SL$^{-1}$ ($R^2 = 0.9721 \pm 0.0070$; $n = 9$).
instantaneous FL relation point, and represents the instantaneous elastance (or reciprocal compliance) of the cell.

Figure 8B shows representative superimposed instantaneous elastance curves in isometric contraction under low and high preloads, and Fig. 8C shows the same in isotonic mode. Increasing preload slows isometric relaxation but does not affect the time to peak elastance. Time to peak elastance was significantly longer in isotonic mode (low afterload) than isometric mode (high afterload) of contraction (Table 2).

Figure 9 shows FL relation curves in physiological work-loop style contractions and corresponding instantaneous elastance curves during contractions with different afterloads. Despite the fact that both curves follow the same FL trajectory only during early isometric contraction and late isotonic relaxation, the elastance profiles allow one to identify a slowing in time to peak contraction and faster relaxation with the lower afterload. This is consistent with the behavior observed with isometric and isotonic contractions, shown in Fig. 8 and Table 2, and illustrates the utility of the instantaneous elastance concept for characterization of single-cell contractile behavior.

To illustrate the utility of working with cells that are suspended from CF only (i.e., not in contact with the coverslip), Fig. 10 shows representative FL relation curves of auxotonic and isometric contractions in a cell that was not lifted off the poly-HEMA-coated coverslip. ESFLR in auxotonic contraction dissociates from the isometric case, as a consequence of resistance to shortening caused by cell-coverslip interaction. This dissociation becomes larger as the amount of shortening increases. Although this behavior could be interpreted as “shortening deactivation,” it is clearly not an expression of processes intrinsic to the cell.

DISCUSSION

Cell-CF Attachment

With the improved attachment of intact, isolated cardiomyocytes to CF we can, for the first time, present data that investigate ESFLR in single isolated cardiac cells. The attachment, after 2 min of pacing, is sufficiently stable and robust that reproducible force measurements can be conducted during a 20-min time window and over a large range of physiologically relevant SL. Because internal shortening still takes place, there will be viscoelastic links between CF and contractile filaments. This is, however, highly reproducible, and we found no slippage within the investigated range of SL once CF were firmly attached. Previous studies, using the same batch of CF but no pacing protocol for improved mechanical connection, supported a limited range of active force (19, 33). To improve CF attachment, Yasuda et al. (37) used graphite-reinforced carbon (GRC) fibers, which are thought to have enhanced surface charges. Although we used non-GRC fibers, we could improve CF attachment by pacing cells, after initial adhesion, for 3 min at 2–4 Hz. The initial adhesion is regarded to be due to electrostatic force between CF and the cell membrane surface (19), although a mechanical interaction component may exist. The mechanisms underlying the improvement of CF attachment by pacing remains to be studied.
End-Systolic FL Relation

Our results show clearly that within the range of SL studied, ESFLR in single ventricular cardiomyocytes, isolated from guinea pig, is near linear and independent of preload or afterload. It has been reported previously that larger mammals (e.g., rabbit and dog) show a linear end-systolic pressure-volume relation (ESPVR) (10, 28), whereas smaller rodents (e.g., mouse or rat) have convex ESPVR (9, 32). The relation between the intrinsically three-dimensional (3D) processes that underlie ESPVR characteristics and the one-dimensional nature of ESFLR is nontrivial. Thus the fact that the ESFLR of papillary muscles, even if isolated from larger mammals, can have a concave shape has been attributed to the dimensional mismatch of underlying preparations (5, 11). Similar considerations make the extrapolation from the present observation of a linear ESFLR in cardiomyocytes to 3D characteristics of guinea pig heart difficult. In addition, the range of preloads assessed in this study was limited (by CF detachment forces) to SL of up to 2.05 μm in isometric contractions. Projections on ESFLR shape at SL beyond this point (such as described in papillary muscle experiments, at SL of up to 2.2–2.4 μm) should be avoided (5, 11).

A potentially interesting divergence from 3D behavior (which involves load dependency of ESPVR) is that ESFLR in guinea pig single isolated cardiomyocytes is virtually load independent (as shown in Figs. 5 and 6) over a range of SL that may be viewed as physiologically relevant. In previous studies involving in vivo and ex vivo whole organ and native tissue preparations, a decrease in contractility was observed in shortening muscle compared with isometric contraction (2, 20, 29). This has been termed shortening deactivation and gives rise to a shallower ESFLR in contracting compared with isometric muscle.

One of the factors that may explain cell-tissue differences in the load dependency of ESFLR is the mechanical resistance of extracellular viscoelastic elements, such as connective tissue, in native myocardium (29). Single-cell preparations are therefore of significant interest for mechanical studies, because they allow one to study the intrinsic mechanical properties of the actual contractile building blocks of the heart in the absence of confounding matter. Lack of extracellular viscoelastic influences is, however, not normally achieved unless cells are fully removed from the solid surface of the perfusion chamber. If that is not done, external friction adds to the load on myocytes, and contractions that involve shortening will result in shortening muscle compared with isometric contraction (2, 20, 29). This has been termed shortening deactivation and gives rise to a shallower ESFLR in contracting compared with isometric muscle.

One of the command functions is derived at different loading states. From use of the auxotonic length change in control conditions as a “generic” source signal shape, from which all other PZT command functions are derived at different loading states. Thus ES contraction timing (T_max) was determined as the point of peak auxotonic contraction. This time approximation would be justified by the conventional time-varying elastance model, which assumes that the profile of elastance curves is load independent (30). However, as shown in Fig. 8 and Table 2, main differences in the time course of elastance changes under high and low preloads and afterloads are time-to-peak contraction and relaxation time constants. Lowering afterloads in isotonic contraction lengthened time to peak and sped up relaxation. The mechanism underlying this behavior has been suggested to be linked to a shortening-induced decrease in Ca^{2+} affinity of myofilaments (1, 18, 36). This result is consistent with previous findings comparing free shortening and auxotonic contractions in single cells (33). Similarly, a reduction in preload of isometric contraction sped up relaxation, and the underlying mechanism may be linked to the length dependency of myofilament Ca^{2+} affinity (1). This result is also consistent with previous findings in trabeculae at SL compatible with the range studied presently (12). We did not observe significant effects of changing preload on relaxation time constants of isotonic contractions (Table 2). Therefore, shortening-induced decrease of Ca^{2+} affinity and length-dependent increase of Ca^{2+} affinity may have balanced out, and further studies are required to assess this.

FL Control

Adaptive feed-forward control systems have been used in multicellular preparations (4, 22), because this method tends to be more stable and noise resistant than real-time feedback systems. However, there are potential limitations that arise from use of the auxotonic length change in control conditions as a “generic” source signal shape, from which all other PZT command functions are derived at different loading states. Thus ES contraction timing (T_max) was determined as the point of peak auxotonic contraction. This time approximation would be justified by the conventional time-varying elastance model, which assumes that the profile of elastance curves is load independent (30). However, as shown in Fig. 8 and Table 2, time to peak elastance and relaxation rate can be affected by preload and afterload in single cells. The elastance curve of a work-loop style contraction, which is a complex combination of both isometric and isotonic contractions, is quite different in shape from that in auxotonic contraction (Fig. 9). Such discrepancy between the assumed contractile profile, used to shape the PZT command (source signal) and the actual contractile profile, causes deviation from perfect isometric or isotonic behavior. To address this problem, direct FL feedback control is required. However, such feedback control requires high-precision noise filtering of the length signal to avoid amplification of noise, which is no trivial challenge (21). On the other hand, feed-forward control, as implemented in the present study, is relatively simple and free from undesirable noise amplification. With the use of gain optimization and introducing minor timing offsets to address the time-to-peak
discrepancy, the present results show that the desired shapes of isometric, isotonic, and work-loop style contractions can be implemented with at least the same level of accuracy as that provided by current feedback systems (21). Also, the resulting elastance curves are principally comparable with those observed in whole heart preparations (30) so that we regard the discrepancy mentioned above as nonlimiting.

Given the dimensional difference between pressure-volume (P-V) and FL work-loops (as seen in whole heart vs. isolated trabeculae or cells, respectively), one cannot simply relate the pseudo-isobaric phase of P-V loops to the isotonic phase in FL relations. Thus isobaric ejection (i.e., a decrease in chamber volume at constant pressure) would translate into auxotonic FL changes (reduction in myocardial force during contraction-induced decrease in chamber dimensions, according to Laplace’s Law). However, in the real heart, ejection is not isobaric, and pressure toward the end of ejection is higher than at the beginning (aortic pulse pressure differences). The FL work loops presented are simplifications, therefore, that may help to illustrate the scope of this technique and aid comparison between cells. More realistic FL relations can be extracted from computational models of the deforming heart, if they include sufficiently detailed information on cardiac histomorlemal features (2b, 24). Interestingly, those computed FL loops bear similarity to measured cardiac surface deformation loops (17). Future research must be directed at comparing cell responses to “simplified” and “physiologically shaped” FL command sequences, more representative of the in situ setting.

**Active Force**

Active force per unit cross-sectional area in single isolated guinea pig cardiomyocytes is smaller than that in multicellular preparations. Previous studies showed that single guinea pig myocytes produce active forces of ~5.3 mN/mm² at SL < 1.9 μm (26), whereas papillary muscle preparations produce maximum forces in excess of 40 mN/mm² (38). Several points should be taken into consideration when comparing single cell and tissue data. First, papillary muscle studies usually assess maximum force at SL of 2.2–2.4 μm (13, 35). Although our CF attachment technique was improved to apply higher preloads, it did not support absolute forces in excess of 2.5 μN (CF would detach from cell membrane). This corresponds to a normalized force of ~16 mN/mm² and is usually observed at SL < 2.2 μm. Second, many papillary muscle studies were performed at room temperature, where contractility is higher than at body temperature (38), used in our study. Third, there may be factors inherent to multicellular preparations that affect contractility, such as paracrine effects, for instance, from the endothelium (2a).

In conclusion, we developed a dynamic FL control system that uses a pair of compliant, computer-controlled, and PZT-positioned CF to dynamically control the mechanical environment of isolated intact cardiomyocytes. Using this system, we studied characteristics of ESFLR and elastance curves in single cells under various loading conditions. We found that ESFLR is virtually load independent in single cells at SL from 1.85 to 2.05 μm. This load independency of contractile profiles is consistent with previous findings in whole heart and papillary muscle. The present system employs fully suspended cells and is suitable for the study of cardiac mechanics at the level of single intact myocytes.

**ACKNOWLEDGMENTS**

We thank Doug Tillotson and colleagues from IonOptix Corporation, Milton, for invaluable technical support and Rebecca A. B. Burton for expert laboratory assistance.

**GRANTS**

This study was supported by a project grant from the British Heart Foundation (BHF). G. Iribe holds a PhD Training Grant from Eisai Co., Ltd. P. Kohl is a BHF Senior Research Fellow.

**REFERENCES**


