

# Mechanical characterization of cells and tissues at the micro scale

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**Abstract**— Cell-substrate interactions are relevant for a number of biological and clinical applications e.g. to determine the effectiveness of medical implants. Cells are natural transducers that respond to and sense signals originating in their microenvironment. One important cell signaling mechanism is known as chemo-mechanical transduction. This refers to the use of external mechanical cues to initiate internal biochemical cellular processes and *vice versa*. One key factor to characterize and understand these interactions is the evaluation of the mechanical forces present at the cell-substrate interface. Recent advances in the micro and nanotechnology fields have allowed the development of new tools for the measurement of cellular and tissue forces. These tools have provided a means to study extremely low cellular and subcellular forces (pN- $\mu$ N) as well as detailed small-scale tissue mechanics. This paper will review some of the most significant approaches to characterize the mechanical properties of cells and tissues at the micro-scale. Material properties, device fabrication, and design issues will be discussed.

**Keywords**— Cell-substrate interactions, Cellular mechanics, Mechanotransduction, Microdevice, Micro-scale.

**Resumen**— Las interacciones célula-sustrato juegan un papel fundamental en gran número de aplicaciones biológicas y clínicas. Las células son transductores naturales que sensan y responden a señales en su entorno fisiológico. Uno de los mecanismos más importantes empleados en la caracterización de interacciones celulares es la transducción químico-mecánica, la cual se basa en la implementación de señales externas que se aplican a la célula con el fin de inducir diversos procesos bioquímicos al interior de ésta y viceversa. Los avances alcanzados en el campo de la micro y nanotecnología han permitido el desarrollo de nuevas herramientas para medir fuerzas a nivel celular o incluso sub-celular (pN- $\mu$ N), y dilucidar la mecánica de los tejidos en la escala micrométrica. La presente revisión literaria describe algunos de los micro-dispositivos empleados actualmente para caracterizar las propiedades mecánicas de las células y tejidos en la micro-escala.

**Palabras clave**— Interacciones célula-sustrato, Mecánica celular, Mecanotransducción, Micro-dispositivo, Micro-escala.

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## I. INTRODUCTION

It has been widely documented that cellular behavior can be influenced by external factors related with the surrounding microenvironment. Recent advances in the micro and nano-technology fields have encouraged the study of the effect of different factors (e.g. topography and chemistry of the substrate) on cellular behavior. One key factor to characterize and understand the cell-substrate interactions is the evaluation of the mechanical forces presented at this interface. Cells bind to the extra cellular matrix (ECM) (*in vivo*) or to the cell culture substrate (*in vitro*) via transmembrane proteins called integrins, which are adsorbed as a layer onto the substrate. Integrins and

other cytoplasmatic proteins form large clustered structures called focal adhesions, which act as signal transducers of the ECM environment and regulate the cytoskeletal conformation. These focal adhesions play a fundamental roll in cell spreading and migration, and can be modulators of other cellular functions such as proliferation and differentiation [1- 4]. The cell not only exerts mechanical forces on the ECM (for example contractile forces during spreading and traction forces during migration), it also senses and responds to them, inducing structural remodeling of the ECM. The characterization of these forces can lead to a better understanding of cellular biomechanics, and how this can affect cell adhesion,

migration, proliferation, and differentiation [1, 5-8]; thus improving the knowledge of the physiology of human tissues, and the possibility to predict and control the cellular behavior under determined parameters.

The first approach for measuring cellular forces was developed in the 80's by Harris *et al.* They studied a measurement method based on the observation of the wrinkles and folds formed by the traction forces of cells (chick heart fibroblast, PTK-1, liver parenchyma cells and pigmented retinal cells among others) cultured on cross-linked silicone rubber films ( $\sim 1\mu\text{m}$  thickness) sitting on top of a liquid silicone layer [27, 1, 5]. While providing a foundation for measuring cellular forces, this method presents several disadvantages since it provides only a qualitative measurement of the forces, low spatial resolution due to the limitations of a flat surface (making the characterization of forces at individual focal adhesion points more difficult), and the complex relationship between the wrinkle or fold geometry and the force magnitude.

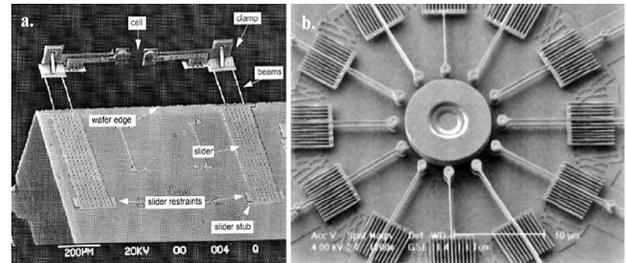
Different researchers have proposed the implementation of elastic gels or membranes with randomly embedded sub-micron fluorescent particles. In these structures, the force is measured according to the displacement of the particles, produced by the deformation of the substrate due to the cellular forces. The complexity of this model is still an issue, due to the 3-D character of the matrix and random particle distribution, which leads to superposition of particles. Additionally, the models did not provide unique solutions [1, 9]. This method was improved through the implementation of regular arrays of fluorescent beads fabricated by electron beam lithography instead of randomly distributed particles. This homogenous distribution simplified the calculation of the force, but the superposition between the displaced beads was still a problem [5, 10].

Taking advantage of these first developments and of the new tools provided by micro and nano technology, it has been possible to improve the quality and efficiency of techniques used to measure cell forces. This review will be focused on studying some of the most significant devices for characterizing the mechanical properties of cells and tissues at the micro scale. Two different groups will be discussed: 1) silicon MEMS (micro-electro-mechanical systems) sensors fabricated by micromachining techniques; and 2) polymer-based sensors fabricated by soft lithography techniques.

## II. SILICON MICROMACHINED SENSORS

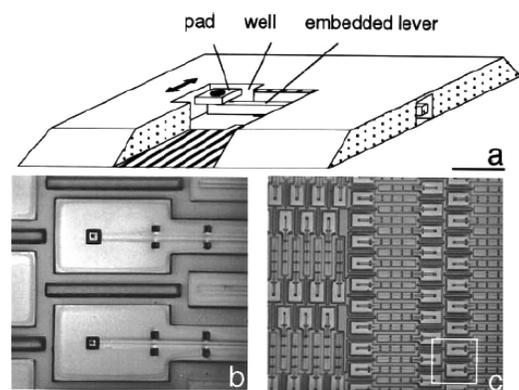
Using photolithography, surface and bulk micromachining techniques, several silicon-based

microdevices for dynamical evaluation of cellular forces have been fabricated. The designs include horizontal and vertical microcantilevers (arrays or single distribution), platforms and microgrippers among others (Fig. 1) [1, 3, 5, 11-18].



**Fig. 1.** Design of some cell transducers. **a.** Heart cell force transducer proposed by Lin *et al.* (from [17]). **b.** Multiple passive and active cantilevers beams (from [18]).

In the late 1990's, Galbraith and Sheetz used horizontal polycrystalline silicon microcantilevers with single crystal silicon cell attachment pads on its free end, to evaluate chicken embryo fibroblast and fish keratocyte traction forces [13, 14]. The array of microcantilevers was equitably distributed along the substrate, and each microcantilever was isolated in a well to allow the characterization of single cells (Fig. 2). The adhesion of the cell to the walls or bottom of the well produced a displacement of the microcantilever, which was measured by light microscopy ( $0.2\mu\text{m}$  of resolution). The magnitude of the force was proportional to the deflection and spring constant of the microcantilever (according to Hooke's law). Due to its simplicity, the method presented some disadvantages, since the horizontal orientation of the microcantilever restricts the measurement to one axis, and the fact of having only one microcantilever per well limited the measurement of the force to one single location under the cell (low spatial resolution). It is also important to note that the implementation of cell attachment pads can alter the mechanical properties of the microcantilevers.



**Fig. 2.** **a.** Schematic view of the microdevice (scale bar =  $10\mu\text{m}$ ). **b.** detail of the pads on the microcantilevers (scale bar =  $10\mu\text{m}$ ). **c.** Image of the Microcantilever array (scale bar= $1\text{mm}$ ) [13].

Yang *et al.* developed a chip to measure cell force response to large stretches and indentations in monkey kidney fibroblast (MKF) [19-21]. The chip was formed by a horizontal microcantilever with a probe in its free

end used to indent or stretch the cell, two parallel flexible beams that were used to sense the cell force response, and a piezoelectric actuator to move the probe in the x axis to indent or stretch the cell (Fig. 3).

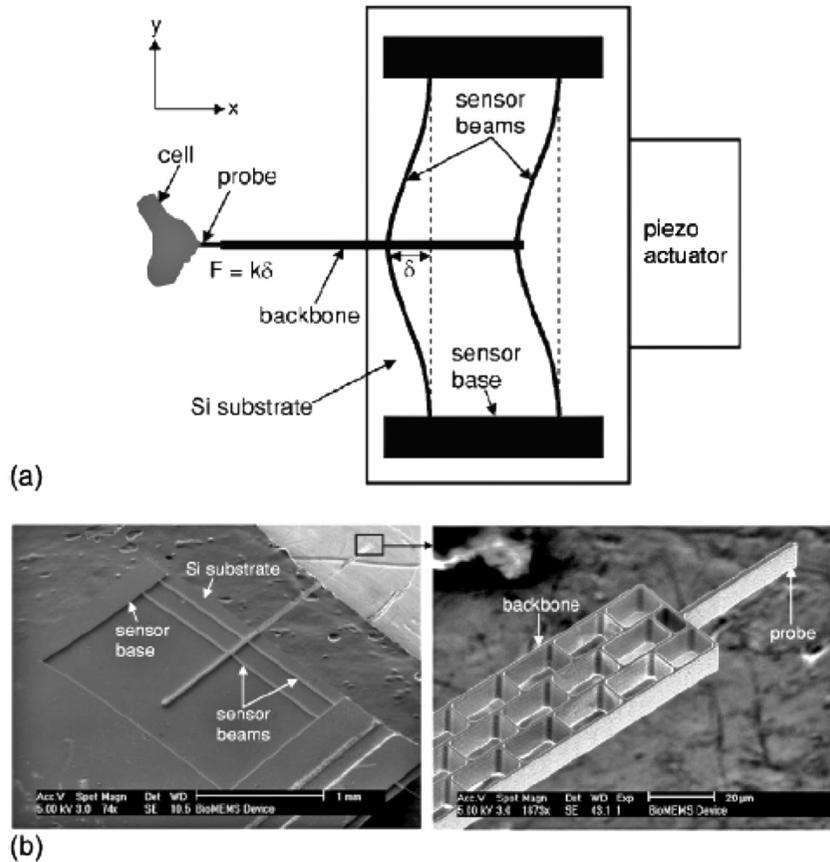


Fig. 3. (a) Schematic view of the chip. (b) SEM images of the sensor beams (left) and the microcantilever (right) [19].

The microcantilever and the beams were fabricated from single-crystal silicon by using a Single Crystal Reactive Etching and Metallization (SCREAM) process. Similar to the design of Galbraith and Sheetz, this method presents several disadvantages due to the implementation of a single microcantilever and its horizontal orientation. The design of this chip was improved by implementation of just one flexible sensor beam and a reference point fixed to a parallel rigid bar; in this case the microcantilever including the probe where located perpendicular to the sensor beam (Fig. 4). In this way the chip was able to measure two component forces. However, the chip presented limitations for measurements in liquid environments since its structure could be damaged by the capillary forces presented when immersing into or emerging from the liquid.

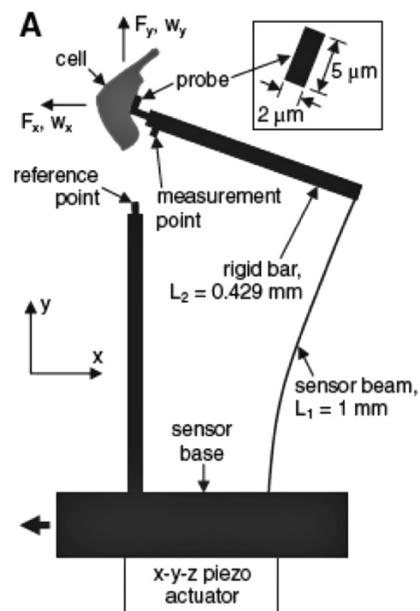


Fig. 4. Schematic view of the chip modified [21].

Subsequent studies employed vertical microcantilever arrays [5, 22], which improve the spatial resolution by having a higher number of points to measure forces on the same cell. The vertical orientation of the microcantilevers also increased the degrees of freedom of the model due to the microcantilever displacement in more directions. Additionally, by using this type of design it was possible to make characterization of different cells at the same time, and it was possible to observe the force direction according to the displacement of each microcantilever at any time [22].

Petronis *et al.* developed three different substrates with vertical silicon microcantilevers arrays [5]. A thin SiO<sub>2</sub> layer on top of the microcantilevers was used as cell attachment pads, which had larger lateral dimensions than the underlying microcantilever but smaller size than the cells being evaluated, to improve the spatial resolution. The flexibility of each substrate depended on its geometry (width and length) (Fig. 5). Dynamical characterization of hTERT BJ-1 fibroblast and HSVEC vascular endothelial cells cultured on the substrates was performed. Fluorescence microscopy was used to determine the deflection of the microcantilevers. The results suggested the possibility of obtaining single substrates with areas of different elastic properties by combination of the three geometries proposed.

One interesting applications of microcantilevers to characterize tissues has been proposed by Xi *et al.* (2005). A silicon microdevice with a thin SiO<sub>2</sub> coating was fabricated using SCREAM processes [23]. The microdevice was composed by a horizontal microcantilever and a rigid post, with a self assembled layer of neonatal ventricular myocytes in between. The self assembled layer was formed by culturing cells on a Cr/Au rectangular substrate between the microcantilever and the post. To assure that the Cr/Au did not introduce mechanical stress to the cantilever, a poly-N-isopropylacrylamide (PNIPAAm) sacrificial layer was

used during the cell culture process. The PNIPAAm was removed once the myocyte layer was formed, by placing the device at room temperature, since the PNIPAAm could be liquefied at room temperature due to its low solid-liquid phase transition temperature ( $>32$  °C) (Fig. 6). The displacement of the microcantilever was measured by using microscopy and digitalized video images. This microdevice avoids manual isolation and integration of muscle tissue for its characterization. However, it is important to characterize the effect of vibratory movement on the estimation of the microcantilever deflection, and the force components in the y-axis.

Besides the possibility to characterize the mechanics of the cell-substrate interactions, some microdevices allow mechanical stimulation of cells to evaluate their behavior under loading conditions, resembling the physiological environment of the tissues. Previous studies have reported that mechanical stimulation can induce different cell responses, such as increased proliferation, protein production, differentiation, synthesis of new biomolecules, and changes in cell morphology [3, 24, 28-30]. Scuor *et al.* developed a platform to study the biomechanical response of single cells under biaxial stretching [3]. The microdevice fabricated from polycrystalline silicon via PolyMUMPs foundry process, consists of a circular plate divided in four quadrants to position the cell and a four-link mechanism (rigid beams) attached to the platform by means of revolute joints. The platform was connected to 12 sets of comb-drive actuators to operate the cell stretcher (Fig. 7a and 7c). The comb-drive actuator was designed based on the force equilibrium between electrostatic force and a spring force (Fig. 7b). Additionally, six folded springs were connected to the device to prevent lateral instability of the comb-drives [25]. The displacement in the platform was proportional to the driving voltage, and the device could operate in air or even immersed in water. This approach could be used potentially to study pericardium cells, which are mainly subjected to biaxial stretching loads.

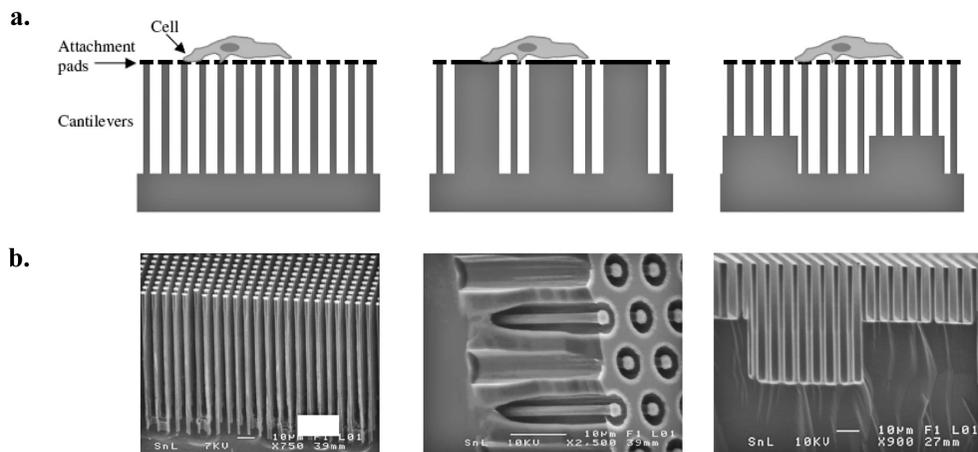


Fig. 5. a. Schematic view of the three designs evaluated. b. SEM images of each designed substrate showed in (a). [5].

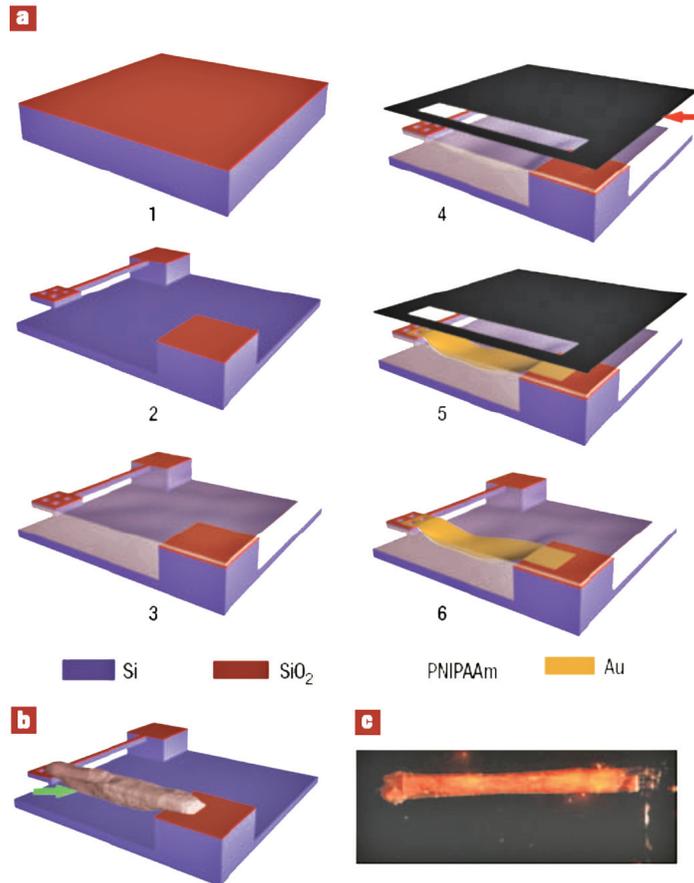


Fig. 6. a. Diagram of the fabrication process of the microdevice. b. Self-assembled myocyte layer in between the microcantilever and the rigid post. c. Actine filament staining with Rhodamine-phalloidin. [23].

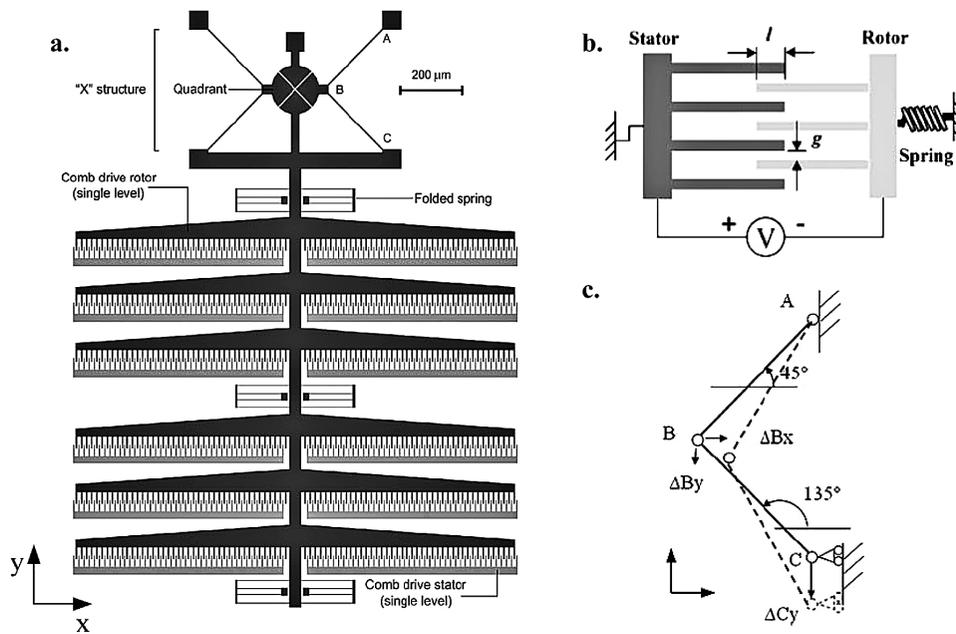


Fig. 7. a. Biaxial cell stretcher [3]. b. Schematic view of a comb-actuator concept [25]. c. Kinematic principle of the platform [3].

Finally, the implementation of microgrippers has also been proposed for mechanical characterization of tissues. One advantage of the implementation of these microdevices is the possibility to avoid previous preparation of the sample (excision, drying and fixation), or even achieve an *in vivo* characterization of the tissue. Menciassi *et al.* developed a teleoperated micro robotic instrument to measure soft tissue properties and pulse during microsurgery procedures [11, 12]. The instrument was composed of a microgripper which served as force sensor and mechanical stimulator, a three-degree of freedom motorized manipulator which moved the microgripper, a fiber optic microscope to visualize the microgripper position, a haptic interface that provided force feedback, and a PC-base control unit (Fig. 8). The microgripper was initially fabricated with electroplated nickel coated with gold via LIGA. A later version made with a superelastic alloy (Ni50.8Ti49.2) was obtained by using a wire microelectron discharge machining ( $\mu$ EDM), improving the mechanical properties of the device since the LIGA microgripper presented insufficient stiffness compared to that of some tissue samples. The force was sensed by semiconductor strain gauges located on the arms of the microgripper.

Even though silicon is widely used to fabricate MEMS, it is not the most adequate option for many mechanical testing applications due to its extremely brittle character, limited flexibility, and poor interaction with biological tissues. Additionally, silicon is a non-transparent substrate, which could complicate the observation and characterization of the cells cultured on it.

### III. POLYMER-BASED SENSORS FABRICATED BY SOFT LITHOGRAPHY TECHNIQUES

Soft lithography techniques have allowed the fabrication of microcantilever arrays from elastomeric polymer materials. This technique allows rapid and low cost fabrication of identical substrates. One example is the work proposed by Tan *et al.*(2003), which described the implementation of substrates with a PDMS posts array to characterize traction forces exerted by bovine pulmonary artery smooth muscle and NIH/3T3 mouse fibroblast cells, with a spatial resolution of  $9\ \mu\text{m}$  (Fig. 9) [26]. The substrates were fabricated by replica molding. Initially a master was created by photolithography, then a negative mold was fabricated from PDMS using soft lithography; finally the negative mold was used to micromold the PDMS post array. The deflection of the microcantilevers was measured by fluorescence microscopy using a regularly spaced grid of coordinates that represented the ideal undeflected positions of the posts as a reference. Substrates with different stiffness and different isotropic characteristics were obtained by modifying the geometry of the patterns (oval post arrays). The resolution of this method was limited by the deviation of unattached (to the cell) posts from the ideal grid ( $0.2\ \mu\text{m}$ ), and the local compliances generated when the cells spread down the length of the posts. These local compliances were eliminated by implementation of fibronectin pads stamped on to the tips of the posts using microcontact printing. However the spatial resolution of this method was limited ( $9\ \mu\text{m}$ ) due to the low density of microposts under the cell, which also seemed to affect cell adhesion and migration. Du Roure *et al.* (2005) improved the spatial resolution of this method by using an array with higher density of microposts (Fig. 10) [22].

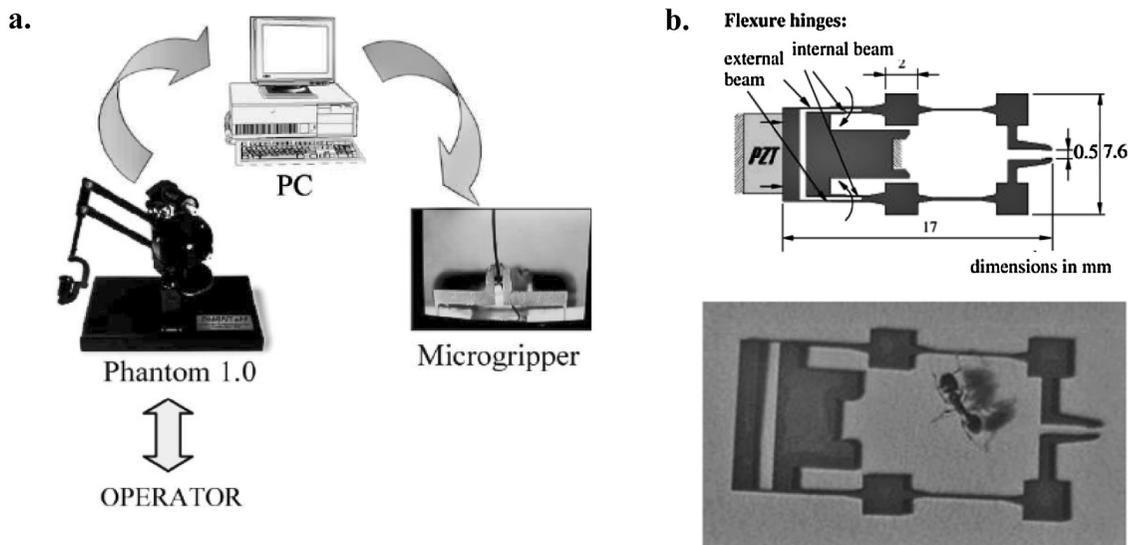
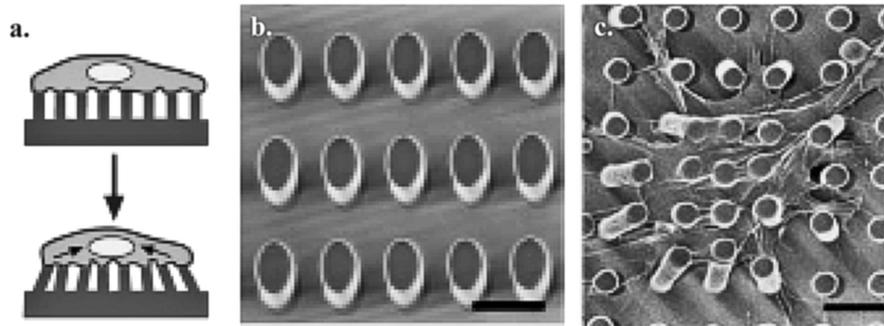
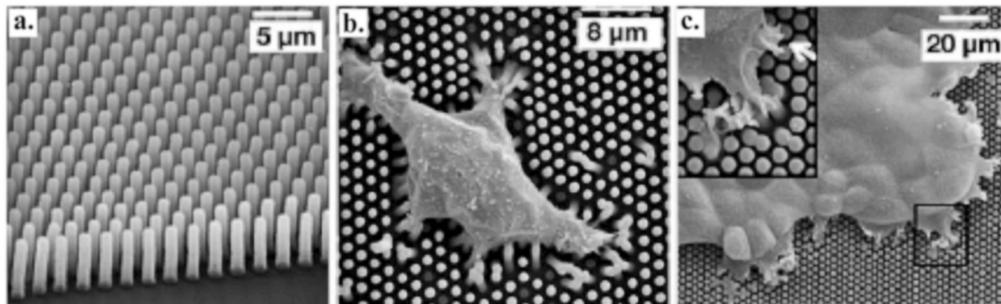


Fig. 8. a. Scheme of the teleoperated micro robotic instrument. b. Images of the electroplated nickel microgripper. [11, 12].



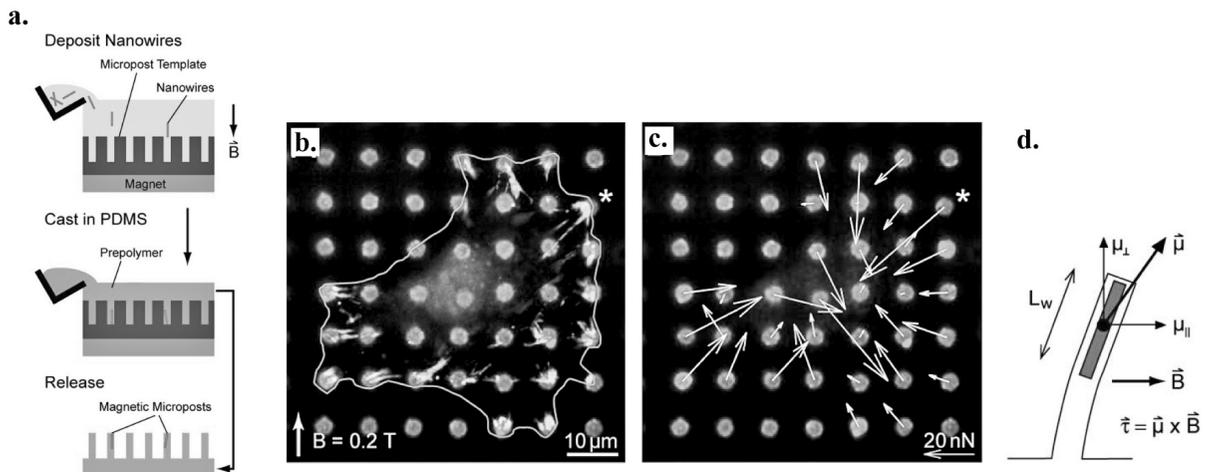
**Fig. 9.** a. Scheme of a cell cultured on the array. b. Array of post with oval cross section to introduce anisotropic stiffness (scale bar = 10µm). c. SEM image of a bovine pulmonary artery smooth muscle cell attached to the posts (scale bar = 10µm) [26].



**Fig. 10.** SEM images of the high density posts array. a. PDMS post array. b. Individual cell lying on the substrate. c. Cell monolayer on the substrate. [22].

Following the same line, Sniadecki *et al.* reported the development of a magnetic microposts array to track changes in the traction forces exerted by living cells due to external forces. The array was obtained by casting a Co nanowires (350 nm in diameter, and 2-7 nm in length) suspension into previously patterned molds (circular microwells of 3 µm diameter, 10 µm length, and 9 µm center to center spacing) at a ratio of 1 nanowire per

200 posts. A vertical magnetic field was used to align the nanowires along the long axis of the posts. After that a PDMS solution was casted into the mold to embed the nanowires. The non-magnetic posts (the ones without Co nanowires) were used to track the forces by optical inspection (using a spring model), while the magnetic posts were used to apply forces to the cells via magnetic actuation of these posts (Fig. 11) [31].



**Fig. 11.** a. Diagram of the fabrication process of magnetic microposts array. b. and c. Immunofluorescent images of a NIH 3T3 cell on top of the microdevice, after applying a 0.2-T horizontal field for 10 minutes. Green stain (focal adhesions), red stain (microposts), and blue stain (cell nuclei). d. Magnetic post actuation [31].

In spite of the great advantages of the implementation of polymers for the design and fabrication of force sensors, due to their versatility, biocompatibility and mechanical properties, these materials have not been widely explored for this application. One advantage of using this type of material to fabricate cell force sensors, based on the deformation of these elastic substrates, is the possibility to regulate the elasticity of the substrate without inducing significant chemical changes, by controlling the cross-linking level on the substrates [5]. Additionally the manufacturing of polymer-based microdevices has the advantage of simple low cost processing, and the ability to obtain complex three dimensional microdevices.

#### IV. CONCLUSION

Several materials and designs have been proposed for the fabrication of force sensors for cells and tissues at the microscale. Research on this field has been primarily focused on development of silicon-based sensors. However, silicon presents limitations due to its extremely brittle character, limited flexibility, poor interaction with biological tissues, and its non-transparent surface. Polymer-based sensors could overcome these limitations due to their versatility, biocompatibility, mechanical properties, and simple low cost processing. There is limited literature on the implementation of polymers for these kinds of sensors. The unique properties of polymers suggest that future research on this area should aim to studying different materials and designs for the fabrication of polymer-based sensors to characterize cell and tissue micromechanics.

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#### REFERENCES

1. Sniadecki N.J., Desai R.A., Ruiz S.A., Chen C.S. Nanotechnology for cell-substrate interactions. *Annals of Biomedical Engineering*, 34, 59-74, 2006.
2. Chicurel M.E., Singer R.H., Meyer C.J., Ingber D.E. Integrin binding and mechanical tension induce movement of mRNA and ribosomes to focal adhesions. *Nature*, 392, 730-733, 1998.
3. Scour N., Gallina P., Panchawagh H.V., Mahajan R.L., Sbaizero O., Sergio V. Design of a novel MEMS platform for the biaxial stimulation of living cells. *Biomedical Microdevices*, 8, 239-246, 2006.
4. Van Vliet K.J., Bao G., Suresh S. The biomechanics toolbox: experimental approaches for living cells and biomolecules. *Acta Materialia*, 51, 5881-5905, 2003.
5. Petronis S., Gold J., Kasemo B. Microfabricated force-sensitive elastic substrates for investigation of mechanical cell-substrate interactions. *Journal of Micromechanics and Microengineering*, 13, 900-913, 2003.
6. Galbraith C.G., Sheetz M.P. Forces on adhesive contacts affect cell function. *Current Opinion in Cell Biology*, 10, 566-571, 1998.
7. Kong H.J., Polte T.R., Alsberg E., Mooney D.J. FRET measurements of cell-traction forces and nano-scale clustering of adhesion ligands varied by substrate stiffness. *Proceedings of the National Academy of Science of the United States of America*, 102, 4300-4305, 2005.
8. Benigno K.A., Dembo M., Kaverina I., Small J.V., Wang Y. Nascent Focal Adhesions Are Responsible for the Generation of Strong Propulsive Forces in Migrating Fibroblasts. *The Journal of Cell Biology*, 153, 881-887, 2001.
9. Dembo M., Oliver T., Ishihara A., Jacobson K. Imaging the traction stresses exerted by locomotion cells with the elastic substratum method. *Biophysical Journal*, 70, 2008-2022, 1996.
10. Balaban N.Q., Schwarz U.S., Riveline D., Goichberg P., Tzur G., Sabanay I., Mahalu D., Safran S., Bershadsky A., Addadi L., Geiger B. Force and focal adhesion assembly: a close relationship studied using elastic micropatterned substrates. *Nature Cell Biology*, 3, 466-472, 2001.
11. Mencias A., Scalari G., Eisenberg A., Anticoli C., Francabandiera P., Carrozza M.C., Dario P. An instrumented probe for mechanical characterization of soft tissue. *Biomedical Microdevices*, 3, 149-156, 2001.
12. Mencias A., Eisenberg A., Carrozza M.C., Dario P. Force sensing microinstrument for measuring tissue properties and pulse in microsurgery. *IEEE/ASME Transactions on Mechatronics*, 8, 10-17, 2003.
13. Galbraith C.G., Sheetz M.P. A micromachined device provides a new bend on fibroblast traction forces. *Proceedings of the National Academy of Science of the United States of America*, 94, 9114-9118, 1997.
14. Galbraith C.G., Sheetz M.P. Keratocytes pull with similar forces on their dorsal and ventral surfaces. *The Journal of Cell Biology*, 147, 1313-1323, 1999.
15. Hilt J.Z., Gupta A.K., Bashir R., Peppas N.A. Ultrasensitive Biomems sensor based on microcantilevers patterned with environmentally responsive hydrogels. *Biomedical Microdevices*, 5, 177-184, 2003.
16. Fauver M., Dunaway D.L., Lilienfeld D.H., Craighead H.G. Microfabricated cantilevers for measurement of subcellular and molecular forces. *IEEE transactions on biomedical engineering*, 25, 891-898, 1998.
17. Lin G., Pister K.S.J., Roos K.P. Surface micromachined polysilicon heart cell force transducer. *Journal of Microelectromechanical Systems*, 9, 9-17, 2000.
18. Bao G., Suresh S. Cell and molecular mechanics of biological materials. *Nature Materials*, 2, 715-725, 2003.
19. Yang S., Saif T. Micromachined force sensors for the study of cell mechanics. *Review of Scientific Instruments*, 76, 44301-44301, 2005.
20. Yang S., Saif T. Reversible and repeatable linear local cell force response under large stretches. *Experimental Cell Research*, 305, 42-50, 2005.
21. Yang S., Saif M.T. Force response and actin remodeling (agglomeration) in fibroblasts due to lateral indentation. *Acta Biomaterialia*, 3, 77-87, 2007.

22. Du Roure O., Saez A., Buguin A., Austin R.H., Chavrier P., Silberzan P., Ladoux Benoit. **Force mapping in epithelial cell migration.** *Proceedings of the National Academy of Science of the United States of America*, 102, 2390-2395, 2005.
23. Xi J., Schmidt J.J., Montemagno C.D. Self-assembled microdevices driven by muscle. *Nature materials*, 4, 180-184, 2005.
24. Miyazaki H., Hasegawa Y., Hayashi K. A newly designed tensile tester for cells and its application to fibroblast. *Journal of Biomechanics*, 33, 97-104, 2000.
25. Chen C., Lee C., Lai Y.J., Chen W. Development and Application of Lateral Comb-Drive Actuator. *Japanese Journal of Applied Physics*, 42, 4059-4062, 2003.
26. Tan J.L., Tien J., Pirone D.M., Gray D.S., Bhadriraju K., Chen C.S. Cell lying on a bed of microneedles: an approach to isolate mechanical force. *Proceedings of the National Academy of Science of the United States of America*, 100, 1484-1489, 2003.
27. Harris A.K., Wild P., Stopak D. Silicon rubber substrata: a new wrinkle in the study of cell locomotion. *Science*, 208, 177-179, 1980.
28. Martin R.B., Burr D.B., Sharkey N.A. *Skeletal Tissue Mechanics* Springer-Verlag, New York 1998, 225.
29. Brighton C.T., Fisher J.R. Jr, Levine S.E., Corsetti J.R., Reilly T., Landsman A.S., Williams J.L., Thibault L.E. The biochemical pathway mediating the proliferative response of bone cells to a mechanical stimulus. *The Journal of Bone and Joint Surgery*, 78, 1337-1347, 1996.
30. Brighton C.T., Wang W., Seldes R., Zhang G., Pollack S. Signal transduction in electrically stimulated bone cells. *The Journal of Bone and Joint Surgery*, 83, 1514-1523, 2001.
31. Sniadecki N.J., Anguelouch T.Y., Lamb C.M., Liu Z., Kirschnert S.B., Liu Y., Reich D.H., Chen C.S. Magnetic microposts as an approach to apply forces to living cells. *PNAS*, 104, 14553-14558, 2007.