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Medicine in Novel Technology and Devices 13 (2022) 100107

Contents lists available at ScienceDirect

Medicine in Novel Technology and Devices

journal homepage: www.journals.elsevier.com/medicine-in-novel-technology-and-devices/

Full Length Article

Microfabricated platforms to investigate cell mechanical properties

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ARTICLE INFO

Keywords:
BioMEMS
Single cell
Mechanical properties
Microdevices

ABSTRACT

Mechanical stimulation has been imposed on living cells using several approaches. Most early investigations were conducted on groups of cells, utilizing techniques such as substrate deformation and flow-induced shear. To investigate the properties of cells individually, many conventional techniques were utilized, such as AFM, optical traps/optical tweezers, magnetic beads, and micropipette aspiration. In specific mechanical interrogations, micro-electro-mechanical systems (MEMS) have been designed to probe single cells in different interrogation modes. To exert loads on the cells, these devices often comprise piezo-electric driven actuators that attach directly to the cell or move a structure on which cells are attached. Uniaxial and biaxial pullers, micropillars, and cantilever beams are examples of MEMS devices. In this review, the methodologies to analyze single cell activity under external loads using microfabricated devices will be examined. We will focus on the mechanical interrogation in three different regimes: compression, traction, and tension, and discuss different microfabricated platforms designed for these purposes.

1. Introduction

Cell mechanical characteristics, such as elastic, viscoelastic, and shear modulus, are important in a variety of cell activities and functions, including cell growth, division, motility, and adhesion [1–4]. Due to its relevance in several applications such as cell separation [5], disease diagnostics [6–8], immunological status monitoring [9–11], and drug screening [12,13], measuring cell mechanical characteristics has piqued the interest of both academics and industry [14]. As a result, techniques for measuring the mechanical characteristics of cells that are precise, robust, and sensitive are in great demand. Mechanobiology studies the connections between mechanical stimuli and cellular biology, including cell processes for sensing, transducing, and responding to mechanical stimuli, and cellular mechanical property characterization [15, 16]. Mechanical forces, both intrinsic and extrinsic, have a major influence on cell behavior and tissue homeostasis such as tissue remodeling [17, 18]. The evaluation of cell deformation in response to mechanical force over an extended period, which may be described by the theory of stress and strain, is required for assessing the mechanical characteristics of cells. Investigating the mechanical properties of cellular components and their relationship can help us understand the overall mechanical characteristics of cells.

Mechanical stimulations have been imposed on live cells using different methods [19,20]. Most early research was conducted on groups of cells, utilizing techniques such as substrate deformation [21,22], in which cells are grown on a deformable substrate, and flow-induced shear [23], in which a fluid running over a culture of cells causes the cells to experience shear stress. Due to the intrinsic variability of cells, most cell mechanical characteristics are heterogeneous [24], and therefore examining cells in groups only yields average responses rather than revealing the complicated responses that individual cells have. With recent technological advancements, many approaches were created to examine single cells [20]. These approaches include atomic force microscopy (AFM) [25,26], optical traps/optical tweezers (OT) [27], magnetic beads [28], and micropipette aspiration [29] among others. These techniques allow for the manipulation of single cells and can be utilized to investigate cell heterogeneity.

Microstructure system is a term used to describe more sophisticated cell probing techniques. To apply force on the cells, these devices often have actuators that attach directly to the cell or move a structure to which

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https://doi.org/10.1016/j.medntd.2021.100107
Received 7 October 2021; Received in revised form 1 December 2021; Accepted 8 December 2021
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the cell is attached. Uniaxial and biaxial pullers, micropillars, and cantilever beams are examples of microdevices [19]. Microstructure-based technologies have increased the precision, efficiency, and consistency of different cell manipulation and characterization activities, through higher force and displacement resolutions. Additionally, microstructure devices have enabled new forms of cell research. Microsensors and actuators have typical feature sizes that range from sub-micrometers to hundreds of micrometers, which are comparable to single-cell sizes. Because of its unique properties such as size matching to single cells and the capacity to generate/measure microscale movements and forces, microstructure devices have been recognized as excellent instruments for cell manipulation and characterization [30]. Owing to its scale, microstructures have demonstrated displacement and force resolution down to the sub-nanometer and sub-nano newton levels [31], respectively, allowing on-chip sensors to reliably detect microscopic cell deformation and low cellular forces. Despite its small size, a microdevice may perform various tasks in cell manipulation and characterization (for example, micro grasping, cellular force detection, and cell deformation measurement) [32, 33]. Furthermore, materials frequently utilized in microdevice construction (e.g., silicon, silicon dioxide/nitride, and polymers) are biocompatible, posing no biological risk to the cells being controlled or measured. Moreover, microdevices' suitability for batch production processes enables the manipulation and characterization of many cells simultaneously, resulting in higher manipulation/characterization throughput and more reliable statistical data. The advantages of microfabrication (such as batch manufacturing and chip integration) make them an appealing option for studying single-cell dynamics. Microdevices have been used to mechanically describe hamster and monkey fibroblasts [34, 35], canine kidney cells [36], cardiac myocytes [37], mouse zona pellucida oocytes, and embryos [38] among other cells.

Cells in the biological environment experience different types of force regimes, including compression, traction, and tension (Fig. 1). Cells that are subjected to flow also experience flow-induced shear stress. Cells respond differently to each regime, so researchers have developed various platforms to investigate these responses. In the compression mode, microtubules are the most dominant parts of the cell that respond, while in the tension mode, actin filaments are involved. In the traction mode, integrins and focal adhesions try to stabilize the cell. It is worth mentioning that the origin of these forces is also different. Tension and compression forces originate from outside of the cells by neighbor cells; while traction forces are active stress responses from cells to their surrounding matrix. Naturally, the techniques to measure these forces are different. This paper reviews microdevices that can measure the mechanical properties of cells in response to these forces, which has not previously been reviewed with this perspective. Here we describe the method and discuss the advantages and disadvantages of these devices. It is also worth noting that platforms that study fluid-induced shear stress in cells fall within the group of microfluidic devices and are not covered in this review.

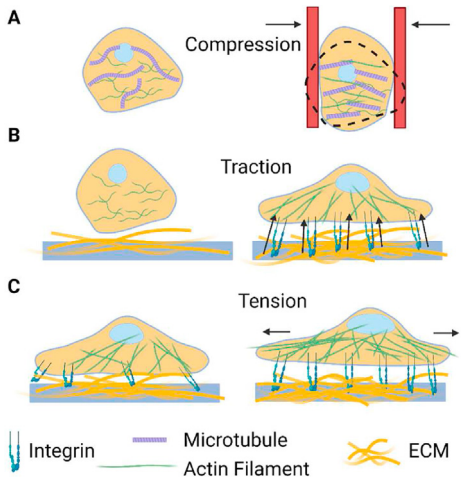

Fig. 1. Cells are subjected to different regimes of mechanical stimuli: A) compression, B) traction, and C) tension. In each condition, cells react in different ways to dissipate the force or strengthen the structure to maintain the cell and tissue integrity.

1.1. Microdevices to study tension

Cells in various organs in our body are subjected to stretch at different strain and strain-rate levels. The mechanism that cells use to withstand these forces and the way they dissipate the stress is the interest of many researchers. These techniques apply to a monolayer of cells or single-cell pairs [19] with different approaches; however, in this paper, we review the microfabricated devices used to interrogate the cell response to tension. Study of these forces at a single-cell level can decipher the underlying mechanism that later interprets the tissue behavior. The methods to measure these forces mostly consist of two beams of which one of them serves as the actuator and the other one serves as the sensor. Based on the techniques of actuation and sensing, they can provide different resolutions and precision (Table 1).

Serrell et al. designed a biocompatible MEMS-based device for quantitative force-displacement measurements of adherent cells. The function of the device revolves around a circular single-cell platform that is divided into two parts. A cell is placed on the platform and manipulated using a picolitre dispensing instrument, which pushes the cell into place with media, enabling adhesion to the platform. Once adhesion is established, displacement is applied to one half of the platform while the other half is mechanically linked to a sensor that can measure the force on the cell. An off-chip micromanipulator provides large, linear displacement that hooks into the device using a probe tip. The sensor is a series of cantilever beams, with a stiffness determined and calibrated by AFM. This study evaluated the adhesive properties of fibroblasts, with de-adhesion occurring around 1500 nN of force at 25% strain. This device successfully evaluated the adhesion forces of a cell to a sensing substrate. The large displacement and high resolution are major benefits, as well as the ability to test cells in biologically relevant conditions. A major drawback of this method is the throughput due to single cell placement on a device designed for one test per experiment. Additionally, it was reported that the cellular imaging could not be effectively conducted with the current system [34] (Fig. 2A). A platform capable of bi-axial testing was presented by Scuor et al. using a comb drive capacitor which could be driven piezoelectrically, thermally, or magnetically. The platform had a unique design that applied bi-axial actions. No measurements on cells were conducted, but the platform had a computationally determined stiffness of 17.5 N/m. There were issues with the introduction of liquids with the device and the effects on the magnitude of displacement [39] (Fig. 2B).

Vikram Mukundan et al. fabricated an electrostatic comb-drive actuator that was implemented in biologic ionic aqueous media for cell studies. The actuator was fabricated on a silicon wafer with an ion etching process, metal layer evaporation, lift-off, and a wet etching process. Through optimizing two different electrodes geometries, the stiffness of the suspension beams, and the location of the cell-binding site, the device allows independent voltage signals at each comb electrode and ensures zero current in the substrate. Madine–Darby Canine Kidney (MDCK) cells were deployed to demonstrate the fidelity of the actuator by applying forces to live adherent cells attached to a collagen pretreated gold pad. The stretching process of the cell-cell junction was video captured, and the displacement was measured using an optical image tracking algorithm. To achieve quasi-static stiffness measurements, a 100 nm/s strain rate was applied. Working in ionic media could not be eliminated due to inherent material properties that lead to electrochemical corrosion and limit its ability to be used for longer period cell studies and certain cell types [36] (Fig. 2C).

Table 1
Methods to measure cell mechanics in the tension mode.

Method	Cell type	Parameters studied	Pros	Cons	Ref.
Stretcher Platform	• Fibroblast	• Adhesion force	• Single cell • 40 nm resolution	• Throughput • Up to 25% strain	3
MEMS Platform with "comb" capacitor drive.	• No measurement	• No measurement	• Bi-Axial	• Did not test cells • Small displacement (3 μm) • Conditional Effects	36
Electrostatic comb-drive actuator	• Madine–Darby Canine Kidney (MDCK)	• Stiffness	• Differential electrode	• Working in ionic media • Electrochemical corrosion	5
Silicon micromachined device	• Madine–Darby Canine Kidney (MDCK)	• Stiffness	• Shear and tension	• Monolayer	37
Stretchable electronics	• Madine–Darby Canine Kidney (MDCK) • Human alveolar basal epithelial adenocarcinoma (A549)	• Electrical cell-substrate impedance (ECIS)	• Real-time • Label-free	• Monolayer • Different effective strain	45
Two-photon polymerization (TPP)	• Skin cancer cells (A431)	• Rupture stress	• Accurate strain and strain-rate • High-resolution • Single-cell pair	• Poor image quality	46

A silicon micromachined device was implemented by Garcia et al. that can apply and sense tensile and shear forces in an epithelial cell monolayer. The device features two cell adhesion planks that are each supported by two sets of folded beams that act as springs with known spring constant. Once the monolayer is formed on the two planks, the actuating side is positioned using a tungsten needle attached to a three-axis micromanipulator to stretch it and the forces within the monolayer are recorded by tracking the deformation of the beams. With this device, the mechanics of Madin-Darby canine kidney (MDCK) cell epithelia in shear and tension were investigated. Under tension, the monolayer experienced a higher maximum force and had a lower relaxation time constant when stretched on a high stiffness device (beam thickness of 11 μm) than a medium stiffness device (beam thickness of 8 μm). This was theorized to be the result of the viscoelastic behavior due to the inherently higher loading rate on the stiffer device. Under shear stretch, however, the monolayer experienced similar maximum shear forces on both devices, indicating that different structures may control resistance to shear and tensile loads. Under cyclic stretching conditions with shear loading, it was found that the relaxation time constant decreased with each application of stretch, indicating that cyclic shear loading may inhibit the ability of epithelia to resist shear stress. While this device allows for the application of both tensile and shear load, which is uncommon in many similar devices, the fabrication tolerances limit the ability to accurately measure the force and allow only for finding relative force differences between devices with different stiffness [40] (Fig. 2D).

The combination of electronics with stretchy and flexible materials has resulted in a significant surge in new technological advancements and applications. For applications such as brain interface [42, 43], epidermal sensing [44, 45], and cardiac electrotherapy [46], the interaction of flexible and stretchy devices with biological tissues is receiving special attention. Mechanically stretched cells and cell monolayers may be electrically sensed using stretchable microelectrodes on an ultra-elastic substrate [47, 48]. With a unique interlaced meander design coupled to the microelectrodes and an ultra-elastic substrate of Poly (dimethylsiloxane) (PDMS) with Young's modulus of 50 kPa, up to 35% cyclic stretch is possible. The platform allows for real-time electrical cell-substrate impedance (ECIS) monitoring of cell monolayers without the need for labels. A uniaxial mechanical strain of more than 20% raised the electrical impedance of Madin-Darby canine kidney (MDCK) cell monolayers. However, Human alveolar basal epithelial adenocarcinoma (A549) cell monolayers, which lack established cell adhesion, demonstrated a consistent reduction in electrical impedance across the whole applied strain range of 35% [41] (Fig. 2E).

With the rapid advancement of the microfabrication process, Esfahani et al. used a two-photon polymerization (TPP) method to directly print the whole structure at once. This device has two islands, one for the actuation and one for the sensor. A pair of cells are deposited on the islands using the Eppendorf cell isolation setup. When a mature junction forms between the cells, they are strained until failure. The stretch test process was captured, and the displacement was analyzed using a custom-made MATLAB code. Based on the stiffness of the beams underneath the islands, this device can capture cellular forces up to a few nN. Skin cancer cells (A431) were used to study the cell response under different strain rates. It was concluded that cell behavior is strain rate dependent since at higher strain rates cell junction ruptured while at lower strain rate cells dissipated the force [24] (Fig. 2F).

1.2. Microdevices to study compression

In-plane compression of epithelial tissues occurs often during adult life and embryonic development because of both inherent and extrinsic stresses [49, 50]. These pressures are critical for shaping complex tissues which are formed during developmental morphogenesis and are essential to the function of many organs. Epithelia in the airway experience periodic area changes during normal breathing and longer-term compression during diseases such as asthmatic bronchial constriction [51]. Another important feature of compression is its nature of origin. The compression stress traces its origin to extracellular forces exerted by either neighboring cells or external stimuli to drive several morphogenetic activities involving tissue bending and folding throughout embryonic development, including the formation of the optic cup [52], gut villi [53], and cortical convolutions in the brain [54]. Therefore, the study of cell response under compression is important.

Cell deformability for flowing cells such as RBCs and cell stiffness for muscle and heart cells during compression is the main subject of research to develop and implement tools to compress cells. These methods mostly used suspended single cells captured by a gripper or tweezer. Fabricating soft grippers and high-throughput methods are the main challenges of these devices. Table 2 summarizes these methods together with their advantages and disadvantages.

Walker et al. used a high throughput microfluidic electromagnetically actuated MEMS μHammer to trap and compress cells [55]. The strain magnitude (42% and 69%) and duration of squeezing (10 μs and 100 μs) were evaluated to study the effects of the compression on membrane permeability, apoptotic induction, and proliferation of human neural progenitor cells (hNPCs). The magnitude of applied strain significantly affects cell membrane permeability shortly after compression, whereas increasing the duration of strain increased early apoptosis in cells 24 h after compression. The high throughput ability, consistent testing, rapid analysis using propidium iodide, and engineered controls make this a robust method for testing the effects of compression. However, the lack of force quantification is a downside (Fig. 3A). A soft robotic device for the

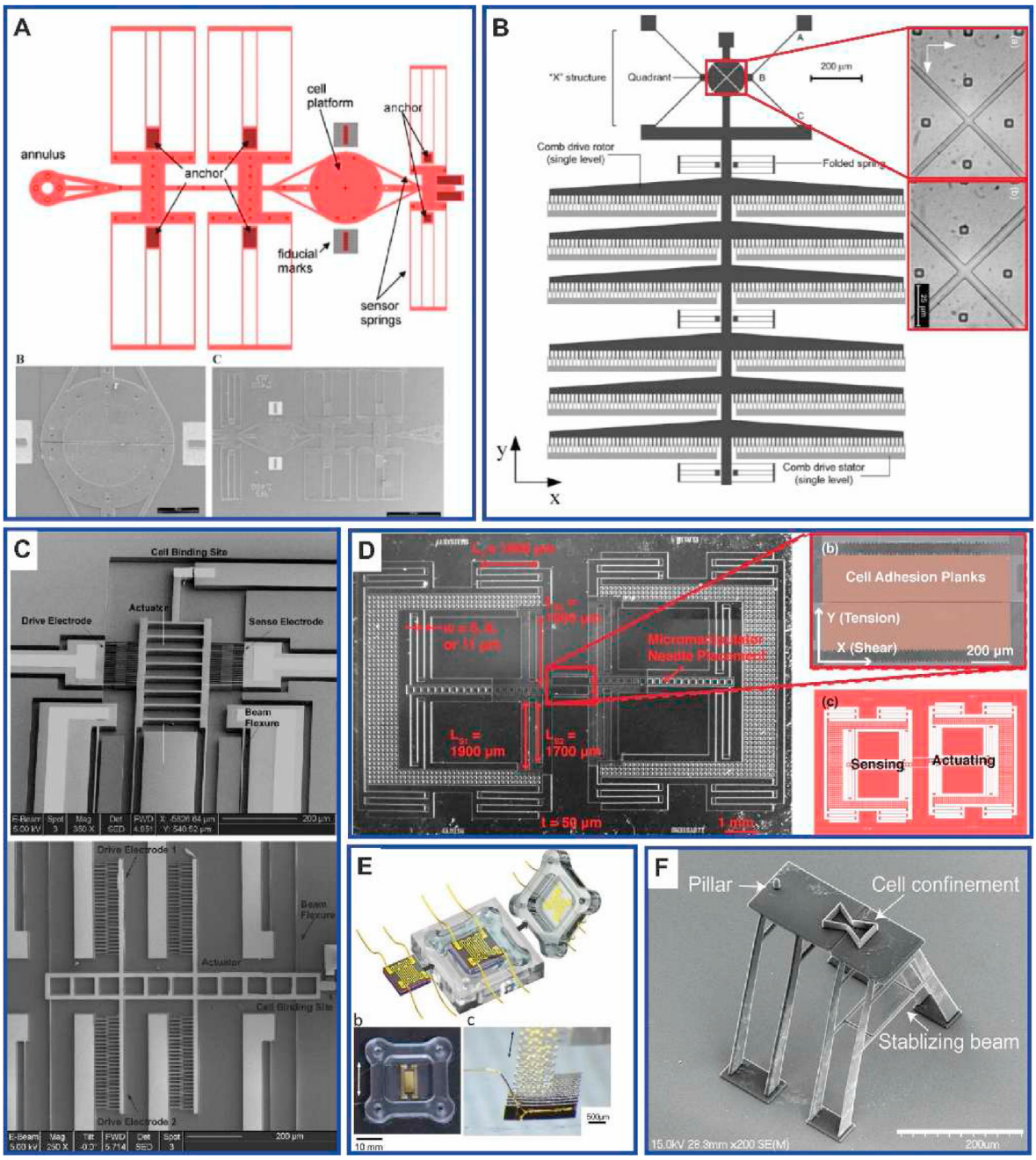

Fig. 2. Microstructures that can capture cell characteristics in the tension mode. A) Flexible MEMS beams. Reproduced with permission from Ref. [34] Copyright Springer. B) Bidirectional mechanism comb drive capacitor. Reproduced with permission from Ref. [39] Copyright Springer. C) Electrostatic comb-drive actuator. Reproduced with permission from Ref. [36] Copyright IEEE. D) Silicon micromachined flexible beams. Reproduced with permission from Ref. [40] Copyright IOP Publishing. E) Stretchable microelectrodes on an ultra-elastic substrate. Reproduced with permission from Ref. [41] Copyright Elsevier. F) 3D printed microstructure. Reproduced with permission from Ref. [24] Copyright National Academy of Sciences.

mechanical characterization of 3D biological samples has been designed by Parreira et al. The device uses microscale optomechanical actuators (μOMAs), which shrink when exposed to a laser to drive a flex-tensional mechanism that pushes a piston-like end-effector to compress the tissue sample. The end effector compresses the tissue sample against a cantilever beam fabricated from poly (ethylene glycol) diacrylate (PEGDA) with known stiffness to measure the force applied to the sample. With this device, the stiffness of a spheroid cluster of human embryonic kidney (HEK) cells was measured to 2.45 kPa. While the device is not as sensitive as other measurement techniques such as electrostatic comb drives, it has a simple design and interfaces with biological tissues well due to the relatively soft materials it is fabricated from Ref. [56] (Fig. 3B). Barazini et al. produced an electrothermally actuated single-cell squeezing device to perform mechanical characterization of brewing ale and lager yeast cells at three different fermentation phases. Cell compression was induced by a MEMS squeezer, and displacement measurements were taken using optical microphotographs. Across all fermentation phases, ale cells ruptured under an average force of 0.28 ± 0.05 μN and displayed a midpoint stiffness of 4.8 ± 1.0 μN/μm, whereas lager cells ruptured at 0.47 ± 0.10 μN and had a stiffness of 5.3 ± 0.9 μN/μm. This device displayed very high displacement and imaging resolutions (10 nm) and was compatible while submerged in water. However, the maximum compression was limited to 2.5 μm. Additionally, the low throughput nature of single-cell placement is a drawback [57] (Fig. 3C).

Table 2
Methods to measure cell mechanics in the compression mode.

Method	Cell type	Parameters studied	Pros	Cons	Ref.
Electromagnetically actuated μHammer	• Neural Progenitor	• Apoptosis	• Control time of compression • High Throughput • Consistent Test • Rapid Analysis	• No force measurement	14
Electrothermal MEMS actuator	• Yeast • "Red Ale" • "SMA"	• Rupture force • Pre-rupture stiffness • Post-rupture stiffness	• Underwater conditions • High-resolution set-up	• 2.5 μm displacement • Low throughput • 30 min estimated cell placement each	16
Microscale optomechanical actuators	• Human embryonic kidney (HEK)	• Young's modulus	• simple design and interfaces with biological tissues	• Not as sensitive as other measurement techniques	15
Microgripper	• Benign prostate cells BHP and malignant prostate cells PC-3 and LNCaP circulating tumor cells (CTCs)	• Young's Modulus • stiffness	• Small size • Single cell • Simple design	• Measure biological sample up to 9.7 kPa	20
MEMS tweezers	• SUM159PT (a triple-negative breast cancer cell line)	• Size, stiffness, and viscosity	• Good imaging • Single cell	• Throughput	21, 22
MEMS tweezers	• No measurement	• Measuring stiffness and viscosity	• Analytical model	• Just feasibility study	23
Microgripper	• Human red blood cells (RBCs)	• Deformability	• Simple design • Analytical model	• Potential damage introduced by heat • Limited opening size	24
Nanoprobe	• Monkey kidney fibroblast cell	• Force response to stretch	• Simplicity	• Its service life • Post-experiment cleaning	25
Nano pin	• Agarose gel (0.6%) and human lung carcinoma A549 cells	• Stiffness	• Normal growth after experiments	• Complicated design and setup	26

During the past several decades, microgrippers have been widely developed, and more recently, novel synthesis techniques [63, 64] have allowed designers to handle tissue via selective, non-isotropic compliance [65]. In one study, the authors designed a MEMS microgripper to measure the stiffness of cells to identify early signs of cancer metastasis. Circulating tumor cells (CTCs) tend to be softer than non-malignant cells and therefore measuring their stiffness is a way to identify them in blood. The device features two polysilicon arms, one of which is fixed while the other can move by applying AC and DC voltage to rotary comb-drive actuators. Once the cell is gripped between the arms, an AC voltage applied to the electrodynamic actuators causes the mobile arm to oscillate, cyclically squeezing the cell. The stiffness of the cell can be found by either tracking the displacement of the cell as it is squeezed through the capacitance shift in the electrostatic actuators or by increasing the actuation frequency until the torsional resonant frequency of the mobile arm is reached, both of which are dependent on the stiffness of the cell. With this device, the elastic moduli of benign prostate cells BHP and malignant prostate cells PC-3 and LNCaP were 2797, 1401, and 287 Pa, respectively, demonstrating that malignant cells are less stiff than non-malignant cells [66].

The combination of microfluidics with MEMS tweezers enables high-throughput measurements with subcellular imaging. Perkin et al. designed a microfluidic device with a side aperture that can be used to capture a SUM159PT cell (a triple-negative breast cancer cell line) with a MEMS tweezer and study its size, stiffness, and viscosity. During an 80% compression cycle, the resonance frequency (related to cell stiffness) increased while the amplitude (connected to viscosity) decreased [58, 67] (Fig. 3D). A new approach for evaluating the viscoelastic characteristics of soft materials has been presented by Giamberardino et al. This method relies on the use of a microsystem with a flexible 4-bar linkage that has low stiffness and serves the tissue sample as the connecting rod. The stiffness of the sample is calculated in the static mode while the viscosity is investigated in the dynamic mode. This method can measure the stiffness and the viscosity coefficient of the tissue sample to detect illnesses in living creatures [59] (Fig. 3E). Cauchi et al. designed a horizontal electrothermal microgripper that can open to 9 μm at 3 V applied voltage, which provides a suitable solution for studying the deformability characterization of human red blood cells (RBCs) that have an average diameter of 8 μm. The microgripper was designed based on applying voltages to two parallel arms of different widths. This leads to generating temperature differences between the two arms and causes a bending moment in the direction of the cold arm. The microgripper was fabricated using PloyMUMPs to provide layers of silicon structure as the substrate to support the microgripper actuator, composed of polysilicon and gold metal. The efficiency of this microgripper for use in cell studies has not been proved and potential damage introduced by heat to the cell membrane needs further investigation [60] (Fig. 3F). A force sensor designed by Yang et al. consists of a probe and a flexible beam to stimulate and measure cell force response. This sensor shows its simplicity and versatility for the study of cell mechanics that is comparable with some existing techniques such as AFM or glass needles. The sensor was fabricated using a modified single-crystal reactive etching and metallization (SCREAM) process, and its stiffness was calibrated with an AFM cantilever. Prior to use, the probe was coated with fibronectin to enhance cell adhesion. Monkey kidney fibroblast cells were used to demonstrate the function of the force sensor by applying 2 μm lateral displacement on the cell membrane for 20 min. A drawback is that this sensor may not be able to survive the capillary force during the taking-out-of-liquid process, so its service life is a concern. Moreover, the post-experiment cleaning process for this customized sensor was not included in this paper [61] (Fig. 3G).

Momoko Kumemura et al. designed a Nanopin sensor based on resonance frequency measurement for studying mechanical characterization of individual adherent cells. The Nanopin consists of a displacement sensor, a comb drive actuator, and a sensing probe. Like the working mechanism of nanotweezers, the actuator can oscillate the sensing tip with nanometer displacement. The output signal is collected and analyzed through a Lock-in-amplifier and LABVIEW program. Incorporated with the UnipicK + single-cell collection system, the Nanopin was calibrated and positioned precisely in the z-axis before contacting the cell or gel surface. Agarose gel (0.6%) and human lung carcinoma A549 cells were used in the study. A549 cells were cultured on non-coated, and Poly-L-lysine (PLL) coated dishes and were indented 1.5 μm after the initial contact. A constant resonance frequency was observed on cells with PLL coated dish; however, resonance frequency and amplitude increased on the non-coated dish. Stiffness of 0.02 N/m was calculated based on a simple mass-spring-damper model after analyzing data. Cells showed normal growth after experiments, demonstrating that

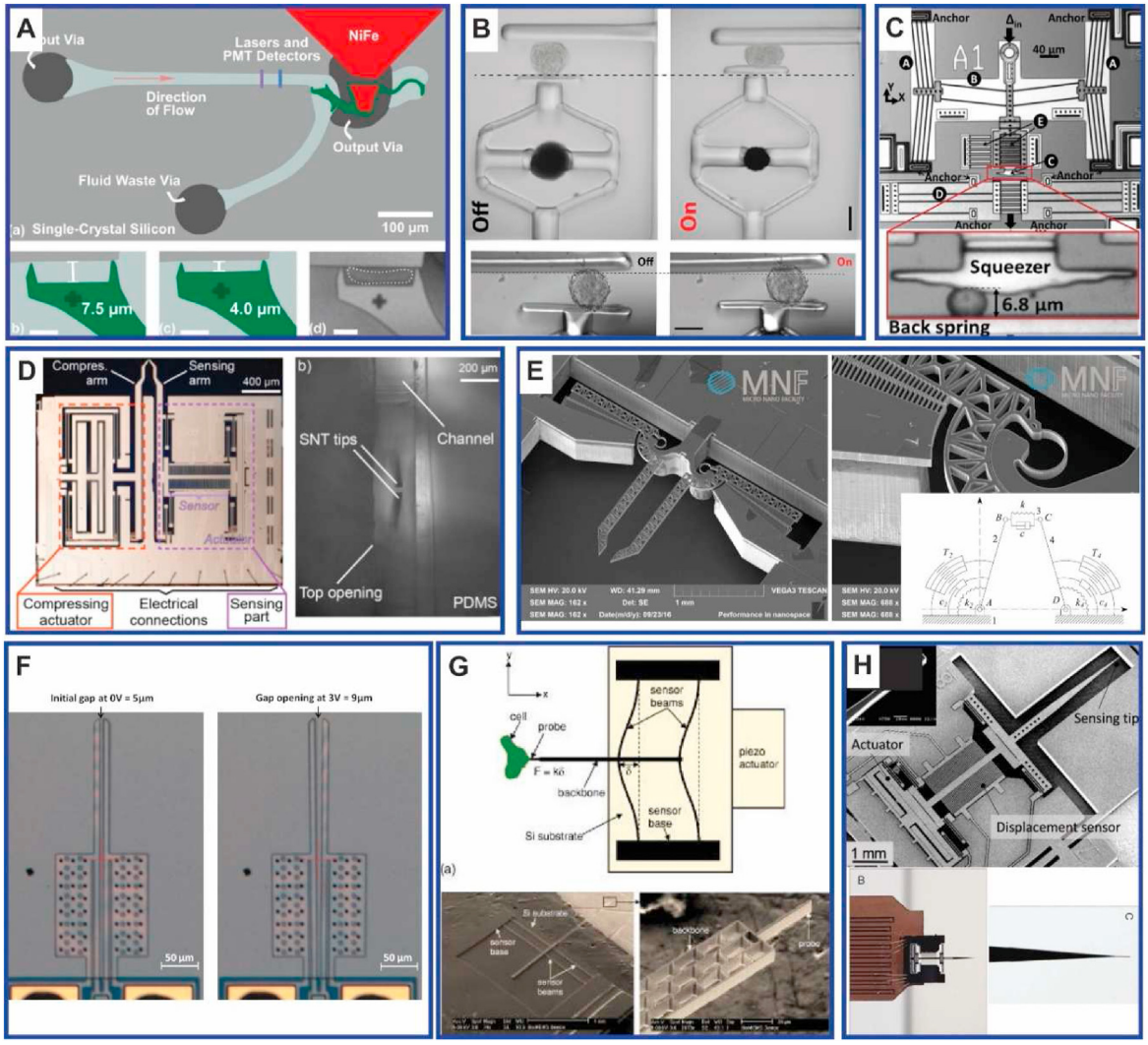

Fig. 3. Microstructures that can capture cell characteristics in the compression mode. A) μHammer. Reproduced with permission from Ref. [55] Copyright IEEE. B) Optomechanical actuator. Reproduced with permission from Ref. [56] Copyright Frontier. C) Electrothermally actuator. Reproduced with permission from Ref. [57] Copyright © Taylor and Francis. D) Microfluidic chip with a MEMS tweezer. Reproduced with permission from Ref. [58] Copyright Nature Publishing Group. E) Flexible 4-bar linkage. Reproduced with permission from Ref. [59] Copyright MDPI. F) Horizontal electrothermal microgripper. Reproduced with permission from Ref. [60] Copyright MDPI. G) MEMS flexible beams. Reproduced with permission from Ref. [61] Copyright AIP. H) Nanopin. Reproduced with permission from Ref. [62] Copyright Cell Press.

Table 3
Methods to measure cell mechanics in the traction mode.

Method	Cell type	Parameters studied	Pros	Cons	Ref.
Spring-like sensor	• Fibroblasts (3T3) • Cancer-associated fibroblasts (CAF05) • Human colon (FET) • Human lung epithelial carcinoma (A549)	• The maximum force	• The force resolution of around 1 nN • 3D culture	• Cultured at room temperature at least 10min	27
MEMS cantilever force sensor	• Bovine Aortic Smooth Muscle Cells (BAOSMC)	• The average force of a single focal adhesion	• Electric resistivity of the piezoresistive • 1 nN resolution	• Only capable of FA force measurement	28
Magnetic microposts	• NIH 3T3	• Traction force	• Focal adhesion size, traction force, and the strain energy • Actuation mechanism	• Cannot control the direction of casted nanowires • Hard to control the density of nanowires in each micropost	32
Piezo-phototronic light nano-antenna (PLNA) array	• Cardiomyocytes	• Spatial distribution of force on a contracting cardiomyocyte	• Dynamic real-time imaging	• Complicated design and setup	33
MEMS force transducer	• Cardiomyocytes	• Maximum contractile force • Maximum tensile stress	• Measuring of contractile forces in heart cells	• Process of clamping and gluing	34

this prototype could measure cell mechanical properties without damaging cells [62] (Fig. 3H).

1.3. Microdevices to study traction forces

Traction forces are another type of cellular force exerted by cells at the focal adhesion sites to the substrate and extracellular matrix (ECM). Cells sense the substrate rigidity and adopt their cellular function such as migration and differentiation. Therefore, quantitative analysis of these forces allows us to better understand the underlying mechanisms and mechanotransduction pathways in cell decisions. The techniques to measure these forces mainly rely on surface modification such as growing cells on micropillars or cantilevers with piezo sensors. In addition, growing cells between two beams and measuring their deflections is another innovative method to capture contraction or traction. Furthermore, since the traction force is an inherent property of cells, it can be measured with and without external loads. Several techniques have been invented to measure traction force, of which we review microfabricated-based methods (Table 3).

Emon et al. designed a mechanical sensor that quantitatively measures the single-cell force, several discrete cell forces, and the tissue stiffness. This platform uses one highly sensitive soft spring to sense the cellular force fluctuation producing a force resolution on the order of 1 nN. Another stiff spring and two grips are used to hold the cell-ECM mixture. The liquid cell-ECM forms into a capillary bridge between the springs and transfer the contractile force to the soft spring when cells pull the collagen fibers in the ECM. They found that the maximum force of fibroblasts (3T3) cells was 20 nN in 20 h after cell seeding while the maximum force of cancer-associated fibroblasts (CAF05) cells was around 50 nN with a sharp increment between 16 h and 18 h. The time-resolved force of human colon (FET) and human lung epithelial carcinoma (A549) cancer cells were also measured via this platform. Interestingly, they cocultured FET cells with CAF05 cells to mimic the tumor microenvironment (TME) on the platform and measured the time-resolved force of this cancer model. They found that the cocultured cells have a larger change of force output and ECM stiffness. Besides the advantage of the accurate measurement of force changes on cell and ECM stiffness changes, this structure can also study cells in 3D culture which is closer to the physiological microenvironment compared to 2D culture. Due to the vulnerable soft spring, they used gelatin (solid at room temperature and liquid at 37 °C) as a sacrificial layer before the cell-ECM tissue was formed. This means that cells should be cultured at room temperature for at least 10min during the polymerization of collagen which may be harmful cells [68] (Fig. 4A).

In another study, a MEMS force sensor was fabricated to dynamically show the traction force changes of adhesive cells when they interact with substrates. The authors used the deformation of the cantilevers to display the force changes of cells when they adhered to substrates. Different from other groups which extract the force and stiffness from the displacement changes directly, they used the changes of electric resistivity of the piezoresistive layer to show the cellular forces. The resistivity is measured as a voltage signal based on the Wheatstone bridge circuit and instrumentation amplifier. The force resolution of this sensor is around 1 nN and its temporal resolution is approximately 2.5 μs? Bovine Aortic Smooth Muscle Cells (BAOSMC) were cultured on the cantilever to test the sensor. Their results showed that the average force of a single focal adhesion is about 7 nN and this force disappeared in microseconds by adding trypsin-ethylenediaminetetraacetic acid. They also tested the traction force changes when the focal adhesion is decomposed by trypsin and showed that the bending force on the cantilevers was released. The advantage of this sensor is that it can detect the force changes rapidly and accurately. However, it can only be used for testing the force of focal adhesion between cells and substrates [69] (Fig. 4B).

Microposts and micropillars have been widely used to study traction forces generated by different cell types [73–75]. Cells are seeded on a bed of posts to adhere and proliferate, and traction forces at focal adhesions bend the posts. By calculating the post displacement and its stiffness, the traction force is obtained. Magnetic microposts are used to not only measure the force changes of focal adhesion during the cell growth and proliferation but also monitor the cell responses under the external forces. These microposts are made by casting the solution of nanowires and PDMS into the mold and it can induce the external force on cells by torquing the nanowires under a magnetic field. Sniadecki et al. tested the cell response to the external forces on NIH 3T3 using this platform, including the focal adhesion size, traction force, and strain energy. By controlling the magnetic field, they tested the cell response under one step and multiple steps of external force. The local focal adhesion size increases under both the single and multiple actuations. Furthermore, the traction force exhibits a sudden loss on the cell boundary in the first minutes of applying the external force. The advantage of this setup is that it can apply both external forces on cells and detect the cell response to those external forces. The drawback is that they cannot control the direction of casted nanowires which induces an uneven external force on cells. Otherwise, it is also hard to control the density of nanowires in each micropost. Since the pillar dimension (D = 3 μm) is larger than the nanowires (D = 350 nm), it's hard to cast only one nanowire in one pillar [70] (Fig. 4C).

Zheng et al. designed a piezo-phototronic light nano-antenna (PLNA) array, which consists of InGaN/GaN nanopillars and utilizes the piezo-phototronic effect to visualize traction cell contraction forces in contracting cardiomyocytes. When the nanopillars are strained, their photoluminescence efficiency decreases, resulting in a drop in luminescence which can be visualized under a confocal microscope. The device has a spatial resolution of 800 nm and a temporal resolution of 333 ms, experiences almost no photobleaching, and can be used on living cells. With this device, the authors observed that the spatial distribution of force on a contracting cardiomyocyte is heterogeneous, with contracting forces being higher at the perimeter of the cell and lower near the nucleus [71] (Fig. 4D).

A MEMS force transducer system has been proposed by Lin et al. for measuring contractile forces in heart cells. The device has two free-standing polysilicon clamps each supported by a pair of microbeams on which cells are glued with a silicone sealant. Cell contraction causes the beams to bend, from which the contractile force is found using the deflection and spring constant of the beams. With this device, the authors found a maximum contractile force of 12.6 μN and maximum tensile stress of 23.7 mN/mm^2. In addition, it was found that the contractile force increased as the free calcium concentration in the culture media increased. This device was the first MEMS device that allowed for measuring contractile forces in heart cells but had limitations due to the process of clamping and gluing cells to the device poorly reflecting the physiological environment of the cell [72] (Fig. 4E).

2. Conclusion and future perspectives

Internal and external forces play an important role in cell functionalities such as cell migration, proliferation, and differentiation. These forces are mainly in three modes; compression such as cell contraction, tension such as skin stretch, and traction such as cell-ECM connection. Cells respond with different parts of the cytoskeleton based on the nature of the force to either dissipate the force or strengthen the tissue. Investigating how cells respond to these forces will help us to decipher the underlying mechanisms and find better therapeutic options. Many techniques have been designed and implemented to measure cellular forces at different levels of which microscale structures provide a better physiological environment besides their higher accuracy and resolution. The invention of silicon microfabrication led researchers to design miniature devices to capture small forces. Due to the fabrication complexity and limited methods of force measurement, they had to use specific materials and complicated design that led to low efficiency and sometimes not biocompatible devices. With the advent of 3D printers, fabrication of any design with different (biocompatible) materials is

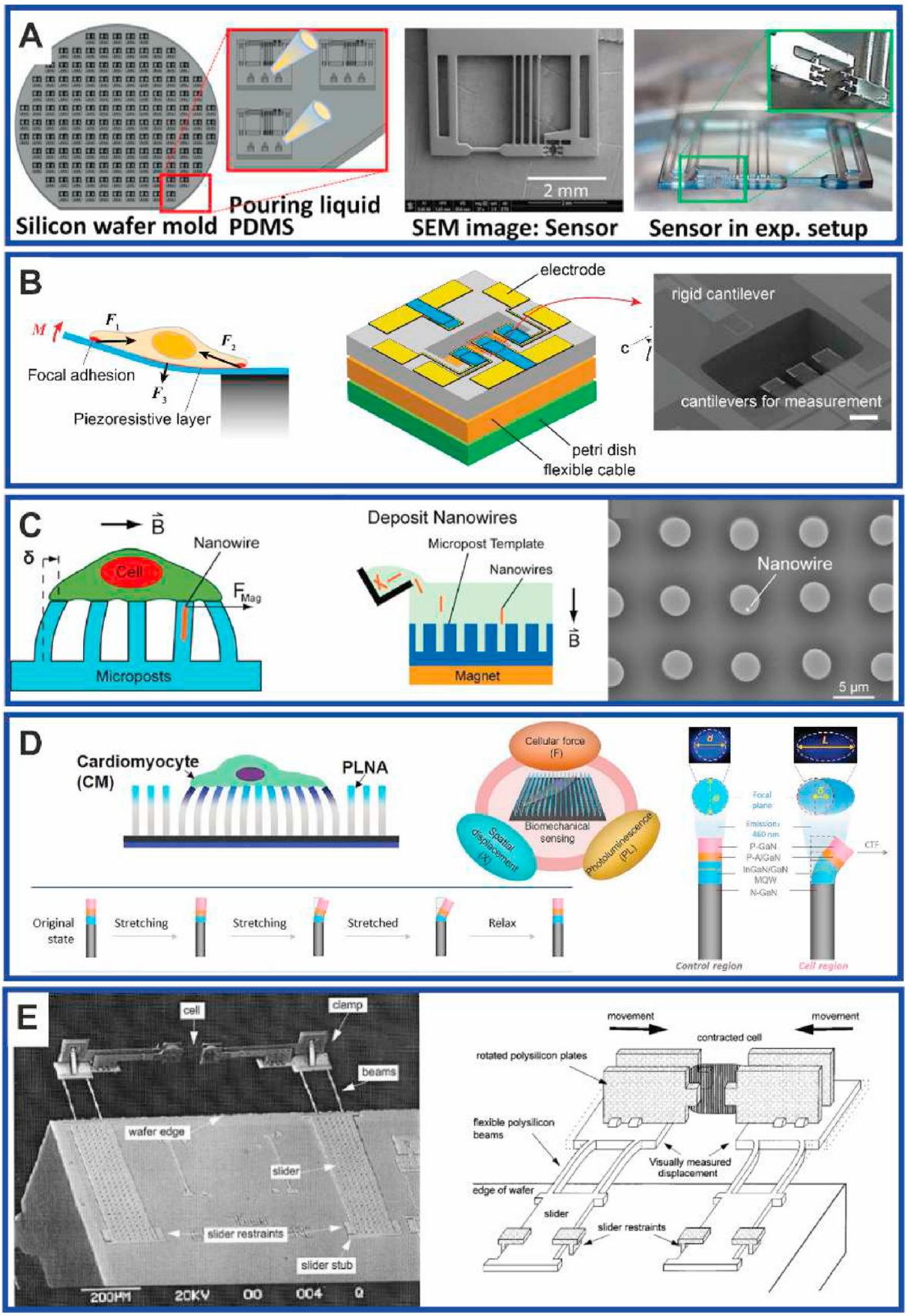

Fig. 4. Microstructures that can capture cell characteristics in the traction mode. A) Flexible cantilever beams. Reproduced with permission from Ref. [68] Copyright Science (AAAS). B) Piezo-resistive cantilever beams. Reproduced with permission from Ref. [69] Copyright IOP Publishing. C) Magnetic microposts. Reproduced with permission from Ref. [70] Copyright National Academy of Sciences. D) Piezo-phototronic light nano-antenna (PLNA) array. Reproduced with permission from Ref. [71] Copyright Science (AAAS). E) MEMS force transducer. Reproduced with permission from Ref. [72] Copyright IEEE.

possible that allows us to design simple yet precise and accurate devices. Among the 3D printers, the TPP method gives us the best fabrication resolution using biocompatible materials. This method uses two-photon excitation and provides rapid fabrication with a nano meter scale.

Though advances have been made in microfabrication and 3D printing, there are still remaining issues to be considered. An important evaluation criterion for biomedical devices is the level of throughput. Most of the current techniques are low in throughput. The low level of throughput may not be a significant bottleneck for a small number of laboratory experiments. However, if that device is deployed for screening purposes, researchers should consider methods that allow for high throughput measurements. Imaging of the cellular structures is still challenging for some microfabricated devices, particularly for devices fabricated from silicon. Other methods use transparent crosslinked polymers to fabricate cellular scaffolds for force measurement. The polymeric materials are often auto-fluorescent, elevating background noise and thus reducing image quality. Research work to address this will require the development of new materials for microfabrication that enable high-quality imaging. This will help in deciphering the underlying mechanism of cellular response to external and internal forces. Specifically, capturing changes in signaling molecules in response to a load, creep, or relaxation will pave the way for investigating the function of mechanosensors.

Funding resources

We acknowledge the funding support from the NSF (Awards 1826135, 1936065), the NIH National Institutes of General Medical Sciences P20GM113126, and P30GM127200. We acknowledge funding support from the Nebraska Collaborative Initiative and EPSCoR FIRST award. G.M. and J.R. are funded by the NSF Graduate Research Fellowship (Awards 2034837, 2034837).

Credit author statement

R.Y. and A.M.E. conceived the idea. A.M.E., G.M., J.R., H.Z., X.J., B.T.S., and J.R.B wrote the manuscript. R.Y. and A.M.E. revised and wrote the final version of the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We acknowledge the authors and the publishers of the papers from which we reprinted the figures.

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