Rheology in the Biological Sciences

Alison E. Patteson and Paul A. Janmey

Abstract

Rheology is the science of how materials deform and flow and is a critical aspect of understanding the biomechanical functions of cell and tissue. Historically, scientists have designed simple and cost-effective instruments for assessing the mechanical properties of biological materials to inform their functionality. Cells and tissue are heterogeneous and possess complex mechanical properties. Yet, simple instruments such as falling ball viscometers and torsion pendulums, can often accurately capture and measure different aspects of how biological materials deform that are relevant to physiological conditions. Here we review the application of simple, home-built instruments suitable for probing the viscoelastic properties of biological materials, underscoring the importance of creativity and innovation of experimental tool design in the field of biomechanics.

1. Introduction

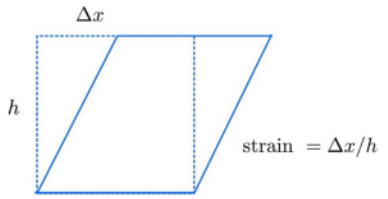
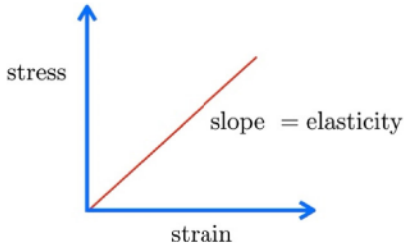
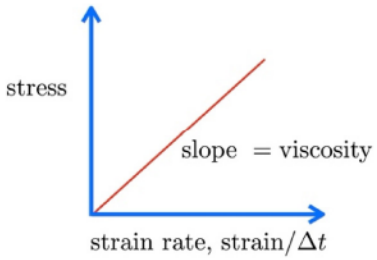
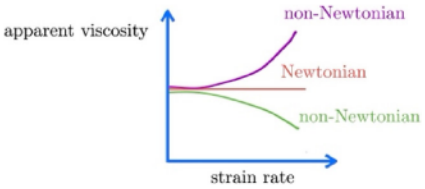
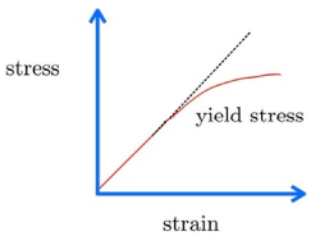
Some form of rheology has been used in biomedical research for centuries, dating back at least to the Greek physicians who diagnosed cancer partly by the appearance of abnormally stiff tissues. The term rheology stems from the Greek 'rheo' meaning to flow. During the Scientific Revolution, Robert Hooke formalized the concept of elasticity in 1678 to link the force applied to an elastic medium and its deformation, and Isaac Newton developed the Law of Viscosity in 1687 to describe the response of an incompressible fluid to shear stress. Most biological materials have complex material properties that are not fully captured by a single elasticity constant or viscosity and can be described as viscoelastic materials that behave in part like solids and in part like fluids in response to external forces (Table 1). Some of the first measurements of non-Newtonian viscosity, in which the viscosity is not a constant but depends on shear rate, were done by centrifuging metal particles through the cytoplasm of plant cells, and perhaps the earliest modern day microrheology instrument, consisting of an electromagnet driving a magnetic particle in a soft material visualized on a microscope slide, was used to quantify the viscoelastic properties of the cell interior more than 100 years ago (6). Much of what is known about the rheology of soft tissues, cells, and biopolymer gels was first measured using simple lab-built instruments before the development and widespread accessibility of commercial rotational rheometers in the 1970's (7), which were based on the earlier Weissenberg rheogoniometer (8). As commercial rheometers became more accurate and more sophisticated, enabling viscoelastic measurements at extremely small strains and over frequency ranges of many decades, they also became somewhat less accessible to the researchers and laboratories interested in the viscoelastic properties of biological materials, and especially in the changes that occur in these materials when they become diseased or when they are subjected the stresses and deformations that caused damage.

The heterogeneous structure of biological tissues, the large strains that occur during physiologically relevant deformations of soft tissues, and the limited temperature range over which biological materials function renders some of the sophistication of modern rheological instruments less useful for their characterization than they are in the characterization of stiffer, more crystalline, or otherwise more homogeneous and ordered materials that are more commonly studied by rheological instruments. Non-traditional uses of commercial rheometers that perhaps compromise some of the precision of the instruments but allow for multiaxial deformations of soft materials that more closely mimic the physiological context have recently been revied elsewhere (9). In this article, we discuss some of the creative uses of simple built instruments that probe the biologically relevant aspects of biomaterials, with an emphasis on those that can be built with minimal expense (Fig. 1). We focus on methods for macroscopic rheology, and a comparison of methods for microrheology of single cells is available elsewhere (10). We also note here that there are challenges associated with experimentally measuring material properties. These challenges have been described elsewhere, and we refer the reader to the review by Ewoldt, Johnston, and Caretta for reference (9).

2. Applications of rheology to biological samples

The cytoplasm of nearly all cells can transform between liquid-like and solid, gel-like phases (11-13), and these mechanical features have been recognized for nearly a century (6), before even the proteins responsible for gelation of the cell were identified. The cytoskeleton gives cells its dynamic architecture and is primarily responsible for the mechanical properties of cells (14) (Fig. 2). When proteins such as actin and myosin that form filaments in muscle cells were also found in non-muscle cells (15, 16), and the other cytoskeletal filaments, microtubules, and intermediate filaments, were identified and purified, a large number of studies using methods of soft matter physics began to characterize the conditions under which gelation occurred, and to quantify

TABLE 1. Common material properties and terms to describe biological materials. For full details, we recommend (36).

Mechanical properties and terms	Description	Schematic
Shear strain	Commonly defined as relative displacements between parallel planes of a material	
Shear stress	Force applied over a material area to deform material; Units, Pascals (Pa)	
Elasticity/Young's moduli/ Elastic modulus	In simple Hookean solids, stress is linear with deformation (strain). If deformation is in shear, the relevant quantity is the shear modulus G. If deformation is elongational, the quantity is the Young's modulus E.	
Viscosity	In simple Newtonian fluids, shear stress is linear with the rate of shear deformation. Viscosity is the proportionality constant.	
Viscoelastic material	Material whose shear stress depends on both shear deformation and shear deformation rates. Silly putty is a classic example. At short times, it holds shape like a solid but will flow over long time periods under the force of gravity (image).	
Non-Newtonian Viscosity	Viscosity that is not constant, but varies with shear-rate.	
Yield Stress	Stress at which a material deforms plastically, which means material cannot return to its original state when stress is removed	

the mechanical strength of the materials formed by cytoskeletal polymers. Nearly all of the fundamental aspects of gelation by cytoskeletal polymers were first discovered using simple equipment, such as falling ball viscometers, torsion pendula, hydrostatic pressure devices, or gravity-based indentation devices. Only later were commercial rheometers used

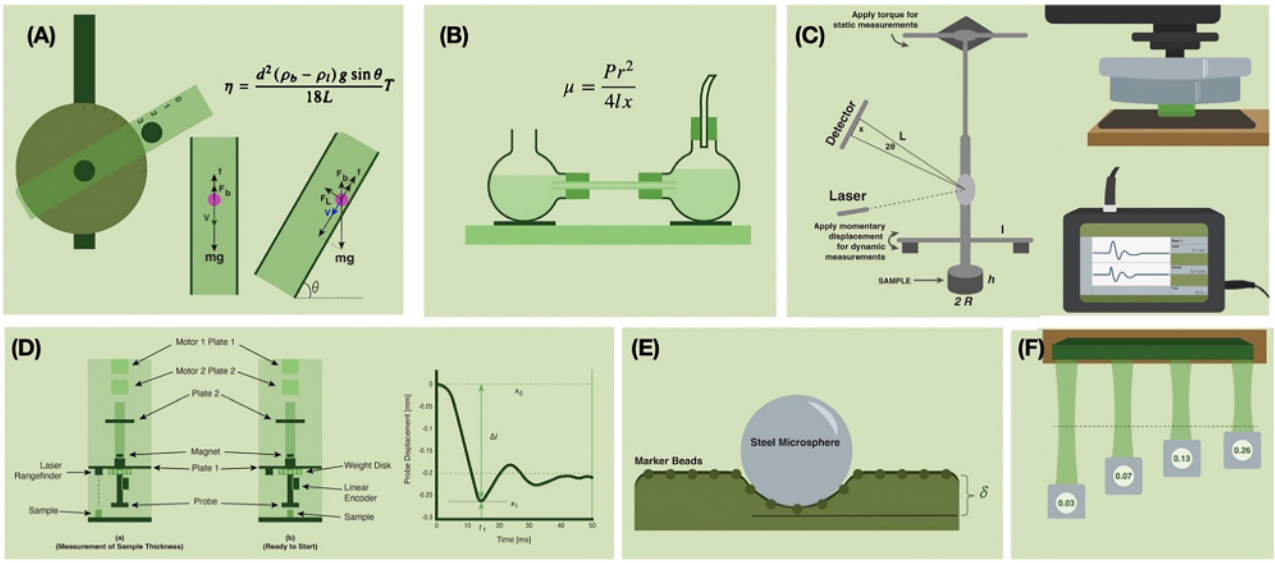
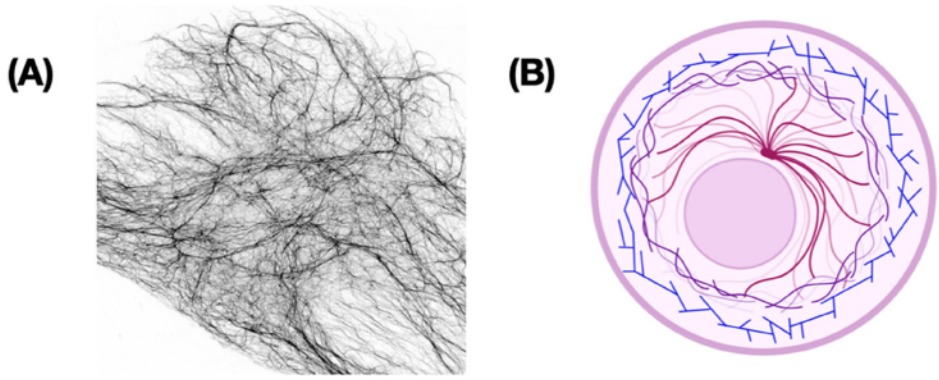


FIGURE 1. Basic instruments for quantifying the mechanical properties of biological materials. The designs include: (A) Falling ball viscometer; (B) Pressure-controlled devices; (C) Torsion rheometers; (D) Uniaxial-impact torsion devices; (E) Surface indentation by gravity of a sphere on a soft surface; and (F) Uniaxial stretching of gels with different amounts of crosslinker.

to study actin- microtubule- and intermediate filament- networks. Today, there are multiple non-invasive diagnostic devices in use to detect stiff tissues as an early reporter of disease, such as the use of ultrasound and magnetic resonance elastography to characterize the development of cancer (17).

3. Falling ball viscometers

Pioneering studies of actin filament rheology were done using newly developed instruments that could measure viscoelasticity of the soft fragile gels they formed (18, 19). One challenge of this method is that it required several milliliters of sample, and other methods were developed to minimize the material



Microtubules

Stiffest cytoskeletal polymer (e.g. highest persistence length, 1 mm)

Susceptible to buckling

Tracks for organelles and maintains cell polarization

Filamentous (F)-actin

Intermediate persistence length (10 μm)

Interacts with myosin motors to generate cellular forces

Networks modulated by many cross linking proteins and biochemical signals

Intermediate filaments

Softest cytoskeletal polymer (e.g. smallest persistence length, 1 μm)

Significantly stiffens at high strain

Protects the cytoskeleton and nucleus from damage

FIGURE 2. The cytoskeleton of the mammalian cell, which strongly impacts the mechanical properties of the cell. The main cytoskeletal polymers are F-actin, microtubules, and intermediate filaments, which each have distinct mechanical properties and persistence lengths that reflect polymer stiffness. (A) Confocal images of mouse embryonic fibroblast, labeled for intermediate filaments. (B) Schematic diagram of cytoskeletal properties and their primary functions. Several crosslinking molecules and molecular motors act to strengthen the cytoskeleton and allow it to generate force. Schematic created with Biorender.

needed. These provided quantitative assessments of viscosity, as well as the yield stress of biopolymer solutions and gels. The most employed method involves falling ball viscometry, in which a dense metal particle is placed on top of a sample held within a thin capillary tube and then released to drop through the sample under the force of gravity (20) (**Figure 1**A). The stress that the particle imposes on the sample is easily calculable from the mass and size of the particle and the gravitational field, and the rate of fluid flow around the particle can be measured based on the time it takes the particle to drop from a given distance. The stress imposed by the bead can be lessened by tilting the sample to acute angles, in which case the ball rolls rather than falls, enabling detection of gels with very small yield stresses. Once the falling ball reaches a steady terminal velocity, which for nearly all applications occurs very soon after its release, the viscosity η is calculated from the expression

$$\eta = \frac{d^2\ (\rho_b - \rho_l) g \sin\theta}{18L} T$$

where d is the diameter of the ball, ρ_b and ρ_l are the densities of the ball and liquid, respectively, g is the acceleration of gravity, θ is the angle of the capillary with respect to the gravitational field, and T is the time for the ball to drop a distance L.

This method was first developed for simple Newtonian fluids, and its application to non-Newtonian liquids as well as gelling materials is fraught with potential artifacts due to the complicated strain fields around the falling ball and possible interactions with the capillary wall. Nevertheless, the method has been useful for characterizing conditions under which cytoskeletal proteins gel and for identifying factors that alter this gelation. **Figure 3** shows how falling ball viscometry was used to detect the conditions under which neurofilaments form viscoelastic networks and how the combined effects of actin filament severing and cross-linking proteins control the conditions under which actin filaments would form an elastic network. **Figure 3**A shows that the time course of gelation of a neurofilament suspension depends on the ionic conditions of the solvent and the effect of divalent cations that crosslink the filaments. **Figure 3**A also shows that neurofilaments are more efficient in network formation than are the intermediate filaments GFAP, derived from glial cells, rather than neurons. **Figure 3**B shows that a viscous suspension of actin filaments transforms into a gel as it is titrated with increasing amounts of the crosslinking protein filamin C. When the filament length is decreased by addition of the active-filament-severing and crosslinking protein villin, more crosslinker is needed to transform the shorter filaments into a three-dimensional elastic network. These studies and many like them were among the first to show how actin binding proteins and other cytoskeletal factors alter the conditions under which elastic networks formed. These networks could be formed of a single filament type, or in some cases showed that two different cytoskeletal filaments, such as microtubules and neurofilaments, could act together to link each other into a composite network that was not formed by either filament system alone (21, 22).

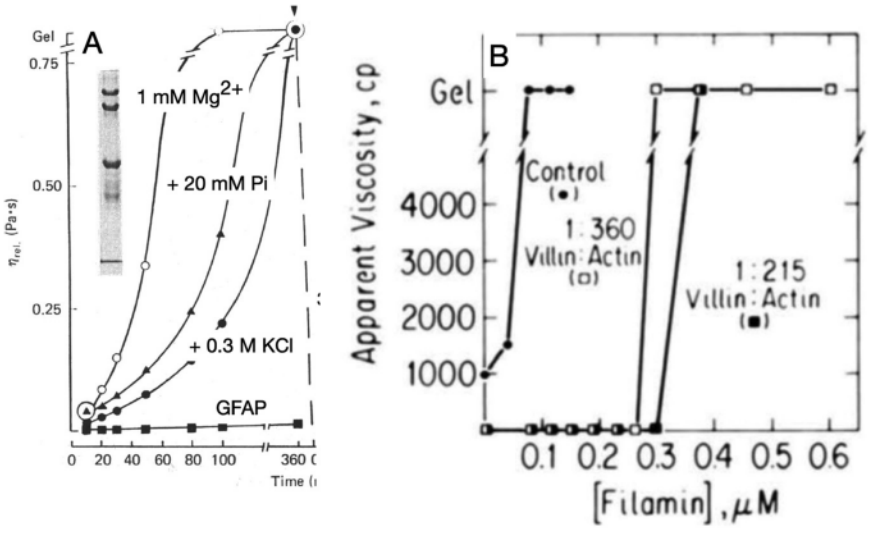


FIGURE 3. Gelation of neurofilaments and actin filaments measured by falling ball viscometry. A: Purified neurofilaments were incubated in solvents containing different ionic conditions, and the resulting increase in viscosity, leading to formation of a gel monitored as a function of time. Glial fibrillary acidic protein (GFAP), in contrast to neurofilaments, did not gel under these conditions. B: Actin filaments are incubated with the cross linking protein filamin C and the actin filament severing protein villin at different molar ratios. Decreasing the actin filament length by villin increased the amount of cross linking protein required to form a gel. Reproduced with permission from (3, 4).

4. Pressure devices

One of the first studies of elasticity in actin-based crosslinked networks used purified actin and actin crosslinking proteins to create either viscous liquids or elastic gels, with a finite yield stress. These studies used a device developed in the 1920s (23) (**Figure 1**B) in which a sample is placed within a capillary connected between a chamber that can be pressurized by pushing a piston into liquid in contact with the gel and a chamber in equilibrium with the atmospheric. The yield stress of the gel is detected by monitoring displacements of particles within it and relating it to the hydrostatic pressure imposed (24). The modulus of rigidity μ at which the gel yields is given by the expression $\mu = Pr^2/4lx$ where P is the pressure at when the gel yields, r and l are the radius and length of the capillary, and x is the distance that a fiducial marker within the gel moves just at the yield point.

These measurements allow an accurate estimate of the yield stress of the network, but do not reliably measure its elastic modulus, since the determination of x is likely to be variable between different samples. However, the device is highly sensitive to proteins that introduce crosslinks between actin filaments and to changes in solution conditions that alter actin gelation. One early study showed that a non-muscle actin crosslinking protein, now called filamin A, was more efficient in forming actin gels than a similar protein purified from muscle, now called filamin C, which was in

turn more efficient than gelation by the motor protein myosin (24).

5. Torsion pendulums

While falling ball viscometers can measure the yield stress of gels, the elastic moduli of materials are more easily measured by torsion pendulums (Fig. 1C). By changing the moment of inertia of the pendulum arm, it is possible to vary the frequency of measurements over approximately two orders of magnitude. This capacity is illustrated in a study of gelatin gels made in solvents with different amounts of glycerol to vary the solvent viscosity and study the ratio of shear storage and loss moduli and gelatin gels as a function of frequency. The practical range of frequencies in the pendulum used in this study was from 1 to 100 radians/s allowing confirmation that the time temperature superposition principle applied also to these biopolymer materials (25).

The design of the torsion pendulum enables both static stresses and an oscillatory strain to be imposed simultaneously. By twisting a calibrated wire that suspends a pendulum a known amount, a constant shear stress can be applied to the sample and the strain resulting from that stress defines a static shear modulus. In addition, a small oscillatory strain can be imposed by deflection of the pendulum arm allowing a low amplitude dynamic measurement to be superimposed on a static stress. In this way the so-called differential shear modulus, a low strain modulus measured on the sample held at a variable and possibly large shear strain could be measured. For well cross-linked samples with minimal shear creep, it is possible to adjust the static stress to impose a specific static strain on top of which the low strain oscillatory measurement could be made.

This capacity is illustrated in early studies of biopolymer networks. Figure 4A shows a typical damped oscillatory displacement caused by a momentary impulse to the pendulum arm that induces free oscillation at a frequency that depends on the moment of inertia of the pendulum, the size of the sample, and its viscoelastic parameters. The shear storage (G') and loss (G'') moduli at a radial frequency w are calculated from the expressions:

$$G'(\omega) = w^2 I/b\,(1 + D^2/4\pi^2) \text{ and}$$
$$G''(\omega) = w^2 I/b\,(D/\pi)$$

where I is the moment of inertia, $b = \pi R^2/2h$ is the form factor of the disc-shaped sample with radius R and height h, and D is the logarithmic decrement in oscillation amplitudes = $ln\ (A_n/A_{n+1})$ where A is the amplitude of the nth oscillation.

The impulse to the pendulum arm can be imposed either while the sample is at rest or subjected to a static torsion caused by twisting the wire. Figure 4B shows how the shear storage modulus of a macrophage extract gel, composed mainly of actin filaments and their cross linking proteins, depends on the magnitude of the shear strain. The macrophage gel strongly stiffens as the shear strain increases up to approximately 5%, but then the gel ruptures abruptly at strains above 7%. Such a gel might be difficult to measure by conventional stress controlled rheometers, which without prior knowledge of the elastic modulus expected, initially impose an arbitrary stress to reach a target maximal strain, but that might overshoot the strain, and thereby rupture the network before measurement.

In Figure 5A a crosslinked fibrin gel is deformed to variable shear strains and then the differential shear modulus is measured, illustrating the strong shear strain stiffening effect of fibrin. The pendulum allows the constant strain to be reversed, to measure possible hysteresis in the sample. For samples that exhibit a finite shear creep within the time needed to do the oscillatory measurement, which is typically a few seconds, a constant strain cannot be imposed by the pendulum, but a constant stress can be imposed, on top of which the differential shear modulus is measured. This capability is shown in a study of neurofilament networks (Fig 5B) which unlike fibrin gels are non-covalently cross-linked rather than covalently bonded and illustrates the even more striking sheer strain dependence of the shear modulus of neurofilament networks, and their ability to resist large stresses. Measurements of differential shear modulus at constant stresses are advantageous because the oscillatory measurements are within their linear range, but imposed on a highly deformed sample, and provide data that are more compatible with theoretical modeling (26).

A functional torsion-rheometer can be built at home for a minimal cost. The first torsion-rheometers were designed with a plate hanging from a wire, which offered minimal friction when applying torque to a sample (see Fig. 1C). The oscillations

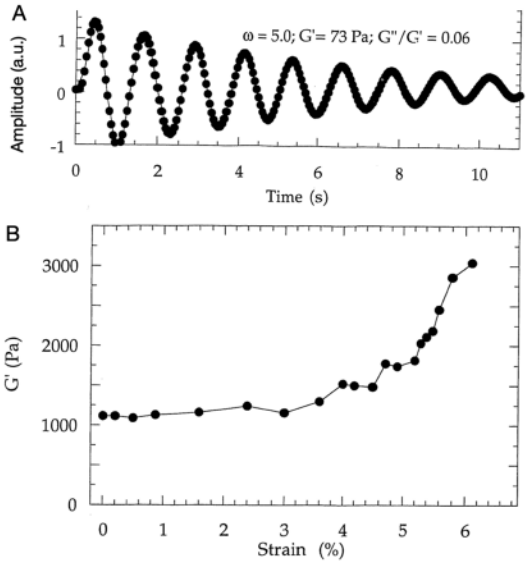


FIGURE 4. Measurements of viscoelasticity using a torsion pendulum. A: The free damped oscillation of a polymerized actin sample after a momentary imposition of < 2 % strain. The storage modulus is calculated from the frequency and the loss modulus by the decay of successive peak amplitudes. B: The shear storage modulus of a macrophage extract at increasing levels of static strain above which a small oscillatory strain is imposed. Reproduced with permission from (5).

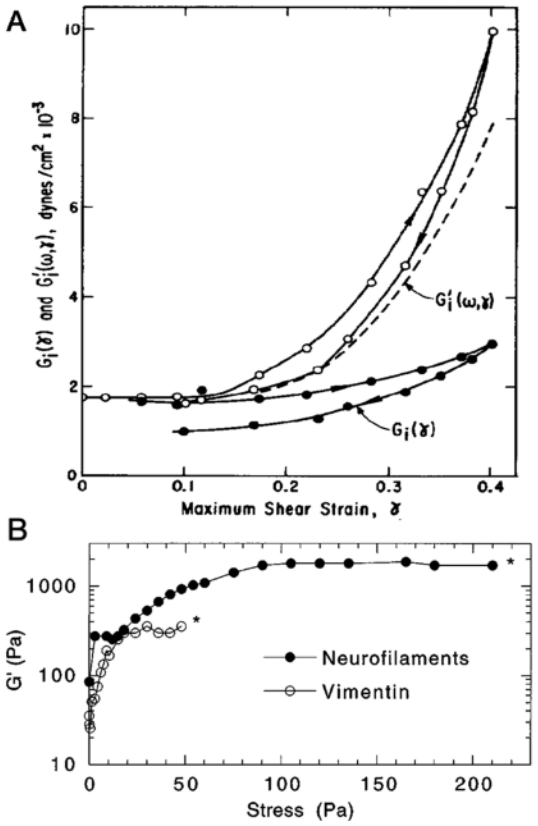


FIGURE 5. Differential shear moduli of fibrin and neurofilament gels at increasing shear strain amplitudes. A: A covalently cross-linked fibrin gel was deformed by static torsion in a pendulum to a constant strain, and a small oscillatory deformation was superimposed by a small impulse on the pendulum arm. B: Differential shear modulus of non-covalently cross-linked neurofilament gels at increasing levels of sheer stress. Reproduced with permission from (1, 2)

of the plate can be measured precisely using a mirror, laser, and a photodetector. An alternative design was recently described, which required fewer experimental components. Namely, this 'pocket rheometer' requires essentially only an angular sensor to be coupled to the plate that applies torsion to the sample (27). The accuracy of this design depends on the accuracy of the sensor and friction between the plate and the sensor, but a range of different frequencies and strain amplitudes can be applied to measure the elastic and loss moduli of different gels (such as agar and phantom tissue constructs) with minimal equipment.

6. Uniaxial-impact torsion devices

The same ideas on which the torsion pendulum is based can also be used to construct an instrument to measure the dynamic viscoelastic repose after a pulse of uniaxial deformation (28). Figure 1D shows a device in which a weight is rapidly dropped onto the surface of a soft material, in this example the flesh of an apple. The apparent Young's modulus and viscous loss are calculated from the known mass of the probe, the amplitude of indentation, and decay of the displacement amplitude. The electronics of this device allows very rapid detection of motion, allowing measurement of relatively stiff samples (>MPa).

7. Surface indentation by spheres

A versatile and perhaps even simpler method to measure the elastic modulus of the surface of soft biomaterials involves placing a dense metal sphere with radii in the range of a millimeter onto the surface of a flat sample and then measuring the indentation of the surface by conventional microscopy (Figure 1E). The density difference between the metal probe and the biological sample, which typically has a density very close to that of water, imposes a stress due to the force of gravity on the bead that can range from $O(10)$ μN to $O(100)$ μN depending on the radius of the bead and the density of material from which it is made. The diameters of easily available commercial spheres range from 400 μm to 1 mm (29). The resulting surface stresses imposed by these spherical indenters are conveniently in a range that can put significant deformation but generally not break hydrogels of biological tissues which have Young's moduli in the range of $O(10)$ - $O(100)$ Pa. Some advantages of this method are the lack of need to calibrate force sensors, since the stress is accurately defined by the size and density of the particle, and the resulting strain can be accurately measured by conventional fluorescence microscopy because the typical displacement underneath the millimeter sized bead is in the range of a few 100 microns and easily be measured accurately by refocusing on the maximally displaced markers (30-32). Multiple millimeter or sub millimeter beads can be placed on a single sample, and simultaneously the local stiffness can be measured over a wide area.

In the simplest case, the Young's modulus of the sample E is calculated from the expression

$$E = \frac{3\ 1-\nu^2 F}{4R^{0.5}\ \ 1.5}$$

where ν is the Poisson's ratio of the sample (commonly near 0.5 for incompressible tissues, but often variable for hydrogels) F is the force applied by a bead of radius R and δ is the distance of maximal indentation.

Some disadvantages of the method are that calculation of an apparent Young's modulus from the displacement requires the use of a Hertz relation for which surface adhesive forces and Poisson ratios need to be measured or assumed (33) and the thickness of the sample needs to be accounted for in the calculation of elastic modulus (32). However similar issues apply to any measurement of surface elasticity using an atomic force microscope or other indenting probe, and the formalisms for interpreting force indentation curves by these methods are very well developed. An excellent summary of

the use of bead indentation for biomaterial rheology is available (31).

8. Uniaxial stretching

Perhaps the simplest measure of elastic modulus, suitable for structures such as tissues or stiff hydrogels that can be gripped at both ends, is to measure their elongation when forces are applied by hanging weights at one end. This method is analogous to laboratory demonstrations illustrating Hooke's law, in which increasing masses lead to increasing elongation of a spring from which a spring constant can be derived. The utility of this simple method is demonstrated by its use to measure the Young's modulus of polyacrylamide gel substrates (**Figure 1F**) in the landmark study that demonstrated how important substrate stiffness is to cell morphology and motility (34).

9. Conclusions and Perspectives

In this article, we have described many different types of creative and home-built designs to measure mechanical properties of biological materials. We covered simple early devices, such as falling ball viscometers and torsion pendulums that have helped us better understand the viscoelastic behavior of soft tissues, cells, and biopolymer gels. Even though commercial rheometers are more sophisticated and advanced, the complexity and heterogeneity of biological materials often require more adaptable and accessible methods. We are inspired by the do-it-yourself (DIY) culture and the possibility of new creative designs. DIY-design, brought to the attention of public in particular during the shelter-in-place orders of COVID-19 have generated new techniques, as recently described by Hossain and Ewoldt (35). These methods not only offer a practical alternative for researchers with limited resources but also encourage creativity and innovation in experimental design.

Authors

Paul Janmey is Professor of Physiology at University of Pennsylvania. Paul received is A.B. in Chemistry and Philosophy at Oberlin College in 1976 and received a Ph.D. in Physical Chemistry at University of Wisconsin, 1982. His lab studies several aspects of cell mechanics, including how cells sense and respond to the stiffness of soft materials (usually hydrogels linked with cell adhesion proteins), mechanical properties of cytoskeletal polymers, and how cell membrane structure mediated by inositol phospholipids lead to production of signals that remodel the cytoskeleton.

Alison E. Patteson is an Associate Professor of Physics at Syracuse University. Her research focuses on the soft matter physics of cell motility and biofilm development. Alison is a native of Lancaster County, PA and received her B.S. in Physics and Mathematics from Kutztown University in 2011. Her Ph.D. was in Mechanical Engineering at the University of Pennsylvania (UPenn), studying active complex fluids and bacteria motility with Prof. Paulo Arratia from 2011-2016. Her postdoctoral research was with Prof. Paul Janmey at UPenn on the vimentin cytoskeleton and confined cell motility (2016-2019). Alison took a faculty position in 2019 at Syracuse University. She is a recipient of the APS Dissertation Award in Statistical and Non-linear Physics (2018), NIH Outstanding Investigator Award (2021), and APS Maria Goeppert Mayer Award (2024). Alison is also a Cottrell Scholar (2023) and Sloan Research Fellow (2023). She loves mechanobiology, working with students, and college basketball.

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