

Blowing bubbles to study living material

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Cavitation rheology measures the pressure at which a bubble blown in soft material suddenly expands rapidly. It is gentle enough to allow stiffness measurements of biological materials in vivo.

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Living materials respond to stresses, or deformation forces, in profound and surprising ways. Bones become weak if they don't carry weight. Muscles and soft tissues atrophy, or shrink, if exercise is limited. Whether stem cells differentiate into hard or soft tissue can strongly depend on the stresses they experience during growth. Therefore, to promote or direct the growth of healthy tissue—the challenge of tissue engineering—or to limit the growth of unwanted tissue such as cancerous tumors, scientists must understand the stresses that biological materials experience as they grow and live.

Environment and scale matter

To learn about effects of stress in materials, researchers explore how a material responds to being stretched or compressed. That response, in both living and inanimate materials, is typically characterized by a property called the elastic modulus, E , which describes the stiffness of a material. Wiggle gelatin desserts have a low elastic modulus, whereas steel has a high elastic modulus. But how does one obtain the quantity, particularly for a living material?

The question presents two key challenges. First, mechanical properties such as the elastic modulus are usually determined by measuring the amount of force per unit area needed to stretch or compress a given material. That task is not easy for a tissue or cell that exists as part of a living being. In reality, what is known about the mechanical properties of living tissues has been deduced from experiments conducted on nonliving specimens extracted from their living host. We know that when tissues are removed from their native state their mechanical properties change, but we know little of tissues' *in vivo* properties.

The second challenge is related to length scales. Tissues are beautiful examples of hierarchical materials, in which structures and geometries at various scales correspond to a number of different properties and functions. To understand the connection between hierarchy and elastic properties, imagine that you are living in a cell. Your environment is a complex arrangement of nucleic acids, biopolymers, proteins, fatty acids, and other components, assembled in organelles that have specific functions. At the microscopic cellular length scale, if you push on some parts, you will feel the stretchiness of the cell membrane; push on other parts and you will feel the stiffness of structural microtubules. Now imagine that you are larger, say 100 cells across, about the size of a grain of salt. At that size, if you push and pull, you will feel the stiffness resulting from the cells trying to pull against or with each other. Exactly how the components of

all those cells combine to define stiffness depends on the shape and organization of the group of cells. So the elastic modulus of the whole material is not simply derivable from the individual parts. All soft condensed matter is inhomogeneous on some length scale, so averaging over the material gives an incomplete picture. And since living materials are extremely hierarchical, it is important to measure properties at a variety of length scales and locations—not a simple endeavor.

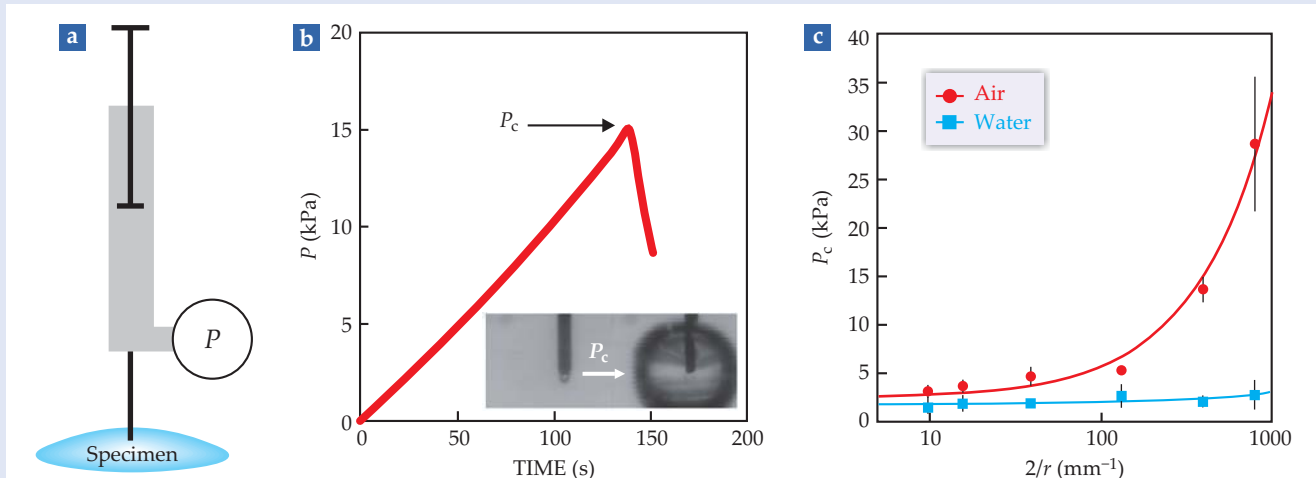
Cavitation rheology is a new experimental method that we and our colleagues have developed for measuring mechanical properties of soft elastic materials, living and synthetic, across a broad size range of 0.1–1000 μm . The technique is in its early stages, in particular concerning measurements of scale dependence, but we, with Jessica Zimmerman, have already made *in situ* measurements of the vitreous humor in bovine eyes.

Bubble growth takes off

Cavitation rheology measures the pressure it takes to blow a bubble or other defect inside a soft material. More precisely, it relies on a phenomenon in which a bubble will suddenly expand once a critical pressure is reached. That jump in size is something that you have experienced when you've tried to inflate a balloon. At first, it's very difficult and you get red in the face, and then, suddenly, the balloon gets larger with increasing ease. The sudden expansion of the balloon is well understood, though the standard result for the balloon's radius assumes that the balloon has a thickness much smaller than its radius. However, integration of the standard result yields the pressure–growth relationship for a small bubble inside a solid with a large volume.

A key difference that distinguishes small bubbles from balloons is that the elasticity of the solid into which the bubble is introduced is not the only factor resisting bubble growth; a second contribution is provided by the surface tension γ between the air in the bubble and the surrounding material. In fact, the critical pressure P_c for sudden growth of a bubble of radius r can be expressed as $P_c \approx E + 2\gamma/r$. A plot of P_c versus $1/r$ for a homogeneous material (for which E is not a function of r) reveals E through its γ -intercept.

In a cavitation rheology experiment, a syringe needle with radius r creates a defect inside a soft material (see panel a of the figure). As the medium inside the syringe is compressed, the pressure climbs at the tip of the needle until the critical pressure is reached. At that point the bubble size jumps and the pressure drops. That the critical event is sig-



The principle of cavitation rheology and some results. (a) In a cavitation rheology experiment, the cavitation medium—air or water—is injected into a synthetic or living material. The pressure at the tip of the syringe is recorded using a pressure sensor. (b) The plot shows pressure versus time for an experiment conducted on a polyvinyl alcohol hydrogel. The pressure at the tip of the needle increases until it reaches a critical pressure P_c , after which the pressure drops off and the bubble expands dramatically, as shown in the inset photo. For the data run photographed, the syringe needle radius r is about 200 μm . (c) Shown here is the critical pressure for cavitation in PVA as a function of $2/r$. When air is used as a cavitation medium, the significant surface tension between air and the hydrogel causes the critical pressure to rise for smaller syringe radii. With water, the critical pressure is nearly independent of radius. The curves represent linear fits through the data, distorted by the logarithmic x-axis; their y-intercept gives the elastic modulus for the hydrogel.

naled by a pressure drop is a key advantage of cavitation rheology because it usually eliminates the need to visualize the bubble growth. Hence measurements can be made in non-transparent materials such as tissues in a living host. Occasionally, though, it is useful or even necessary to track the bubble growth.

Another nice feature of cavitation rheology is that it can be easily applied to materials that are otherwise difficult to handle. Panel b of the figure shows experimental results for a polyvinyl alcohol hydrogel, one of several synthetic soft materials that we and coworkers used in initial attempts to characterize soft materials. The plot shows an initial rise in pressure and the sudden drop at P_c . Within a mere 0.1 s after P_c was obtained, the bubble size increased dramatically.

The most suitable length scales for learning about cellular materials and the molecular organization of synthetic materials are small—less than 10 μm . However, as needle radius decreases, the surface tension between air and the material causes the critical pressure to rise significantly. To overcome the challenge of separating surface and elastic contributions to P_c at small length scales, an experimenter can use fluids to induce cavitation. Panel c of the figure, for example, shows experiments on the PVA hydrogel in which water replaced air as the cavitation medium. The critical pressure is lower for the water experiments than for air because of the nearly-zero surface tension between water and PVA, which itself is more than 80% water. Indeed, P_c is almost independent of needle size.

Applications

In our experiments on the vitreous humor in bovine eyes, we measured the elastic moduli for vitreous samples both in the eye and removed from the ocular cavity. As anticipated, the elastic modulus for the vitreous humor in the ocular cavity was different from—in fact, approximately five times as large as—the value determined from extracted samples; the experiment clearly indicates the importance of determining mechanical properties of soft tissues in their native, living environment.

Similar measurements are under way for a wide range of living tissues, including the lens, liver, and brain. What we learn of the tissues' mechanical response could, in the long term, lead to new diagnosis standards, better understanding of some diseases, and new materials that can prevent traumatic damage. On the other hand, some of the knowledge we gain about in situ materials could be used immediately. The engineering of new tissues or tissue replacements and assessment of those materials after implantation are usually based on ex vivo measurements. Techniques capable of determining the mechanical properties of those materials in situ could help tissue engineers improve those synthetic replacements to give a better match to the tissues they are designed to replace.

The mechanical properties of biological materials have long been studied, and it is now clear that they strongly influence the growth and development of cells and tissues over a broad range of length scales. Because those properties change when living material is removed from its host, exploration must focus on quantitative measurements in vivo. Scientists will no doubt develop a number of promising techniques. Cavitation rheology, one step in the right direction, demonstrates that simple, fascinating physics can lead to new knowledge with broad medical applications.

Additional resources

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