

A FEASIBILITY STUDY ON MEMS TEST-STRUCTURES FOR ANALYSIS OF BIOLOGICAL CELLS AND TISSUE

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Abstract- *This research explores the feasibility of developing Microelectromechanical Systems (MEMS) for use as test platforms to study the characteristics of biological cells and tissues. MEMS technology can allow for the study of biological cells and tissues on the cellular level using very small sample sizes as well as having the potential for automation and reduced costs. The advantages and limitations of current experimental techniques are evaluated to investigate the role that MEMS can play in performing biological analysis. In addition, a conceptual MEMS testing device is presented here that will allow for the direct measurement of cellular elasticity and adhesion.*

I. Introduction

Determining the mechanical properties of biological materials, including cells, tissues, and fluids, is important to the advancement in medical research. Current experimental techniques operate primarily using macro-scale equipment, however advances in microelectromechanical systems (MEMS) technology allow researchers to conduct experiments on the micro or even nano-scale. MEMS also offer the potential for improved efficiency and repeatability by automating processes. This paper will discuss current testing methods and demonstrate the need for MEMS based test structures to perform biological analysis of mechanical properties. Further, a proposed surface micromachined test mechanism is developed that will allow for the direct measurement of cellular elasticity and adhesion.

II. Current Testing Methods

Mechanical properties of cells can be determined using a variety of techniques including atomic force microscopy, micromanipulation, and rheoscopy. Each method is described below and has its own strengths and weaknesses.

Atomic Force Microscopy (AFM), originally developed for the study of surface sciences, has emerged as a useful tool for conducting studies on living biological materials. Its operating principle is described in detail in [1,2] and will not be discussed here. AFM can be used to map surfaces and derive mechanical properties such as adhesion, and Young's modulus. These mechanical properties can be determined with very high accuracy by generating force versus distance curves with the AFM and then using the appropriate data analysis to extract the information. The

advantages of AFM are that it can be operated in many modes and environments depending on the need and the material being studied. It may be used on materials ranging in size from molecules to tissues, and even fluids in both gaseous and liquid environments. In addition, the samples require very little preparation. A disadvantage to AFM is the difficulty in maintaining and operating the equipment. There are many potential sources of error that are not easily eliminated, including the sensitivity and calibration of the photodiode, calibrating to determine the spring constant of the AFM cantilever, and the unknown tip geometry of the cantilever. Accounting for all of these factors can be costly and time-consuming, but otherwise, the results for cell elasticity cannot be accurately determined [1]. In addition, extracting the mechanical property data from the force versus distance curves can be computationally expensive [2].

Micromanipulation is a technique used in [3-7] in which individual cells and tissue fibers are subjected to various forces to measure their mechanical properties. This technique primarily uses probes or micropipettes precisely positioned with robotic micromanipulators to apply loads in tension or compression to the cells. The force applied is measured with a commercial force transducer, and the operation is observed through a microscope. Micromanipulation techniques allow for the study of very small particles subjected to forces ranging from 1 μN to cell disruption [3]. In [5], cell indentation or "poking" is used to perform viscoelastic measurements on F9 mouse embryonic carcinoma cells. It was determined that the carcinoma cells are ~20% more resistant to indentation than the control cells. Researchers in [4] determined the force required to burst baker's yeast cells by squeezing them between two parallel plates. The forces required to burst the cells varied from 55 μN to 175 μN , much higher than previously reported values required to burst mammalian cells, 1.5-4.5 μN . In addition to compressive forces, tensile loads may also be applied. In [6], fibroblasts from rabbit patellar tendons were adhered to the ends of micropipettes and then pulled apart to determine elasticity and Young's modulus. Yu and Nelson took a slightly different approach to cell manipulation and automated the process of DNA injection using visual servoing [7]. Cells were held in micromachined holders to aid with the injection. The precision of these setups depends on the abilities of the

micropositioning and force transducers that are chosen. Mahsmoushy *et al.* [4] report motion accuracy of $\pm 0.2 \mu\text{m}$ and force transduction of $\pm 490 \mu\text{N}$ full scale at a resolution of $0.01 \mu\text{N}$.

Another micromanipulation technique to determine the elasticity of cells to deformation and how they recover is aspiration. By inducing suction with a micropipette, the cells are pulled into the tube, deforming from their original shape. The suction pressure is released and the cell is observed as it is expelled and returns to its original shape. Results from this type of experiment yield data on cell deformation and recovery [9]. Aspiration is a good technique for observing the reaction of individual cells and the applied force is easy to calculate, as it is the product of the aspiration pressure and area of the micropipette. One limiting factor is the unknown contact forces between the pipette and the cell.

A rheoscope may be used to determine the adhesion forces of cells to a substrate and also to observe the effects of shear stress on the cells. The device, described in [8] operates by inducing a shear stress on cells and observing the effect it has on the cell attachment. This technique operates on multiple cells in a culture at a time and uses an indirect method of force transduction. The advantages of this technique are that it allows for the direct observation of dynamic forces to the cells. The disadvantages are that the applied force is not directly measured, but rather is estimated from the rotational speed of the plate. This can make it difficult to characterize the true mechanical properties. It is also difficult, if not impossible, to observe the effects on one single cell before and after the forces are applied.

MEMS offer the possibility of moving these measurements into the micro and nano-scale, allowing for new advances and understanding in the field of biomechanics. An example of biological analysis that has been successfully conducted using a MEMS device is given by Lin *et al.* who developed a MEMS device to measure the force produced by a single heart cell [10]. The cell was isolated and glued inside a mechanical clamp at the ends of two micromachined cantilever beams. The cell was then excited using a calcium solution and the contraction force caused the micromachined cantilevers to deflect. By measuring the beam deflection, the cells were found to produce $\sim 12 \mu\text{N}$ of force. These results match those conducted by another study that performed a similar experiment using a carefully hand constructed apparatus with hand-cut cantilever beams that were manually assembled [11]. The MEMS force transducer, while still requiring some manual manipulation to place the heart cell into the device, requires far less tedious experimental setup than that in [11], and because the MEMS devices are bulk

manufactured, the devices will all have almost identical properties.

III. Test Structure for Tensile Analysis

A. Conceptual Device

A MEMS device is proposed that will perform similar functions as those using AFM, micromanipulation, rheoscopy, and aspiration techniques to apply loads to biological samples in order to measure elasticity and adhesion properties. This device applies a tensile load to the sample with one end fixed, thus it can be thought of as a MEMS “tensile testing machine”. A conceptual drawing of the device is shown in Fig. 1. The actuator used is a scratch drive actuator (SDA). This type of actuator is explained in detail in section IV below. The cell is attached across two plates, one fixed and the other moving. The force from the actuator pulls the plates apart and the cell is stretched. By measuring the displacements of the device, the mechanical properties of the cell may be determined.

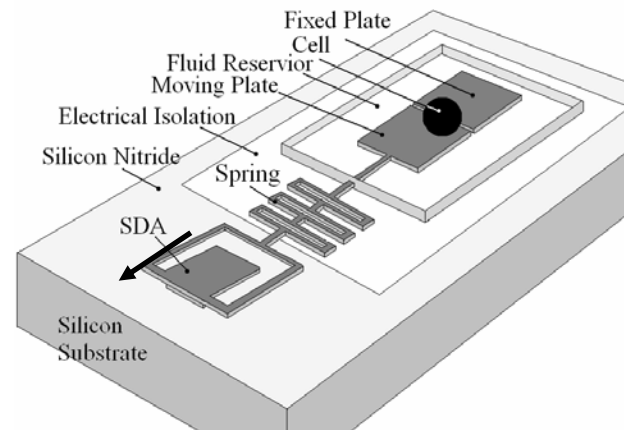


Fig. 1. Conceptual drawing of MEMS tensile testing machine. A scratch drive actuator (SDA) is shown applying a tensile force to a single cell.

B. Operating Principle

The operating principle of the device is shown schematically in Fig. 2(a). The actuator exerts a force on the system thus displacing the linear spring and pulling on the cell. In the micromanipulation techniques described in [3-6], a force transducer is used to measure the level of force being applied to the sample. In order to determine the force being applied to the sample in this system, it is necessary to include an intermediate spring. Simply calibrating the actuator to determine its force output for a given actuation signal will not suffice because friction will be present in the system. At this small scale, friction can be an overriding factor in system behavior and is difficult to predict numerically. Therefore, the net force produced will be determined by measuring the spring displacements at points A and B. The net force, F_{net} , is the force exerted by the actuator minus any losses incurred, such as those due to friction. It is assumed that the spring constant of the

surface micromachined spring, K_S , is known. By measuring the displacements X_1 and X_2 at points A and B, the net force exerted by the actuator, F_{net} , and the elasticity of the biological sample, K_C , may be determined.

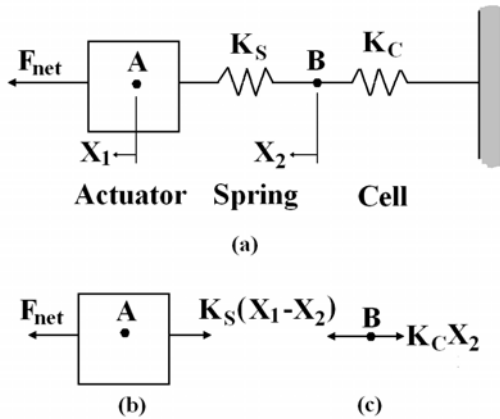


Fig. 2. (a) Schematic of the operating principle of the proposed MEMS tensile test device. (b) and (c) With measurements at points A and B, the relationship between the force and displacements is obtained.

Figure 2(b) examines the forces acting on point A. From the forces acting on point A, the following relationship may be derived to find the net force,

$$F_{net} = K_S(X_1 - X_2) \quad (1)$$

Similarly, by considering the forces acting at point B in Fig. 2(c), the relationship between the measured displacements X_1 and X_2 is used to estimate the elasticity of the biological cell sample, K_C .

$$K_C X_2 = K_S(X_1 - X_2) \quad (2)$$

In addition, the force and displacement data may be used to construct a stress-strain model for the cell. Assuming the material exhibits elastic behavior, the Young's modulus may be found.

This technique is similar to that used to study forces generated by an electrostatic microengine [12]. In this example, the forces exerted on the drive pin were calculated from measuring the position vectors of the moving components of the microengine. By knowing these position vectors, their corresponding velocities, the geometry and masses of the device components, the forces are calculated.

IV. Design Issues

A. Actuator

In order to provide the tensile loading on the sample, an actuator must be chosen that can supply the appropriate

range of forces with sufficient resolution. There are a variety of MEMS actuators available for this purpose. Because it is desirable in the scope of this project to design the device such that it is compatible with the surface micromachining processes available at Sandia National Laboratories, two such actuators are considered here. An electrostatic microengine, such as that pictured in Fig. 3, is able to produce forces over a range of 4 nN to 27 μ N on the drive pin, depending on the rotational velocity [12]. The force that is then transmitted to the device would be determined by the gear train. The actuation force can be increased by utilizing micromachined gears as is shown in Fig. 3, in which the microengine is driving a micromirror. The microengine is limited in range of motion and is rather large which and can dominate the available design space on the die. In the figure, the full size of the engine is not shown.

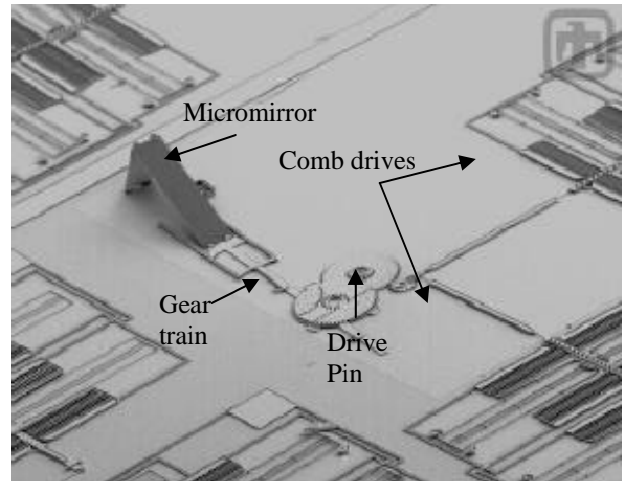


Fig. 3. An electrostatic microengine is used to drive a micromirror. The force is transmitted from the electrostatic comb drives to the mirror using a gear train [12].

An alternative to this actuator is the scratch drive actuator (SDA) like that depicted in Fig. 1. These actuators, pictured in Fig. 4 are compact and able to produce very large forces, up to 250 μ N for one actuator [13]. These devices are also easily arrayed to create even higher forces reaching the mN range.

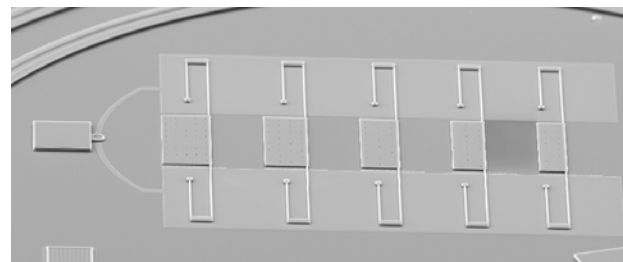


Fig. 4. Scratch drive actuators fabricated at Sandia National Laboratories using the SUMMIT™ V surface micromachining process.

Figure 5 depicts the operation of a SDA. These actuators consist of a flat plate suspended above the substrate by folded spring arms. There is a short bushing projecting down from the plate. The devices are electrostatically actuated and rely greatly on frictional forces to produce in-plane linear motion. As a voltage is applied across the substrate and the plate, the plate is pulled down and the bushing flexes out, resulting in an incremental “step”. This process is repeated to result in actuation over large distances. The size of each step is dependent upon the plate geometry, the applied voltage and signal frequency. Each step is on the order of 10 nm. The devices shown in Fig. 4 exhibit step sizes ranging from 4-16 nm for a plate size of 200x85 microns, with step size increasing with the voltage applied. These step sizes correspond to incremental force increases of 18-60 N [14].

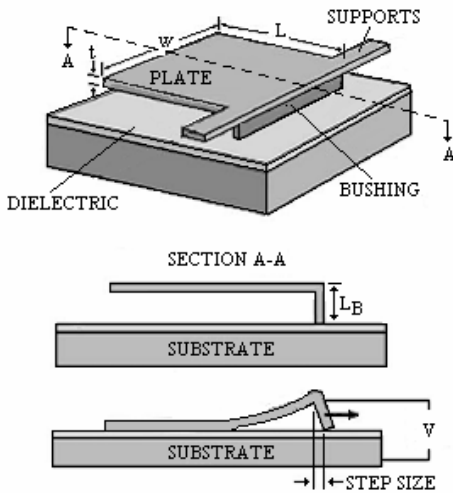


Fig. 5. Schematic of Scratch Drive Actuator and its operation. As a voltage is applied across the device and the substrate, the actuator produces a stepwise motion [14].

B. Spring

The micromachined spring is used in series with the actuator to assist in determining the net force that is being applied to the cell. The proposed method requires prior knowledge of the spring constant K_s . Using beam-bending analysis, this can be easily calculated. Assuming that a box-spring is used, as is shown in Fig. 1, then the analysis is as follows. Figure 6 shows the geometry of the box-spring [13].

To determine the displacement of the box-spring structure, first consider each of the long beams separately. The deformation, dt , of a beam in three point bending is known from simple beam mechanics [13].

$$dt = \frac{FL^3}{192EI} \quad (3)$$

E is the Young’s modulus of the material and I is the bending moment of inertia. From this relationship, it is possible to determine the spring constant of n -multiple boxes.

$$K_s = \frac{96EI}{nL^3} \quad (4)$$

Li *et al.* used finite element analysis in conjunction with the above equations to determine the spring constant in order to conduct force measurements on SDAs [13].

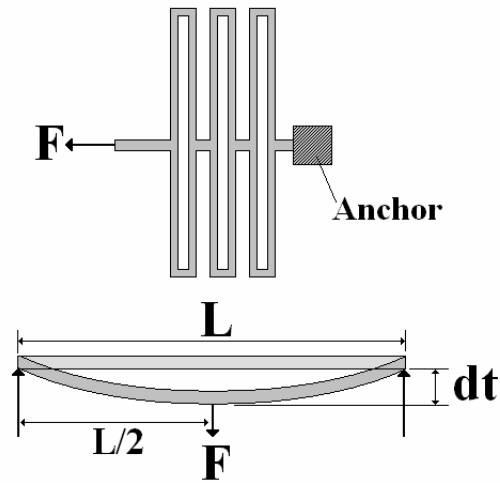


Fig. 6. The deformation of a box-spring under a loading F may be considered as simple three-point bending of a beam.

C. Measurement

There are several optical methods that may be employed to track the displacement of the points A and B. One way is to use optical imaging and feature tracking, such as Sandia National Laboratories MEMScript software used in [14]. This software was able to track displacements of points of interest with resolution of 1-2 microns. Another optical method that promises nanometer accuracy to measure displacements is to use optoelectronic laser [12].

A method that may be employed with the above optical measurement schemes is the use of compliant mechanisms to amplify the displacements such that they can be more accurately measured. An example of this type of device is the stoke multiplier developed by Kota and Hetrick and shown in Fig. 7 [15,16]. This device uses a series of compliant micromachined beams to provide mechanical advantage and amplify an input displacement by 20 times [16]. This device may be used in conjunction with another MEMS actuator that has a limited stroke, such as a surface micromachined microengine, to increase its actuation range.

A similar device may be designed for the MEMS tensile test machine to amplify the displacements such that they may be easier to detect and improve measurement resolution.

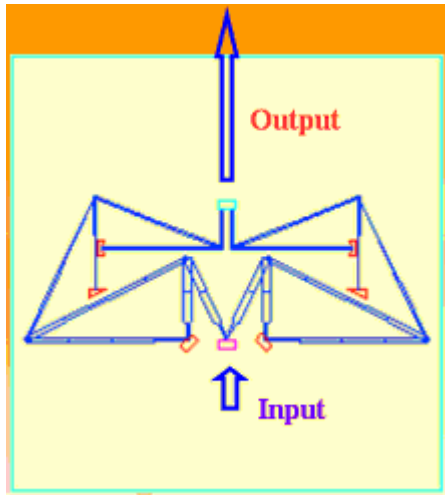


Fig. 7. Picture of a MEMS stroke amplifier that increases the displacement of the input through a series of compliant beams [16].

D. Device Fabrication

The MEMS tensile test device is designed such that it may be fabricated using the SUMMiT™ V surface micromachining process with bioMEMS modifications developed by Sandia National Laboratories [17]. Figure 8 shows all of the available layers in the process including the modifications for bioMEMS applications.

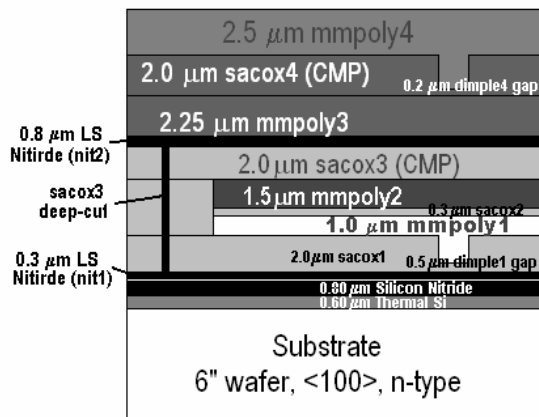


Fig. 8. Process flow for Sandia National Laboratories surface micromachining process SUMMiT V with BioMEMS modifications [17].

This process was created specifically to enable the fabrication of microfluidic channels. It uses polysilicon as the structural material, silicon oxide as a sacrificial material, and silicon nitride to construct the fluid channels. Another benefit of this process is that it allows for the interaction of electromechanical and fluidic devices as

demonstrated by the cellular manipulation device described in [17].

The actuator, spring, and plates can be constructed in the first two structural layers, mmpoly1 and mmpoly2. The cell and its fluid can be contained in a fluid reservoir created using the silicon nitride layers. The depth of the silicon nitride channels may be a limiting factor on the size of the samples considered.

E. Cell Handling

A crucial aspect of using the device will be determining how to isolate and attach a single cell or tissue sample of interest. Cell isolation has been primarily done by-hand, which can be a very tedious task. Finding a way to automate the process would be greatly beneficial.

Once a sample has been isolated and placed into the device, it must be secured. The attachment needs will depend on the type of biological sample being studied. Some may be attached mechanically using clamps or glue, as in [6,10,11]. The effect that the glue and clamping mechanisms have on the samples is unknown. In other studies where micromanipulation techniques were employed, mouse embryonic carcinoma cells are prepared on a glass slide coated with bovine serum albumin (BSA) to promote adherence [5]. In other cases, it may be beneficial to grow a cell culture directly onto the device, which would require placing the device in an incubator.

Cell handling is critical to the development of MEMS based test structures such as the one proposed here. Currently, there is no single solution to this issue, however multiple methods are being investigated.

V. Conclusions and Future Work

The work-in-progress presented here provides a background analysis for the development of innovative MEMS test structures to perform biological analysis and sample manipulation. While current methodology is able to provide some information regarding the mechanical properties of cells and tissue, MEMS may offer the ability to further that knowledge. Also presented, is the concept and design of a MEMS tensile testing machine to determine elastic and adhesive properties of cells. Challenges remain to design a device that is capable of providing the appropriate force to the cell, developing an efficient method of cell handling, and being able to accurately measure the displacements. Future work includes development of a prototype device and design of other biological test structures.

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