A force transducer for measuring mechanical properties of single cardiac myocytes

C. Tasche, E. Meyhofer, and B. Brenner. A force transducer for measuring mechanical properties of single cardiac myocytes. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H2400–H2408, 1999.—We have described a transducer design capable of recording forces generated by single cardiac myocytes with sufficient temporal resolution to detect force responses to rapid length changes. Our force sensors were made from thin steel foils that act as cantilevers whose bending is monitored by reflection of a laser beam. Deflection of the laser beam is measured by a differential photodiode detector. A small, 50-µm-thick tungsten needle attached to the free end of the steel foil allowed us to glue single cardiac cells to the force transducer. The transducers have compliances of ~0.02 mN and resonance frequencies between 2 and 3 kHz. The resolution is ~18 nN rms at a detector bandwidth of 16 kHz, so we were able to resolve 0.2% of the maximum isometric force (~12 µN) developed by a single cardiac myocyte. We have demonstrated that the transducer is well suited to analysis of mechanical properties of single ventricular myocytes, for example, the recording of isometric forces and rate constants of force redevelopment after rapid release-restretch maneuvers.

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IT WAS OUR AIM to gain further insight into the molecular mechanism of cardiac muscle contraction and its regulation by analyzing the mechanical behavior of cardiac myocytes. In skeletal muscle systems, rapid release-restretch maneuvers and various other mechanical measurements have been used for this purpose (e.g., Ref. 6). To adapt these analyses to cardiac muscle it was necessary to work with isolated single heart cells, because within the myocardium the cardiac myocytes are arranged in a complex network, which makes it impossible to accurately determine forces and length changes at the level of the sarcomere in multicellular preparations. In addition, multicellular preparations are often plagued by diffusion problems. For example, it is difficult to prevent the formation of substrate and product gradients during contraction or to completely exchange regulatory proteins into the filament lattice. It was therefore necessary to work with so-called skinned cardiac myocytes, cells in which the sarcolemma, sarcoplasmic reticulum, and other organelles have been made permeable or disrupted with a detergent to gain access to the filament network. Recording mechanical parameters from single skinned cardiac cells is, however, considerably more difficult than working with skeletal fibers, largely because of the comparatively small size of the cardiac myocytes.

Isolated rat cardiac cells have a length of ~70–170 µm and diameters between 20 and 40 µm (15), often with irregular cross sections. They develop maximal isometric forces of ~12 µN (28). The rate constant of force redevelopment (6) following rapid release-restretch maneuvers is ~5 s⁻¹ after a length change of 20% of the total cell length in 2.5 ms (28). Basic experiments, such as isometric force measurements, require a force sensor system that resolves forces below 0.1 µN yet is sufficiently stiff such that isometric conditions are maintained and sarcomere length is changed by less than ~0.1%. Other important design parameters are the mechanical resonance frequency and damping of the force transducer, which limit the bandwidth over which measurements and length perturbations can be carried out and therefore determine the minimum temporal change that can practically be detected. For example, to determine the rate constants of force redevelopment, we needed a force sensor with a resonance frequency well in excess of 1 kHz to prevent mechanical oscillations of the transducer in response to the rapid release-restretch maneuver.

Mechanical measurements on single cardiac myocytes have been carried out with a variety of transducer designs. Several laboratories (1, 10, 14, 15, 21, 22) have used commercial capacitive force transducers from Cambridge Technology (Watertown, MA). The most sensitive model can resolve submicronewton forces, but the resonance frequency is only ~100 Hz. Other transducer designs for cardiac myocytes and myofibrils use optical fibers (2, 13, 19, 20, 27), suction pipettes (4, 7, 8), glass needles (25, 26), and microfabricated polysilicon beams (17, 18) as cantilevers. Another system for measuring forces of myofibrils is based on current-carrying wires placed in a magnetic field (16). Various techniques have been used to detect the bending of the different cantilever systems. Some authors have used strain gauges (25, 28); others have measured the displacement of the cantilever tip by video analysis (17, 18, 26), optoelectronically (16), or with a laser beam that projected the image of a slit or the blackened glass needle onto a differential photodiode (4, 7, 8, 12). The problem with published force transducer designs for single heart cell mechanics is that their resonance frequencies were lower or their compliances higher than acceptable for our measurements (see also summary in Ref. 20). Whereas the force transducer design developed by Iwazumi (16) is well suited for measurements on single myofibrils, it appeared to be difficult to adapt this design to the geometry and the higher forces...
generated by single cardiac myocytes. We believe that there was no suitable system available. Thus we have adapted the transducer design used in atomic force microscopy (AFM) (3) to the requirements of force measurements on single cardiac myocytes.

Most AFM sensors utilize small silicon cantilever beams with dimensions in the range of several micrometers. They have high resonance frequencies (often >10 kHz) and high stiffness (~10–1,000 N/m). With the appropriate detection system (i.e., laser beam deflection, tunneling current, interferometer), these sensors can reach the required nanoNewton resolution necessary for the proposed experiments on cardiac myocytes. Despite these excellent properties, AFM sensors are not well suited for measurements on cardiac myocytes. First, the diameters of single cardiac cells approach the dimensions of the cantilever of typical AFM sensors. It is therefore difficult to attach single myocytes exactly to the end of the beam and to accurately define the loading and the effective length of the cantilever. Second, the glue necessary to attach the cell must be distributed over a relatively large area of the beam such that its mechanical properties are changed. Third, AFM sensors are fragile. Removal of glue residue and cell debris from the beam is not possible, and a new sensor is required for each cell. Here we have described a force transducer system that is based on a simple AFM design but that uses rugged steel and tungsten cantilevers instead of a silicon beam. We have characterized in detail the mechanical and electrical properties of our transducer and demonstrated that it is well suited for measurements of isometric forces and rate constants of force redevelopment of single skinned cardiac cells.

**DESIGN CONSIDERATIONS**

**Design principles.** Our force sensors are made of thin steel foils that serve as cantilevers. Bending of the cantilever is monitored by reflecting a stabilized laser beam off the surface of the steel foil. When a force is applied to the sensor, the cantilever bends and the laser beam is deflected from its initial position. Displacement of the centroid of the reflected laser beam is monitored with a dual-photodiode detector (SPOT-2D, UDT Sensors, Hawthorne, CA). As a laser source we employed a system (Schäfer and Kirchhoff, Hamburg, Germany) with a TOLD 9140 diode (Toshiba Electronics Europe, Düsseldorf, Germany) that emits at a wavelength (λ) of 688 nm. To increase the beam pointing stability, the diode is coupled via a lens into a single mode fiber. The output of the single mode fiber (~8 mW) is collimated to a beam with a 1-mm diameter and Gaussian intensity distribution. We used a parallel beam geometry to have flexibility in choosing the distances among the collimating optic of the fiber, the force sensor, and the position detector. The displacement of the laser beam in the plane of the active area of the photodiode detector is magnified ~50 times compared with the displacement of the cantilever tip.

We attached small needles or pins (~1 mm long) with cyanoacrylate glue (Uhu, Bühl, Germany) to the free end of the steel foils because we did not want to directly immerse the force-sensing steel foils into the physiological buffer solutions. Only the free ends of needles were submerged in buffer, where they served as well-defined attachment points for isolated cells. Thus the laser beam did not pass through buffer, and we avoided possible artifacts in the displacement records produced by scattering objects such as air bubbles and particles in solution, scratches in the glass of the recording chamber, or protein adsorption to the surface of the steel foils. All components of the force transducer were mounted together in an aluminum block to obtain high mechanical stability. A schematic of the transducer design is shown in Fig. 1A.

**Material and geometry of the force sensor.** A material with a plane surface, good reflectivity, and a high Young's modulus was required for the cantilever so that we could manufacture beams with the required stiffness and high sensitivity. The force sensor should have a compliance of ~0.01–0.02 mN to maintain nearly isometric conditions for single cardiac myocytes while measuring forces in the microNewton range. Single cardiac muscle cells reach maximal isometric forces of ~12 µN. Thus, assuming a cell length of 120 µm and the chosen sensor compliance, we expected changes in total cell length of 120–240 nm, corresponding to 0.1–0.2%.

Our cantilever beams were made from steel foils (Goodfellow, Bad Nauheim, Germany or Hasberg, Bernau, Germany) with a thickness of 25 or 75 µm. We cut them from larger sheets to the required size with a sharp paper cutter and assembled the individual components of the force transducer by gluing them together with cyanoacrylate glue (Uhu, Bühl, Germany). Both foils yielded suitable sensors, but the thicker foil has less surface structure and reflects better, and therefore makes sensors of higher sensitivity possible. Because cantilevers from 75-µm-thick steel foil were too stiff, we increased the compliance to ~0.02 mN by cutting a rectangular aperture with a laser (λ = 780 nm, E = 800 J; Spectra Physics, Darmstadt-Kranichstein, Germany) into the beam. In Fig. 1B the geometry of a force transducer (diameter 75 µm) is shown. Typical numerical values of dimensions for steel foils with a thickness of 75 µm were as follows: b = 2 mm (total width of transducer element), b* = 100–150 µm [width of compliant element of transducer (when constructed with aperture)], L = 4 mm (free overall length of transducer), l1 = 3 mm (total length of transducer foil), l2 = 1 mm (length of tungsten needle), and l3 = 1 mm [length of compliant element of transducer (length of rectangular aperture)]. With 25-µm-thick steel foils, we used the following dimensions: b = 2 mm, L = 3 mm, l1 = 2 mm, and l2 = 1 mm. In this case we required no aperture to increase compliance.

For our biological measurements we needed to attach ventricular myocytes to the force sensors (see also above). We chose to lengthen the force sensors with needles of tip diameters slightly larger than the width of the cells. A material with a very high Young's modulus is best for this purpose to maximize the sensitivity of the transducers by making the needles as
stiff as possible. We selected tungsten needles with a diameter of 50 µm and a length of 1 mm, because tungsten has a high Young's modulus (340 GPa).

Detector. We used a differential photodiode as a position sensor. A simplified scheme of the electronic design of the detection system is shown in Fig. 1. An operational amplifier (OPA2111, Burr Brown, Filderstadt, Germany) with 10-kΩ feedback resistors (metal film resistors) converts the photocurrents into voltages (current-to-voltage gain is 10 V per mA). Feedback capacitors (ceramic, 1 nF) prevent gain peaking and limit the bandwidth of the signal from the photodiode to 16 kHz. An instrumentation amplifier (AD 624, Analog Devices, Munich, Germany) subtracts the voltages and amplifies the difference signal by a factor of 33 or 100. We used a gain of 33 to be able to calibrate the force transducer over a wide range. For biological measurements, a gain of 100 is required for sufficient sensitivity. The bandwidth of the electronic noise can be further controlled by low-pass filtering the output of the instrumentation amplifier.

Calibration of the force transducer. We used the following strategy to determine the force sensitivity. Because the force sensor is too small, too sensitive, and too difficult to operate in a horizontal orientation, we could not calibrate the force sensitivity by suspending small weights from the tip. Instead, we exerted forces on our sensor with a second force transducer (“calibrating transducer”) of known properties. The compliance was determined by moving the tip of the force sensor through known distances with a piezoelectric transducer and recording the resulting output signal.

The calibrating transducer was designed to be ~50 times more compliant and much larger than the actual force sensor such that it can be readily calibrated with a set of weights. This transducer consists of a thin brass cantilever with a needle at the free end. Two semiconductor strain gauges (Micro Engineering II, Upland, CA) that are mounted with cyanoacrylate glue to the brass beam are used to record the deflection of the transducer. Strain signals from the gauges are converted with a standard Wheatstone bridge to voltage signals. The system was calibrated with different weights made from tungsten wire or aluminum foil, which were suspended at the needle tip of the horizon-

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**Fig. 1.** A: schematic of force transducer. A parallel laser beam (heavy dotted line) is emitted from the optics of the single mode fiber and reflected off the cantilever (steel foil) toward the displacement detector. The laser beam, in x-y plane of force transducer, hits the cantilever at a 35° incidence angle. A steel foil is attached to the apparatus with cyanoacrylate glue. Application of force to the needle tip initiates bending of cantilever and deflection of laser beam in z-direction. Spot displacements are measured with a position sensitive differential photodiode. Distance between cantilever and detector is 183 mm. B: geometry of force transducer made from 75-µm-thick steel foil. Typical dimensions were b = 2 mm (total width of transducer element), b* = 100–150 µm (width of compliant element of transducer, with aperture), L = 4 mm (free overall length of transducer), l₁ = 3 mm (total length of transducer foil), l₂ = 1 mm (length of tungsten needle), l₃ = 1 mm (length of compliant element of transducer = length of rectangular aperture), with a tungsten needle 50 µm in diameter. Glass plate provides a flat surface with a well-defined edge. C: electronics for position detection. Two operational amplifiers (OPA2111) and feedback resistors (R₁ and R₂) form current-to-voltage (I-V) amplifiers to convert photocurrents (I₁ and I₂) from photodiode detector (PD) into voltages (V₁ and V₂). Transimpedance gain (G) is 10 V/mA. Bandwidth of I-V converters is limited by pole formed by feedback resistors and capacitors (C₁ and C₂). An amplified differential output voltage (Vout; gain 33 or 100) is provided by a precision instrumentation amplifier (AD 624 SD). Low-pass filter at output of instrumentation amplifier (R and C) is used to reduce bandwidth of electronics.
tally oriented brass cantilever. The masses of the weights ranged from 0.75 to 11.62 mg, corresponding to forces of 7.4 and 114 μN. The calibrating transducer has a sensitivity of 70 mV/μN and a compliance of ~0.8 m/N, and it behaves very linearly over the calibrated range of forces.

To calibrate the force sensitivity of the sensor, the calibrating transducer was mounted to a piezoelectric translator (P-844.10, Physik Instrumente, Waldbronn, Germany), which in turn was attached to a mechanical micromanipulator (M-461, Newport, Darmstadt, Germany). The needles of both force sensors were aligned one parallel to the other, and the tips were connected with a carbon fiber orientated perpendicular to the transducers. The carbon fiber, 9-μm thick and ~200-μm long, was glued with a polyurethane varnish (Blue Peter, International Farbenwerke, Hamburg, Germany) to the tips of the needles. Rectangular force pulses with varying amplitude were introduced by moving the base of the calibrating transducer with the piezoelectric translator. The responses of both force transducers were recorded simultaneously (see inset, Fig. 2A), and the force sensitivity was determined from these data.

### SYSTEM PROPERTIES

Technical data and geometry of two force transducers are summarized in Table 1.

Force sensitivity. A typical calibration of a force sensor made from 75-μm-thick steel foil is displayed in Fig. 2A. Drift in the baseline of the calibrating transducer (Fig. 2A, inset, top trace) had little influence on the calibration of the force transducer because we only analyzed the amplitudes of the rectangular pulses. The relationship of the output of calibrating transducer versus force transducer was linear over the entire range we examined (up to 40 μN). At a gain setting of 33 the sensitivity of this transducer was 0.45 V/μN, and it behaved very linearly ($r^2 = 0.9996$). Because the reflectivity of the steel foil varies over the surface of the cantilever, the shape of the intensity profile of the laser spot and, consequently, the sensitivity of the sensor are dependent on where exactly the laser beam is reflected off the cantilever. The collimating optic, force sensor, and position detector have therefore been mounted permanently together in a rigid aluminum block. If for some reason the position of the force sensor relative to the laser beam were changed, the calibration would have to be repeated.

Compliance. Characterization of the transducer compliance is illustrated in Fig. 2B. For this procedure, the tip of the force sensor was displaced defined distances by coupling the tip to a piezoelectric translator. A typical response of the force sensor to rectangular displacement pulses is shown in Fig. 2B, inset. From the relation of force output versus tip displacement we determined stiffness or compliance. The force transducers that we fabricated had compliances between $1.9 \times 10^{-2}$ and $2.6 \times 10^{-2}$ m/N.

The compliance of the force sensors are, in contrast to their sensitivity, not altered after changes in the relative position of force sensor and laser beam. Also, the compliance does not vary when the tip of the needle is submerged into the solution of the recording chamber, and it does not change with the form of the meniscus or the position within the chamber. However, positional signals are affected by such alterations. For example, evaporation of fluid from the recording chamber can therefore cause slow shifts in the baseline of long-term measurements.

Resonance frequency. We measured the resonance frequency of our force sensors by exposing them to a sudden unloading by retracting a piezoelectric translator from the force transducer tip such that the force sensor became suddenly unloaded and began to oscillate. Such a calibration maneuver is shown in Fig. 2C. In Fig. 2C, inset, an expanded view is shown of a freely vibrating transducer. The resonance frequency of a 75-μm-thick steel foil with an attached tungsten needle submerged in solution and a geometry such as that shown in Table 1 was 2.33 kHz. The resonance frequency with the tip of the needle in air deviated by ~1%.

Resolution. The noise of the output voltage of the force transducer system for a 75-μm-thick steel foil was ~150 mV peak to peak (p-p), corresponding to a displacement of 2.1 nm and a force of 110 nN. Therefore, we can resolve 0.34 nm root mean square (rms) and 18 nN rms at a signal bandwidth of 16 kHz. The noise of the transducer consists of several components, including 1) photon shot noise, which represents the statistical fluctuation in a steady state and finite photocurrent; 2) intensity fluctuations of the output of the laser; and 3) dark noise that originates in the electronics necessary to amplify and condition the output signal. Neither photon shot noise nor dark noise limits the resolution of our force transducers. Also, the noise was independent of whether a cell was attached or not.

### Table 1. Transducer specifications

<table>
<thead>
<tr>
<th>Geometry</th>
<th>Thickness of Steel Foil</th>
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<tr>
<td>b, mm</td>
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<tr>
<td>b*, μm</td>
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</tr>
<tr>
<td>l1, mm</td>
<td>2</td>
</tr>
<tr>
<td>l2, mm</td>
<td>1</td>
</tr>
<tr>
<td>l3, mm</td>
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<tr>
<td>Sensitivity (gain factor 100), V/μN</td>
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<tr>
<td>Resolution (signal bandwidth 16 kHz)</td>
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<tr>
<td>rms nN</td>
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<tr>
<td>Linear range</td>
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<tr>
<td>Applied force, μN</td>
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<tr>
<td>Tip displacement, μm</td>
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</tr>
<tr>
<td>Compliance, mN</td>
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</tr>
<tr>
<td>Resonance frequency, kHz</td>
<td>3.0</td>
</tr>
</tbody>
</table>

b, Total width of transducer element; b*, width of compliant element of transducer (when constructed with aperture); L, free overall length of transducer; l1, total length of transducer foil; l2, length of compliant element of transducer (length of rectangular aperture); l3, length of tungsten needle; rms, root mean square.
vibrations were reduced by mounting the system onto an antivibration table such that they did not contribute detectable signals to the noise of the transducer.

The photon shot noise has an amplitude of $8 \text{ mV}_{\text{p-p}}$, whereas dark noise contributes with an amplitude of $9 \text{ mV}_{\text{p-p}}$ to the total noise signal ($150 \text{ mV}_{\text{p-p}}$). At low frequencies ($<10 \text{ kHz}$) the dark noise in our electronics is due to Johnson noise originating in the feedback resistors. There is additional noise at higher frequencies that is amplified because of the large bandwidth of the instrumentation amplifier. If we limit the bandwidth of the output signal with a 10-kHz RC low-pass filter (see Fig. 1C), this noise can be reduced to $1.2 \text{ mV}$.

The total noise is dominated by the fluctuations in the output beam of the diode laser. Using a test configuration without the force transducer, we observed 1) that the laser output fluctuates on the order of $0.05\% \text{ p-p}$ of the total intensity and 2) that the noise components of the two halves of the beam reaching the individual diodes of a photodetector are not well correlated. Therefore, the noise components in the photocurrents of the diode does not cancel; rather, they add in square. On the basis of the individual photocurrents of $0.2 \text{ mA}$, a transimpedance gain of $10,000 \text{ V/A}$, and the 100-fold gain of the instrumentation amplifier, we calculated an output noise on the order of $141 \text{ mV}$ (see Fig. 1C), which agrees well with the observed noise in the signal.

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Fig. 2. Transducer calibration. A: force sensitivity. A transducer made of 75-µm-thick steel foil was calibrated by exposure to rectangular force pulses with amplitudes ranging from 1 to 40 µN. Inset: calibration procedure with 3-µN force pulses. Top trace: applied force pulses ($F_{\text{app}}$); output of calibrating transducer ($V_{\text{out}}$). Main plot shows output signal of force transducer vs. output of calibrating transducer (means ± SD, n = 6). Force transducer sensitivity is given by slope and is $0.45 \text{ V/µN}$ at a gain of 33. B: compliance. Inset: amplified output signal ($V_{\text{out}}$) of force transducer system (bottom trace) in response to displacement pulses generated by moving transducer tip with a piezoelectric translator (top trace). Main plot shows resulting relationship between force and applied displacement ($\Delta L$; means ± SD, n = 6). Forces were calculated from output signals using sensitivity calibration. Slope of straight line is $49.8 \text{ N/m}$, corresponding to a compliance of $-0.02 \text{ mN}$. Relation was linear over entire range ($r^2 = 0.9996$). Tip displacements ranged between 45 nm and 1 µm. C: resonance frequency. A piezoelectric translator was used to displace the transducer tip. Rapidly retracting the piezoelectric translator from the force transducer system (bottom trace) initiates oscillations, starting at 1st marked position (vertical dashed line). Top trace: movement of piezoelectric translator. Bottom trace, deflection of force sensor. Peak (2nd marked position) in deflection of force sensor results from braking adhesive forces at contact between tips of the force transducer and piezoelectric translator. Resonance frequency can be determined directly from frequency of oscillation. Inset: expanded view of oscillations. During all calibration procedures, tip of force transducer was submerged in solution. Arrow, moment of braking adhesive forces.
Clearly, fluctuations in the laser beam overwhelm all other noise sources and therefore represent a main limitation for increasing the resolution of our transducer system.

**BIOLOGICAL APPLICATIONS**

To demonstrate the functionality of our force transducer, we performed some biological measurements on single cardiac muscle cells. We attached single skinned rat ventricular myocytes to our force transducer and imposed rapid release-restretch maneuvers on these cells while relaxed or calcium activated. These examples confirm that our system is well suited for physiological measurements and is capable of accurately resolving isometric forces and rate constants of force redevelopment.

**Experimental protocol.** For our physiological measurements we modified an inverted microscope (DMIL, Leitz, Wetzlar, Germany). The standard microscope stage was replaced by a 280 × 350-mm square, 20-mm-thick aluminum plate without affecting the optical performance of the microscope. This stage was firmly attached to the microscope and supported with four solid aluminum posts from the surface of an antivibration table (Melles Griot, Bensheim, Germany) on which the entire setup rested. On this new microscope stage we assembled the force transducer, a piezoelectric translator, and a manipulator for a series of small troughs so that we could visualize single myocytes with the aid of the inverted microscope and attach them between the force transducer and piezoelectric translator. The force transducer system and piezoelectric translator were mounted to separate mechanical micro-manipulators, which allowed us to precisely manipulate them. A glass capillary with a 1-mm-long tungsten needle was attached to the piezoelectric translator. A schematic of the setup is shown in Fig. 3A.

For measurement of isometric forces and rate constants of force redevelopment, a single cell was mounted between the needles attached to the force sensor and piezoelectric translator. A suspension of enzymatically isolated cells (modified from Refs. 9 and 11) and skinned cells (modified from Ref. 5) was inspected at a magnification of 500 or 800, and suitable myocytes were selected and attached to the ends of the force transducer and piezoelectric translator in the following manner. First, the tips of the tungsten needles were coated with a thin layer of a one-component polyurethane varnish. In preactivating solution (modified from Ref. 5) the varnish-coated tips were then lowered and gently pressed onto the ends of a selected cell. The varnish was allowed to polymerize for 5 min, and subsequently the cell was washed twice in preactivating solution. The sarcomere length was adjusted to

![Fig. 3. A: schematic showing setup of apparatus used for measuring isometric force and force redevelopment of single skinned ventricular myocytes. Cells were mounted with aid of a compound microscope between the tungsten needles of force sensor and piezoelectric translator using a 1-component polyurethane varnish. Piezoelectric translator is used to apply length changes to cardiac myocyte. B and C: biological application, with force responses to rapid release-restretch maneuvers. Amplitudes of length changes were 20% of total cell length. In B, cell was relaxed (pCa 8); in C, cell was activated (submaximal activation, pCa 5.33). Cell length, 69 μm; cell diameter, 18.4 μm. Top traces: displacements during 4 release-restretch maneuvers. Bottom traces: force signals. Sarcomere length, 2.3 μm; temperature, 12°C; ionic strength, 170 μM.](http://ajpheart.physiology.org/)

Downloaded from http://ajpheart.physiology.org/ by 10.220.32.246 on October 14, 2017
2.3 µm with the aid of a reticle in the ocular. Myocytes were activated by altering the free calcium concentration in the physiological bathing solution (modified from Ref. 5). These exchanges were accomplished by rapidly switching between troughs with different experimental solutions.

Release-restretch experiments were performed according to a procedure modified from a published method (6). Myocytes were cycled between isometric and isotonic conditions through rapid length changes of ~20% of total cell length introduced by the piezoelectric translator. After a 120-ms interval of unloaded shortening, the cells were rapidly restretched to their original length and held under isometric conditions until active force redeveloped to a steady state. The bandwidth of the displacement signal was limited to ~200 Hz to avoid oscillations of the force transducer. Force redevelopment after rapid release-restretch was interpreted as the result of redistribution of cross bridges between non-force-generating and force-generating states. In Fig. 3, B and C, registrations in preactivating and activating solution are shown. Records in both preactivating (pCa 8) and activating solution (modified from Ref. 5; pCa 5.33) are from the same myocyte. Under preactivating (relaxing) conditions only a small passive response and no active force redevelopment was detectable. When the same myocyte was activated in a solution containing a free calcium concentration of ~4.7 µM (pCa 5.33), it repeatedly developed isometric forces of ~8 µN, and the rate constant of force redevelopment was ~3 s⁻¹.

DISCUSSION

The design and operation of a novel force transducer system for measuring mechanical properties of single skinned ventricular myocytes has been described. The arrangement presented here is mechanically and electrically simple. It consists of a laser diode, a steel foil with an attached tungsten needle, a dual-photodiode detector, two current-voltage (I-V) converters, and an instrumentation amplifier. In response to external forces, the steel foil bends and the laser beam, which is reflected off the steel foil, is deflected. Deflections of the beam are detected with the dual-photodiode detector. We calibrated our force transducers to force and displacement levels exceeding those maximally generated by single myocytes by more than fourfold. The output signal behaved very linearly, and the compliance was constant over the entire calibration range.

An important advantage of this system is its versatility. It is easy to fabricate transducers with different compliance and resolution by varying the geometry and material of the cantilever. Therefore, the transducer can be adapted to different kinds of experiments. Cantilever foils with various lengths or widths can be easily made from stock steel foil by cutting them out with sharp scissors or a paper cutter. The cantilevers can be further modified by etching them in acidic solutions or by cutting them with a focused laser. In designing a transducer for the mechanical analysis of single cardiac cells, we explicitly used this versatility to balance sometimes counteracting requirements for the force sensors such as low compliance, high sensitivity, high resonance frequency, high reflectivity, mechanical rigidity, size, and longevity.

A limitation of the use of steel foils as cantilevers is their optically imperfect surface. Part of the reflected laser beam is scattered, which leads to a significant background signal in its Gaussian intensity distribution. Such a background reduces the sensitivity of the laser detection system. In addition, the optical quality varies over the surface of the cantilever, and it is therefore possible that the intensity distribution within the spot becomes different if the position of the steel foil relative to the laser beam is changed. It is necessary to recalibrate the sensitivity of the force transducer after a removal of the cantilever from the transducer assembly.

A second limitation of our transducer design is the sensitivity of the baseline signal of the transducer to changing solution levels in the recording chamber. For example, solution changes when activating skinned myocytes bring about a shift in the baseline signal, and evaporation of solution will lead to slow drifts. These problems can be avoided by completely submerging the cantilever. Our measurements, however, were not affected by such baseline shifts because we subjected our cells to release-restretch maneuvers. This allowed us to determine the zero force level during each release. Therefore, we decided to only immerse the tip of the tungsten needle to avoid other problems, such as protein adsorption with deterioration of the cantilever surface and glue joints, that are associated with submerging the entire cantilever.

Our force sensor system is also sensitive to air currents. Typically, we observed a shift in the baseline of ~0.16 µN/s. Simple experiments with a screen around the setup showed that the effects of air movement can be significantly reduced. Again, in our experimental protocol, slow drift had little influence on the measurements. Under our experimental conditions the time course of the drift was slow in comparison to the time course of the force redevelopment, so it had very little effect on the shape of force redevelopment curves. For convenience we refrained from shielding the setup from air currents; however, shielding might be necessary under conditions in which smaller forces need to be recorded over a longer duration.

The resolution of our force sensor is limited to ~18 nN rms and 0.34 nm rms. Two factors determine this limit: 1) the sensitivity and 2) the noise floor of the detector system. It is possible to increase this resolution limit, while keeping all other parameters unchanged, by relative simple means. The sensitivity in the current setup is limited by the imperfect reflection of the laser beam off the steel cantilever. Simple calculations show that the sensitivity should be more than fivefold increased if the reflected spot has a Gaussian intensity profile without a (scattered) background. One strategy to improve the resolution would be to polish and coat the steel foils with reflective layers (e.g., silver), similar to procedures employed in the
production of optical mirrors. Alternatively, one could simply try to find (steel) foils with a better reflectivity. For example, when we used 75-µm-thick foil instead of 25-µm-thick foil, we could increase the sensitivity by 16-fold (see Table 1). The noise floor of the position signal is determined by the fluctuations in the beam of the diode laser. This noise can be decreased by reducing the bandwidth of the I-V converters. Because the mechanical resonance frequency of the transducer is \( \sim 3 \) kHz, the bandwidth of the electronics could be reduced from 16 kHz to 3–4 kHz without a loss of temporal information and the signal-to-noise ratio would improve more than twofold. Reductions of the intensity fluctuations of the laser with laser intensity stabilizer (liquid crystal or acoustooptical devices) might also be possible but would be considerably more complex and expensive. The most effective way to increase the resolution without altering the mechanical properties of the force transducer would be to improve the optical properties (reflectivity) and reduce the bandwidth of the output signal to the mechanical resonance frequency of the cantilevers.

Force transducers for the proposed mechanical experiments on single cardiac muscle cells require low compliances of \(-2 \times 10^{-2}\) mN to ensure quasi-isometric conditions and resonance frequencies \(>1\) kHz to prevent oscillations during release-restretch maneuvers. We designed our transducers to exactly satisfy these mechanical criteria. The compliance of all of our cantilevers was between 1.9 and 2.6 \( \times 10^{-2}\) mN. Only the compliances of capacitive force sensors and those used in atomic force microscopy have similar (adequate) values. Other systems that have been developed to investigate muscle mechanics at the single cell level are characterized by transducer compliances that are much higher. For example, optical fibers (2, 13, 19, 20, 27) or other cantilever beam systems (7, 8, 26, 28) all have compliances that are larger by a factor of 10–10³ than is acceptable for quasi-isometric conditions.

Our force sensors have resonance frequencies between 2 and 3 kHz and are underdamped. Because of the small size and the high stiffness, these sensors cannot be critically damped by being immersed in aqueous solution. Therefore, unstable oscillations must be avoided by limiting length perturbations to a band of frequencies well below the resonance frequency of the sensor. The force transducer from Cambridge Technology includes one model (406A) with comparable sensitivity but insufficient temporal resolution (resonance frequency 100 Hz). Therefore, these transducers are quite unfit for mechanical measurements on cardiac myocytes employing rapid releases or length changes. Although the electromagnetic transducer design (16) and microfabricated force transducers (3, 23, 24) can reach resonance frequencies well above 1 kHz, it would be inappropriate to attach single cardiac cells to them.

In summary, our force transducers are well suited to measure forces developed by cardiac myocytes with nanoNewton resolution. Our force sensors are stiff enough to maintain essentially isometric conditions (\(\Delta L < 0.2\%\) of total cell length) even when the cells are maximally activated. The resonance frequency of our system is \(\sim 2–3\) kHz, providing sufficient temporal resolution to accurately follow force redevelopment after rapid length changes. Exemplary biological measurements on skinned single cardiac cells demonstrated that our system has ample stability as well as sufficient spatial and temporal resolution for recording of force signals in response to the proposed mechanical perturbations.

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Address for reprint requests and other correspondence: B. Brenner, Medical School Hanover, Dept. of Molecular and Cellular Physiology, Carl-Neuberg-Str. 1, D-30625 Hanover, Germany (E-mail: brenner.bernhard@mh-hannover.de).

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REFERENCES


