

Micro-scale force-transducer system to quantify isolated heart cell contractile characteristics [☆]

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Abstract

A custom-designed microelectromechanical force transducer, with a volume of less than 1 mm³, is being developed to quantify forces generated in isolated cardiac muscle cells. A single heart cell will be attached to flexible, hinged polysilicon plates submerged in a nutrient solution. As the cell contracts, the plates will bend, and the contractile force can be measured based on the known spring constant of the plate and the amount of deflection. Prototype structures have been fabricated and have been mechanically tested. We have demonstrated that living rat heart cells can be attached to polysilicon using a commercial silicone sealant. We have also observed that polysilicon is an inert material when exposed to cardiac cells and their saline environment, and has no detectable effect on the cells themselves.

Keywords: Force transducer; Heart cells; Micomechanics

1. Introduction

The purpose of this research endeavor is to study the passive and active mechanical properties of individual heart cells. By taking advantage of microelectromechanical systems (MEMS), we have designed a novel, custom-made force transducer that will permit force and stiffness measurements on isolated living cardiac myocytes. With this device we intend to elucidate the complex physiology of cardiac muscle at higher fidelities than previously attainable.

Up to now, the force transducers used to determine the contractile characteristics from cardiac cells have been relatively large in scale compared to the cells themselves [1-3]. The bending portion of most macro transducers used to obtain readings of important quantities such as force development and complex (oscillatory) stiffness modulus (stiffness/area) is usually orders of magnitude greater in mass than the specimen. Thus, these massive systems are inherently limited in frequency response and sensitivity and must be positioned outside

the cell's saline bath. Forces due to surface tension cannot be ignored in these cases, and can make the force and stiffness data obtained from cells difficult to interpret.

The force transducer that we are designing will eliminate some of these problems by shrinking the entire system to less than 1 mm×1 mm×1 mm. This smaller system will allow higher frequency measurements to be made and can be fully submerged in the saline bath. With our force transducers, we intend to measure the complex stiffness modulus from isolated rat cardiac cells at a range of oscillatory frequencies in excess of 1 kHz, as opposed to the hundreds of hertz previously attainable. With such a high-frequency measurement, we hope to resolve some of the key issues in the source of muscle force development at the molecular level. Other workers have performed experiments of this type at lower frequencies and with larger, multicellular preparations [3,4]. However, the interpretation of the data is very difficult due to the confounding variables of the extracellular collagen matrix and attachment compliance inherent in the larger specimens.

Additionally, we expect to resolve micronewton direct twitch forces (i.e., transient response) from isolated rat cardiac cells in a 1 kHz bandwidth. Usually, cell mem-

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branes need to be removed to ensure strong attachment to most probes. Also, the data gathered from contractile forces in response to a chemical Ca^{2+} stimulus or an electrical stimulus have been restricted to demembrated preparations and bandwidth-limited transducers [5,6]. Known methods for measuring the force exerted by a contracting muscle cell involve rather elaborate set-ups consisting of suction micropipettes, glass cantilever beams, carbon fibers, or aluminium T-clips attached to commercially available force transducers [1,9]. Most of these methods are invasive and tear or puncture the cell membranes upon attachment to the transducer; none has demonstrated reliable repeatable measurements from intact cells at high frequency. Our force transducer may allow the membranes to remain in place for more physiologically realistic measurements, and will operate at a higher sensitivity and wider bandwidth than other transducers currently used.

2. Materials and methods

To date, we have designed and fabricated several prototype structures using a commercially available MEMS process that offers two structural layers and two sacrificial layers (MCNC, Electronic Technologies Division, Research Triangle Park, NC 27709, USA). Previous structures range from single cantilever beams to suspended hinged plates [10]. The current design utilizes two $1.5 \mu\text{m}$ thick polysilicon plates that are rotated 90 degrees out of the surface of the wafer using microfabricated polysilicon hinges as described in the literature [11]. The $200 \mu\text{m}$ long plates vary in width from 80 to $160 \mu\text{m}$ and incorporate spring locks for further support. The rotated plates are water assembled in the rinse tank after release. They are mounted on sliders that allow for the adjustment of the spacing between these plates to accommodate various cell lengths (Fig. 1).

The cell will be manually attached to the ends of the rotated plates using a commercially available silicone sealant. The cell is then externally electrically stimulated to contract, pulling the plates inwards. Plate deformation will be measured using a digital-output video camera; the spring constant of the plates is calculated from their dimensions. The resulting force will be measured at various levels and rates of excitation.

Variations on the basic structure have been fabricated, including a 'honeycomb' version in which the cell will be mounted on one side and the glue will be applied on the back side (Fig. 2). This way the glue can seep through the large holes in the grid and fix the cell to the grid. This is a one-step process, while the device in Fig. 1 requires two steps (cell and glue application

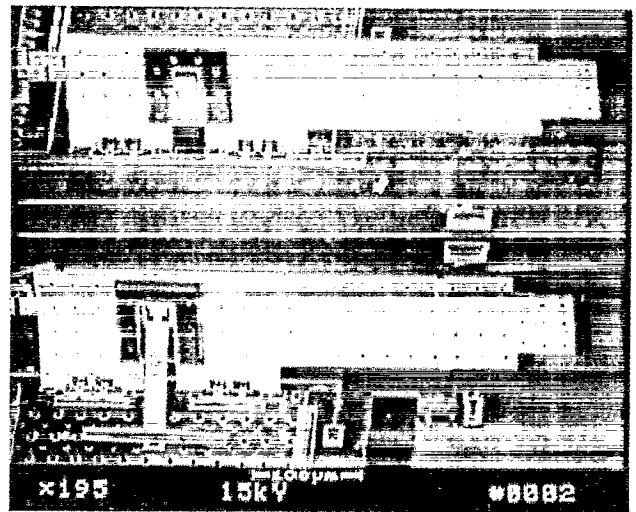


Fig. 1. SEM micrograph of current force transducer design. Spring locks support the rotated plates (bottom left corner). Sliders (bottom edge, middle) are used to allow the flat polysilicon plates to roll forward to adjust for cell size and restrict motion along one direction. Displacement is measured visually with the aid of a video camera. The rotated plate shown here is $200 \mu\text{m} \times 80 \mu\text{m}$, not including the hinges and spring lock.

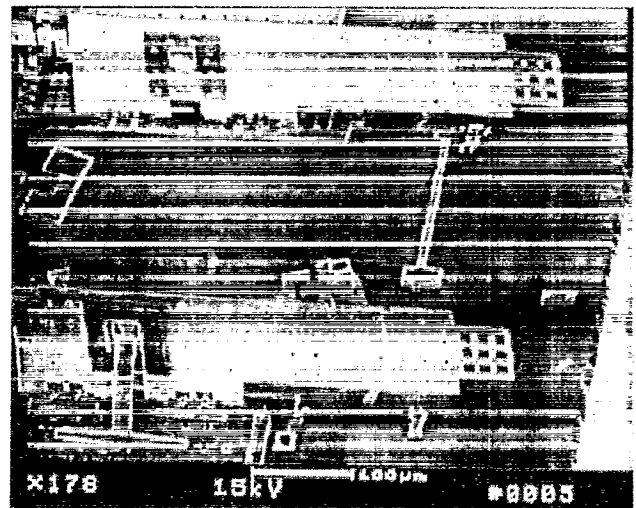


Fig. 2. SEM micrograph of a variation on the current force transducer design. The grid has holes $10 \mu\text{m} \times 10 \mu\text{m}$ large. This variation allows for easier cell attachment by allowing the cell to be positioned in front of the plate while the glue is applied to the back. Glue can seep through the holes and attach the cell to the grid simultaneously.

to the front side separately). Variations in which no glue is required have also been fabricated [12].

3. Results and discussion

To date, we have demonstrated that cells will stick to polysilicon using a commercial silicone sealant (Dow Corning silicone rubber sealant). We have observed that live rat cardiac cells will adhere to polysilicon layers deposited on a silicon substrate [10]. Recently we have actively attached cardiac cells to a rotated

polysilicon plate. Using metal probes, glue was placed in a $50\ \mu\text{m} \times 50\ \mu\text{m}$ area on a $200\ \mu\text{m} \times 200\ \mu\text{m}$ polysilicon plate and fixed cardiac cells were placed in the glue. To test the strength of the bond, a metal probe was used to move a part of the cell that extended past the edge of the hinged plate. The cell and plate remained rigidly stuck together, such that moving the cell also moved the plate.

Prototype test structures were used to test the biocompatibility of silicon with cardiac cells as well as the flexibility of silicon. Polysilicon freestanding plates were submerged in 500 mM KCl for approximately one month and showed no signs of chemical degradation or erosion [10]. Experiments have also been done in which the current version of the force transducers is present in a nutrient solution with isolated cardiac myocytes for several hours; there is no evidence of adverse effects on the cells due to the silicon or damage to the silicon due to the saline environment. Also, the glue itself had no adverse effects on the cells or the silicon structures during the course of the experiment. These results were expected.

The $200\ \mu\text{m}$ long plates were mechanically displaced in air using metal probes attached to micromanipulators. It was found that $200\ \mu\text{m} \times 80\ \mu\text{m}$ plates (e.g., Fig. 1) could sustain an average deflection of $130\ \mu\text{m}$ at the tip of the cell-attachment site before failure. This corresponds to a maximum force of $1.1 \times 10^{-4}\ \text{N}$ and a maximum strain of 0.73%, which is comparable to the ϵ_{max} reported elsewhere [13]. The $200\ \mu\text{m} \times 160\ \mu\text{m}$ beams could bend an average of $142\ \mu\text{m}$ before breaking; $F_{\text{max}} = 2.4 \times 10^{-4}\ \text{N}$, $\epsilon_{\text{max}} = 0.8\%$. Approximately 80% of the structures sustained fracture at the junction between the $200\ \mu\text{m}$ plate and the first hinge. On some occasions, after fracture the remaining portion of the rotated plate still stood vertically, indicating the strength and robustness of the hinges and spring locks.

4. Future prospects

Ultimately strain-sensitive resistors and electronics will be integrated with the structures into a single unit, completing an entire force transducer data-acquisition system that is still on a sub-millimeter scale and fully submersible in saline solution, thus decreasing the mass of the system and eliminating surface-tension problems (Fig. 3). 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. It has already been demonstrated that integrated circuits can be combined with the same three-dimensional hinged structures used so far [14]. 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

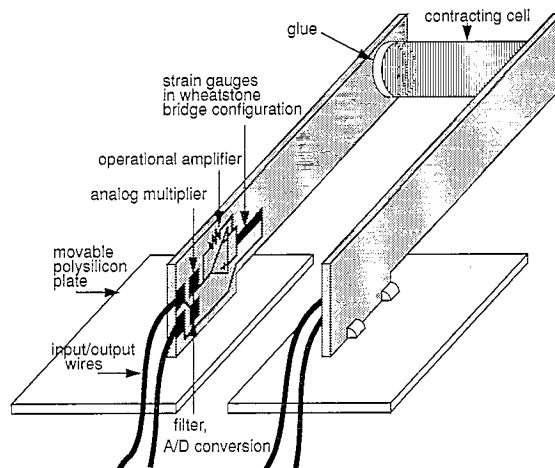


Fig. 3. Schematic drawing of future version of force transducer. Ideally we would like to integrate electronics with the mechanical structures to complete an entire system capable of making measurements in solution. Only the input and output wire would emerge from the saline bath.

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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