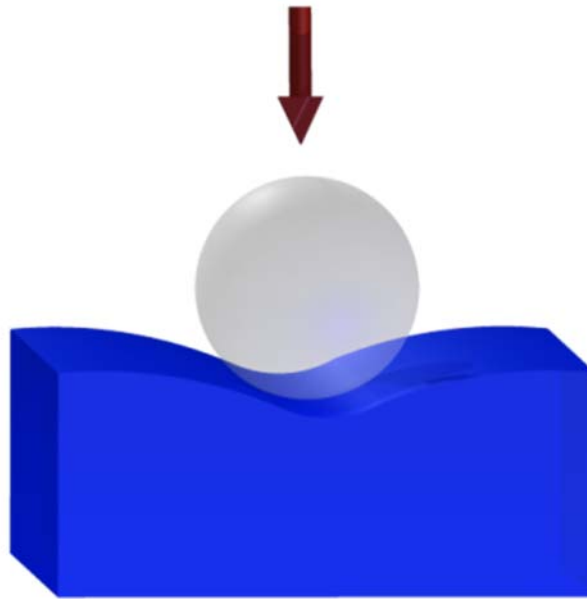




Indenter



Instruction Manual

Institut für Angewandte Physik
AG Prof. Tilman Schäffer
Universität Tübingen

Assistant room number: C9 A38
Experiment room number: C9 P31

Table of Contents

Table of Contents.....	2
I. Preparation.....	2
1. Introduction	3
2. Solid Mechanics	3
3. Elasticity, Elastic-plasticity and Viscoelasticity	6
4. Contact Mechanics.....	9
5. Laboratory.....	11
A. Tensile Test	11
B. Compression Test.....	12
C. Indentation Test.....	12
D. Fabrication of PDMS Sylgard 184 and Sylgard 527 Samples.....	13
6. Protocol.....	14
References	15

I. Preparation

In order to conduct this experiment, knowledge of the following topics should be acquired by reading this instruction manual:

- o Principles of mechanical testing methods
- o Interpretation of stress-strain curves
- o Elasticity, plasticity and viscoelasticity
- o Dynamic mechanical analysis
- o Relationship between force and indentation for different indenter geometries

You must be able to answer the following questions before the experiment in order to pass the lab day:

- How can mechanical properties of a material be measured?
- What is the definition of the elastic modulus?
- What is the “yield point”?
- What is the characteristic of plastic deformation?
- What is the difference between elasticity and viscoelasticity? How does one measure viscoelastic properties?
- What is the difference between spherical, conical and cylindrical indentation?

1. Introduction

From the perspective of biomedical engineering, the human body consists of different types of tissue, which strongly vary in their material and structural composition. Tissue is generally composed of three basic elements: cells, fibers and intercellular substance. The proportion and arrangement of these elements are characteristic for each tissue and determine its mechanical properties [1]. Tissue covers a wide range of mechanical properties. For example areolar tissue with abundant intercellular substance and a loosely arranged network of sparse fibers exhibit a more viscous behavior [2]. In contrast skeletal muscle tissue, arranged in strong bundles of fibers, can resist large deformations and is also able to generate active forces by contraction [3]. Bone tissue mainly serves as a structural supportive framework and has a rigid material consistency [4].

Detailed knowledge of mechanical properties of biological tissue is of great interest in many regards. It has been noted that alteration of mechanical properties are correlated with the pathological state of several human diseases like cancer and, therefore, could serve as a biomarker to develop new diagnostic tools [5-8]. Also several cellular processes like adhesion [9], migration [10], proliferation [11] and differentiation [12] are affected by the surrounding mechanical microenvironment. This is an important factor, which must be taken into account in the development of new biomaterials for the use as an implanted device [13].

This practical experiment should give you a fundamental understanding of mechanical properties of a material and how they could be determined by standard engineering methods.

2. Solid Mechanics

The mechanical properties of a material are characterized by its response to an applied load. To determine these properties various experimental methods exist, which differ in the type of applied load (Figure 1). In all these testing methods the loading force causes deformation of the sample.

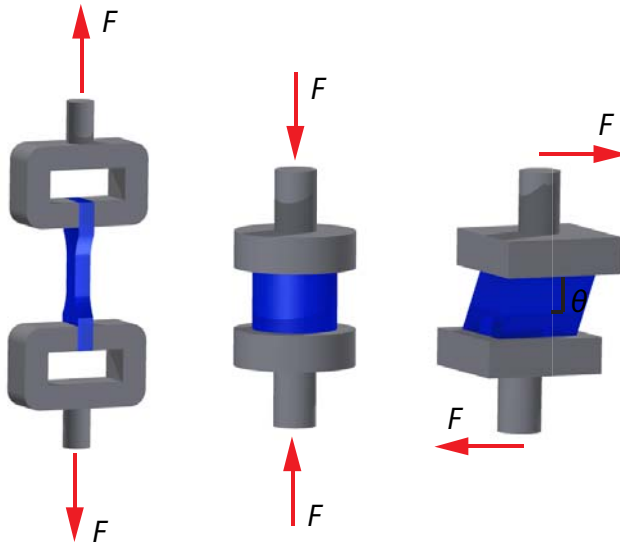


Figure 1. Testing methods to obtain mechanical properties of a material. The sample is subjected to an external load in form of tension (left), compression (middle), or shear (right panel), leading to deformation of the sample.

To illustrate the relationship between load and deformation, Figure 2A shows a simple configuration, where a tensile force is applied to one end of a bar, which is fixed at the opposite side. Thereby the force is assumed to be evenly distributed over the cross-sectional area of the bar. The tensile stress σ is then defined as the ratio of applied force F to cross-sectional area A of the sample: $\sigma = F/A$. The tensile strain ε is the relative displacement ΔL with respect to the undeformed sample's length L_0 : $\varepsilon = \Delta L/L_0$. The relationship between stress and strain is typically plotted in a so called “stress-strain curve” and is characteristic for a material (Figure 2B).

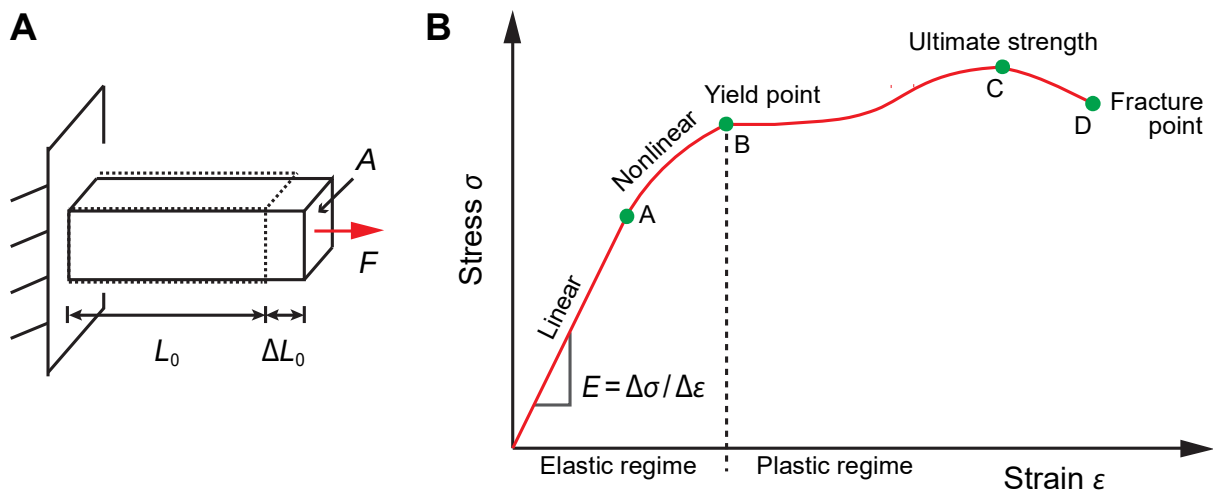


Figure 2. (A) Tension of a solid material and (B) the corresponding stress-strain-curve.

For small deformations the material follows Hooke's law, so the strain is directly proportional to the applied stress. This proportionality can be expressed in form of a parameter denoted as Young's modulus or elastic modulus E :

$$E = \frac{\sigma}{\varepsilon}. \quad (1)$$

In case of an applied shear force the shear modulus G is used and gives the relation between shear stress and shear strain. The material's response to uniform pressure is described by the bulk modulus K , which is defined as the ratio of change in applied pressure to fractional change in the sample's volume. For isotropic materials these three moduli uniquely determine their linear-elastic properties and can be converted into each other.

Beyond a certain strain (Figure 2B, point A) the material deforms nonlinear with increasing stress, but still behaves elastic up to a certain point defined as "yield point" (Figure 2B, Point B). After exceeding this point, the material enters the plastic regime, where the deformation is irreversible and the material retains permanently deformed after unloading. In the case of perfect plasticity there is no increase in stress during deformation, the material flows at constant load. In most materials the stress in the plastic regime increases after a short period with increasing strain, which is known as strain hardening.

The stress increases until the ultimate strength of the material is reached (Figure 2B, point C). At further increase of the strain, most materials form a neck and the stress decreases until ultimately fracture occurs (Figure 2B, point D). During necking the cross-sectional area of the specimen decreases and, therefore, the stress slightly drops.

The shape of the stress-strain curve depends on the structural and chemical composition of the material. The structure of polymers for example strongly depends on the degree of polymerization and on the temperature. Below its glass transition, a polymer exhibits a stress-strain curve (Figure 3), which shows the characteristics of a brittle material. Brittle materials do not undergo plastic deformation and directly fail after they have exceeded the elastic regime. For semi-crystalline polymers the stress abruptly drops beyond the yield point due to the formation of a neck. In the regime of cold drawing the neck extends over the length of the sample with increasing strain and the polymer chains are getting straightened. The polymer chains are now parallel orientated to the load, which causes stiffening of the polymer. This phase of strain hardening continues until the polymer fails. The extension of a material, which deforms plastically without fracture, is often associated with the term of ductility. For temperatures above the glass transition, polymers have the stress-strain characteristics of elastomers, which are amorphous polymers and can be deformed elastically to very large strains.

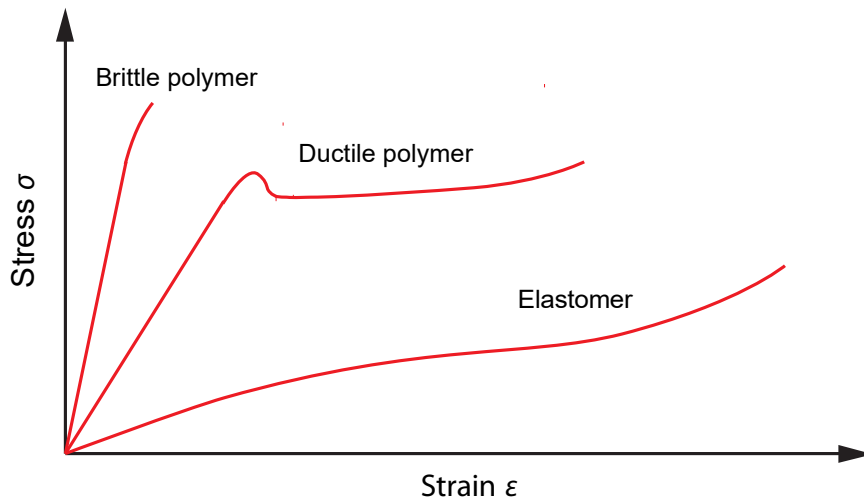


Figure 3. Stress-strain behavior of polymers can be brittle, ductile or elastomeric.

3. Elasticity, Elastic-plasticity and Viscoelasticity

In Figure 4 the basic differences between a linear elastic, nonlinear elastic, elastic-plastic and viscoelastic behavior of a material with respect to loading and unloading are demonstrated. Elasticity in general describes the material's ability to recover its original shape and size after the applied load has been removed. In elastic materials, the strain energy is completely stored as potential energy, so that the loading and unloading paths coincide with a linear (Figure 4A) or nonlinear (Figure 4B) shape. In the case of elastic-plasticity only the elastic component of the material recovers, and a permanent deformation remains after the load has been released (Figure 4C). If the elasticity is explicitly time, frequency, and temperature dependent, it is called viscoelasticity. The material has both elastic and viscous characteristics. Generally, viscosity describes the resistance of a fluid to flow. Honey, for example, is more "viscous" than water, because due to the larger amount of internal friction it flows slower than water. The stress in a purely viscous materials is proportional to the strain rate, $\sigma = \eta \dot{\epsilon}$, with the viscosity η .

The stress-strain relationship of a viscoelastic material is a function of the applied strain rate, and exhibits hysteresis as a result of energy dissipation (Figure 4D). The area between the loading and unloading path is the amount of dissipated energy and is greater for smaller strain rates.

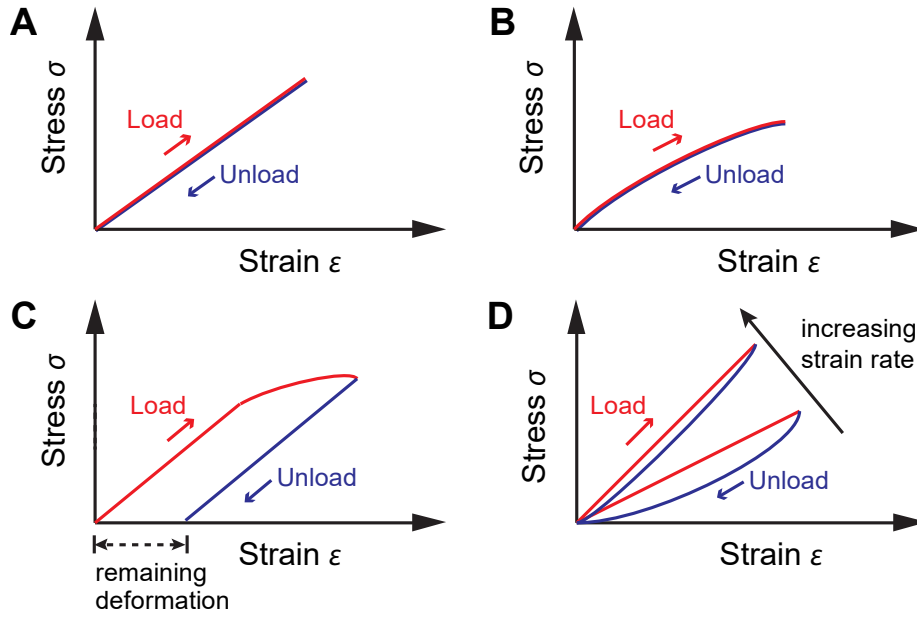


Figure 4. Material's stress-strain behavior due to loading and unloading in the case of (A) linear elasticity, (B) nonlinear elasticity, (C) elastic-plasticity, and (D) viscoelasticity.

There exist different experimental methods to obtain viscoelastic material properties. Two basic static experiments are the creep test and the stress-relaxation test. If a viscoelastic material is subjected to a constant stress σ_0 , the strain $\epsilon(t)$ continuously increases over time (Figure 5A). This time behavior of the strain is known as creep and can be expressed by the creep compliance $(t) = \epsilon(t)/\sigma_0$. In the case of a constant induced strain the stress relaxes over time (Figure 5B). The relationship between stress $\sigma(t)$ and constant strain ϵ_0 is given by the stress relaxation modulus $G(t) = \sigma(t)/\epsilon_0$.

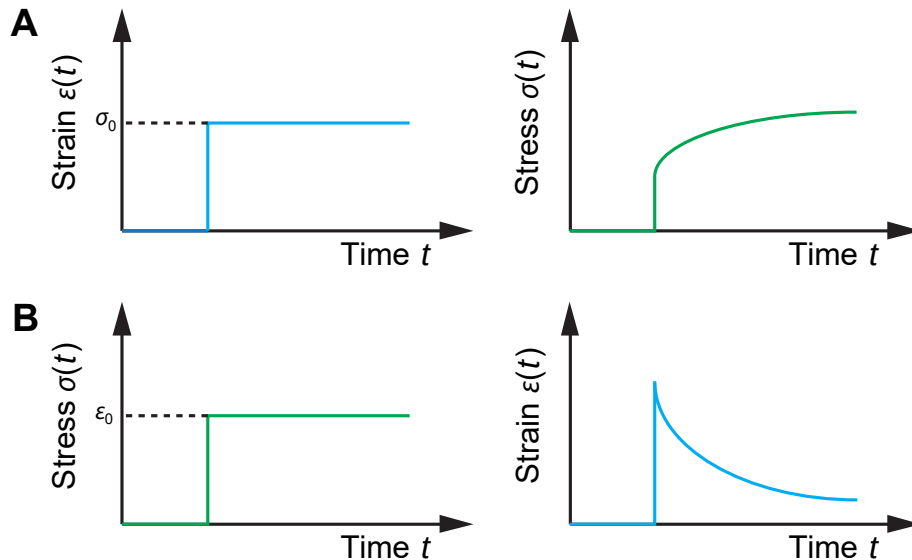


Figure 5. Viscoelastic material behavior. (A) Creep of the strain due to an applied constant stress. (B) Stress relaxation after a constant strain is induced.

A further approach to characterize viscoelastic materials is based on analyzing the material's response to a dynamical excitation (Figure 6). If, for example, a harmonic strain $\epsilon(t) = \epsilon_0 e^{i\omega t}$ with a certain amplitude ϵ_0 and frequency $\omega = 2\pi f$ is induced, the stress response $\sigma(t) =$

$\sigma_0 e^{i(\omega t + \phi)}$ is also harmonic with the same frequency as the strain, but phase-shifted by the phase angle ϕ . For a purely elastic material the phase angle is $\phi = 0$ and for a purely viscous material $\phi = \pi/2$.

The complex dynamic modulus E^* is then defined as

$$E^* = \frac{\sigma(t)}{\varepsilon(t)} = \frac{\sigma_0 e^{i(\omega t + \phi)}}{\varepsilon_0 e^{i\omega t}}. \quad (2)$$

Applying Euler's formula, $e^{i\phi} = \cos \phi + i \sin \phi$, the equation can be transformed into

$$E^* = \frac{\sigma_0}{\varepsilon_0} \cos \phi + i \frac{\sigma_0}{\varepsilon_0} \sin \phi \quad (3)$$

$$\Leftrightarrow E^* = E'(\omega) + iE''(\omega), \quad (4)$$

where $E'(\omega) = \sigma_0/\varepsilon_0 \cdot \cos \phi$ is the storage modulus and $E''(\omega) = \sigma_0/\varepsilon_0 \cdot \sin \phi$ the loss modulus. The storage modulus represents the energy stored, and the loss modulus the energy dissipated during an oscillation cycle. There is a simple relationship between storage and loss modulus, which is expressed in the loss tangent $\tan \phi = E''/E'$.

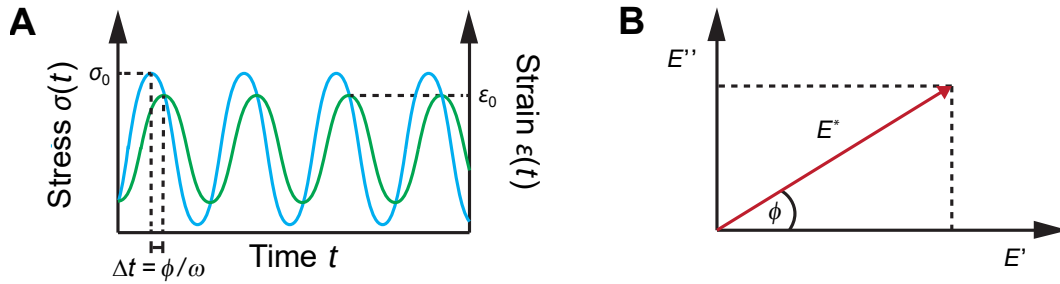


Figure 6. Dynamic behavior of a viscoelastic material due to an applied harmonic strain. (A) Phase shift between the applied strain and the resulting stress. (B) Vector diagram of the complex dynamic modulus E^* . The loss tangent, $\tan \phi$, is the ratio of storage modulus E' to loss modulus E'' .

To describe linear viscoelastic behavior phenomenologically there are various models using a combination of spring and dashpot, which are in the simplest case connected in series (Maxwell model) or in parallel (Kelvin-Voigt model), see Figure 7. The spring represents the elastic component and the dashpot represents the viscous damping component of the system.

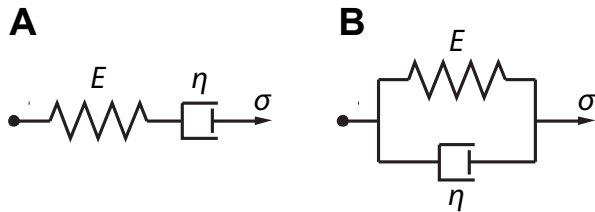


Figure 7. (A) The Maxwell model and (B) the Kelvin-Voigt model.

In more complex models like the standard linear solid model there is a combination of components connected in series and in parallel.

4. Contact Mechanics

Besides the testing methods illustrated in Figure 1, it is also possible to obtain mechanical properties of a material by indentation. In indentation experiments an indenter of defined geometry is approached to a sample's surface and penetrates into the sample, usually until a predefined force is reached. The relationship between force and indentation is described by contact models. Historically, in 1882 the German physicist Heinrich Hertz developed an analytical solution to solve the contact problem between two elastic spheres of radius R_1 and R_2 (Figure 8) [14].

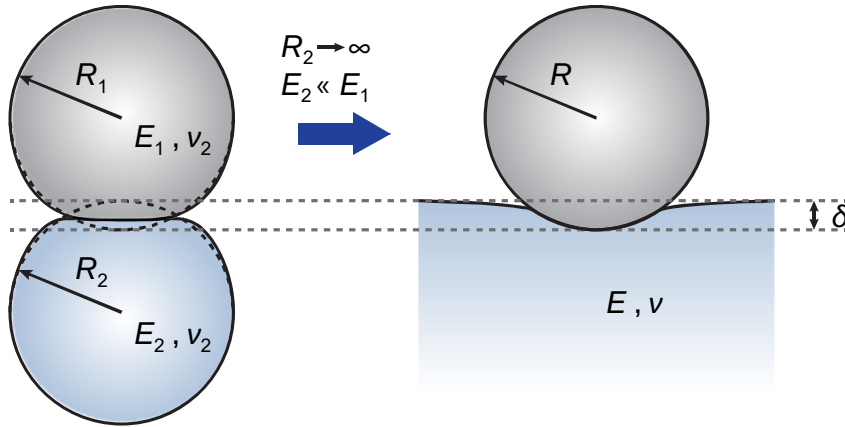


Figure 8. Hertz contact model of two elastic spheres and the transition to spherical indentation.

Based on the assumption that the penetration depth is much smaller than both spheres' radii, the force F between the contact surfaces and the indentation δ is given by:

$$F = \frac{4}{3} E_{\text{eff}} \sqrt{R_{\text{eff}}} \delta^{3/2} \quad (5)$$

with the effective elastic modulus E_{eff} :

$$\frac{1}{E_{\text{eff}}} = \frac{1 - \nu_1^2}{E_1} + \frac{1 - \nu_2^2}{E_2} \quad (6)$$

and the effective radius R_{eff} :

$$\frac{1}{R_{\text{eff}}} = \frac{1}{R_1} + \frac{1}{R_2}, \quad (7)$$

where E_1, E_2 are the elastic moduli and ν_1, ν_2 the Poisson's ratios of the two bodies, respectively. The Poisson's ratio ν describes the relation of transverse strain to axial strain and is a dimensionless material constant. For an incompressible material the Poisson's ratio is $\nu = 0.5$, which is the case for the PDMS polymers investigated here. Setting one sphere radius to infinity and assuming E_1 considerably larger than E_2 , the contact between a rigid sphere and a flat elastic half-space is obtained. Sneddon extended the Hertz model to cylindrical and conical indenter geometry (Figure 9) [15]. The formulas are summarized in the following table:

Tip geometry	Force-indentation relationship
Spherical	$F = \frac{4}{3} \frac{E}{1 - \nu^2} \sqrt{R} \delta^{3/2}$
Conical	$F = \frac{2}{\pi} \frac{E}{1 - \nu^2} \tan \alpha \delta^2$
Cylindrical	$F = 2 \frac{E}{1 - \nu^2} R \delta$

Table 1. Relationship between force and indentation for various tip geometries based on Sneddon's modified Hertz model. Here, E is the elastic modulus and ν the Poisson's ratio of the half space, R the radius of the sphere or the cylinder, and α the half cone angle of the indenter (see Figure 9).

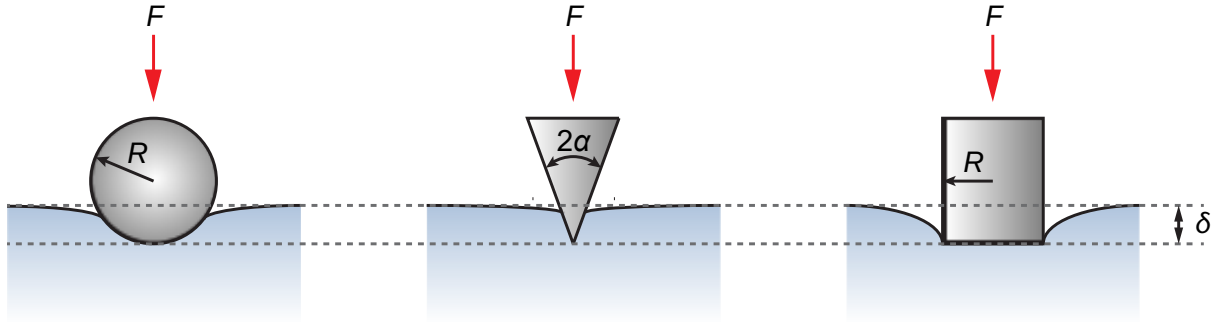


Figure 9. Indentation of a soft sample by a spherical, conical, and cylindrical indenter.

The experimental setup for indentation tests is typically built up of a force transducer attached on a displacement device, which enables the approach to the sample's surface. At the force transducer an indenter of defined geometry is mounted. The force transducer can be seen as a Hookean spring, so that the force on the indenter tip is given by

$$F = k \Delta d, \quad (8)$$

where Δd is the relative deflection and k the spring constant of the force transducer. The indentation δ of the sample is then

$$\delta = \Delta z - \Delta d \quad (9)$$

with the relative z -displacement Δz . In the case of a very soft sample compared to the spring constant of the force transducer, this simplifies to $\delta \approx \Delta z$. Recording force and displacement while approaching the indenter towards the sample will result in a so-called force-distance curve. One approach to determine the elastic modulus E from the force-distance curve is to transform the equations in Table 1 so that the force-indentation relationship becomes linear. For example, in the case of a spherical indenter, Equation (5) becomes

$$F^{2/3} = \left(\frac{4}{3} \frac{E \sqrt{R}}{1 - \nu^2} \right)^{2/3} \delta. \quad (10)$$

This can be expressed as

$$F^{2/3} = a \cdot \delta, \quad (11)$$

where a is the slope. If the model is fit to the data, plotting the force-distance curve in form of Equation (11) should give a linear correlation between force and indentation so that the slope can be determined by a linear least square fit.

5. Laboratory

The measurements will be performed with a commercial testing machine Zwicki Line 500 (Roell GmbH, Ulm). The maximal load of the force transducer is 5 N. The force transducer has a connection pin where a gripper, a compression plate, and various indenters can be attached. Be careful when you exchange these tools and don't overload the force transducer. In the configuration of the machine you can setup the sampling rate, integration time of the displacement device and the force transducer. The optimal settings are indicated for each test.

It is recommended to prepare the samples for the different measurements all together at the beginning of the experiment.

A. Tensile Test

The specimen for a uniaxial tensile test has typically the standardized dog-bone-like shape (Figure 10) with either a circular or rectangular cross-section. The gage length L_0 marks the part of the specimen where the elongation is measured. The cross-section of the ends (called shoulders) is typically larger than of the gage section to enable a rigid grip.

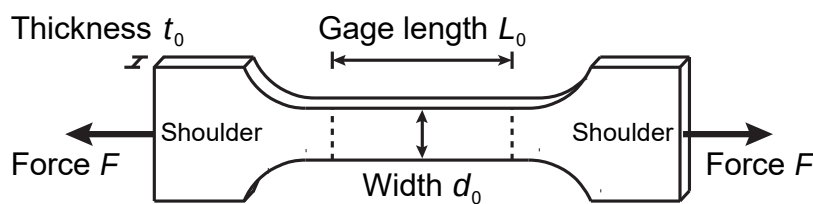


Figure 10. Tensile specimen with a rectangular cross-section of $A_0 = d_0 \cdot t_0$.

- Prepare tensile specimens out of polydimethylsiloxane (PDMS) with widths of $d_0 = 1 \text{ mm}$, 2 mm , and 4 mm . The gage length is $L_0 = 14.3 \text{ mm}$ in all cases. PDMS is a silicone polymer and widely used as material for medical applications including implant devices because of its good biocompatibility. The fabrication of the PDMS samples is described in detail below (section D. Fabrication of PDMS Sylgard 184 and Sylgard 527 Samples).
- Perform a tensile test by recording the force and displacement during elongation. Use following setting:

Sampling rate	50 Hz
Integration time (force)	80 ms
Integration time (z-displacement)	20 ms
z-velocity	30 mm/min

- Calculate the stress and strain from force and displacement values and plot the stress-strain curve for the PDMS sample using a suitable program (e.g. Microsoft Excel). This step should be done in the laboratory already.
- Explain the shape of the curve.
- Estimate the upper limit of the linear elastic regime and calculate the elastic modulus from the slope using a suitable program (e.g. Microsoft Excel).

B. Compression Test

- Prepare Sylgard 527 samples (see section D. Fabrication of PDMS Sylgard 184 and Sylgard 527 Samples) for the compression experiment and determine their dimensions (Figure 11).

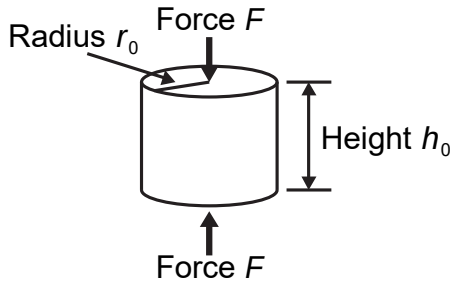


Figure 11. Cylindrical sample for compression test.

- Record the force and displacement during loading and unloading of the sample.

Settings:

Sampling rate	100 Hz
Integration time (force)	80 ms
Integration time (z-displacement)	10 ms
z-velocities	10 mm/min, 50 mm/min, 90 mm/min, 130 mm/min

- Repeat it for different loading/unloading velocities. Determine the strain rate from the loading velocity and the distance traveled, $\dot{\epsilon}(t) = d\epsilon/dt = v/L$.
- Convert the force-displacement curves into stress-strain curves and discuss their shape. How does the shape of the curve change with increasing strain rate?
- Determine the elastic modulus for each strain rate and plot elastic modulus vs. strain rate using a suitable program (e.g. Microsoft Excel).
- Lower the force transducer until a certain deformation of the sample is reached. Then modulate the z-movement sinusoidal and record force/displacement.

Settings:

Sampling rate	100 Hz
Integration time (force)	80 ms
Integration time (z-displacement)	10 ms
z-displacement oscillation frequency	0.2 Hz and 1 Hz

- Repeat it for different oscillation frequencies. Determine the phase shift ϕ between stress and strain and calculate the storage modulus E' and the loss modulus E'' for the different frequencies.

C. Indentation Test

- Prepare PDMS samples (see section D. Fabrication of PDMS Sylgard 184 and Sylgard 527 Samples) for the indentation test.
- Record force-distance curves on the PDMS sample with a spherical indenter, cylindrical indenter, and two conical indenters with different half cone angles. For that bring the indenter tip into contact with the sample surface. This could be easily done

by monitoring the force. Retract the tip around 1 mm and perform a force-distance curve with a force distance of 1.5 mm.

Settings:

Sampling rate	100 Hz
Integration time (force)	40 ms
Integration time (z-displacement)	10 ms
z-velocity	30 mm/min

- c) Make photographs of the four indenters with a stereo microscope and copy them for later analysis. At home characterize their tip geometry. Which dimensions of the spherical, conical and cylindrical indenter do you need to evaluate your force-distance curves?
- d) Determine the height of the sample. For that plot force vs tool separation and determine the contact point.
- e) Determine the maximum force at maximum indentation and compare the values for each type of indenter.
- f) Determine the elastic modulus of the PDMS sample by linearizing the formulas given in Table 1 and performing a least square fit to the linearized force-indentation curves using a suitable program (e.g. Microsoft Excel). This step should be done in the laboratory already.
- g) If you have a different viscoelastic sample, which variable should be kept constant during the indentation experiments to allow best comparison?

D. Fabrication of PDMS Sylgard 184 and Sylgard 527 Samples

Reagent: Sylgard 184 Silicone Elastomer, Sylgard 527 Silicone Dielectric gel

Description: Sylgard 184 Silicone Elastomer is supplied as two-part (base/curing agent) liquid component kit. When the two components are thoroughly mixed, the mixture cures to a transparent, flexible elastomer. The elastic modulus of PDMS strongly depends on the mixture ratio. The PDMS is used to prepare the samples for the tensile and the indentation test. For the compression test the sample consists of Sylgard 527, which is a very soft two-part silicone gel.

Sample preparation steps:

- a) Weight approximately 2 g of PDMS base in a petri dish for the tensile and the indentation test samples. One petri dish in each case. For the compression test sample weight around 2.5 g of Sylgard 527 Part A in a petri dish.
- b) Add the amount of PDMS curing agent to obtain the desired mixture ratio for the tensile test samples (base/curing agent 20:1) and the indentation test sample (base/curing agent 30:1).
Add the amount of Sylgard 527 Part B for the compression test sample. The mixture ratio of Part A and Part B should be 1:1.

- c) Thoroughly mix the two components with a spatula. Clean the spatula thoroughly with tissue paper and ethanol or isopropyl alcohol after each usage.
- d) Fill the mixed polymer in the desired form for the tensile and compression specimen. For the indentation test the mixed polymer can be left in the petri dish.
- e) Degas the mixtures for 20 min in the desiccator to remove air bubbles.
- f) Place the samples in the oven at 80°C. After 40 min the samples are cured to a flexible elastomer.

6. Protocol

The protocol should contain the basic theoretical concepts to understand and evaluate the results obtained during the experiments. The major part of the protocol should be the presentation of the results and their detailed discussion and interpretation. The protocol must contain a signed declaration that the elaboration of the report was performed by you and your group (see below).

Name:

Matriculation number:

I hereby declare that my elaboration (or that of my group) was made independently and is not a copy (even partially) of an existing protocol or of the instruction manual. All external sources (also the instruction manual) have to be cited. I am aware that a violation of this rules is plagiarism and leads to the experiment being graded as failed (grade 5) and can result in an exclusion from the labwork.

.....
Date

.....
Signature

References

1. Fung, Y.C., *Biomechanics : mechanical properties of living tissues*. 1993, New York: Springer-Verlag.
2. Abbott, R.D., et al., *Stress and matrix-responsive cytoskeletal remodeling in fibroblasts*. J Cell Physiol, 2013. **228**(1): p. 50-7.
3. Zatsiorsky, V.M. and B.I. Prilutsky, *Biomechanics of skeletal muscles*. 2012, Champaign, IL: Human Kinetics. xvi, 520 p.
4. Rho, J.Y., T.Y. Tsui, and G.M. Pharr, *Elastic properties of human cortical and trabecular lamellar bone measured by nanoindentation*. Biomaterials, 1997. **18**(20): p. 1325-30.
5. Suresh, S., *Biomechanics and biophysics of cancer cells*. Acta Materialia, 2007. **55**(12): p. 3989-4014.
6. Lekka, M., *Atomic force microscopy: A tip for diagnosing cancer*. Nat Nanotechnol, 2012. **7**(11): p. 691-2.
7. Chaudhuri, O., et al., *Extracellular matrix stiffness and composition jointly regulate the induction of malignant phenotypes in mammary epithelium*. Nat Mater, 2014. **13**(10): p. 970-978.
8. Hoyt, K., et al., *Tissue elasticity properties as biomarkers for prostate cancer*. Cancer Biomarkers, 2008. **4**(4-5): p. 213-225.
9. Discher, D.E., P. Janmey, and Y.L. Wang, *Tissue cells feel and respond to the stiffness of their substrate*. Science, 2005. **310**(5751): p. 1139-1143.
10. Ng, M.R., et al., *Substrate stiffness regulates cadherin-dependent collective migration through myosin-II contractility*. Journal of Cell Biology, 2012. **199**(3): p. 545-563.
11. Wang, Y., et al., *Substrate stiffness regulates the proliferation, migration, and differentiation of epidermal cells*. Burns, 2012. **38**(3): p. 414-420.
12. Park, J.S., et al., *The effect of matrix stiffness on the differentiation of mesenchymal stem cells in response to TGF-beta*. Biomaterials, 2011. **32**(16): p. 3921-30.
13. Huang, G.Y., et al., *Engineering three-dimensional cell mechanical microenvironment with hydrogels*. Biofabrication, 2012. **4**(4).
14. Hertz, H., *Über die Berührung fester elastischer Körper*. Journal für reine und angewandte Mathematik, 1882. **92**: p. 156-171.
15. Sneddon, I.N., *The relation between load and penetration in the axisymmetric boussinesq problem for a punch of arbitrary profile*. International Journal of Engineering Science, 1965. **3**(1): p. 47-57.