Cell Mechanics Meets MEMS

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Introduction

In addition to developing his famous spring equation, Robert Hooke (1635-1703) devised a compound microscope and studied thin slices of cork. Looking at the dead cellular walls of plant tissue, he observed little pores that he termed 'cells' [1]. Even though the term 'cell' stuck, the conceptual visualization of this ubiquitous building block of life has undergone dramatic changes.

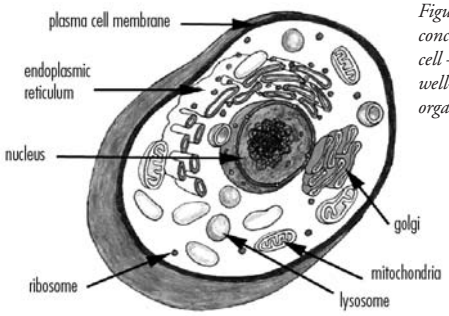

Figure 1. Early conceptualization of a single cell – a static entity with well-defined structure and organelles [2]

The cell was once imagined (Figure 1) as a static, near-spherical volume, with neatly positioned components and fairly constant properties [2]. Mathematically, the cell has been modeled as a liquid droplet: a viscous fluid-filled bag with constant surface tension. The liquid droplet model has been known to yield accurate results for cells in suspension, but does not provide an adequate description for cells adhering to their surroundings [3]. It is now generally accepted that the cell is as an incredibly complex, dynamic entity, continuously adapting and responding to its surrounding environment. Cells actively sense and respond to changes in chemical, topographical, and mechanical cues [4], which are transmitted via soluble chemical signals, adjacent cells, and the surrounding extracellular matrix of proteins.

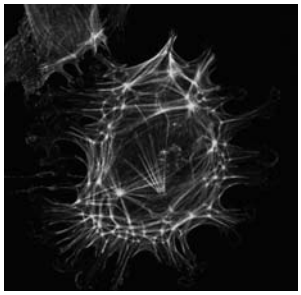

Figure 2. Stained actin cytoskeleton of a single cell [10]

Much work has been done on investigating cellular response to chemical signals, varied surface interface chemistry [5], and surface topography [6]. However, less focus has been placed on the effects of mechanical forces exerted on and by the cell. 'Cellular Mechanobiology' - the application and study of mechanical forces with respect to cell biology - is largely unexplored, and shows potential for driving innovations in cell-based therapy. Mechanical effects on cell proliferation and organization are profound. It has been well known and documented that tissues remodel in response to changes in mechanical forces. In 1892, Julius Wolff described bone remodeling, in which bones change shape, density, and stiffness when mechanical loading conditions are altered [7]. Various loading conditions can also lead to tissue or organ pathologies, including osteoporosis, atherosclerosis, and fibrosis [8]. On a cellular level, mechanical forces have been demonstrated to alter protein expression, and even control differentiation in stem cells [9]. Unfortunately, there is no simple relationship between applied loading and end biological results.

Cell Mechanics And Mechanotransduction

The cell has a number of mechanical elements which comprise the cellular cytoskeleton and define its shape and locomotive abilities. This cytoskeleton is a filamentous network of several molecular components, including actin filaments, microtubules, and intermediate filaments. Actin filaments localize just beneath the cell membrane and in stress fibers that span the cell. Similarly, intermediate filaments and microtubules crisscross the cell, contributing to the cytoskeletal network that mechanically couples the cell membrane to the nucleus and points in between. Figure 2 is an image obtained by fluorescent microscopy in which the actin component of the cytoskeleton has been stained green [10]. This complex structure has been modeled by Ingber, using a "tensegrity" framework [11]. In tensegrity, or 'tensional integrity' model, elastic cables and rigid bars form an extremely flexible, yet stable structure (Figure 3). "The structure is stabilized not because of the strength of the individual members, but because of the way the structure distributes and balances mechanical stresses" [12]. Ingber proposes that actin microfilaments can be modeled as tension elements, and microtubules as the compression elements. This idea has been supported by experimental evidence [13].

The cytoskeleton is physically linked to the extracellular matrix across the cell membrane by receptors called integrins. Thus, mechanical forces applied to the matrix in which a cell resides are physically transmitted via integrins to the cytoskeleton within the cell. In turn, the dynamic cytoskeletal framework is capable of remodeling itself in response to the applied forces. The cytoskeleton aids in the transport of intracellular molecules and the activation of multiple intracellular signaling pathways that control gene and protein expressions. Accordingly, seemingly simple alterations in the organization of the cytoskeleton can result in complex biological responses and dramatically alter the way a cell behaves. In addition to integrins, mechanical forces applied to cells can activate other putative mechanosensors, including stretch-activated ion channels and membrane receptors [8, 14].

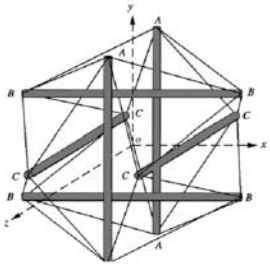

Figure 2. Simplified Tensegrity Model [11]. The combination of struts in compression and elastic fibers in tension keep the overall structure stable.

Experimental Mechanobiology

Traditional techniques in experimental mechanobiology, although varied, fall into two broad categories - passive characterization and active stimulation. Passive characterization techniques are used to determine mechanical properties of the cellular structure while active stimulation seeks to apply mechanical forces and observe the biological response of the cell. Passive characterization includes techniques such as micropipette aspiration, atomic

force microscopy (AFM), laser optical trapping, and magnetic bead measurement. Active stimulation of cells can be carried out by membrane-based stretching, flow-induced application of shear stress, and varying substrate stiffness effects.

I. Passive Characterization Techniques

A. Micropipette Aspiration

In micropipette aspiration, a glass pipette with an internal diameter of 1-10_m is used to deform a cell. The micropipette is manipulated in the cell growth medium such that it is very close to the cell being studied. A vacuum is then applied through the micropipette to the cell that is partially aspirated into the micropipette, as shown in Figure 4. The aspiration length varies with the applied pressure and is used to calculate the rigidity of the cellular membrane and cytoskeleton. This technique can be used to characterize both adherent and non-adherent cells [15].

B. Atomic Force Microscopy (AFM)

In atomic force microscopy, a micro-scale cantilever beam is operated to deform a cell. Based on the deflection of the cantilever, which is often measured by a reflected laser beam (Figure 5), the local stiffness of the cell is measured, and a 'map' of cell stiffness across the cell surface can be generated [16]. Such information can provide valuable insight into the cytoskeletal structure and the effects of environmental parameters upon it.

C. Laser Optical Trapping

The instrument known as 'optical tweezers' makes use of laser to create a potential well, capable of trapping small objects within a defined region. Particles can be attached to the cellular membrane and manipulated laterally across the substrate surface. The laser power required to constrain the particle is directly proportional to the forces being applied to that particle by the cell. In this way, the stiffness of the cell can be measured. Recently, Guck et al. developed an innovative technique, in which the entire cell is stretched by dual optical tweezers. A schematic is shown in Figure 6. By coupling the optical stretcher system with a microfluidic flow chamber, they were able to sequentially stretch and characterize thousands of individual non-adherent cells. Images obtained with a camera enabled them to measure deformation of the cell and correlate the calculated cytoskeletal rigidity with cell type [17].

D. Magnetic Bead Measurement

In this technique, a 4-5_m diameter paramagnetic bead is bound to a live cell. This is done by coating the bead with an extracellular matrix protein or an antibody, which then binds to receptors or other proteins on the cell membrane. An external magnetic field is applied to twist the bead (magnetic bead twisting cytometry), or to apply a displacement to the bead (magnetic bead microrheometry). This is usually done under an optical microscope to observe displacements of the beads [18]. The magnetic field is applied either by a large coil surrounding the sample or with a magnetized needle [19]. In a single cell, the observed displacement can be used to characterize cellular mechanical properties. Additionally, because beads can be bound to specific cell surface proteins, the biological response induced by tugging on these proteins can be studied with this technique.

II. Active Stimulation Techniques

A. Membrane-Based Stretching

In membrane-based stretching methods, cells are grown on a flexible substrate. The substrate is cyclically deformed in some manner. The cells, bound to the substrate by integrin-matrix protein interactions, are stretched by each of the focal contact points. There are two types of stress fields that are currently often used for testing cellular response. One is uniaxial stretching, in which the cells are stretched longitudinally. This is conducted either by stretching an elastomeric substrate in one direction, or by flexing the substrate to create a tensile strain on the convex side. The other type of stress field is biaxial stretching, in which the outer edges of a circular membrane are constrained, and a pressure differential is applied across the membrane [20]. The Flexercell Corporation markets large-scale platforms for these types of experiments [21].

B. Flow-Induced Shear Stress

In vivo, there are several situations in which fluid flow applies shear stress to cells. For example, the flow of blood exerts shear forces on the endothelial cells that line blood vessels. Because of their physiological relevance, experiments aimed at determining the biological effects of flow-induced shear stress on cells are particularly useful. There are a large number of experimental devices applying various kinds of fluid flow to cells. Flow can be pulsatile or steady, and flow chamber geometries are designed to create flow disturbances that simulate complex flow profiles in the vasculature [22]. Microfluidic devices have recently received much attention in this area, due to their ability to apply precise uniform stresses across a specified region. Such microfluidic devices can be used to determine the effect of applied shear on protein expression, or to determine adhesion strength between cells and the substrate [23, 24].

C. Substrate Stiffness

Cells are exquisitely sensitive to the stiffness of the substrate to which they are attached. Adherent cells sense the local elasticity of their matrix by pulling on the substrate via cytoskeleton-based contraction. These tractional forces are tuned by the cell to balance the resistance provided by the substrate. To a certain

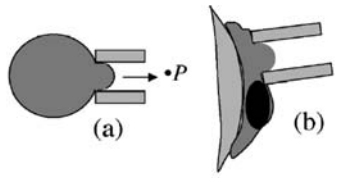

Figure 4. Micropipette Aspiration (adapted from [15]). The first panel is a schematic of a cell in suspension undergoing aspiration. The second is that of a cell adhering to a substrate.

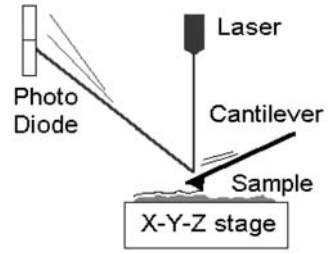

Figure 5. AFM Schematic [16]. A laser beam is reflected off a cantilever. As the cantilever is rastered across a sample, it deflects based on the topography, shifting the reflected beam on the photodiode.

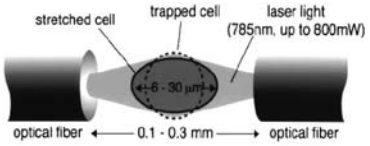

Figure 6. Optical Stretcher system, developed by Guck et al. [17]. Two diametrically opposed laser beams confine the cell in a two dimensional potential well. By narrowing the well, the cell is squeezed.

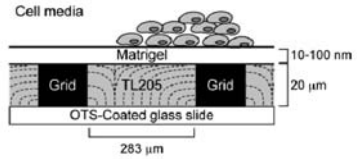

Figure 7. Liquid crystal culture device developed by Palecek et al. [32]. The TL205 is liquid crystalline material, which displays changes in colour depending on deformation.

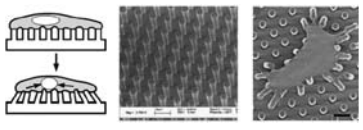

Figure 8. PDMS post device, developed by C.S. Chen et al. to measure cellular forces exerted on a substrate – adapted from [34]. The first panel shows a schematic drawing of the cell exerting forces on the post. The second and third panels are the experimental device itself.

limit, it appears as though the cell attempts to match its stiffness with that of the underlying substrate by altering the organization of its cytoskeleton and possibly through strain-stiffening [25]. Hence, by culturing cells on substrates of different stiffness, one can regulate cytoskeletal structure and tension. The cytoskeleton regulates intracellular signaling and gene and protein expressions. Therefore, the mechanics and topography of the substrate can have profound effects on many cellular processes, for example, growth, movement, and death [4, 26-28].

Mechanobiology And MEMS (Microelectromechanical Systems)

The passive sensing techniques briefly described in the previous section are typically of low throughput. It takes much time to obtain information on a single cell. Given the broad variability that is inherent in cellular properties, detecting statistically significant differences in cell populations can be experimentally challenging. The benefits of a system capable of performing several measurements quickly have been seen in the optical stretching device [17]. The investigators were able to distinguish metastatic cells from healthy cells, based on the rigidity information obtained. This would not have been possible with only a small sample population. Unfortunately, this particular technique is only useful for cells in suspension, precluding its use for adherent cells.

Active stimulation is capable of acting upon an entire population of cells, allowing for observing changes in protein expression. However, none of the experimental setups is able to apply a range of forces to different populations of cells. Each experiment takes much time. Testing a range of mechanical parameters, in combination with chemical stimuli is a daunting task. Despite the demonstrated synergy between mechanical and chemical stimuli, no techniques are available for efficiently manipulating mechanobiological parameters in a combinatorial manner.

Cells are different in sizes. An ostrich egg is a single cell with a diameter of 12cm, while a human red blood cell is orders of magnitude smaller, with a diameter of approximately 8_m. Most cells, however, are in the range of 10-30_m in diameter, an ideal size to study with MEMS devices. The forces involved range from picoNewton in the case of AFM studies to milliNewton for membrane-based stretching. These force magnitudes are within the range achievable by MEMS microactuators or resolvable by MEMS sensors. In many cases, miniaturization of the system will reduce waste chemicals and media, allow for the application of extremely small and precise forces, importantly, increase the number of results possible per experiment, and the number of experiments that can be performed simultaneously.

Microfabricated MEMS and microfluidic devices have already found a niche in the biology community, for example, for improving throughput. Chin et al. developed a microfabricated device for studying stem cell fates [29], which allows quantitative analysis of a large number of samples with ~10,000 wells on a glass coverslip. Leyrat et al. developed a microfluidic analysis system [30] to perform 100 experiments on a single chip that can conveniently vary cell seeding density, adhesive protein type, media composition, and media replacement intervals. The system is also capable of conducting assays within the chip itself, promising a highly useful tool in cell biology. Micro devices for mechanobiology are less popular, but have also received much interest. Currently, most of the platforms are designed as passive characterization tools with a few capable of applying forces to cells.

I. MEMS-Based Passive Characterization Techniques

Microfabricated thin membranes have been used to determine the forces exerted by cells adhering to substrates. As the cells anchor themselves to the substrate, the membrane wrinkles. Algorithms were developed to analyze the deformation. Forces exerted by each of the cells were calculated [31]. One of the major drawbacks is that cells on stiffer substrates cannot be studied because the membranes must have a local stiffness low enough to wrinkle. This drawback has recently been addressed by Palacek et al. with their Liquid-Crystal cell culture system. They created a microfabricated grid, in which liquid crystals were deposited. The liquid crystals are the same material used in flat-screen monitors, changing colors when forces are applied. The whole surface was then covered with an extremely thin layer of a matrix protein mixture (Matrigel). Cells were cultured as shown in Figure 7. This system provides a fairly stiff region upon which the cells are able to grow while providing feedback on the cellular forces involved. The Matrigel layer can be tuned to change surface stiffness and study resulting forces [32].

Galbraith et al., going a step further, designed a microdevice, in which movable, horizontally configured cantilever beams were mounted. Cells were plated onto the beams. Deflections of the beams were tracked as cells migrated [33]. Unfortunately, this device was only able to track forces along a single axis. In order to rectify the problem, Chen et al. developed a simple system to calculate directional cellular forces. They cultured cells on a microarray of low-stiffness polydimethylsiloxane posts and observed the deflection of each post (Figure 8). The results provide an easy basis for comparison of cellular mechanical forces among various cell types. The system is simple and effective, and allows researchers to examine a large number of cells at a time.

Leveraging the concept of vision-based cellular force sensing, Sun et al. developed a silicone elastomer-based cell holding device [35] for characterizing suspended cells. Together with a sub-pixel visual tracking algorithm, deflections of elastic, low-stiffness structures are visually tracked, and material deflections are subsequently transformed into cellular forces. The vision-based cellular force sensing framework established in this study is not scale or cell-type dependent. The device design, visual tracking algorithm, and experimental technique form a powerful platform that permits visually resolving cellular forces in real time with a sub-nanoNewton (26pN) resolution for applications in single cell characterization and manipulation (e.g., automated cell injection [36]).

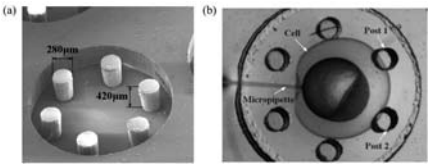

Figure 9. (a) Cell holding device with feature sizes for accommodating zebrafish embryos. (b) Indentation forces applied by the micropipette cause the two supporting posts to deflect [35].

MEMS force sensors have also been developed to measure forces generated by individual cells. Figure 10 shows a surface micromachined device for monitoring the contractile forces of heart cells as they beat [37]. Cantilevers deflect as the cell contracts. The change in cantilever position is monitored via video microscopy, and the forces exerted by the cardiac cells are calculated. Figure 11 shows a MEMS-based two-axis capacitive force sensor [38] that is capable of resolving normal forces applied to a cell as well as tangential forces generated by improperly aligned cell probes. By integrating these cellular microforce sensors into a microrobotic system, mechanical property characterization was conducted on mouse zona pellucida (ZP) [39]. The experimental results quantitatively describe the mechanical property changes during the ZP hardening process.

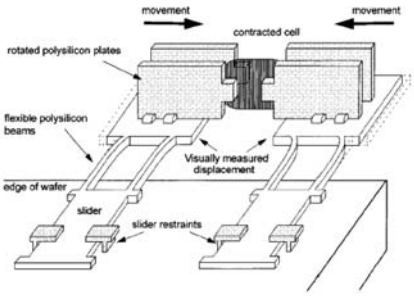

Figure 10. MEMS device for detecting cardiacforces [37].

Figure 11. MEMS capacitive cellular force sensor [38].

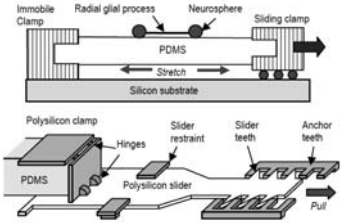

Figure 12. MEMS device to study cerebral cortex neurogenesis [44]. The first panel is a side view, shows the PDMS membrane applying a strain to the radial glial process. The second panel is a view of the sliding clamp and ratchet system.

This study also provided insight into ZP protein structure development, justifying that an increase in the number of cross links of protein ZP1 between ZP2-ZP3 units is responsible for ZP stiffness increase.

II. MEMS-Based Active Stimulation Techniques

The primary technique used to actively stimulate cells at the microscale is for micropatterning proteins onto a substrate and culturing cells in these small areas. The patterned areas constrain the cells to a certain shape. Cells can spread over multiple islands or can be confined to a single island, even in unnatural geometric shapes, such as squares or triangles [40]. Ingber found that cell death or proliferation can be forced through such geometrical constraints, indicating that mechanics plays an important role in cell behavior [41]. This approach has also been used for multicellular work by Bhatia et al. who co-cultured hepatocytes and fibroblasts and found that specific proteins were expressed depending on the geometry of the cellular interactions [42]. Recently, Gopalan et al., used macroscale stretching combined with micropatterning on deformable elastomers [43].

Besides the micropatterning approach, several novel MEMS devices have also been developed to mechanically stimulate cells. Lin et al. studied the effects of mechanical tension on cerebral cortex neurogenesis, using a specially designed micro device. They cultured embryonic brain tissue in a fibrin gel on PDMS. This PDMS construct was then placed in a micromachined ratcheting stretch clamp, as shown in Figure 12. The tissue was then stretched out at set time intervals in order to prevent the tissue cells from adapting to a non-varying strain field by changing focal adhesion positions. Their findings suggest that the induced strains modulate neuronal migration, which is a key factor in cerebral cortex development [44].

Most recently, Scuor et al. developed a biaxial cell stretching device [45], capable of applying two orthogonal strain to a single cell. The device utilizes a geometric configuration capable of applying two strain fields with a single actuator (Figure 13). Biological studies have yet to be performed.

Conclusion

The idea that mechanics influences cell regulation is not new, but recent evidence suggests that it has a far greater impact on cellular response than previously thought. With this new dimension to cellular biology come a number of unique experimental challenges. Applying extremely precise forces to a single cell is challenging in itself, and being able to sweep through a range of environmental parameters, in addition to collecting a statistically significant quantity of data is not possible with standard techniques. Because of the length scales involved, microfabricated devices and systems are ideally suited to tackle these challenges. Using MEMS technology to draw meaningful conclusions on biological problems requires a multidisciplinary approach by scientists and engineers, including collaboration of mechanical, electrical, chemical, and biomedical approaches.

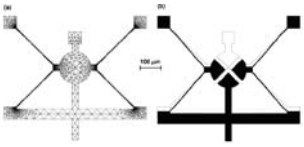

Figure 13. (a) Undeformed Finite Element mesh of biaxial cell stretcher device. (b) Moving the lower arm of the device results in strains in two orthogonal directions [45].

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