Review article

Sandra Pérez-Domínguez, Shruti G. Kulkarni, Carmela Rianna and Manfred Radmacher*

Atomic force microscopy for cell mechanics and diseases

https://doi.org/10.1515/nf-2020-0001

Abstract: Atomic Force Microscopy (AFM) is a powerful technique widely employed in biophysics, for instance to study topography of living cells and cell mechanics. Cell mechanics is a very interesting, biophysical parameter of cells, because it is strongly changed by various cellular processes, for example during cell division, cell movement, differentiation, aging, and also various diseases. Since cancer is a prominent example of changes in mechanical properties of diseases, the concept of the mechanical fingerprint has developed, which makes it possible to distinguish between healthy and diseased cells. In this article we report on various studies of cell mechanics with the AFM. We will first give a brief introduction on AFM principles and operational modes and then we will report on some applications of AFM in the field of cellular biophysics, like discriminating between healthy and cancer cells, as well as distinguishing cancer cells at different stages of malignancy. Overall, we will show that AFM has made a significant contribution in studying the biophysics of cancer and the concept of mechanical fingerprints could find a wide variety of applications in biomedicine and medical diagnostics.

Keywords: Atomic Force Microscope; biophysics; cancer; disease

Zusammenfassung: Das Kraftmikroskop (AFM für Atomic Force Microcope) ist ein vielfältiges Instrument, das immer häufiger in der Biophysik, insbesondere für topographische und mechanische Untersuchungen von Zellen, verwendet wird. Hier ist besonders hervorzuheben, dass das Kraftmikroskop es erlaubt zelluläre Prozesse in physiologischen Bedingungen zu verfolgen. Zellmechanik ist eine sehr interessante, biophysikalische Kenngröße, da sie stark durch verschiedenste zelluläre Prozesse verändert wird. Beispiele hierfür sind Zellteilung, Zellbewegung, Differentiation, Altern, und auch diverse Krankheiten. Da sich bei diversen Krankheiten, Krebs ist hier nur ein prominentes Beispiel, die mechanischen Eigenschaften ändern, hat sich das Konzept des mechanischen Fingerabdrucks entwickelt, also die Idee damit zwischen gesunden und kranken Zellen zu unterscheiden. Wir geben in diesem Artikel einen Überblick über Studien der Zellmechanik mit dem Kraftmikroskop. Zunächst werden wir in die Technik des Kraftmikroskops und einfache Betriebsmodi, etwa zur Abbildung, berichten, um dann über Anwendungen in der zellulären Biophysik zu berichten. Gerade bei Krebszellen hat sich gezeigt, dass man mit Hilfe der Zellmechanik nicht nur zwischen benign und malign, sondern sogar den Grad der Malignizität bestimmen kann. Dieses Beispiel zeigt sehr eindrücklich, dass das Studium der mechanischen Eigenschaften von Zellen und Geweben bereits jetzt hilft Zellen und deren Zustand zu charakterisieren. Das Konzept des mechanischen Fingerabdrucks könnte vielfältige Anwendungen in der Biomedizin und in der medizinischen Diagnostik finden.

Schlüsselwörter: Biophysik; Kraftmikroskopie; Krankheiten; Krebs; Zellmechanik

Introduction

Atomic Force Microscopy (AFM) is a powerful technique to measure topographical and mechanical properties of soft samples including live cells. AFM belongs to the family of scanning probe microscopes, which, as the name suggests, is based on the interaction between a scanning probe (like a tip) and the sample. The precursor of the AFM was the Scanning Tunneling Microscope (STM), developed in the early 1980's to study surface structures of conductive samples (Binnig and Rohrer, 1983). While STM is based on the tunneling current between a metallic tip and a conductive sample, AFM is based on the forces between the tip and the surface (Binnig et al., 1986). Initially, AFM had been mainly used to measure topography of non-biological

Sandra Pérez-Domínguez and Shruti G. Kulkarni contributed equally to the paper.

***Corresponding author: Manfred Radmacher,** Institute of Biophysics, University of Bremen, Otto-Hahn Allee 1, 28359 Bremen, Germany, E-mail: radmacher@uni-bremen.de. https://orcid.org/0000-0001-8744-4541

Sandra Pérez-Domínguez, Shruti G. Kulkarni and Carmela Rianna: Institute of Biophysics, University of Bremen, Bremen, Germany

samples; however, the high-resolution capability of AFM has been increasingly used for biological and biomedical applications as well (Drake et al., 1989). Nowadays, AFM is employed for other applications aside from studying topography, such as cell mechanics (Radmacher et al., 1995), single-molecule interaction force measurements (Florin et al., 1994), and cell-cell interaction studies (Grandbois et al., 2000; Viji Babu et al., 2018).

This review will focus on the study of cell mechanics and elastic properties of cells, which can be investigated with different techniques, like micropipette aspiration (Hochmuth, 2000), optical (Yousafzai et al., 2017; Zhang and Liu, 2008) or magnetic tweezers (Jakab et al., 2002), magnetic twisting cytometry (Laurent et al., 2002), optical stretching (Guck et al., 2001), real-time deformability cytometry (Mietke et al., 2015; Otto et al., 2015), and AFM (Rotsch et al., 1999). AFM has the advantage of allowing mechanical measurements on adherent cells, whereas the majority of other techniques can be applied to cells in suspension. In addition, AFM allows positioning the probe with high resolution for measuring the mechanical properties at different regions of the cell. Recently, a large interest in studying cell mechanics has been raised, mainly because it has a fundamental role in many cellular processes, including cell protrusion, division, migration, differentiation, and morphology (Bohnet et al., 2006; Chen et al., 2004; Lautenschläger et al., 2009; Matzke et al., 2001; McKenzie et al., 2018; Prass et al., 2006; Rehfeldt et al., 2007). Moreover, changes in cell mechanics are strongly related to many diseases, such as cancer, blood, and cardiovascular diseases (Rianna and Radmacher, 2016a). For mammalian cells, it has been shown that cell mechanical properties are mainly determined by the cell cytoskeleton network, where the density and arrangements of filaments, the number of cross-links, activity, and stress generation affect the elastic properties (Brückner and Janshoff, 2015; Moeendarbary and Harris, 2014; Pegoraro et al., 2017; Rotsch and Radmacher, 2000; Schäfer and Radmacher, 2005). The cytoskeleton is mostly made of actin, intermediate filaments, and microtubules. Currently, studies have demonstrated that disruption, disorganization, and deformability of the cytoskeleton play an important role in cancer, even allowing the differentiation between cells in distinct stage of the disease (Ochalek et al., 1988). These changes are commonly related to either a partial loss of actin filaments (Yamaguchi and Condeelis, 2007) or disorganization of microtubules (Pachenari et al., 2014) being the consequence of the lower density of the cellular scaffold.

Moreover, large differences between the mechanical properties of cancerous and healthy cells have been measured using AFM (Abidine et al., 2018; Cross et al., 2008; Lekka, 2016; Li et al., 2008; Prabhune et al., 2012). Lekka and co-workers were the first to use AFM to compare normal and cancer cells, and they found that bladder cancer cells were one order of magnitude softer than their normal counterparts (Lekka et al., 1999). Thereafter, several groups have compared the mechanical properties of cancerous and healthy cells, reaching the consensus that cancer cells are softer than normal (healthy) ones. However, more recent studies have shown that cell mechanics is also strongly affected by the cell's microenvironment, e. g., using materials of different stiffness as cell culture supports; thus, the general paradigm of cancer cells being softer than normal cells can even be inverted (Alibert et al., 2017; Rianna and Radmacher, 2017; Rianna et al., 2017).

In this review, we discuss the applications of AFM in diseases, with a special focus on cancer. We will first give a brief introduction on AFM principles and operational modes, with emphasis on using the AFM to study cell mechanics. Then, we will report the AFM's contribution in the field of cellular biophysics like discriminating between cell's physiological and pathological conditions as well as distinguishing cancer cells at different stages of malignancy. To conclude, we will discuss some future perspectives and possible directions for the widespread use of AFM in the field of biomedicine.

AFM principles

AFM can be used in various modes to image a surface or to study its mechanical properties. The main components of a typical AFM setup are a microfabricated cantilever with an integrated tip, a laser, a position-sensitive photodiode for measuring the cantilever's deflection, and an xyz piezo-scanner for positioning the sample or tip (Figure 1A). The cantilever, which is considered as a spring, is brought in contact with the sample (Figure 1B). The cantilever's deflection is measured using the optical lever scheme. A laser beam is reflected from the back of the cantilever, which is coated with a reflective material, like gold, and detected by a split photodiode. The difference in the photocurrents of the two segments of the photodiode is a measure of the deflection of the cantilever. The position of the cantilever (or of the sample) is accurately moved and controlled by the xyz piezo-ceramic transducer. For mechanical measurements (e. g., to study cell mechanics), the tip gradually approaches and indents the sample until a maximum pre-set loading force is reached. Then, the tip is retracted from the sample and stops at its original position off the surface. During the approach and retraction, the deflection of the cantilever is recorded and displayed in a force curve,

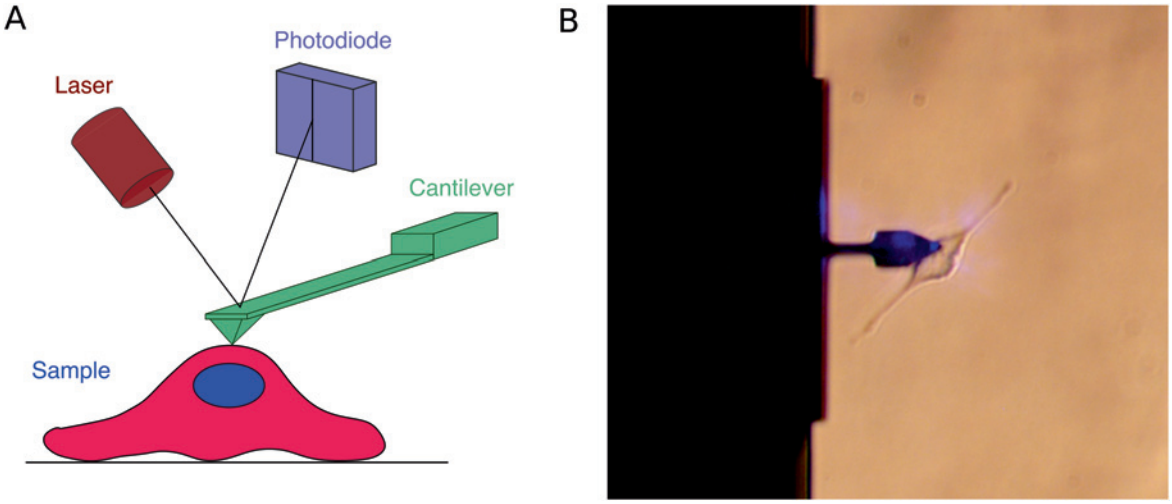

Figure 1: (A) Components of a typical AFM set-up: Laser, Split-photodiode, Cantilever and Sample. (B) Image of an AFM cantilever in contact with a live NIH-3T3 fibroblast in Dulbecco's Modified Eagle Medium with 10% Fetal Bovine Serum and 1% Penicillin-Streptomycin. Experiments were done in 5% CO_2.

which shows the deflection of the cantilever versus the z height (Figure 2).

The elastic properties of the sample can be obtained by analyzing the force, F, versus the indentation, δ. Both quantities can be derived from a force curve: the force, by multiplying the spring constant of the cantilever k with the measured deflection d, and the indentation, by subtracting the deflection from the sample height z:

$$F = kd \tag{1}$$

$$\delta = z - d \tag{2}$$

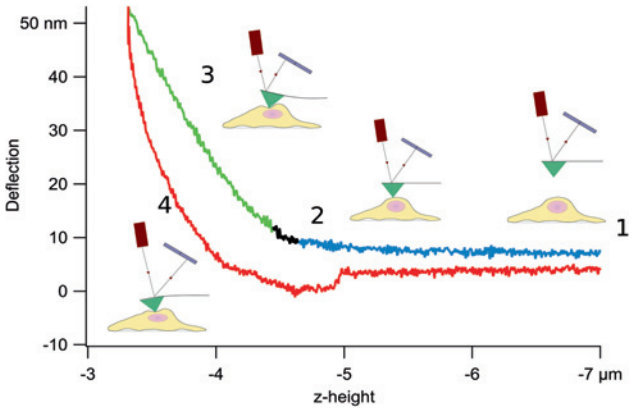

Figure 2: Schematic of an AFM force curve: (1) As the AFM tip approaches the cell, the laser's deflection remains constant, (2) when the tip comes in contact with the cell and starts to indent it, the laser is deflected and its position on the photodiode changes, (3) as the tip continues to indent, the deflection increases and (4) after the deflection trigger threshold is reached (50 nm in this example), the tip is retracted.

AFM of live cells requires certain conditions in order to keep them alive and intact. Biological conditions such as the liquid medium, pH, and temperature should also be maintained during the experiment. When compared to fixed or air-dried cells, live cells are much softer, and special attention must be paid to AFM experimental conditions such as choosing the appropriate cantilever (force constant and tip geometry) and calibrating these two parameters correctly to carry out accurate force measurements. A large variety of cantilevers, which offer many different force constants and tip geometries, are commercially available. The pressure exerted by the cantilever tip on the live cell should be minimized, and at the same time, appropriate force must be exerted to obtain an elastic response. AFM tips can be sharp (such as triangular or pyramidal shape) or relatively blunt (like spherical tips). A sharp probe has the advantage of higher resolution mapping of cells' topography (spatial resolution); however, it may also damage the cell due to higher pressures applied. Hence, when using sharp tips for cell investigations, the spring constant should be very low, to prevent cell damage during measurement. Blunter tips will reduce the possibility of damaging the cell but sacrifices lateral resolution.

Apart from choosing the appropriate cantilever for each measurement, knowing its spring constant k is also crucial. The manufacturer usually only claims a nominal spring constant of the cantilever with a large error margin. Therefore, the spring constant has to be calibrated by measuring the thermal fluctuations of the cantilever and employing Boltzmann's equipartition theorem (Hutter and Bechhoefer, 1993). According to this theorem, the energy in any degree of freedom of the system has to be equal to the

thermal energy due to the absolute temperature of the system.

$$\langle E \rangle = \frac{k_B T}{2}, \tag{3}$$

where E is the energy, k_B is the Boltzmann constant, and T is the temperature. Assuming that the cantilever undergoes mainly bending vibrations at the resonance frequency, we estimate the spring constant from the fluctuations in tip position:

$$\langle E \rangle = \frac{k_c \langle d^2 \rangle}{2}, \tag{4}$$

where E is the energy, k_c is the spring constant, and $\langle d^2 \rangle$ is the mean square displacement of the cantilever. By joining both equations, we can obtain the spring constant:

$$k_c = \frac{k_B T}{\langle d^2 \rangle}. \tag{5}$$

The value $\langle d^2 \rangle$ is measured from the fit of the power frequency spectrum. This can be done with the AFM itself or with a very accurate vibrometer. Some AFM cantilevers can be purchased already calibrated by the manufacturer. A procedure to reduce errors and to obtain mechanical data in a reproducible and standardized manner has been reported recently (Schillers et al., 2017).

Mechanical properties

To measure the mechanical properties of a soft sample, e. g., a cell, the force applied versus the achieved indentation has to be analyzed with an appropriate model from contact mechanics (Figure 3A). In contact mechanics, stress (force per area) and strain (relative length change) are related by the elastic (or Young's) modulus, E. Here, we use the force indentation relations (Figure 3B), which were initially derived by Heinrich Hertz in 1882 (Hertz, 1881), for the case of spherical indenters. The Hertz model can also be applied to parabolic indenters, which are occasionally used as well. Sneddon extended the former model to the case of conical indenters (Sneddon, 1965), whereas Rico and co workers extended it to pyramidal tips (Rico et al., 2005). The geometry of the indenter used during the experiment determines the relation among applied loading force, indentation, tip geometry, and the Young's modulus value of the sample:

$$\text{Parabolic or spherical indenters}: F = \frac{4}{3}\sqrt{R}\,\frac{E}{1-\nu^2}\delta^{3/2} \tag{6}$$

$$\text{Pyramidal indenter:} = \frac{1}{\sqrt{2}}\tan\alpha\,\frac{E}{1-\nu^2}\delta^2 \tag{7}$$

where F is the loading force, R is the radius of the curvature in spherical or parabolic probes, α is the half-opening angle of the cone or pyramidal face angle, and ν is the Poisson's ratio (which is considered 0.5 for cells, since the volume is conserved during compression). Finally, as previously mentioned, δ is the indentation depth and E is the elastic or Young's modulus of the sample.

The Hertz model is based on several assumptions: the sample is considered as an isotropic, homogeneous, and linear elastic material; moreover, it should be flat and infinitely thick. In a strict sense, none of these assumptions are fulfilled for cells; nevertheless, the Hertz model describes the mechanical data of cells surprisingly well and is therefore used widely. However, three points should always be

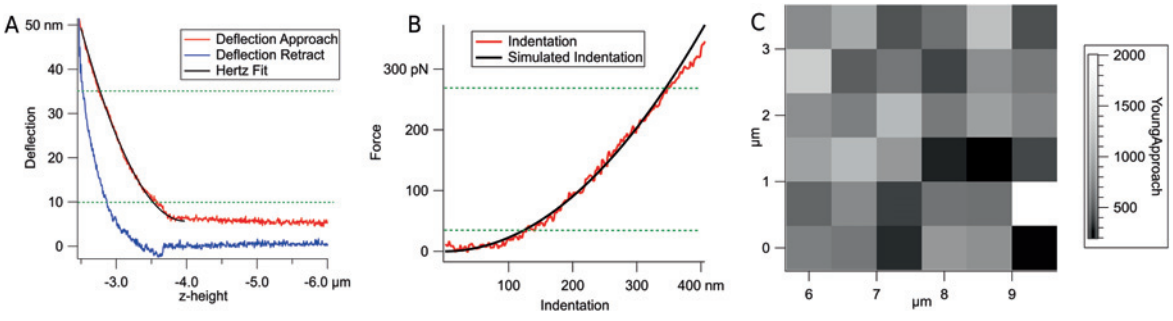

Figure 3: (A) Representation of an AFM force curve: deflection v/s z-height with approach and retract curves in red and blue, respectively. The Hertz fit on the approach curve is displayed in black with a fit range of 10–35 nm (green lines). (B) Representation of an AFM indentation curve: Force v/s indentation in red, where force is calculated using the relation $F = kd$, and then plotted against the indentation calculated using the formula $\delta = z - d$. Force range used to fit it corresponds to the deflection range used in the Hertz fit. (C) Force map, where each point is the Young's modulus calculated from the Hertz fit of the approach curve.

appreciated: the cell's internal structures, its finite thickness, and its viscous contributions. The latter can be easily seen in the hysteresis between the loading and unloading force curves (approach and retract) in the indentation part of the force curve (Kirmizis and Logothetidis, 2010) (Figure 3A).

Another critical parameter to bear in mind while measuring the cell's elastic properties is the indentation depth of the tip. Cells are living systems composed of a large number of different structures, such as organelles, cytoskeleton network, nucleus, etc. Depending on the indentation depth, different internal cellular structures may be compressed. Hence, the Young's modulus reflects the mechanical response originating from several cellular structures. Consequently, the correct choice of indentation depth can be crucial for the identification of pathologically modified cells (Lekka, 2016).

The velocity at which the measurement is performed is also an important parameter in the AFM experiment, which needs to be controlled. Large tip velocity results in high viscous response of the cell, whereas at very low speeds, the cell may undergo internal reorganization or movement, possibly in response to or induced by the indentation of the tip.

AFM applications: cell mechanics and cancer

Cell mechanics is a novel and very important biophysical property of cells, which is strongly connected to many cellular processes, such as migration, differentiation and aging (Bohnet et al., 2006; Chen et al., 2004; McKenzie et al., 2018). In diseases, including cancer, mechanical properties of cells often change, resulting in the notion of the mechanical fingerprint of diseases (Rianna and Radmacher, 2016a; Suresh, 2007).

Cancer is one of the most numerous causes of deaths worldwide. When this disease occurs, cancer cells acquire anomalous abilities, e. g., the formation of metastases (leaving local tissue to migrate through blood vessel to other secondary organs and invading them) and continuous proliferation. Another peculiar change happening during cancer is the change in the mechanical properties of cancer cells. Using different techniques, changes in cancer cell mechanics have been reported, leading to the notion of using cell mechanics as a parameter to discriminate between normal and cancer cells.

The study of cancer mechanics with the AFM has been introduced by Lekka and co workers (Lekka et al., 1999). In this study, they compared the elastic properties of cancer and normal cells and found that bladder and ureter cancer cells were one order of magnitude softer than normal cells. After this study, many more groups have used AFM to compare the mechanics of cancer cells and their normal counterparts.

Our group also contributed to these investigations. In 2012, Prabhune et al. employed AFM to compare the mechanical properties of primary thyroid cells to their malignant counterparts (Prabhune et al., 2012). AFM experiments were performed over three consecutive days after cell seeding to consider the effect of culture time on the elastic properties of both cell lines. It was found that cancer thyroid cells were softer than normal ones, and even over time, the differences between them increased (from ~1.5 to 5 kPa). Using the AFM in imaging mode, they also detected topographical differences between the two cell lines and found that cancer cells were higher than normal cells. Moreover, AFM measurements were complemented with fluorescence microscopy. Staining cell actin filaments with rhodamine-phalloidin, they observed that cancer cells presented fewer stress fibers and single filaments distributed throughout the cell body. Contrarily, primary thyroid cells exhibited thick bundles of actin filaments, running continuously at the basal and nuclear regions. This supports the hypothesis that changes in actin organization are connected to cell mechanics and cancer's mechanoadaptive phenomenon.

As mentioned above, many studies have reported that cancer cells are softer than normal cells (Lekka, 2016; Lekka et al., 1999; Li et al., 2008; Prabhune et al., 2012; Rebelo et al., 2013); however, in the majority of these experiments, conventional cell culture systems using Petri dishes as support, whose mechanical and topographical properties are very different from those in tissues, were used. In order to better mimic the natural cell microenvironment, the mechanics of cancer cells has also been investigated on different stiffness gels, for example, cancer cells have been plated on soft collagen matrices by Staunton et al. (Staunton et al., 2016), where they found that breast adenocarcinoma cells, which invaded the matrix, were stiffer than cells remaining on top of the collagen gel, suggesting that the contractility of actomyosin plays an important role in the first steps of metastatic invasion.

Attempting to further investigate differences in the mechanics of cancer and normal cells mimicking the mechanical properties of native tissues, Rianna et al. measured the viscoelastic properties of healthy and cancer thyroid cells on stiffness-tunable polyacrylamide gels (PA) (Rianna and Radmacher, 2016b). In this study, PA gels with two different stiffness values were used as cell culture supports and Petri dishes were used as control. They found that, in agreement with previous findings, when seeded on

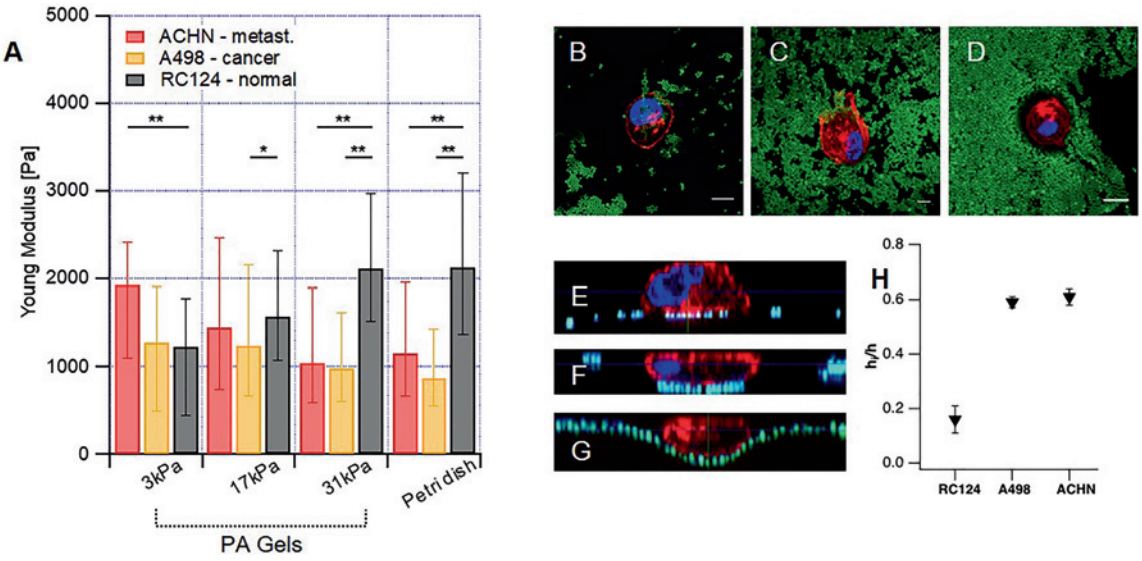

Figure 4: (A) Young's modulus values of normal and cancer cells on substrates with different mechanical properties: 3, 17 and 31 kPa stiffness PA gels and conventional Petri dishes, as a control. Red, orange and black bars stand for metastatic ACHN, cancer A498 and normal RC124 renal cells, respectively. Confocal images of fixed cells with color channels blue for nucleus, green for fluorescent bead and red for actin cytoskeleton are reported. Confocal images (B–D) and confocal side view (E–G) of normal RC124 (B and E), cancer A498 (C and F) and metastatic ACHN cells (D and G) on soft PA gels (3 kPa) with embedded 1 µm diameter green fluorescent beads Scale bars are 10 µm. The index of cell indentation (H) calculated as the ratio between the cell portion indenting the gel (h_i) and the total cell height (h); markers are median values and error bars are standard deviation. Reproduced from Rianna and Radmacher, 2017 with permission from the Royal Society of Chemistry.

Petri dishes, thyroid cancer cells were softer than their normal counterparts; however, the trend was reversed on soft gels, with cancer cells being stiffer than normal ones. Moreover, cancer cell mechanics were independent of substrate rigidity, showing constant values of Young's modulus (1.2–1.5 kPa) on gels with different stiffness, whereas the Young's modulus measured from normal cells increased with substrate stiffness (1.2–2.6 kPa). Thus, normal cells adapt their viscoelasticity to those of the underlying materials, whereas cancer cells are insensitive to the mechanics of the surrounding environment. In agreement with this result, Lin et al. found that most cancer cells did not alter their mechanical properties on matrices of varying stiffness, defining loss of stiffness sensing as a feature of the mechanical phenotype of cancer cells (Lin et al., 2015).

The stiffening of cancer cells on soft gels could be related to their function in metastasis, i. e., when in contact with soft and compliant matrices, cancer cells could attempt to invade and penetrate the gel, as they do with the local matrices in the process of metastatic invasion. This hypothesis is consistent with previous findings, showing that metastatic cancer cells apply high lateral forces on soft PA gels and that applied forces directly correlate with metastatic potential (Kristal-Muscal et al., 2015). Moreover, other studies showed that metastatic cancer cells increase their stiffness while invading into collagen I matrices (Staunton et al., 2016) and proved the role of traction stresses in regulating the mode with which cancer cells invade ECM networks to contribute to cancer metastasis (Aung et al., 2014).

To gain further insights in the relation between cancer cell mechanics on soft gels and metastatic invasive potential, Rianna et al. employed AFM and confocal microscopy to study the mechanical properties and invasive index of normal, cancer, and metastatic renal cells seeded on soft PA gels (Rianna and Radmacher, 2017). In accordance with their previous findings (Rianna and Radmacher, 2016b), they confirmed that cancer cells lost the ability to adapt their stiffness to the rigidity of the underlying support and that they were stiffer than normal cells when seeded on soft gels. Further studies on cancer cell invasion into PA gels by confocal microscopy revealed that the cell indentation index (calculated as the ratio between the cell portion indenting the gel and the total cell height) was higher in metastatic cells than in healthy cells (Figure 4).

Conclusions

The mechanical properties of cells, especially in diseased cells, are a marker of cell state and condition. Thus, studying cell mechanics will lead to an improved

understanding of cellular function and structure. Cell mechanics bridges the biochemical properties of living systems with their biophysical structure, as is becoming apparent in the case of actin cytoskeleton remodeling in cancer cells. In this review, we demonstrated that studying the mechanics of biological systems has vast potential in understanding health and disease.

AFM is an excellent tool to study cell mechanics, mainly for two broad reasons. First, with the combination of its high spatial resolution, including topographic imaging, with its great versatility in determining mechanical properties, mechanical measurements of biological samples can be performed fast and with high sensitivity. A large variety of relevant mechanical parameters can be obtained, and the mechanics of a cell's interaction with its environment can also be studied. There is also a variety of cantilevers to choose from, depending on the biological question and system, the mechanical parameters to be obtained, and the AFM system available. Second, it can be performed under biological conditions and for a wide range of samples, such as living samples in physiological environment. Application of drugs or other treatments during an experiment is also feasible as well as the combination with other broadly used microscopy techniques in biology, such as confocal microscopy.

Thus, AFM is a unique tool for determining the forces of, and between, cells and the mechanical properties of cells. It is entering its mature stage; therefore, AFM is moving from the field of basic research to biomedical applications, which opens up new and exciting applications. It may even pave the road to using the mechanical fingerprint of cells in diseases as a new diagnostic tool.

Research funding: We acknowledge the support of the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 812772, Project Phys2BioMed.

References

Abidine, Y., Constantinescu, A., Laurent, V.M., Sundar Rajan, V., Michel R., Laplaud, V., Duperray, A., and Verdier, C. (2018). Mechanosensitivity of cancer cells in contact with soft substrates using AFM. Biophys. J. *114*, 1165–1175.

Alibert, C., Goud, B., and Manneville, J.B. (2017). Are cancer cells really softer than normal cells? Biol. Cell *109*, 167–189.

Aung, A., Seo, Y.N., Lu, S., Wang, Y., Jamora, C., del Álamo, J.C., and Varghese, S. (2014). 3D traction stresses activate protease-dependent invasion of cancer cells. Biophys. J. *107*, 2528–2537.

Binnig, G., Quate, C.F., Gerber, C. (1986). Atomic force microscope. Phys. Rev. Lett. *56*, 930–933.

Binnig, G. and Rohrer, H. (1983). Scanning tunneling microscopy. Surf. Sci. *126*, 236–244.

Bohnet, S., Ananthakrishnan, R., Mogilner, A., Meister, J.J., and Verkhovsky, A. B. (2006). Weak force stalls protrusion at the leading edge of the lamellipodium. Biophys. J. *90*, 1810–1820.

Brückner, B.R., and Janshoff, A. (2015). Elastic properties of epithelial cells probed by atomic force microscopy. Biochim. Biophys. Acta *1853*, 3075–3082.

Chen, L., Chin, L.C., Ashby, P.D., and Lieber, C.M. (2004). Single-walled carbon nanotube AFM probes: Optimal imaging resolution of nanoclusters and biomolecules in ambient and fluid environments. Nano Lett. *4*, 1725–1731.

Cross, S.E., Jin, Y.S., Tondre, J., Wong, R., Rao, J.Y., Gimzewski, J.K. (2008). AFM-based analysis of human metastatic cancer cells. Nanotechnology *19*, 384003.

Drake, B., Prater, C.B., Weisenhorn, A.L., Gould, S.A.C., Albrecht T.R., Quate, C. F., Cannell, D. S., Hansma, H. G., and Hansma P. K. (1989). Imaging crystals, polymers, and processes in water with the atomic force microscope. Science *243*, 1586–1589.

Florin, E.L., Moy, V.T., and Gaub, H.E. (1994). Adhesion forces between individual ligand-receptor pairs. Science *264*, 415–417.

Grandbois, M., Dettmann, W., Benoit, M., and Gaub, H.E. (2000). Affinity imaging of red blood cells using an atomic force microscope. J. Histochem. Cytochem. *48*, 719–724.

Guck, J., Ananthakrishnan, R., Mahmood, H., Moon, T.J., Cunningham, C. C., and Käs, J. (2001). The optical stretcher: A novel laser tool to micromanipulate cells. Biophys. J. *81*, 767–784.

Hertz, H. (1881). Über die Berührung fester elastischer Körper. J. Reine Angew. Mathematik. *92*, 156–171.

Hochmuth, R.M. (2000). Micropipette aspiration of living cells. J. Biomech. *33*, 15–22.

Hutter, J.L. and Bechhoefer, J. (1993). Calibration of atomic-force microscope tips. Rev. Sci. Instrum. *64*, 1868–1873.

Jakab, A., Schubert, G., Prodan, M., and Forgacs, E. (2002). PCA, followed by two-dimensional nonlinear mapping and cluster analysis, versus multilinear regression in QSSR. J. Liq. Chromatogr. Relat. Technol. *25*, 1–16.

Kirmizis, D. and Logothetidis, S. (2010). Atomic force microscopy probing in the measurement of cell mechanics. Int. J. Nanomed. *5*, 137–145.

Kristal-Muscal, R., Dvir, L., Schvartzer, M., and Weihs, D. (2015). Mechanical interaction of metastatic cancer cells with a soft gel. Procedia IUTAM *12*, 211–219.

Laurent, V.M., Hénon, S., Planus, E., Fodil, R., Balland, M., Isabey, D., and Gallet, F. (2002). Assessment of mechanical properties of adherent living cells by bead micromanipulation: Comparison of magnetic twisting cytometry vs optical tweezers. J. Biomech. Eng. *124*, 408–421.

Lautenschläger, F., Paschke, S., Schinkinger, S., Bruel, A., Beil, M., and Guck, J. (2009). The regulatory role of cell mechanics for migration of differentiating myeloid cells. Proc. Natl. Acad. Sci. *106*, 15696–15701.

Lekka, M., Laidler, P., Gil, D., Lekki, J., Stachura, Z., and Hrynkiewicz, A.Z. (1999). Elasticity of normal and cancerous human bladder cells studied by scanning force microscopy. Eur. Biophys. J. *28*, 312–316.

Lekka, M. (2016). Discrimination between normal and cancerous cells using AFM. BioNanoSci. *6*, 65–80.

Li, Q.S., Lee, G.Y.H., Ong, C.N., and Lim C.T. (2008). AFM indentation study of breast cancer cells. Biochem. Biophys. Res. Commun. *37*, 609–613.

Lin, H.H., Lin, H.K., Lin, I.H., Chiou, Y.W., Chen, H.W., Liu, C.Y., Harn H.I.C., Chiu, W.T., Wang, Y.K., Shen, M.R. et al. (2015). Mechanical phenotype of cancer cells: Cell softening and loss of stiffness sensing. Oncotarget *6*, 20946–20958.

Matzke, R., Jacobson, K., and Radmacher, M. (2001). Direct, high-resolution measurement of furrow stiffening during division of adherent cells. Nat. Cell Biol. *3*, 607–610.

McKenzie, A.J., Hicks, S.R., Svec, K.V., Naughton, H., Edmunds, Z.L., and Howe, A. K. (2018). The mechanical microenvironment regulates ovarian cancer cell morphology, migration, and spheroid disaggregation. Sci. Rep. *8*.

Mietke, A., Otto, O., Giraldo, S., Rosendahl, P., Taubenberger, A., Golfier, S., Ulbricht, E., Aland, S., Guck, J., and Fisher-Friedrich, E. (2015). Extracting cell stiffness from real-time deformability cytometry: Theory and experiment. Biophis. J. *109*, 2023–2036.

Moeendarbary, E. and Harris, A.R. (2014). Cell mechanics: Principles, practices, and prospects. WIREs Syst. Biol. Med. *6*, 371–388.

Ochalek, T., Nordt, F.J., Tulberg, K., and Burger, M.M. (1988). Correlation between cell deformability and metastatic potential in B16-F1 melanoma cell variants. Cancer Res. *48*, 5124–5128.

Otto, O., Rosendahl, P., Mietke, A., Golfier, S., Herold, C., Klaue, D., Giraldo, S., Pagliara, S., Ekpenyong, A., Jacobi, A., et al. (2015). Real-time deformability cytometry: On-the-fly cell mechanical phenotyping. Nat. Methods *12*, 199–202.

Pachenari, M., Seyedpour, S.M., Janmaleki, M., Shayan, S.B., Taranejoo, S., and Hosseinkhani, H. (2014). Mechanical properties of cancer cytoskeleton depend on actin filaments to microtubules content: Investigating different grades of colon cancer cell lines. J. Biomech. *47*, 373–379.

Pegoraro, A.F., Janmey, P., Weitz, D.A. (2017). Mechanical properties of the cytoskeleton and cells. Cold Spring Harb. Perspect. Biol. *9*, a022038.

Prabhune, M., Belge, G., Dotzauer, A., Bullerdiek, J., and Radmacher, M. (2012). Comparison of mechanical properties of normal and malignant thyroid cells. Micron *43*, 1267–1272.

Prass, M., Jacobson, K., Mogilner, A., and Radmacher, M. (2006). Direct measurement of the lamellipodial protrusive force in a migrating cell. J. Cell Biol. *174*, 767–772.

Radmacher, M., Fritz, M., and Hansma, P.K. (1995). Measuring the elastic properties of biological materials with the atomic force microscope. Biophys. J. *68*, A139.

Rebelo, L.M., De Sousa, J.S., Mendes-Filho, J., Radmacher, M. (2013). Comparison of the viscoelastic properties of cells from different kidney cancer phenotypes measured with atomic force microscopy. Nanotechnology *24*, 55102.

Rehfeldt, F., Engler, A.J., Eckhardt, A., Ahmed F., and Discher, D.E. (2007). Cell responses to the mechanochemical microenvironment-Implications for regenerative medicine and drug delivery. Adv. Drug Delivery Rev. *59*, 1329–1339.

Rianna, C., Kumar, P., and Radmacher, M. (2017). The role of the microenvironment in the biophysics of cancer. Semin. Cell Dev. Biol. *73*, 107–114.

Rianna, C., Radmacher, M. (2016a). Cell mechanics as a marker for diseases: Biomedical applications of AFM. AIP Conf. Proc. *1760*, 20057.

Rianna, C. and Radmacher, M. (2016b). Comparison of viscoelastic properties of cancer and normal thyroid cells on different stiffness substrates. Eur. Biophys. J. *46*, 309–324.

Rianna, C. and Radmacher, M. (2017). Influence of microenvironment topography and stiffness on the mechanics and motility of normal and cancer renal cells. Nanoscale *9*, 11222–11230.

Rico, F., Roca-Cusachs, P., Gavara, N., Farré, R., Rotger, M., Navajas, D. (2005). Probing mechanical properties of living cells by atomic force microscopy with blunted pyramidal cantilever tips. Phys. Rev. E *72*, 021914.

Rotsch, C., Jacobson, K., and Radmacher, M. (1999). Dimensional and mechanical dynamics of active and stable edges in motile fibroblasts investigated by using atomic force microscopy. Proc. Natl. Acad. Sci. *96*, 921–926.

Rotsch, C. and Radmacher, M. (2000). Drug-induced changes of cytoskeletal structure and mechanics in fibroblasts: An atomic force microscopy study. Biophys. J. *78*, 520–535.

Schäfer, A. and Radmacher, M. (2005). Influence of myosin II activity on stiffness of fibroblast cells. Acta Biomater. *1*, 273–280.

Schillers, H., Rianna, C., Schäpe, J., Luque, T., Doschke, H., Wälte, M., Uriarte, J.J., Campillo, N., Michanetzis, G.P.A., Bobrowska, J., et al. (2017). Standardized nanomechanical atomic force microscopy procedure (SNAP) for measuring soft and biological samples. Sci. Rep. *7*, 5117.

Sneddon, I.N. (1965). The relation between load and penetration in the axisymmetric boussinesq problem for a punch of arbitrary profile. Int. J. Eng. Sci. *3*, 47–57.

Staunton, J.R., Doss, B.L., Lindsay, S., Ros, R. (2016). Correlating confocal microscopy and atomic force indentation reveals metastatic cancer cells stiffen during invasion into collagen I matrices. Sci. Rep. *6*, 19686.

Suresh, S. (2007). Biomechanics and biophysics of cancer cells. Acta Mater. *55*, 3989–4014.

Viji Babu, P.K.Rianna, C., Belge, G., Mirastschijski, U., and Radmacher, M. (2018). Mechanical and migratory properties of normal, scar, and Dupuytren's fibroblasts. J. Mol. Recognit. e2719.

Yamaguchi, H. and Condeelis, J. (2007). Regulation of the actin cytoskeleton in cancer cell migration and invasion. Biochim. Biophys. Acta *1773*, 642–652.

Yousafzai, M.S., Coceano, G., Bonin, S., Niemela, J., Scoles, G., and Cojoc, D. (2017). Investigating the effect of cell substrate on cancer cell stiffness by optical tweezers. J. Biomech. *60*, 266–269.

Zhang, H. and Liu, K.K. (2008). Optical tweezers for single cells. J. R. Soc. Interface *5*, 671–690.

Bionotes

Sandra Pérez-Domínguez
Institute of Biophysics, University of Bremen, Bremen, Germany

Sandra Pérez-Domínguez is a PhD student at the Institute of Biophysics, University of Bremen, Germany, funded by H2020-MSCA-ITN 2018. She obtained her Bachelor's degree in Chemistry in 2017 and her Master's degree in Molecular and Cellular Biology in 2018 at the University of Zaragoza, Spain.

Carmela Rianna
Institute of Biophysics, University of Bremen, Bremen, Germany

Dr. Carmela Rianna is a post-doctoral researcher at the Institute of Biophysics, University of Bremen, Germany. In 2015 she obtained a PhD at the Department of Chemical, Materials and Industrial Production Engineering, University of Naples Federico II, Italy. Previously, she obtained Bachelor's and Master's degrees in Biomedical Engineering at the University of Naples Federico II. In 2018 she was a post-doctoral researcher fellow for one year at the University of California, Berkeley, Department of Bioengineering, Berkeley, CA, USA.

Shruti G. Kulkarni
Institute of Biophysics, University of Bremen, Bremen, Germany

Shruti G. Kulkarni is an MSCA-ITN Early Stage Researcher and PhD student at Institute of Biophysics, University of Bremen. She obtained her joint Bachelor and Master of Science in Biological Sciences at the Indian Institute of Science Education and Research – Kolkata, West Bengal, India in 2019, with her MS thesis focusing on Biophysics.

Manfred Radmacher
Institute of Biophysics, University of Bremen, Bremen, Germany
radmacher@uni-bremen.de
https://orcid.org/0000-0001-8744-4541

Dr. Manfred Radmacher is a full professor in Biophysics at the University of Bremen. He studied physics at the Technical University in Munich where he also received his PhD in 1993 on applications of the Atomic Force Microscope for the investigation of soft samples. After two years as a Post-Doc at the University of California in Santa Barbara, he returned to the Ludwig-Maximilians University in Munich, where he received his habilitation in 1999. Then he moved to Göttingen to be Professor at the University, until he started his current full professor position in 2002 at the University of Bremen. His main interest are the mechanical properties of cells and the relation between cell mechanics and diseases.