Evaluating Bulk Stiffness of MCF-7 Cells using Micro-scale Composite Compliant Mechanisms

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Abstract— Biomechanical assays offer a good alternative to biochemical assays in diagnosing disease states and assessing the efficacy of drugs. In view of this, we have designed, fabricated and tested a miniature compliant tool to estimate the bulk stiffness of cells, particularly MCF-7 (Michigan Cancer Foundation) cells. The compliant tool comprises a gripper and a Displacement-amplifying Compliant Mechanism (DaCM), where the former helps in grasping the cell and the latter enables vision-based sensing of force. A DaCM is necessary because the field of view of a microscope at the required magnification is not large enough to simultaneously observe the cell and a point on the gripper that move sufficiently to estimate the force. Therefore, a DaCM is strategically embedded within an existing gripper design leading to a composite compliant mechanism. The DaCM is designed using the kinetoelastostatic map technique to achieve a resolution 42 nN. The gripper, microfabricated with SU-8 polymer using photolithography, is within the footprint of about 10 mm by 10 mm with the smallest feature size of about 5 microns. The gripper was tested in air and was found to be satisfactory in grasping and squeezing objects as small as 15 microns in size. However, testing in aqueous medium encountered an unanticipated problem due to buoyancy, which curled the jaws of the gripper up by as much as 40 microns and thus losing contact with the cell that is to be grasped. A design modification is suggested to fix this problem.

Keywords—Compliant grippers; bulk stiffness; cells; manipulation;

I. INTRODUCTION

The Cytoskeleton, which is arguably the principal determining factor for the shape and stiffness of a cell, is a network of microtubules, actin filaments, and intermediate filaments [1]. Cytoskeletal studies are becoming relevant in the case of cancer cells because their incessant division is thought to be facilitated by the cytoskeleton [2]. Hence, one of the ways of treating cancer is to subject it to drugs that affect the cytoskeleton and thereby reducing tumor growth [3]. The efficacy of these drugs is usually assessed using biochemical assays. But lately, exploration of biomechanical assays has begun both as a cost-effective alternative and as a technique that works at the single cell level. Based on this premise, in this work, we explore techniques to measure possible change in stiffness of MCF-7 (Michigan Cancer Foundation) cell-line as a result

of anti-cancer drugs, using miniature grippers that also serve as vision-based force sensors.

It is pertinent to compare mechanical characterization of single cells using miniature grippers and other Micropipette-based techniques. aspiration, optical tweezers, magnetic tweezers, atomic force microscope are the commonly used bio-micromanipulation techniques [4]. These techniques enable application of loads on the cells and thereby assess their mechanical response and constitutive material properties. However, most of them require auxiliary systems such as a suction pump with a high-resolution controller in the aspiration technique, lasers in optical tweezers, etc., and hence are expensive and not easily scalable. There are other biomechanical assays such as an array of micro-posts, substrate stretcher, etc., which are beneficial in assessing the mechanical forces applied by the cells on the substrate but not in direct measurement of their stiffness [2]. Micro-grippers, on the other hand, are simple in construction; are scalable and cost-effective; and are amenable for application of loads on cells and help in measurement of forces.

The simplest form of a micro-gripper is a pair of cantilever fingers that come closer to each other upon actuation. The actuation can be electro-thermal [5], electro-magnetic [6] piezoresistive [7], electrostatic [8], piezoelectric [9], etc. When these actuators are integrated with the gripper and are immersed in aqueous medium in which live cells are tested, their behavior might change or they require special care. Although careful calibration for a given medium is possible [8,9], it increases the complexity and cost. Furthermore, many of them can only grasp but not manipulate. Manipulation of cells, i.e., rolling and squeezing in multiple ways, is important in establishing specificity in changed stiffness of the cells and structural changes that caused the change. In view of this a welldesigned gripper is preferred over mere pair of cantilever beams. In this paper, we present a compliant mechanismbased cell manipulation system that is also used to measure forces and assess the bulk stiffness of the cells.

Figure 1 shows our bio-micro-manipulation system that includes an inverted microscope, a miniature gripper shown in the inset, an extending arm that connects the actuation point of the gripper to an x-y-z micro-positioner,

and a camera. The gripper is immersed in the aqueous medium containing suspended cells. When the arm attached to the micro-positioner is moved, the gripper is actuated to grasp, roll, or squeeze a cell. The image from the camera, in addition to providing a view of the cell held in between the jaws of the gripper, also helps extract the displacement of a portion of the gripper using which the force can be estimated [10]. For this vision-based force sensing to be effective, within the field of view, there has to be at least one point of the gripper (other than the point of actuation and the jaws) with displacement larger than the resolution of the image, which in our case is $0.5 \ \mu m$. None of the gripper designs in our database [11] meet this requirement. In this regard, a composite compliant mechanism consisting of a displacement-amplifying compliant mechanism (DaCM) and a gripper is proposed in this paper. The DaCM, which is described in Section 2, was designed with the help of kinetoelastostatic maps [12].

The compliant gripper integrated with a DaCM was fabricated using lithography technique and it was used to manipulate the cells and thereby estimate their bulk stiffness. Although the gripper was actuated in air, the effect of buoyancy became more prominent when the gripper was tested under the aqueous medium. Some attempts were made to reduce this effect with limited success. However, we present methods to further reduce this effect. The design methodology, fabrication procedure, the setup, testing and discussion comprise the rest of the paper.

II. METHODOLOGY

Figure 2(a) shows a miniature gripper designed to be small enough so that all of it is within the view of the microscope while the cells are manipulated. Cells, during manipulation, are expected to apply a load of the order of 10s of nN [8]. However, for this amount of force none of the points of the gripper respond with a displacement of more than 400 nm, which is difficult to measure using optical microscopy. One of the ways to address this problem is to introduce a DaCM at a strategic location, as shown in Fig. 2(b); a DaCM is embedded in both the jaws of the gripper thereby making the DaCM interact directly with the cell. The DaCM amplifies the displacement at the jaws, as shown in Fig. 2(c); small displacement at the input is amplified to measurably large displacement at the output. Here, our task is restricted to the design of the DaCM because as the role of the gripper is to position and move the DaCMs appropriately. This design of the mechanism allows scaling up by using an array of DaCMs at the jaws of the actuator region as shown in Fig. 2(c) to increase the throughput of the technique; however in this paper it is restricted to one pair of DaCMs. The DaCM is to be redesigned to meet the requirements on the resolution of the force that it ought to detect. That is, the output displacement of the DaCM should be large enough for optical measurement in response to minimum force that is to be resolved. It is to be noted that when the DaCM is designed for the resolving low forces the stiffness of the DaCM is also expected to be low. This feature would be beneficial in gentle handling of the specimen.



Fig. 1. A typical compliant mechanism-based cell manipulation setup.



Fig. 2. (a) An existing design of a gripper (redesigned after [13]). (b) The composite compliant mechanism used for cell-manipulation. (c) The DaCM simulated to demonstrate the amplification (d) A scalable design not discussed in this work.

The overall size and second-level feature sizes of the DaCM, as shown in Fig. 3(a), are determined using the kinetoelastotstatic maps. Kinetoelastostatic maps are drawn in 2D by plotting a non-dimensional quantity capturing the nonlinear static response against a non-dimensional number η , which includes the input force F; average length L, breadth d, and depth b of the beam segments; and the Young's modulus E.

$$\eta = \frac{F s^2}{E b d} \tag{1}$$

where slenderness ratio, $s = \frac{L}{d}$

In this case, out of the two points shown in Fig. 3(b), the output point of the DaCM where an amplified displacement is observed, is chosen and displacement at this point is non-dimensionalised using the average length L of the slender segments if the mechanism. It is to be noted that the kinetoelastostatic map is parameterized with respect to the slenderness ratio s. The map can be generated once s is determined. This necessitates one to determine L. The value of L is estimated based on the constraint that both the DaCMs should be within the field of view of the microscope, which is about 2 mm in diameter. Hence, the size of the compliant mechanism is adjusted such that the size in the y-direction is around 0.7 mm without changing the proportions. The restriction on the overall size of the DaCM restricts the average length of the DaCM. The average length, which is defined as the average of the lengths of all flexible beam segments in the mechanism, given by

$$L = \frac{\sum_{i=1}^{N} L_i}{N_s} = 0.3858 \,\mathrm{mm}$$
(2)

Here, N is the number of beam finite elements and N_s is the number of beam segments of the DaCM (12 in this case).

The next step is to determine the value of the breadth and this is obtained from the choice of the manufacturing. The DaCM is to be manufactured using the photolithography process and this restricts the minimum feature size to 5 μ m. Thus, the value of *s* can evaluated as

$$s = \frac{\sum_{i=1}^{N} s_i}{N_s} = \frac{\sum_{i=1}^{N} \frac{L_i}{d_i}}{N_s} = 77.2$$
 (3)

As shown in Fig. 3(c) a kinetoelastostatic map is generated for s = 77.2, the data point highlighted in the figure is obtained based on the other constraints in the design as discussed next.

In the vision-based force sensing technique the DaCM is expected to have at least 500 nm displacement at its output point. Thus, the minimum value of the non-dimensional displacement is

$$\frac{U_{out}}{L} = \frac{500e^{-9}}{385.8e - 6} = 0.0013 \tag{4}$$

As shown by the data point highlighted in Fig. 3(c) for a given value of U_{out} / L and s there is an unique value of $\eta = 0.8598e$ -3. From Eq. (1), we can see that many combinations of the three parameters F, b and E can be used to make η assume the value obtained from Fig. 3. However, based on the preference of the manufacturing process (photolithography in our case) a commonly used photoresist SU-8 is chosen as the material. Thus, the value of the Young's modulus gets fixed at 4e9 N/m². Now, depending upon the force that the DaCM has to resolve, the thickness of the DaCM can be determined and their ratio is a constant given by

$$\frac{b}{F} = \frac{s^2}{\eta Ed} = \frac{77.2^2}{0.0008598 \times 4e9 \times 5e - 6} = 346.5$$
(5)



Fig. 3. (a) The DaCM where L_X , L_Y represent the overall size and b, L_1 , d_{1-3} represent the second-level features. (b) The DaCM showing the input and output points (c) Kinetoelastostatic map corresponding to the DaCM shown in (a).

The DaCM, if desired to resolve a force of 10 nN then its out-of-plane thickness has to be about $3.5 \,\mu m$ as per (5). Although, thickness of 3.5 µm is achievable using photolithography technique, the DaCM would deform outof-plane substantially due to its self-weight as observed from Fig. 4. The DaCM was simulated for out-of-plane deformation for different values its out-of-plane thickness using Abaqus finite element software. It is observed from Fig. 4 that the out-of-plane deformation reduces when the thickness of the DaCM is increased. In order to reduce the out-of-plane deformation, the DaCM can be made very thick (aspect ratio >20), which is possible with SU-8 polymer. However having thickness of the gripper close to the size of a cell (about $15 \,\mu$ m) is beneficial especially for imaging the deformation that the cell undergoes. Thus, the thickness of the DaCM was restricted to 15 µm. The resulting DaCM can resolve a force of 42 nN, as per (5). In the kinetoelastostatic map technique, the proportions of the mechanism are held fixed. Therefore, once the overall size and cross-section size (here depth = $15 \mu m$) are determined, all the dimensions (i.e., the lengths of all other segments and their cross-sections) are scaled as per the proportions. Once the dimensions of the DaCM are determined, the actuator region on the gripper is scaled appropriately to accommodate them.

Figure 5 shows the overall design of the composite compliant mechanism that is designed for manipulation. It is to be noted that at certain places, pockets were created in order to reduce the effect of buoyancy as well as reduce the deformation due to the self-weight of the gripper. It is to be observed from Fig. 5 that the overall dimension of the mechanism is within a footprint of 10 mm \times 10 mm while the DaCM is within a footprint of about $1 \text{ mm} \times 1$ mm. The actuator region being large is beneficial in setting up the experiment, especially while positioning the extended arm of the micro-positioner with the input point of the gripper. The multi-scale composite compliant mechanism can be used for not only measuring the forces involved in manipulating the cell but also the bulk stiffness of the cell. This feature of the mechanism is discussed next.

In the device shown in Fig. 6 the DaCM moves when the gripper is actuated. In this case, the points on the actuator region and points of amplified displacement have the same amount of displacement. This implies that the DaCM is not deformed but has merely undergone rigidbody displacement. Between these two points (points on the actuator and the points on the DaCM) a change in the displacement can occur only when there is a force at the input point of the DaCM. Thus, when an image is acquired using the camera attached to the microscope, change in the displacements between these two points are estimated in order to estimate the load that the gripper is applying on the cell.

The relation between the displacement at the output point of the DaCM and the input force is determined through finite element analysis a priori. Figure 7 shows points obtained by simulating the DaCM for different values of the input force. The simulated points are fitted with a cubic curve. This pre-calibrated curve is used in estimating the forces that is applied by the gripper onto the cell that is grasped.



Fig. 4. Out-plane deformation that the mechanism undergoes due to its self-weight.



Fig. 5. Final design of the composite compliant mechanism.



Fig. 6. Scheme to evaluate the displacements at certain points of the mechanism to estimate the involved during manipulation.



Fig. 7. Pre-calibrated curve to estimate the force applied on the cell depending on the displacements at the points of amplified displacement of the two DaCMs.

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III. FABRICATION

Figure 8 shows the composite compliant mechanism fabricated using the photolithography process applied onto a 15 µm thick SU-8 material. Usually, photolithography is carried-out on a Si-based substrate but this choice is not suitable for the case of grippers because the manipulation is to be carried out under an inverted microscope. One of the ways to address this problem is to isolate the grippers after fabricating them on the Si-based substrate and then placing them under the microscope. But, in that case, handling becomes an issue. As an alternative, glass substrate was chosen and grippers were fabricated on it, and used for manipulation along with it. In case of the gripper, except the pads, all the remaining beam segments have to move when the gripper is actuated. Under such conditions to avoid friction, all the parts except the pads have to be at a certain distance from the substrate. In order to facilitate this requirement; as shown in Fig. 9, a sacrificial layer (AZ 4562, a positive photoresist) was spincoated to occupy the glass substrate partially.

After the sacrificial layer is spin-coated, baked and developed, SU-8 was spin-coated such that it occupies the remaining area of the substrate as well as creates a layer on the top of the sacrificial layer; this is shown in Fig. 9. Next, the sample is baked using a hot plate. Following the baking, the sample is exposed under UV light using a chrome mask containing the design of the gripper. In this step, as shown in Fig. 9, the chrome mask is positioned such that the pads of the mechanism are exposed in the portion where the sacrificial layer is absent. After the UVexposure, the sample is baked and during this step, due to the presence of positive photo resist as the sacrificial layer, bubbles get created and this has been observed by Bao, et al. [15]. However, formation of the bubbles was reduced by heating the sacrificial layer for a longer time (> 1 min) during its soft-baking step. Also it was observed that the bubbles were not affecting the grippers and they are removed when the sample is kept in the SU-8 developer. After the post-exposure bake the sample is kept in SU-8 developer to obtain the gripper. It was observed that AZ4562 dissolves in the SU-8 developer, and thereby removes the sacrificial layer. There is no need for an extra step in the fabrication to remove the sacrificial layer. We obtain a SU-8 gripper on glass with the pads of the mechanism firmly fixed onto the glass substrate while rest of its parts is in suspension. The details of the parameters used for spin-coating, baking-temperature, baking-time, exposure-energy etc. are provided in Table 1.



Fig. 8. The Fabricated SU-8 prototype. Close-up of the region where the cell will be positioned for testing is also shown.

Fabrication step	Details of the parameters
Substrate cleaning	Washed it with isopropanol
Sacrificial layer	Spin-coat with AZ4562 at 4000 rotations per minute (RPM) for 40 s yielded a 6.5 µm layer
Pre-baking the sacrificial layer	95 °C for 2-3 mins
Development of the sacrificial layer	Using diluted AZ351B with water (1:4)
SU-8 layer	Spin-coated at 500 RPM for 5 s and then at 1600 RPM for 30 s yielded a 18 µm layer
Pre-baking of SU-8	95 °C for 25-30 mins
Exposure	Flood exposure with 365 nm UV light at an intensity of 300 mJ/cm ²
Post-exposure bake	95 °C for 6 mins
Development	Place in SU-8 developer for 6-8 mins

IV. EXPERIMENTAL SETUP

Figure 10 shows the manipulation setup used for estimating the bulk stiffness of cells, and it consists of a microscope (IX81, Olympus corp.), a micro-positioner (MP-285, Sutter Inc.) and the gripper. The mechanism is placed such that both the DaCMs are in view while the gripper is actuated. An additional digital microscope is used for better visualization of the mechanism but it is not a necessary part of the setup. The micro-positioner is used in actuating the mechanism through a rotary optical encoder (ROE) controller with a step size of 40 nm. The gripper is actuated using an extension arm attached to the micro-positioner and in turn, this extension arm contains a glass pipette which comes in contact with the gripper. A camera (STC-625AS, Sentech Inc.) attached to the microscope is used to capture images when the gripper is actuated. The image taken by the camera would be later used to estimate the force involved in manipulating the cell.

V. DISCUSSION

The gripper was actuated in air and in medium having low density such as isopropyl alcohol as shown in Fig. 11(a). It can be observed in Fig. 11 (a) that the entire DaCM is in plane and the jaws come closer when the gripper is actuated. As shown in Fig. 11 (b) glass beads of 15 µm in diameter were grasped using the gripper. While the glass bead is in contact with the jaws of the gripper visualization is affected due the dry conditions of the actuation. However, when the gripper is actuated under water or the medium used for culturing the cells the effect of buoyancy force becomes significant. An experiment was setup to demonstrate the effect of buoyancy where one on of the two DaCMs is fixed onto the substrate, as shown in Fig. 12(a). As shown in Fig. 12 (b), the DaCM in suspension is in a different plane as compared to the one attached to the substrate. This was evident from the foci planes of the objective lens. However, the effect of buoyancy is reduced when the gripper is immersed in a

liquid with lower density such as isopropyl alcohol as can be observed from Fig. 12(c).

In order to minimize the effect of buoyancy, we have designed a cover-slip as shown in Fig. 13 (a) such that the mechanism would be sandwiched between the glass substrate and the custom-made cover-slip. Figure 13(b,c) shows a computer-aided model of the arrangement of the mechanism. The cover-slip has step of 50 μ m to avoid contact with the mechanism, which would otherwise result in substantial amount of friction. Also, the cover-slip is designed to have two grooves; one to allow the cells to enter the jaws of the mechanism and the other to provide space for the positioner's probe to actuate the mechanism. The cover-slip is attached to the sample using adhesive.







Fig. 10. The compliant mechanism-based manipulation setup.



Fig. 11. (a) Gripper in action. The jaws come closer when the gripper is actuated. (b) A glass bead being grasped using the gripper.







(b)

(a)





Fig. 13. (a) Cover-slip custom made to sandwich the mechanism. (b) View of the assembly of the mechanism along with the cover-slip. (c) Exploded view of the assembly.

The sandwiched assembly of the gripper did bring down the effect of buoyancy quite significantly, but there is still about 40 μ m of displacement observed at the jaws of the gripper, which has to be addressed. One of the ways

to address this problem is to increase the thickness of the gripper to $30 \ \mu m$, which would increase the out-of-plane stiffness quite significantly. The gap between the coverslip and the mechanism could also be narrowed down such that the height of aqueous medium within the assembly is low and this would also reduce the effect of buoyancy. One more possibility is to introduce pockets at the jaws of the gripper thereby reduce the surface area in contact with the aqueous medium, which would also result in lowering the effect of buoyancy.

VI. CLOSURE

In this work, we have demonstrated that a composite multi-scale compliant gripper could enhance the capability of an existing gripper; highlighted the efficacy of the kinetoelastostatic maps in redesigning a mechanism; demonstrated a fabrication procedure to obtain grippers in partial suspension; the grippers were successfully actuated in air and with partial success in aqueous medium. To enable the grippers to work in the aqueous medium few design methods to address the effect of buoyancy were discussed.

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