How much force is needed to kill a single bacterium?

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V. Vadillo-Rodriguez
Department of Applied Physics, University of Extremadura, Avda de Elvas s/n, Badajoz 06006, Spain
E-mail: vvadillo@unex.es

P. Pedraz, C. Flors
Madrid Institute for Advanced Studies in Nanoscience (IMDEA Nanociencia), C/Faraday 9, Madrid 28049, Spain
E-mail: cristina.flors@imdea.org

C. Flors
Nanobiotechnology Unit Associated to the National Center for Biotechnology (CNB-CSIC-IMDEA), C/Faraday 9, Madrid 28049, Spain
E-mail: cristina.flors@imdea.org

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Abstract

The interaction between bacteria and nanomaterials, particularly from a physical or mechanical perspective, has emerged as a topic of significant interest in both science and medicine. Mechanobactericidal nanomaterials, which exert antimicrobial effects through purely physical mechanisms, hold promise as alternative strategies to combat bacterial resistance to traditional antibiotics. High-aspect-ratio nanoparticles and surface topographies are being engineered to enhance their mechanobactericidal properties. However, progress in this field is hindered by an incomplete understanding of how these materials induce mechanical cell death in bacteria. This review examines the role of atomic force microscopy (AFM) nanoindentation in quantifying forces required to rupture the bacterial cell wall. The reported values range from nN to a few tens of nN, depending on the type of bacterium and the experimental conditions used. We discuss the potential effect of AFM tip properties, loading speed, bacterial immobilization strategy or environmental conditions on the measured rupture values. This perspective also highlights the complexities of modeling bacterial cell rupture and the importance of pressure as a parameter for standardizing results across experiments. Furthermore, the implications of these quantitative insights to understand the mechanisms of action of mechanobactericidal nanomaterials are discussed.

1. Introduction: why does it matter?

The physical or mechanical interaction between bacteria and nanomaterials has become a topic of scientific and medical importance. In particular, mechanobactericidal nanomaterials are promising antimicrobial strategies that overcome bacterial resistance to classical antibiotics, since their action mechanism involves a purely physical interaction.[1] Low-dimensional nanoparticles such as nanotubes or nanowires, and high-aspect-ratio topographies nanofabricated on surfaces, are being optimized for their mechanobactericidal action. However, their development is limited by the current lack of understanding of mechanically-induced bacterial death by these materials.[2] These mechanisms depend on the material properties and on environmental factors, and likely involve a combination of weak and strong mechanical interactions that affect bacterial physiology in different ways. To contribute to this debate, recent studies are trying to provide quantitative information about the range of forces involved using atomic force microscopy (AFM) nanoindentation, in which the AFM tip can be considered as a model for a high aspect ratio nanostruture that ruptures the bacterial cell wall layers.[3] While a range of quantitative data on different bacterial species is starting to emerge, the experimental parameters vary considerably and the results are difficult to interpret in a comprehensive way. In this Perspective, we collect and compare recent work on AFM nanoindentation that help in answering the question: how much force is needed to kill a single bacterium? The influence of environmental and experimental parameters on the measured rupture forces are considered, as well as the non-trivial transformation of force values into pressures. Finally, we discuss the implications of these quantitative studies to better understand the mechanisms of mechanobactericidal materials.

2. Comparison of rupture forces obtained by AFM

AFM nanoindentation experiments are typically performed by approaching a tip to an immobilized live bacterium with a certain indentation speed until a maximum force value is reached, after which the tip is retracted. Force-distance and force-indentation curves are obtained, and if rupture occurs, a clear peak is observed on the approach curve, and a rupture force and indentation depth at rupture can be obtained (**Figure 1**). Although it is technically possible to proceed with nanoindentation without prior imaging, imaging of the cells at low force is routinely conducted to ensure that indentations are accurately targeted at specific locations on the cells.

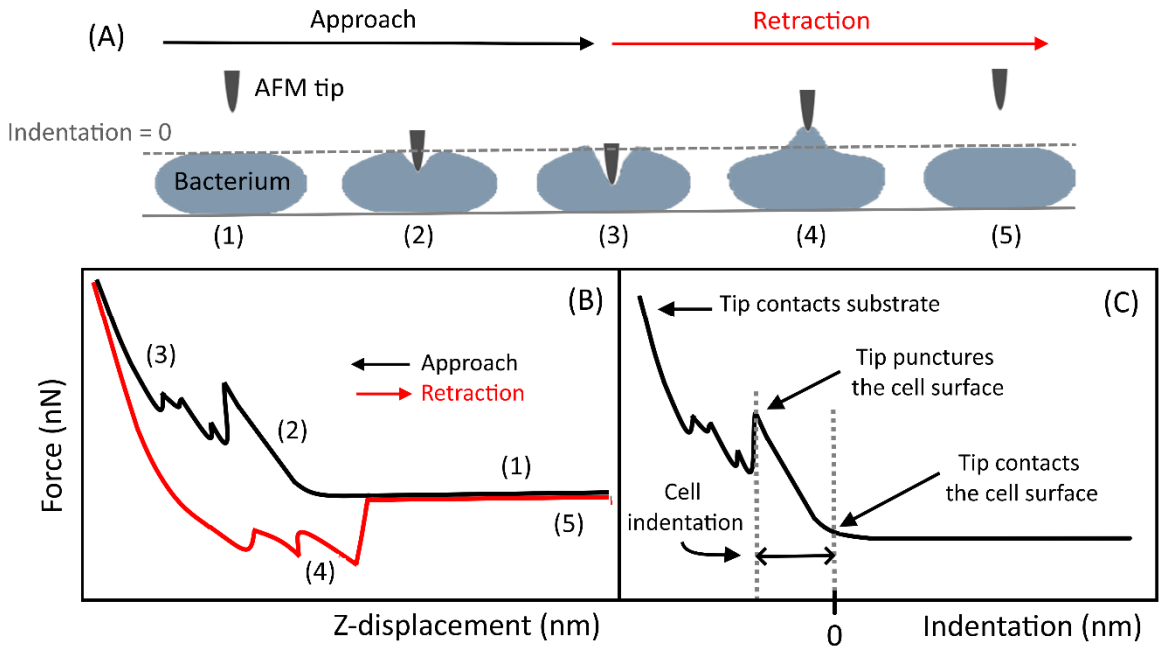

Figure 1. (A) Schematics of an AFM nanoindentation experiment. (B) Typical force-distance and (C) force-indentation curves obtained during the experiment. The AFM tip approaches an immobilized live bacterium at a controlled indentation speed until a maximum force is reached (approach curve), after which the tip is retracted (retraction curve). During the approach, if the bacterial cell is deformed beyond its elastic limit, it ruptures, allowing both the rupture force and the corresponding indentation depth to be measured from the data.

Table 1 shows a compilation of data from the literature regarding AFM rupture experiments on live bacteria. Some important experimental parameters, which may have an impact on the measured rupture force values, are included. For example, bacterial cells are immobilized on a substrate often coated with a material that enhances cell adhesion to ensure the cells remain stationary during indentation. The immobilization method is one of the most crucial aspects in these experiments, as a compromise needs to be found between strong immobilization to withstand the impact from the AFM tip and the negative consequences of this immobilization on bacterial physiology. For instance, it has been reported that higher concentrations of poly-L-lysine (PLL) on the surface result in higher rupture forces.[3c] This observation might be related to the decrease in fluidity of model membranes induced by PLL, and more generally, consistent with the fact that antibiotic compounds affect the mechanical properties of bacteria. The other experimental parameters in Table 1 are discussed below.

For the bacterium *Escherichia coli*, Table 1 shows that the reported rupture force values range from 1 to 25 nN. An intermediate force of about 7 nN is observed for *Pseudomonas aeruginosa*, another Gram negative bacterium. Interestingly, while a rupture force of 1-2 nN is reported for *Salmonella typhimurium*, it is shown that bacteria are still viable after multiple puncturings (i.e. they retain the ability to divide).[3a] On the other hand, it has been shown that rupture forces occurring in the range of 20-25 nN lead to instantaneous bacterial death, which was confirmed by simultaneous detection of a fluorescent viability dye.[3c] Therefore, it is likely that the peaks observed in the force-indentation curves in these reports correspond to different type of events. Rupture force values in the few nN range may correspond to penetration of only the outer membrane of the bacterium, which would be consistent with its ability to repair and ultimately divide, as observed.[3a] It can be seen in Table 1 that experiments that measure the lowest rupture forces in *E. coli* were performed with the softest tips (lowest values of the spring constant, *k*), which would indeed provide the resolution to detect subtle events. Conversely, experiments performed with high *k* tips would have poor resolution in the force-indentation curve, but would provide sufficient force to penetrate the three layers of a Gram negative bacterium, leading to cell death.

Table 1. Compilation of experimental parameters and measured values in published AFM puncture experiments on live bacteria.

Bacterial strain (Gram type and shape)	Immobilization method	Medium	Tip radius [nm]	*k* [N·m^{-1}][a)]	Indentation speed [µm·s^{-1}]	**Rupture force [nN]**	Indentation depth [nm]	Pressure [MPa][b)]	Ref.
S. typhimurium (-; rod)	Antibodies	PBS	25/35[c)]	0.01-0.02	3-6	**1-2**	50-100	0.3	[3a]
E. coli DH10β (-; rod)	PLL 0.001% w/v	PBS 0.01 M	8[a)]	0.6-2	1	**20.3 ± 4.9**	373 ± 157	3.2	[3c]
E. coli DH10β (-: rod)	Cell-Tak	PBS 0.01 M	8[a)]	0.6-2	1	**24.9 ± 4.1**	340 ± 100	4.4	[3c]
B. subtilis CECT356 (+: rod)	PLL 0.002% w/v	PBS 0.01 M	8[a)]	0.6-2	1	**~80**	~175	14.0	[4]

E. coli ATCC25922 (-; rod)	Clean silicon/wrinkled PDMS	PBS	19[c)]	0.022	1-2	**1.04 ± 0.52**	239 ± 124	0.11	[3d]
B. subtilis ATCC6051 (+: rod)	Clean silicon/wrinkled PDMS	PBS	19[c)]	0.022	1-2	**0.74 ± 0.38**	202 ± 93	0.09	[3d]
E. coli CGMCC 1.3373 (-; rod)	PLL 0.01% wt	DI water	40[c)]	0.046	2	**~5**	~85	0.7	[3e]
P. aeruginosa ATCC27853 (-; rod)	Polydopamine 8 mg/ml	PBS	<10[a)]	0.2	[d)]	**6.8 ± 1.4**	[d)]	[e)]	[3f]
S. aureus ATCC700699 (+; cocci)	Polydopamine 8 mg/ml	PBS	<10[a)]	0.2	[d)]	**23.3 ± 19.7**	[d)]	[e)]	[3f]

[a)] Nominal value; [b)] Calculated with the Hertz model using indentation depth values;[5] [c)] Measured by scanning electron microscopy or tip calibration grid; [d)]Not reported; [e)]Indentation depth not available. PBS: phosphate buffer saline, PDMS: polydimethylsiloxane, DI: deionized.

In addition to the experiments included in Table 1, which are all performed in close-to physiological conditions (liquid), two other papers report on AFM measurements of bacteria dried on the surface and imaged at ambient humidity. An early 2010 paper showed that forces above 10 nN are necessary to produce visible and irreversible damage on both *E. coli* and *B. subtilis* (**Figure 2**).[3b] More recently, a value of 34.4 nN was estimated for the rupture of dried *E. coli*.[6] The latter work also explored its mechanical response under different loading conditions to understand the viscoelastic response (see below). Unsurprisingly, rupture values in air are higher than in liquid, owing to a dehydrated cell wall with increased stiffness.

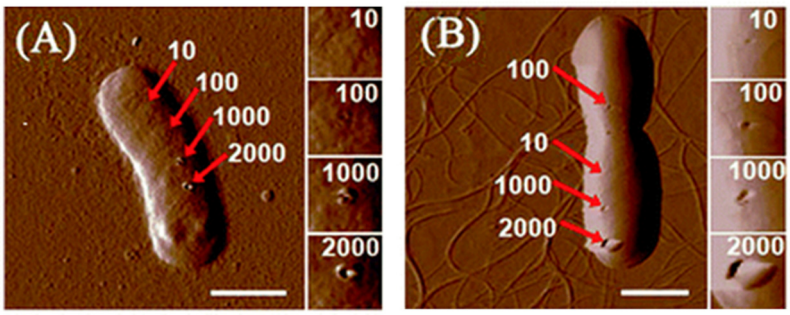

Figure 2. AFM amplitude images of (A) *E. coli* and (B) *B. subtilis* after piercing by a 2 nm AFM tip for 200 times at different locations. Small images on the extreme right are enlarged images of areas punctured by the tip. Numbers represent the applied force in nN. All scale bars are 1 μm. Adapted with permission.[3b] 2010, Royal Society of Chemistry.

2.1 Other experimental factors potentially affecting the rupture values

Besides the method for bacterial immobilization discussed above, Table 1 includes another potentially important parameter associated to sample preparation such as the composition of the medium. Osmolarity of the environment is related to the turgor pressure of the bacterium, which is the outward force generated by the osmotic difference between the cytoplasm and extracellular environment. The bacterial turgor pressure affects its mechanical properties. For example, Han et al show that the cell wall stiffness increases linearly with an increase in turgor.[7] This may contribute to the differences in indentation depth at rupture observed for *E. coli* in PBS of different osmolarities (seldom specified in the literature) or DI water. Additionally, it could potentially lead to unforeseen effects on the measured rupture forces. The mechanical stress induced by turgor pressure could alter the structural characteristics of the bacterial cell wall and membrane, possibly affecting their tensile strength and thus influencing the force at which they fail or break.

Regarding the tip geometry, it would be expected that rupture would occur at lower forces with a smaller tip radius, since the applied force is distributed over a smaller area, increasing the pressure (force per unit area) exerted on the cell. While this seems to be generally the case, systematic experiments on mammalian cells and lipid bilayers show that the differences in rupture force are lower than expected by simply considering the area of the tip.[8] While a few studies have addressed this aspect in the context of bacterial cells, both computationally[9] and experimentally,[3a, 3d] the results are yet inconclusive. Indeed, a key challenge is to properly estimate the contact area between the membrane and the tip, especially at the high indentation depths achieved in bacterial rupture experiments (see section Force vs pressure).

On the other hand, it has been reported that the aspect ratio of the tip has an effect on the overall shape of the force-indentation curve. Pyramidal tips experience more resistance as the tip progresses into the membrane, resulting in multiple peaks in the curve, while the use of sharper high aspect ratio tips results in a cleaner curve with only one peak (**Figure 3**).[3a]

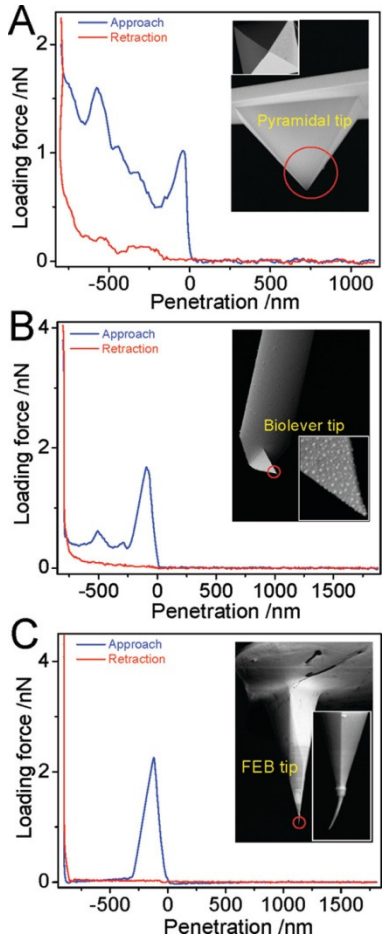

Figure 3. *Salmonella typhimurium* puncturing curves obtained using AFM tips with three different aspect ratios. An SEM image of the AFM tip is shown in the inset for each panel: (A) a pyramidal tip with an aspect ratio of ~1:1, (B) a biolever tip with an aspect ratio of ~1.7:1, and (C) a focused electron beam tip with an aspect ratio of ~10:1. For each tip the portion that pierces bacterial cells is marked with a red circle. Reproduced with permission.[3a] 2009, American Chemical Society.

Table 1 also includes data on the indentation speed, a parameter that has been relatively overlooked in the literature. Bacterial cells are recognized for their viscoelastic properties, displaying traits of both viscous and elastic materials.[10] They deform under stress (exhibiting elastic behavior) and show time-dependent deformation (demonstrating viscous behavior) over prolonged periods. This dual characteristic highlights that their reaction to stress could

vary significantly depending on the rate of stress application. Low indentation speed typically allows for gradual application of force over time, giving the bacterial cell wall more time to deform slowly and distribute stress evenly. As a result, the cell wall may withstand a higher force before reaching its rupture point. Conversely, high indentation speed involves rapid application of force, limiting the time available for the cell wall to deform and distribute stress. Consequently, stress is more likely localized, leading to a quicker exceedance of the tensile strength. To test this hypothesis, a recent study investigated the rupture of *E. coli* under indentation loading speeds ranging from 0.1 to 1.5 $\mu m \cdot s^{-1}$.[6] They observed that, while the indentation depth at rupture decreased with increasing the speed (from ~27 to ~ 11 nm), as expected, the force required to rupture the cell remained relatively consistent, averaging around 34.4 nN across the different loading rates. Interestingly, this consistency might indicate that the cell's mechanical failure is governed more by its material properties, i.e. the types of molecules present in the cell membrane or wall, their arrangement, and bonding types, rather than the dynamics of how quickly the force is applied. Conducting further research in this field would be highly beneficial to support these observations.

2.2 Force vs pressure

Pressure accounts for the area over which force is applied. Bacterial cells may resist a significant force if it spreads over a large area, but could fail under the same force concentrated over a smaller area. While the rupture force is a parameter that can be directly extracted from the force-indentation curves, considering pressures instead of forces would allow an easier comparison between experiments performed with different tips geometries, given that the contact area between the tip and the membrane is known. However, estimating the contact area between the tip and the cell wall is a major challenge in AFM indentation experiments, especially at high indentation depths. The most simplified method considers a circular area that can be directly calculated from the tip radius, which may be reasonable at very low indentation values. The classic Hertz model describes the non-adhesive elastic contacts between a sphere and a sample half-space.[5a] Other more advanced models such as the Johnson-Kendall-Roberts, and Derjagin-Muller-Toropov include (simplified) adhesion forces.[11] All models have limitations such as the assumption of purely elastic deformations or perfectly smooth surfaces. However, due to its simplicity, the Hertz model remains the most widely applied model for the determination of the contact mechanics in the case of AFM nanoindentations of biological samples, despite its limitations.[11-12]

For Table 1, we have used Hertz theory to comparatively estimate pressures at rupture as a function of the applied load, the tip radius and the deformation (indentation depth), which are all experimental parameters. The maximum pressure (p_0) is given by the following Equation 1:

$$\boldsymbol{p_0 = \frac{3F}{2\pi a^2}} \quad (1)$$

where F is the load and a is the contact area radius given by Equation 2:

$$\boldsymbol{a = \sqrt[2]{R\delta}} \quad (2)$$

where R is the tip radius and δ is the deformation.

Rupture pressures for *E. coli* range from 0.1-4 MPa and the differences could be attributed to the different type of events (penetration in the outer membrane vs complete rupture of the cell wall), as discussed above. The lower value is also in accordance with a rupture pressure of ~0.3 MPa for other Gram negative bacteria such as *S. typhimurium* and *P. aeruginosa*, which was estimated using the effective Young Modulus instead of Hertz theory.[3e] It is likely that the Hertz model underestimates the pressure exerted on the cell wall by the AFM tip, as it does not account for adhesive forces that contribute to the overall pressure experienced by the bacterium. The limitations of the currently available models highlight the need for more sophisticated alternatives that account for viscoelasticity, adhesive interactions, and other complex behaviors exhibited by soft biological materials like bacterial cells, especially at high indentation depths. These efforts would contribute towards standardization of these measurements.

2.3 Gram type and cell shape

Gram positive and negative bacteria differ in their cell wall composition. The former have a thick peptidoglycan layer surrounding an internal (cytoplasmic) membrane, and the later have a thinner peptidoglycan layer sandwiched between two membranes. The thicker peptidoglycan in Gram positive bacteria likely makes them more resistant to indentation. While fewer AFM puncture studies have been carried out on Gram positive bacteria, rupture force (and pressure) values seem to be about 3-4 higher than their Gram negative counterparts under similar experimental conditions (Table 1).[13] Reference [4] reports 20 vs 80 nN for *E. coli* and *B. subtilis*, respectively (both rod-shaped), whereas Huang et al[3f] report 6.8 vs 23.3 nN for *P. aeruginosa* (rod-shaped) and *S. aureus* (cocci-shaped), respectively. Finite element simulations have estimated that the rupture force values for Gram-positive bacteria are about 6 times higher than for Gram negative,[2f] reasonably similar to the experimental values found.

Consistent with this trend, it is generally observed that Gram positive bacteria are more resistant to mechano-bactericidal effects than Gram negative.[2d, 2f, 3e, 14]

Another potentially important factor is the shape of the cell. Table 1 shows that most experiments were conducted on rod-shaped (cylindrical) bacteria, with typical lengths of 1-5 µm and 0.5-1 µm diameter. One exception in Table 1 is the cocci *S. aureus*, with a diameter of 0.5-1 µm. While there is currently not enough experimental data to extract meaningful conclusions on shape-dependence, theory predicts that for the same applied external pressure, a rod-shaped bacterium would be easier to break than a spherical cell.[15] This is because the stress distribution in a rod-shaped bacterium is uneven, with higher hoop stress along its cylindrical body. This uneven stress distribution makes it more likely for a rod-shaped bacterium to exceed the tensile strength of its cell wall or membrane and rupture. In contrast, stress in a spherical bacterium is uniformly distributed, allowing it to generally withstand higher pressures before rupturing.

3. Implications for understanding mechano-bactericidal processes

AFM nanoindentation experiments have shown that the relevant magnitude of forces that are necessary to kill a single bacterial cell ranges from a few nN to tens of nN, depending on the type of bacterium and experimental or environmental conditions. These force values are relatively high, and this knowledge has already contributed to shed some light onto mechano-bactericidal mechanisms. For example, it has been ruled out that simple collisions can produce enough force on a bacterium to cause rupture upon contact with a sharp nanostructure.[3b, 3e] Other force-generating mechanisms are gaining importance,[2a] such as capillary force or surface tension at the air-liquid interface,[2g, 16] storage and release of mechanical energy,[2b] or increased membrane abrasion due to flagella-mediated motility.[17] Moreover, an increasing body of evidence suggests that direct cell wall rupture may not be the most frequent mechanism,[2e, 2h, 3b, 14a] and bacterial response to weaker forces can influence metabolic activity, generation of reactive oxygen species, eventually leading to cell death.[2e] Rupture force values have also helped to understand the bactericidal mechanism of carbon-coated $Cu(OH)_2$ nanowires used for water disinfection, which is mainly attributed to hydrodynamic tearing.[3e]

4. Conclusions

The quantification of the force necessary to kill a single bacterial cell is an intriguing basic scientific problem that involves careful sample preparation, complex AFM data acquisition and analysis, and requires advanced knowledge in nanomechanics and bacterial cell wall properties. Several laboratories have managed to produce rupture force data, mostly on Gram negative bacteria, with values ranging from nN to a few tens of nN. Different environmental and experimental conditions were used in these experiments, which likely have an influence on the measured values. While forces are experimental parameters that can be extracted from AFM nanoindentation experiments, transformation into pressure values may allow a certain standardization. However, such calculation is not trivial mostly due to the challenge of estimating the contact area between the tip and the cell wall.

In this perspective, the significance of the rupture force values has been discussed mostly in the context of mechano-bactericidal nanomaterials, a promising strategy that could overcome antibiotic resistance. Indeed, the estimated forces are higher than expected and have led to a reevaluation of the possible mechano-bactericidal mechanisms of action, i.e. how can nN forces can be generated at the bacterium-material interface and what is the role of weaker mechanical interactions. Beyond mechano-bactericidal materials, the question of bacterial cell wall penetration may also be relevant to other contexts, such as the crossing of the bacterial cell wall by nano and micromotors.[18]

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Author Biographies

Dr. Virginia Vadillo-Rodríguez earned her bachelor's degree in Physics from the University of Extremadura, Spain, in 1999 and completed her PhD at the University of Groningen, Netherlands, in 2004. She then pursued postdoctoral research at Penn State University, USA (2004-2005), and at the University of Guelph, Canada (2006-2008). In 2009, she returned to the University of Extremadura and is now a full professor there. Her research has focused on using AFM to characterize bacterial cell surfaces, assess the mechanical/structural properties of proteins, study the impact of nano/micro-topographical surface structures on bacterial adhesion, and explore the mechanisms of natural antimicrobial compounds.

Dr. Patricia Pedraz received her MSc in "Nanostructured Materials for Nanotechnology Applications" in 2013 by the University of Zaragoza (Spain) and obtained her PhD in Physics of Condensed Matter and Nanotechology at Universidad Autónoma de Madrid in 2017. She is currently a research staff member at IMDEA Nanoscience, Madrid, Spain, where she works as an atomic force microscopy (AFM) specialist. Her work has been focused on the study of tribology and lubrication at the nanoscale, as well as the physical and mechanical characterization of soft matter by AFM.

Cristina Flors is a Research Professor at IMDEA Nanoscience in Madrid. She received her PhD in Chemistry from Institut Químic de Sarrià in Barcelona in 2004, and moved to KU Leuven (Belgium) as a postdoctoral fellow. In 2008 she started her independent research career at the University of Edinburgh (UK) and moved to Madrid in 2012. Cristina's work is focused on developing novel tools to study biology at the nanoscale.

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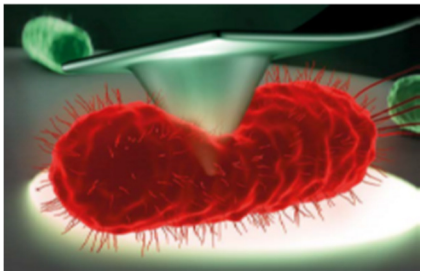

The quantification of the forces necessary to rupture the cell wall of a bacterium is relevant to understand, for example, the action mechanisms of mechanobactericidal nanomaterials. This perspective collects and compares recent work on bacterial nanoindentation beyond rupture using atomic force microscopy. The influence of environmental and experimental parameters on the measured rupture forces are discussed, as well as the non-trivial transformation of force values into pressures.

[1] a) N. Lin, P. Berton, C. Moraes, R. D. Rogers, N. Tufenkji, *Adv Colloid Interface Sci* **2018**, *252*, 55-68; b) A. Roy, K. Chatterjee, *Nanoscale* **2021**, *13*, 647-658; c) K. Modaresifar, S. Azizian, M. Ganjian, L. E. Fratila-Apachitei, A. A. Zadpoor, *Acta Biomater* **2019**, *83*, 29-36; d) D. P. Linklater, V. A. Baulin, S. Juodkazis, R. J. Crawford, P. Stoodley, E. P. Ivanova, *Nat Rev Microbiol* **2021**, *19*, 8-22.

[2] a) S. Hawi, S. Goel, V. Kumar, O. Pearce, W. N. Ayre, E. P. Ivanova, *ACS Appl Nano Mater* **2022**, *5*, 1; b) D. P. Linklater, M. De Volder, V. A. Baulin, M. Werner, S. Jessl, M. Golozar, L. Maggini, S. Rubanov, E. Hanssen, S. Juodkazis, E. P. Ivanova, *ACS Nano* **2018**, *12*, 6657-6667; c) S. Zhao, Z. Li, D. P. Linklater, L. Han, P. Jin, L. Wen, C. Chen, D. Xing, N. Ren, K. Sun, S. Juodkazis, E. P. Ivanova, L. Jiang, *Nano Lett* **2022**, *22*, 1129-1137; d) J. Jenkins, J. Mantell, C. Neal, A. Gholinia, P. Verkade, A. H. Nobbs, B. Su, *Nat Commun* **2020**, *11*, 1626; e) M. I. Ishak, J. Jenkins, S. Kulkarni, T. F. Keller, W. H. Briscoe, A. H. Nobbs, B. Su, *J Colloid Interface Sci* **2021**, *604*, 91-103; f) A. Valiei, J. F. Bryche, M. Canva, P. G. Charette, C. Moraes, R. J. Hill, N. Tufenkji, *ACS Appl Mater Interfaces* **2024**, *16*, 9614-9625; g) A. Valiei, N. Lin, J. F. Bryche, G. McKay, M. Canva, P. G. Charette, D. Nguyen, C. Moraes, N. Tufenkji, *Nano Lett* **2020**, *20*, 5720-5727; h) F. Viela, I. V. Ortega, J. J. Hernandez, I. Rodriguez, S. Moreno-Da Silva, A. López-Moreno, E. M. Pérez, C. Flors, *Small Sci.* **2023**, *3*, 2300002; i) T. E. Catley, R. M. Corrigan, A. J. Parnell, *ACS Omega* **2023**, *8*, 14873-14883; j) A. Pirouz, I. Papakonstantinou, M. Michalska, *Front Chem* **2024**, *12*, 1354755.

[3] a) Z. Suo, R. Avci, M. Deliorman, X. Yang, D. W. Pascual, *Langmuir* **2009**, *25*, 4588-4594; b) S. Liu, A. K. Ng, R. Xu, J. Wei, C. M. Tan, Y. Yang, Y. Chen, *Nanoscale* **2010**, *2*, 2744-2750; c) A. Del Valle, J. Torra, P. Bondia, C. M. Tone, P. Pedraz, V. Vadillo-Rodriguez, C. Flors, *ACS Appl Mater Interfaces* **2020**, *12*, 31235-31241; d) S. L. Arias, J. Devorkin, J. C. Spear, A. Civantos, J. P. Allain, *ACS Appl Bio Mater* **2020**, *3*, 7974-7988; e) L. Peng, H. Zhu, H. Wang, Z. Guo, Q. Wu, C. Yang, H. Y. Hu, *Nat Commun* **2023**, *14*, 5734; f) L. Z. Y. Huang, Z. L. Shaw, R. Penman, S. Cheeseman, V. K. Truong, M. J. Higgins, R. A. Caruso, A. Elbourne, *ACS Appl Bio Mater* **2024**, *7*, 344-361.

[4] A. Del Valle, PhD thesis, Universidad Autonoma de Madrid **2020**.

[5] a) H. Hertz, *J. Reine Angew. Math.* **1882**, *92*, 156–171; b) K. L. Johnson, *Contact Mechanics*, Cambridge University Press, **1985**.

[6] R. Y. Siddiquie, K. Sharma, A. Banerjee, A. Agrawal, S. S. Joshi, *J Mech Behav Biomed Mater* **2023**, *145*, 106048.

[7] R. Han, W. Vollmer, J. D. Perry, P. Stoodley, J. Chen, *Nanoscale* **2022**, *14*, 12060-12068.

[8] M. R. Angle, A. Wang, A. Thomas, A. T. Schaefer, N. A. Melosh, *Biophys J* **2014**, *107*, 2091-2100.

[9] L. Liu, S. Chen, X. Zhang, Z. Xue, S. Cui, X. Hua, B. Yang, H. Yan, C. Liu, J. Wang, Z. Zhang, W. Yu, F. Wu, W. Xu, V. P. Lehto, T. Yue, Y. Liu, Y. Yu, T. Wang, J. Wang, *Sci Adv* **2020**, *6*.

[10] V. Vadillo-Rodriguez, J. R. Dutcher, *Soft Matter* **2011**, *7*, 4101-4110.

[11] M. Krieg, G. Fläschner, D. Alsteens, B. M. Gaub, W. H. Roos, G. Wuite, H. E. Gaub, C. Gerber, Y. F. Dufrene, D. Müller, *Nat. Rev. Phys.* **2019**, *1*, 41-57.

[12] S. V. Kontomaris, *Micro and Nanosystems* **2018**, *10*, 11-22.

[13] We take with caution the data reported in reference 3d, as the immobilization strategy used is unlikely to provide enough adherence to endure the force exerted by the AFM tip, potentially leading to artifacts.

[14] a) A. Agarwal, H. L. Senevirathna, S. H. Koo, C. S. L. Wong, T. S. K. Lim, F. C. Ng, F. Anariba, P. Wu, *Sci Rep* **2023**, *13*, 13290; b) S. Pogodin, J. Hasan, V. A. Baulin, H. K. Webb, V. K. Truong, T. H. Phong Nguyen, V. Boshkovikj, C. J. Fluke, G. S. Watson, J. A. Watson, R. J. Crawford, E. P. Ivanova, *Biophys J* **2013**, *104*, 835-840; c) V. K. Truong, N. M. Geeganagamage, V. A. Baulin, J. Vongsvivut, M. J. Tobin, P. Luque, R. J. Crawford, E. P. Ivanova, *Appl Microbiol Biotechnol* **2017**, *101*, 4683-4690.

[15] a) F. P. Beer, E. R. J. Johnston, J. T. DeWolf, *Mechanics of Materials* 7th ed., McGraw-Hill Education, **2014**; b) A. P. Chatterjee, A. Dasgupta, A. N. Chatterjee, *J Theor Biol* **1988**, *135*, 309-321.

[16] a) A. Valiei, N. Lin, G. McKay, D. Nguyen, C. Moraes, R. J. Hill, N. Tufenkji, *ACS Appl Mater Interfaces* **2022**, *14*, 27564-27574; b) Y. Zhao, H. Cheung, Y. Lam, J. Tang, H. Li, Z. Yang, J. H. Xin, *Adv. Funct. Mater.* **2024**, *34*, 2314581.

[17] X. Liu, M. I. Ishak, H. Ma, B. Su, A. H. Nobbs, *Small* **2024**, *20*, e2310149.

[18] a) A. Somasundar, A. Sen, *Small* **2021**, *17*, e2007102; b) M. A. Ramos Docampo, *Adv Biol (Weinh)* **2023**, *7*, e2200308; c) M. A. Ramos Docampo, O. Hovorka, B. Stadler, *Nanoscale* **2024**, *16*, 2432-2443.