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Novel Microelectromechanical System Force Transducer to Quantify Contractile Characteristics from Isolated Cardiac Muscle Cells

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ABSTRACT

A custom-designed microelectromechanical system (MEMS) force transducer, with a volume less than 1 mm^3, is being fabricated to measure force development in isolated cardiac muscle cells to elucidate the physiology of muscle contraction. A single heart cell is attached to flexible, hinged polysilicon plates submerged in a nutrient saline solution. As the cell contracts, the plates bend, and the contractile force can be measured based on the known spring constant of the plate. The amount of deflection is measured by piezoresistive, ion-implanted strain gauges placed at the base of the plates. Prototype structures have been fabricated and have been mechanically tested using probes. We have demonstrated that living rat heart cells can be attached to polysilicon using a silicone sealant. Polysilicon is an inert material when exposed to cardiac cells and their saline environment and has no effect on the cells themselves.

The measurement of contractile force in the micronewton range from single ventricular cells isolated from rat hearts has proven difficult with current transducer technology. Previously, the force transducers used to determine the contractile characteristics from isolated heart cells have had to be positioned outside the cell's saline bath and attached to the cells via complex and relatively massive structures which inherently limit frequency response and sensitivity.[1-3] By necessity slender pipettes or needles must enter the bath and are subject to surface tension forces that can be greater than that of the cell force. Thus, the force and stiffness data obtained are often difficult to interpret.

By taking advantage of microelectromechanical systems (MEMS) we have designed a novel, custom-made force transducer which permits force and stiffness measurements on isolated living heart cells. This force transducer eliminates some of these problems by shrinking the entire system to less than 1 mm^3. This smaller system allows higher frequency measurements and can be fully submerged in the saline bath. With this transducer, we will be able to not only measure direct force, but also the complex stiffness modulus from isolated rat cardiac cells at a range of oscillatory frequencies in excess of 1 kHz, well above our currently attainable limit of 100 Hz. With this higher fidelity, we hope to correlate our stiffness results to the key events in the crossbridge cycling scheme in which molecular actin and myosin work together to generate force in muscle.[4,5] Other workers have performed experiments of this type at lower frequencies.[3,6] However, the interpretation of the data is difficult due to the confounding variables of the extracellular collagen matrix and attachment compliance.

The direct measurement of twitch forces (*i.e.,* transient response) up to 50 μN from isolated rat cardiac cells in a 1kHz bandwidth is also feasible. Usually, cell membranes must be removed to ensure strong attachment to most probes, and the data gathered from contractile forces in response to a Ca^{2+} stimulus has been restricted to demembranated (skinned) preparations and bandwidth-limited data acquisition systems.[7,8] Known methods for measuring

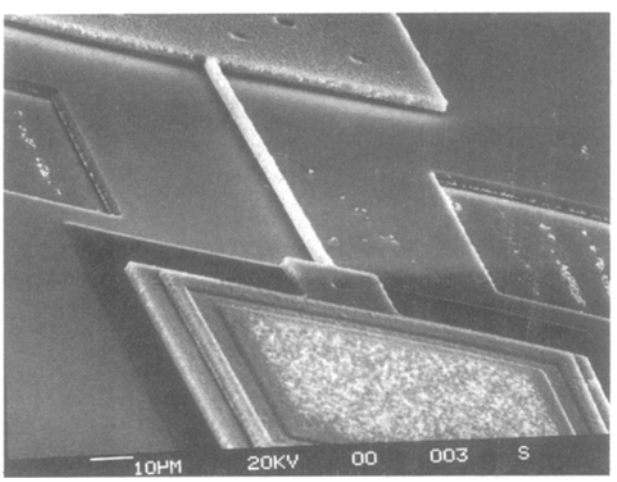

Fig. 1. Perspective view of a first version test structure. Beam is 100 μm long and 2 × 2 μm in cross section.

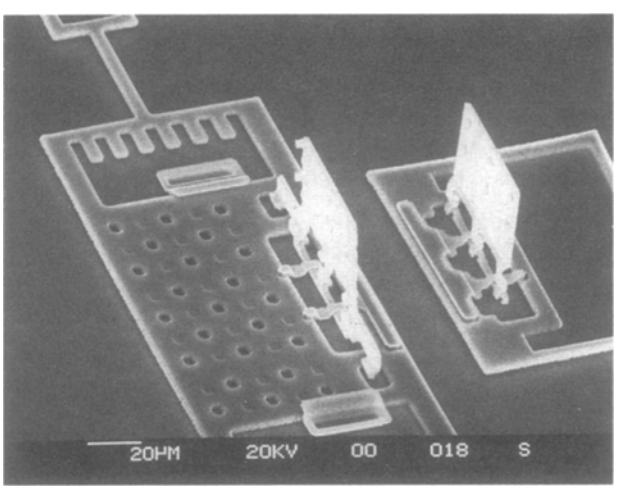

Fig. 2. SEM photograph of second version force transducer. Hinged 50 × 50 μm plates are water assembled; one plate is free to slide while the other is fixed to the substrate.

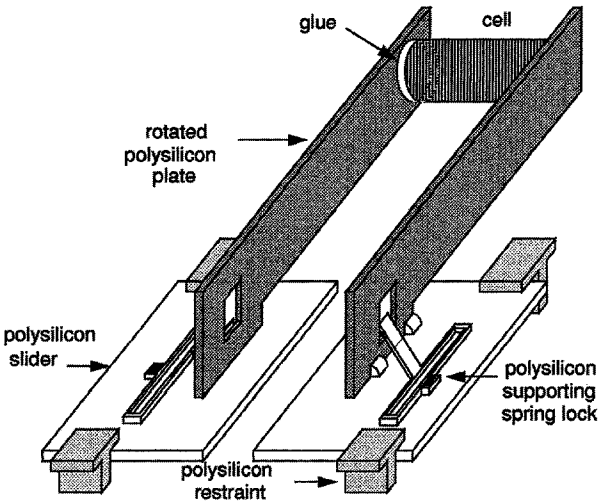

Fig. 3. Schematic drawing of current force transducer design (version 3). Structure will be water assembled and can accommodate various cell lengths.

cropipettes, glass microneedles, and more elaborate setups such as aluminum T-clips attached to commercially available force transducers.[1,9-11] Our force transducer should permit the membranes to remain intact for more physiologically realistic measurements, and will operate at a higher sensitivity and wider bandwidth than other transducers currently used.

Experimental

We have designed and fabricated several prototype structures using a commercially available MEMS process (MCNC, Research Triangle Park, NC). The first version of the force transducer consists of a polysilicon beam of varying length (100-200 μm) with a 2 × 2 μm in cross section. One end of the beam was attached to a 100 × 100 μm freestanding pad while the other end was attached to the substrate (Fig. 1). It was designed such that a cell could be attached at each end to a freestanding pad. When the cell is externally electrically stimulated to contract, it deflects the beams. The amount of deflection is proportional to the force exerted using Hooke's law: $F = kx$, where F = force, k = known spring constant of the beam, and x = beam displacement measured visually using a calibration grid located near the beam. This grid consists of 5 μm aluminum lines and spaces allowing 1 μm resolution.

A second version of the force transducer was developed in response to the need for vertical cell-attachment sites. This force transducer utilizes two polysilicon plates that are rotated 90° out of the surface of the wafer using microfabricated polysilicon hinges.[12] No manual assembly is required because structures assemble automatically in the final rinse step of the process. One rotated plate is attached to a sliding polysilicon plate suspended above the substrate by a cantilever beam (Fig. 2). On contraction, the beam bends and the amount of deflection is measured visually.

The current design (version 3) has a structure similar to version 2 and operates under the same premise. Instead of beams, longer rotated plates are used and incorporate spring locks for futher support. The rotated plates are mounted on sliders that allow for the adjustment of the spacing between these plates to accommodate various cell lengths (Fig. 3). The cell is glued to the plates using a commercially available silicone sealant. Once the cell is attached to the plates, it is externally electrically stimulated to contract, pulling the plates inward. Plate deformation is measured using a video camera, and the spring constant of the plates is calculated using the dimensions of the plates. The resulting force is measured at various levels and rates of excitation.

Force Calculation

We can compute the force exerted via Hooke's law, with k the known spring constant of the beam. For long thin isotropic beams, the spring constant can be written

$$k(a,b,L) = \frac{a^3 bE}{4L^3} \quad [1]$$

where E = Young's modulus (a material constant), L = length of beam, and a and b are the dimensions of the cross section of the beam (or plate).[13] Depending on processing conditions for polysilicon, $E_{poly} = 100$ GPa.[14] Thus, for the 100 × 2 × 2 μm beam used in version 1 of the force transducer, $k = 0.4$ N/m. Given a minimum resolvable displacement of 1 μm in the beam (using the calibration grid) we can calculate the minimum force we expect to resolve

$$F_{min} = kx_{min} = \frac{0.4\text{N}}{\text{m}} \times 1\ \mu\text{m} = 0.4\ \mu\text{N} \quad [2]$$

A large ventricular cardiac cell is ~ 150 μm long and on contraction the cell can shorten to at most 30% of its original length. Assuming the cell shrinkage is equal on both sides, this indicates that Δx = 25 μm per side, corresponding to a cellular force of $F = 10^{-5}$ N for a 100 μm beam. Similar calculations can be done with version 2 and 3 transducers, resulting in sensitivities ~ 4 μN given a 1 μm minimum displacement.

To calculate the maximum force sustained before fracture, we must take into account the strain limit of polysilicon, which has been measured to be as high as $\epsilon_{max} = 1.7\%$ according to Fan *et al.*[15] The surface strain along a beam is described by

$$\epsilon(x) = \frac{a}{2\rho\ (x)} \quad [3]$$

where a = height of the beam (2 μm), and ρ = radius of curvature of the beam

$$\rho(x) = \frac{EI}{F(L\text{-}x)} \quad [4]$$

where E = elastic modulus and I = moment of inertia for a cantilever beam. With

$$I = \frac{a^3 b}{12} \quad [5]$$

then

$$\epsilon(x) = \frac{aF\ (L\text{-}x)}{2EI} \quad [6]$$

The maximum strain occurs at the base of the beam (*i.e.*, $x = 0$). Hence

$$\epsilon_{max} = \epsilon(0) = \frac{aFL}{2EI} \quad [7]$$

with $\epsilon_{max} = 1.7\% = 0.017$

$$F_{max} = \frac{(0.017)\ 2EI}{aL} \quad [8]$$

For a 100 μm beam, $F_{max} = 2.3 \times 10^{-5}$ N. Similar calculations can be done with version 2 and 3 force transducers, resulting in maximum forces up to 4×10^{-4} N. In terms of expected forces from the cardiac cells themselves, Hofmann and Moss measured the maximum tension in rat ventricular myocytes and found it to be 1.41 mg = 1.41×10^{-5} N.[7] Thus, a 100 × 2 × 2 μm beam should not break during operation.

Results and Discussion

We have demonstrated that cells will stick to polysilicon in which the silicone sealant was applied to a wafer with a 3200 Å polysilicon layer (Dow Corning silicone rubber sealant). Some isolated cells placed on top of the wafer became fixed when the glue was applied with a probe. After drying, the cells could not be removed with a second probe. Using the version 1 test structures, we have developed a silicone sealant application technique for depositing the glue in a localized area comparable to the cross section of a typical cardiac cell (approximately 20 × 40 μm).

The version 1 structures also were used to test the biocompatibility of silicon with cardiac cells as well as the flexibility of silicon. The force transducers were submerged in 500 *mM* KCl for approximately a month and showed no signs of chemical degradation or erosion. Further, experiments in which the polysilicon plates are present in a nutrient solution with isolated cardiac myocytes for several hours show no evidence of adverse effects.

The beams were mechanically displaced outside solution and showed varying amounts of flexibility. Using probes we found that the 100 μm beams could sustain a deflection up to 40 μm before failure. This corresponds to a maximum force of 1.6×10^{-5} N and a maximum strain of 1.2%, comparable to the ϵ_{max} found in the literature.[15] The 200 μm beams can bend as far as 80 μm before breaking; $F_{max} = 4 \times 10^{-6}$ N, $\epsilon_{max} = 0.6\%$. Using Eq. 8, we expect $F_{max} = 1.13 \times 10^{-5}$ N for a 200 μm beam if $\epsilon_{max} = 1.7\%$.

Using the version 2 structures, we studied the reliability and repeatability of water assembly of rotated plates. About 30% of the structures assemble in the final deionized (DI) water rinse, but after drying only a few plates remain standing. Most fell and remained fixed such that probing in an attempt to resurrect the plates resulted in cracked or broken structures. Assembly with probes was also done in a liquid environment. For liquid assembly, the wafer was immersed in a petri dish of methanol immediately following the release etch and DI water rinse. The liquid environment eased assembly but, on drying, most plates fell back down as the last drop of methanol evaporated. Surface tension on the 50 × 50 μm plates in methanol is probably responsible for pulling these loose hinged plates down. Thus, in version 3 spring locks are placed strategically near the hinges to ensure that rotated plates stay in position after the wafer is dried.

Future Work

The latest (version 3) test structures have been fabricated. The force on the plates can be calibrated using a probe attached to a microammeter driven by a known current source; this calibration can be done in a liquid environment. In the future, strain sensitive resistors and electronics will be integrated with the structures into a single unit, completing an entire actuator and force transducer data acquisition system that is still on a submillimeter scale and fully submersible in saline solution, thus decreasing the mass of the system and eliminating surface tension problems (Fig. 4). This technological advance allows the measurement of force and stiffness modulus at significantly increased fidelity and permits rigorous investigations of cardiac functions not previously feasible. Given the noise limits in the sensors and the mechanical bandwidth of the system, we should be able to resolve nanonewton forces in a 10 kHz bandwidth. For example, for a 1 kΩ resistor (strain gauge) at room temperature, we model the noise characteristics using the Johnson (thermal) noise equation

$$\bar{v}_{\text{noise (thermal)}} = \sqrt{4k_B\,TR\,(\text{BW})} \approx 0.4\ \mu\text{V} \qquad [9]$$

We expect a reasonable operational amplifier to produce ~ 100 nV(Hz)$^{-1/2}$ of noise, so for a 10 kHz bandwidth

$$\bar{v}_{\text{noise (op-amp)}} = \frac{100\ \text{nV}}{\sqrt{\text{Hz}}} \times \sqrt{10\ \text{kHz}} = 10\ \mu\text{V} \qquad [10]$$

There are many other sources of noise, such as current noise, etc. However, once we take all these effects into account, we can estimate the overall noise to be around twice that of the op-amp, (assuming that all other noise accounts for about 10 mV).

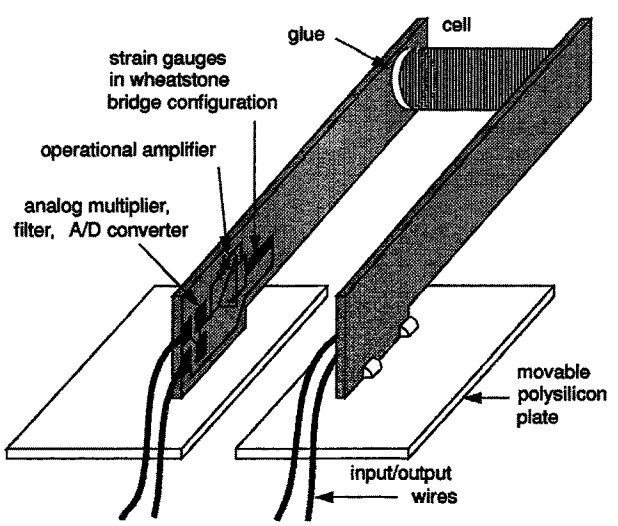

Fig. 4. Schematic drawing of the future version of the force transducer. All electronics and sensing elements are integrated on-chip and fully submersible in saline solution.

Thus

$$\bar{v}_{\text{noise}} \approx 20\ \mu\text{V} \qquad [11]$$

This noise level gives us a lower limit on the minimum detectable voltage change (ΔV). We can obtain a reasonable value for the minimum detectable strain

$$\epsilon_{\text{detectable}} = \frac{\Delta V}{GV_o} \qquad [12]$$

where G = gauge factor = 20 for polysilicon. Setting $\Delta V = 20\ \mu$V (the noise level) and V_0 = input voltage = 1 V, then $\epsilon_{\text{detectable}} = 10^{-6}$. From the minimum strain we can determine the minimum detectable force. In general

$$F_{\text{detectable}} = \frac{Ea^2b}{6L} \times \epsilon_{\text{detectable}} \qquad [13]$$

where E = elastic modulus, a and b are dimensions of the cross section of the beam. Rearranging,

$$\epsilon(F) = \frac{aFL}{2EI} = \frac{aFL}{2E} \times \frac{12}{a^3b} = \frac{6FL}{Ea^2b} \qquad [14]$$

With $E = 10^{11}$ Pa, $a = b = 2\ \mu$m, $L = 100\ \mu$m, and $\epsilon_{\text{detectable}} = 10^{-6}$, we expect $F_{\text{detectable}} = 1.33 \times 10^{-9}$ N. A similar analysis can be done using the dimensions of the beams in version 2 and 3 force transducers, also resulting in minimum detectable forces on the order of nanonewtons.

It has been demonstrated that integrated circuits can be combined with the same three-dimensional hinged structures used thus far.[16] Thus, the leap to total integration between the mechanical force transducer, strain gauges, and signal processing is reasonable. With this kind of a force measurement system we can begin to uncover the fundamental processes in muscle contraction with a much higher resolution than that offered by currently available technology.

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