Biophysical Reviews (2019) 11:667–670
https://doi.org/10.1007/s12551-019-00597-0

COMMENTARY

Some thoughts on the future of cell mechanics

Jochen Guck[1]

Received: 2 September 2019 / Accepted: 3 September 2019 / Published online: 16 September 2019

Cell mechanics research is at a crucial juncture in its decades-old history (for a good historical perspective, see for example Pelling and Horton 2008). One of several reasons that research on the mechanical properties of cells has been actively pursued for many decades is the functional link between cell mechanics and the cell cytoskeleton, a dynamic structure made of different protein filaments and their accessory proteins. This cytoskeleton is involved in such important biological processes as cell migration or cell division and characteristically altered in many diseases such as cancer. Whenever a cell changes its function or becomes pathologically altered, the cytoskeleton restructures, which inevitably leads to telltale mechanical changes. In this sense, one can use a mechanical test to *feel* for cell functional changes. This premise is attractive, because it permits the unbiased, non-destructive, and sensitive investigation of cell interior processes and potentially even the diagnosis and treatment of disease (Di Carlo 2012; Guck and Chilvers 2013). Cell mechanics researchers frequently point to this possibility when motivating their research, despite the increasingly obvious problem that decades-long efforts have not led to a single routine application of cell mechanics in biological research or clinical labs. So, why is this?

Is there a problem with the fundamental premise just sketched? Not very likely—there are too many basic research reports that support this link convincingly. Have biologists and medical researchers been swept away by the huge success of molecular biology and are, thus, simply not interested in seemingly trivial physical properties of entire cells? Maybe some, but there are enough counterexamples from personal experience. And especially the best and most forward-looking biologists are keenly aware of the current limitations of molecular biology and open to "think physics". The success of Dresden-style biophysical research testifies to this. In my opinion, the reason for this "failure" of cell mechanics to deliver so far is down to three aspects: technology, standards, and control.

What is wrong with cell mechanics technology? There are many different ways of applying a known mechanical stress to cells and quantifying the resulting deformation in order to extract elasticity or viscosity of cells (Darling and Di Carlo 2015). The ingenuity of researchers has run wild on this task. From very direct and robust approaches, such as micropipette aspiration, and the gold standard of cell mechanical measurement, nano-indentation using atomic force microscopy, to analyzing the motion of tracer particles in the cytoplasm or at the cell surface, using magnetic or optical forces (see a recent paper comparing such techniques (Wu et al. 2018)), all the way to "listening" to the thermally excited sound waves bouncing around in cells by Brillouin microscopy (Scarcelli et al. 2015)—the possibilities seem exhaustively covered. And using all of these approaches, a very substantial body of solid findings has been built up: the basic premise of the link between functional changes and mechanical measurement holds. So, what is wrong?

One fundamental problem with standard cell mechanics technology is that the number of cells that can be measured in a reasonable amount of time is too low. Measuring a few tens or even hundreds of cells per hour simply does not compete with the throughput of, say, a fluorescence-based flow cytometer—a standard tool found in most biological labs around the world, used very much for the anticipated purpose of cell mechanical analysis. Analyzing thousands of cells in seconds, or actually more importantly, millions of cells in a few minutes, is where it is at. Only this throughput opens the door to such fascinating applications as identifying a handful of cells of interest in a large heterogeneous population of cells—think of picking out the true stem cells amongst already differentiated cells or searching for circulating tumor cells in blood. The good news is, the techniques to screen cell populations for their mechanical fingerprint with a throughput approaching that of flow cytometers are becoming available (Gossett et al. 2012; Byun et al. 2013; Otto et al. 2015;

✉ Jochen Guck
jochen.guck@mpl.mpg.de

[1] Max-Planck-Institut für die Physik des Lichts &
Max-Planck-Zentrum für Physik und Medizin, Staudtstr. 2,
91058 Erlangen, Germany

Lange et al. 2015; Nyberg et al. 2017; Ahmmed et al. 2018; Armistead et al. 2019; Guillou et al. 2016; Adamo et al. 2012; Myers et al. 2016). There are now a sizable number of different approaches, all using microfluidic lab-on-chip systems, able to measure more cells in a single day than have been measured with all other standard techniques taken together—ever.

With this most important problem about to be out of the way, there is still the technological problem that the standard techniques are too difficult to use by non-experts. It is no use if an advanced engineering or optics degree is required to do the measurement, because most biological or medical researchers will not have that. So, an obvious next thing that has to change is to have techniques that are so simple to use that literally anyone can operate them. Here, again, the recent lab-on-chip approaches seem ideally suited to deliver.

The second big obstacle to widespread use of cell mechanics measurements in biology and medicine is the lack of standards. Each method probes the cell in different ways. Some push from the outside, some interrogate properties directly in the interior. Some use tiny stress such as fluid flow and over a large cell area, others apply immense pressures on tiny spots leading to non-linear responses. And then, there is the question of time-scale. Since cells are viscoelastic, it absolutely matters whether a cell is prodded quickly on a sub-second timescale or whether the stress persists for many seconds or minutes. Because of this variety in approach, the values reported with different techniques can differ by orders of magnitude even for the identical cell being measured (Wu et al. 2018). This must be truly bewildering to the interested non-expert. In my opinion, what we have to start using consistently as a community, if we want to have a real impact where it counts, is standardized, inert reference particles that are mixed into cell samples and co-measured in order to have an absolute standard to compare different measurements with. With this purpose in mind, we have recently introduced microbeads made of poly-acrylamide with the size (10–20 μm) and the mechanical properties (roughly 0.1–10 kPa) of cells that are purely elastic (Girardo et al. 2018). So, the elastic modulus extracted with any technique should yield exactly that known value and can then be used to compare cells with. These beads are freely available to anyone who is interested in trying them out. Such a standardized calibration particle would then also lay the basis for establishing a repository for cell mechanics data. A public database where anyone can upload their (standardized) results for everyone else to mine. Of course, making raw data public is increasingly required when submitting research papers to journals, and such a database would fulfill this purpose. Similar databases obviously exist for genomic, proteomic, and transcriptomic, etc. data and have greatly facilitated molecular biology research. Why not do the same for cell mechanics data? We live in the age of big data and artificial intelligence analysis, and cell mechanics research should profit from the tremendous advances in this area. The new high-throughput techniques mentioned above are ideally suited to produce large, standardized datasets for exploration. Who knows what correlations can be found to identify completely new areas of applications or disease diagnosis?

The third, and most difficult obstacle to wide-spread acceptance and application of cell mechanics research, in my opinion, is the current lack of control. We are very good in demonstrating correlations. Cells undergoing mitosis stiffen, cells from a cancer patient are softer than samples from a healthy donor, leukocytes change their mechanics when activated, and so forth. But, is this an important feature of the process? Or is this simply something that also happens and has no further relevance? An epiphenomenon? An essential part of establishing causality in biology are loss-of-function and gain-of-function experiments. For example, if we could arbitrarily change cell mechanics ad lib and without touching any other process (the "how" is the big question), then we could stiffen otherwise compliant cancer cells and see whether that alleviates some of the symptoms of cancer (prevention of cancer metastasis?). Of course, cell mechanics is tightly linked with many other processes so that identifying knobs and switches that only change mechanics is a difficult challenge, and not unlikely impossible. However, with the new high-throughput technology at hand and by pooling standardized data from many different labs, there is an opportunity we need to pursue. The possibility to screen RNAi libraries for a mechanical phenotype has already been demonstrated (Toyoda et al. 2017; Rosendahl et al. 2018) and is currently being expanded. The same can and will be done for FDA-approved drug libraries or small-chemical compound libraries. The high-throughput techniques are there to handle the massive number of measurements in a reasonable time. Another possibility opens up by correlating information from a cell mechanics database (see above) with all the other omics-based databases. This approach could be called "mechanomics" (Ciucci et al. 2017). In such a way, important genes, proteins, etc. can potentially be identified that have the same mechanical effect across many different cell types, species, and perturbations. And as a third option, the ability to sort for mechanical phenotypes, already feasible with passive high-throughput sorters available today (Beech et al. 2012), permits the separation of mechanically distinct subpopulations, which can then be pipelined to further single-cell analysis such as molecular profiling (mass cytometry) or sequencing (scRNA-seq). Maybe we come across a magical switch to soften and stiffen cells in any situation? At least, we will identify a lot of new targets for further exploration in cell biology. Just think of what the identification of blebbistatin as a regulator of myosin activity in a blebbing screen (Straight et al. 2003) has done to our understanding of cell mechanics, as an example.

As I said in the beginning, there are several reasons why one might want to do cell mechanics research. Clearly, the

promise of cell mechanical phenotyping for label-free, unbiased cell functional characterization and diagnosis of disease is simply too tempting to not push towards application seriously. And there are some efforts underway already that have a clear clinical slant (Plodinec et al. 2012; Tse et al. 2013; Toepfner et al. 2018; Surcel et al. 2019). But also, basic research into cell mechanics is valuable in its own right, and there will likely be new insights into cell biological processes coming from our increased ability to quantify physical properties of cells and their compartments. The rising importance of phase separation and phase transition in biology—an area crying for quantitative physical characterization—is one such example. This is the time to make cell mechanics research count. We now have the right tools in place. All we need to do is to buckle up and go for it. The future of cell mechanics research looks bright.

Acknowledgments I want to thank M. Kräter, D. Soteriou, and M. Kubankova for the critical reading of the manuscript and B. Baum, C. Cannistraci, D. Di Carlo, E. Chilvers, E. Darling, D. Discher, B. Fabry, J. Goetz, P. Janmey, W. Lam, S. Manalis, D. Robinson, A. Rowat, U. Schwarz, A. Surcel, T. Sulcheck, K. Tanner, and V. Zaburdaev and all members of my group for important recent discussions. I also want to acknowledge the important work by many others in the cell mechanics community whose work I have failed to mention explicitly in this text.

References

Adamo A, Sharei A, Adamo L, Lee B, Mao S, Jensen KF (2012) Microfluidics-based assessment of cell deformability. *Anal Chem* 84(15). American Chemical Society):6438–6443. https://doi.org/10.1021/ac300264v

Ahmmed SM, Bithi SS, Pore AA, Mubtasim N, Schuster C, Gollahon LS, Vanapalli SA (2018) Multi-sample deformability cytometry of cancer cells. APL Bioeng 2(3):032002–032015. https://doi.org/10.1063/1.5020992

Armistead FJ, Gala De Pablo J, Gadêlha H, Peyman SA, Evans SD (2019) Cells under stress: an inertial-shear microfluidic determination of cell behavior. *Biophys J* 116(6). Biophysical Society:):1127–1135. https://doi.org/10.1016/j.bpj.2019.01.034

Beech JP, Holm SH, Adolfsson K, Tegenfeldt JO (2012) Sorting cells by size, shape and deformability. Lab Chip 12(6):1048–1051. https://doi.org/10.1039/c2lc21083e

Byun S, Son S, Amodei D, Cermak N, Shaw J, Kang JH, Hecht VC et al (2013) Characterizing deformability and surface friction of cancer cells. Pnas 110(19):7580–7585. https://doi.org/10.1073/pnas.1218806110

Ciucci S, Ge Y, Durán C, Palladini A, Jiménez-Jiménez V, Martínez-Sánchez LM, Wang Y, et al. 2017. "Enlightening discriminative network functional modules behind principal component analysis separation in differential-omic science studies." *Scientific Reports* 7 (March). Nature Publishing Group: 43946. doi:https://doi.org/10.1038/srep43946.

Darling EM, Di Carlo D (2015) High-throughput assessment of cellular mechanical properties. *Annu Rev Biomed Eng* 17(1). Annual Reviews):35–62. https://doi.org/10.1146/annurev-bioeng-071114-040545

Di Carlo D (2012) A mechanical biomarker of cell state in medicine. *Jala* 17(1). SAGE Publications):32–42. https://doi.org/10.1177/2211068211431630.

Girardo S, Nicole Träber K, Wagner G, Cojoc C, Herold R, Goswami RS et al (2018) Standardized microgel beads as elastic cell mechanical probes. J Mater Chem B 456. The Royal Society of Chemistry:3. https://doi.org/10.1039/C8TB01421C

Gossett DR, Tse HTK, Lee SA, Ying Y, Lindgren AG, Yang OO, Rao J, Clark AT, Di Carlo D (2012) Hydrodynamic stretching of single cells for large population mechanical phenotyping. Proc Natl Acad Sci U S A 109(20):7630–7635. https://doi.org/10.1073/pnas.1200107109

Guck J, Chilvers ER (2013) Mechanics meets medicine. Sci Transl Med 5(212):212fs41. https://doi.org/10.1126/scitranslmed.3007731

Guillou L, Dahl JB, Lin JG, Barakat AI, Husson J, Muller SJ, Kumar S (2016) Measuring cell viscoelastic properties using a microfluidic extensional flow device. *Biophys J* 111(9). Cell Press):2039–2050. https://doi.org/10.1016/j.bpj.2016.09.034

Lange JR, Steinwachs J, Kolb T, Lautscham LA, Harder I, Whyte G, Fabry B (2015) Microconstriction arrays for high-throughput quantitative measurements of cell mechanical properties. *Biophys J* 109(1). Elsevier):26–34. https://doi.org/10.1016/j.bpj.2015.05.029

Myers DR, Qiu Y, Fay ME, Tennenbaum M, Chester D, Cuadrado J, Sakurai Y et al (2016) Single-platelet nanomechanics measured by high-throughput cytometry. Nat Mater 16(2). Nature Research):230–235. https://doi.org/10.1038/nmat4772

Nyberg KD, Hu KH, Kleinman SH, Khismatullin DB, Butte MJ, Rowat AC (2017) Quantitative deformability cytometry: rapid, calibrated measurements of cell mechanical properties. Biophys J 113(7):1574–1584. https://doi.org/10.1016/j.bpj.2017.06.073

Otto O, Rosendahl P, Mietke A, Golfier S, Herold C, Klaue D, Girardo S et al (2015) Real-time deformability cytometry: on-the-fly cell mechanical phenotyping. Nat Methods 12(3):199–202. https://doi.org/10.1038/nmeth.3281

Pelling AE, Horton MA (2008) An historical perspective on cell mechanics. Arch Eur J Physiol 456(1):3–12. https://doi.org/10.1007/s00424-007-0405-1

Plodinec M, Loparic M, Monnier CA, Obermann EC, Zanetti-Dallenbach R, Oertle P, Hyotyla JT et al (2012) The nanomechanical signature of breast cancer." *Nature Nanotechnology*. Nat Publ Group 7(11):757–765. https://doi.org/10.1038/nnano.2012.167

Rosendahl P, Plak K, Jacobi A, Kraeter M, Toepfner N, Otto O, Herold C et al (2018) Real-time fluorescence and deformability cytometry." *Nature Methods*. Nat Publ Group 15(5):355–358. https://doi.org/10.1038/nmeth.4639

Scarcelli G, Polacheck WJ, Nia HT, Patel K, Grodzinsky AJ, Kamm RD, Yun SH (2015) Noncontact three-dimensional mapping of intracellular hydromechanical properties by Brillouin microscopy. Nat Methods 12(12):1132–1134. https://doi.org/10.1038/nmeth.3616

Straight AF, Cheung A, Limouze J, Chen I, Westwood NJ, Sellers JR, Mitchison TJ (2003) Dissecting temporal and spatial control of cytokinesis with a myosin II inhibitor. *Science (New York, NY)* 299(5613). American Association for the Advancement of Science):1743–1747. https://doi.org/10.1126/science.1081412

Surcel A, Schiffhauer ES, Thomas DG, Zhu Q, DiNapoli KT, Herbig M, Otto O et al (2019) Targeting mechanoresponsive proteins in pancreatic cancer: 4-hydroxyacetophenone blocks dissemination and invasion by activating MYH14. *Cancer Research*, January American Association for Cancer Research, canres.3131.2018. https://doi.org/10.1158/0008-5472.CAN-18-3131

Toepfner N, Herold C, Otto O, Rosendahl P, Jacobi A, Kräter M, Stachele J et al (2018) Detection of human disease conditions by single-cell morpho-rheological phenotyping of blood. *eLife* 7(January). eLife sciences publications limited):e29213. https://doi.org/10.7554/eLife.29213

Toyoda Y, Cattin CJ, Stewart MP, Poser I, Theis M, Kurzchalia TV, Buchholz F, Hyman AA, and Muller DJ. 2017. "Genome-scale single-cell mechanical phenotyping reveals disease-related genes involved in mitotic rounding." *Nature*

Communications, October Springer US, 1–10. doi:https://doi.org/10.1038/s41467-017-01147-6.

Tse HTK, Gossett DR, Moon YS, Masaeli M, Sohsman M, Ying Y, Mislick K, Adams RP, Rao J, Di Carlo D (2013) Quantitative diagnosis of malignant pleural effusions by single-cell mechanophenotyping. Sci Transl Med 5(212):212ra163. https://doi.org/10.1126/scitranslmed.3006559

Wu P-H, Aroush DR-B, Asnacios A, Chen W-C, Dokukin ME, Doss BL, Durand-Smet P et al (2018) A comparison of methods to assess cell mechanical properties. Nat Methods 15(7):491–498. https://doi.org/10.1038/s41592-018-0015-1