Seminar II

Elasticity of biological gels

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Abstract

In the seminar we discuss the elastic behavior of biological gels, which is a major constituent of living organisms. We focus on the difference of their elastic moduli and some unusual behavior which can be seen in vivo. We also derive a theory of semiflexible cross-linked polymer gels and its dependence on concentration of filaments.
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1 Introduction

It is inside humans to understand the nature and its constituent parts. Our ancestors have uncovered the hidden world of cells and fascination about them continues. Cells are a world of their own, crawling, pushing its way through pores and obstacles, while constantly changing its shape and elasticity to adapt to a complex environment [1]. Such a behavior could not be possible without constantly adapting cytoskeleton, the so-called active cell frame, made of biological gels. Cytoskeleton is essential for a variety of cellular processes, including mechanoprotection, motility and division [1, 2, 3]. To understand those mechanical processes it is unavoidable to study the elasticity of biological gels.

2 Gels

A polymer solution in water (or gas), linked with bonds, is called a gel [4]. Gelation takes place when the number of bonds between polymers is large enough to form a single cluster which expands through whole volume. Formation of a gel can be explained in terms of percolation theory. The transition between solution and gel is sudden, so we can determine concentration bond threshold for gelation. The cross-links which connect the polymers can be physical or chemical. A physical gel is formed by overlapping and knotting of the polymers such as household gelatin. In this case, the transition between gel and sol is temperature-depandent. On the other hand, a chemical gel consists of polymer chains permanently connected with covalent bonds. Rubber is one example of a chemical gel connected with sulphur covalent bonds. There exist different kinds of chemical crosslinkers, which can be seen in Figure 1.
2.1 Biological gels

Biological gels are found inside living organisms. They are abundant in different tissues and affect their physical properties dramatically. To understand the behavior of individual tissues it is therefore essential to study the physical properties of these constituent parts. Biological gels are found in a space between cells in the so-called extracellular matrix, which is essential to tissue stability and integrity. They are present also inside cells, building up the cellular cytoskeleton, which provides the cell with structure, shape and protection [2, 5]. In Figure 2, eukaryotic cells can be seen, and their constituent parts are illuminated. Cytoskeleton also forms structures such as flagella (euglena) and cilia (paramecium) used for locomotion.

Figure 2: The eukaryotic cell. Actin filaments are shown in red, cytoskeleton, microtubules in green, and nuclei are in blue [6].
Compared to artificial materials, biological networks are deformed at very small external stresses. For example, natural rubber and synthetic polymer networks can be reversibly deformed at applied stress on the order of $10^4 \sim 10^7$ Pa, and can sustain deformations up to 10 times of their initial size. On the other hand, biological materials are easily deformed at much lower stress, between $10^{-1} \sim 10^2$ Pa for strains $\gamma \leq 1$ [5]. Such dramatic difference in behavior of polymeric and biological networks reflects the difference in the elastic response of individual macromolecules or filaments in the networks. In polymeric networks chains are highly coiled such that their extension results in reduction of the number of available chain conformations. Therefore, the elastic response of these networks is entropic in nature. In biological networks, filaments of a network are almost fully extended between crosslinks. For such networks, their deformation can involve both filaments’ bending, stretching and twisting, where the latter can be either entropic or enthalpic in nature [5].

The cell cytoskeleton is composed mainly of three classes of protein fibers of very different stiffness: actin, microtubules and intermediate filaments [1]. All of them can be ordered into sophisticated assemblies by helper proteins known as crosslinkers, bundles, capping and severing proteins [1]. They form active network, which can sustain all the tasks cells are supposed to do. A substantial specialization is nevertheless necessary for all constituents of the cytoskeleton. Whereas microtubules are mainly involved in transport and the process of cell division, actin and intermediate filaments contribute to its viscoelasticity.

Beside biological networks, cells also contain elements which consume energy [1]. Such a system is called an active gel. Inside them one can find filaments undergoing treadmilling polymerization or molecular motors, both of which can actively change the structure of the system [1].

**2.1.1 Actin filaments**

Actin is a very important protein present in many different cell types. It plays a variety of roles in the cytoskeleton of the eukaryotic cells [7]. They belong to a group of microfilaments, which are the tiniest filaments of the cytoskeleton. Protein actin is a single chain of approximately 375 amino acids and forms structure called G-actin (“G” stands for globular). G-actin units can assemble into a long string called F-actin (“F” stands for filamentous). It forms an asymmetric double-helical filament built by a treadmilling-polymerization mechanism [1]. The formation of F-actin can be seen in Figure 3.

![Figure 3: The formation of a F-actin from a G-actin units.](image)
Mechanical response of the actin networks is determined by the long persistence length of about 16 μm. One of the most important functions of this protein is that it is used as a rail for myosin motors inside muscles, which make muscles contract [7].

3 Elasticity

Every material deforms under external force. Elasticity is a property of a solid material to return to the initial shape when external forces are removed. There exist different reasons for elasticity for different materials. For example metals and other solid materials the lattice size changes when the force is applied on them. When the force is removed, the lattice relaxes to the ground minimal-energy state. This type of elasticity is called energy elasticity. On the other hand, elasticity of polymer chains is entropic in nature. Because of chain stretching, conformation space is decreased, and so is the entropy. As a result, free energy is increased and therefore energy is needed to stretch polymer materials. The elasticity of soft materials often has both energetic and entropic contributions, depending on its state of strain. Entropic contributions tend to be relatively large when the system is only moderately deformed, whereas energetic contributions may become important at large deformations [7]. For little strains, stress is still linear dependant.

4 Experimental methods - Microrheology

The elasticity or compliance of homogeneous materials is determined by applying stresses and measuring the resultant strains. In the regime of linear response, the ratio between strain and stress is called elastic modulus and it can be also frequency dependent. Due to softness of cytoskeletal networks, scientists developed special microrheological methods to determine their mechanical response [1]. In the experiment, magnetic particles were dispersed in the network specimen, and a oscillating magnetic force was applied on them. Induced motion was observed through another tracer particle which enabled a more-detailed investigation of local heterogeneities and stress propagation [1]. A schematic of such a experiment is shown in Figure 4.

![Figure 4: Induced oscillations to determine frequency dependent susceptibility](image)

The thermal motion of single particles is determined by either video microscopy or high-speed interferometric detection [1]. With this method, modulus from $10^{-3}$ to $10^2$ Pa can be measured.

5 Concentration dependence shear modulus of gels

In this section we develop a linear elasticity model to describe a densely cross-linked gel and entangled solution. The model is still entropic in origin and it illustrates how the shear modulus
depends on polymer concentration, in our case protein actin. In the model, the gel consists of chain segments with length $L_e$ equal to the distance between crosslinks. The sketch of the model can be seen in Figure 5.

![Figure 5: A sketch for our model of elasticity. Cross-links are represented with a dark green. $\xi$ is an average mesh size.](image)

On it we apply a uniform shear deformation characterized by an angle $\theta$. In the continuum limit, the energy per segment reads

$$\mathcal{H} = \frac{1}{2} \kappa (\nabla^2 u)^2 + \frac{1}{2} \tau (\nabla u)^2, \quad (4.1)$$

where the first term describes bending of the chain, $\kappa$ being its bending modulus. The second part corresponds to a stretching of the segment of tension $\tau$. $u(x)$ describes the (transverse) deviation of the chain from a straight conformation along the $x$ axis. We also assume the contour length of the chain $L_\infty$ is constant, and because of that following expression holds

$$L_\infty - L \approx \frac{1}{2} \int (\nabla u)^2 \, dx. \quad (4.2)$$

Thermal fluctuations $\nabla u$ determine the equilibrium length $L$ of the individual chain. The chain deviation can be expanded in a Fourier series

$$u(x) = \sum_q u_q \sin(qx). \quad (4.3)$$

Equipartition theorem

$$\langle \mathcal{H} \rangle = E = \frac{k_B T}{2}, \quad (4.4)$$

can be used to determine the mean square amplitude of fluctuations $\langle u_q^2 \rangle$. Using that and Eq. (4.3) inserted into Eq. (4.2) it quickly follows

$$L_\infty - L \approx k_B T \sum_q \frac{1}{\kappa q^2 + \tau}. \quad (4.5)$$

Because we want to calculate to the first order in tension, we can use Taylor expansion of the fraction. Consistent with fixed ends of the chain segment, wave vectors $q = \pi/L, 2\pi/L, ...$ are included. After evaluating sums we can write the average end-to-end distance of the chain

$$L = L_\infty - k_B T L^2 + k_B T L^4 \frac{1}{6\kappa} + k_B T L^4 \frac{1}{90\kappa^2} \tau. \quad (4.6)$$
The second term represents the equilibrium contraction of the end-to-end distance at finite temperature. The last term gives the linear relationship between the applied tension and extension $\delta L$ of the chain segment beyond its relaxed length. For small deformations we can differentiate Eq. (4.6) and we get

$$\tau \sim \frac{\kappa^2}{k_B T L^3} \delta L \quad (4.7)$$

For a network, we consider $L \rightarrow L_e$, which is the entanglement length for a network. Extension now scales to angle between former parallel planes as $\delta L \sim \theta L_e$. We also change tension with applied stress $\sigma \sim \tau / \xi^2$, where $\xi$ is average mesh size. Stress is therefore given by the following relation

$$\sigma \sim \frac{\kappa^2}{k_B T \xi^2 L_e^3} \theta = G' \theta. \quad (4.8)$$

We have denoted elastic shear modulus by $G'$ in comparison to $G''$, which represents losses. The characteristic mesh size for a network of stiff chains is given by [8]

$$\xi \sim \frac{1}{\sqrt{a c_A}} \quad (4.9)$$

where $c_A$ is the concentration of actin monomers of size $\alpha$ [6]. We can see that mesh size decreases with increasing concentration of actin monomers. For a densely cross-linked gel, entanglement length and mesh size are comparable $L_e \cong \xi$. Finally we get the concentration behavior of shear modulus

$$G' \sim \frac{\kappa^2}{k_B T} (a c_A)^{5/2}. \quad (4.10)$$

This expression holds for densely cross-linked gels.

For a solution of semiflexible chains $L_e$ can become substantially larger than $\xi$ [8]. In this case, entanglement length reads

$$L_e \sim \left( \frac{\kappa}{k_B T} \right)^{1/5} (a c_A)^{-2/5}, \quad (4.11)$$

and thus

$$G' \sim \kappa \left( \frac{\kappa}{k_B T} \right)^{2/5} (a c_A)^{11/5}. \quad (4.12)$$

This theory was tested by experiments, which show similar behavior of elastic modulus as a function of action concentration. Results can be seen in Figure 6.
6 Elastic behavior of actin networks

In this section, we discuss elastic properties of biological gels such as cross-linked and entangled actin networks. Understanding these gels is important, because they are located in almost every eukaryotic cell as a mechanical component and they play an important role in cellular mechanoprotection, motility and cell division [2]. Their elasticity of these networks helps us understand how forces are generated inside cells and transmitted to the surroundings. As we already said, actin molecules polymerize inside cell in a long rigid molecules called F-actin. Despite their rigidity, these filaments still undergo thermal fluctuations and their persistence length is

\[ l_p = \frac{k}{k_B T} = 17 \mu m. \]  

(5.1)

The average size of a cell is about 30 microns. Biological gels that are consistent of a entangled solutions of F-actin are a well-studied model for semiflexible polymer networks. They are more elastic at small concentrations as compared with other, more traditional flexible networks at similar concentrations [2]. Elasticity depends not only on the F-actin concentration, but also on concentration of the proteins that bind to the actin molecules, forces them to align into bundles or cross-link them into networks. Small changes of cross-linker density dramatically changes the elasticity of F-actin gels compared to the physically entangled flexible networks, on which altering the cross-linker concentration does not have a significant influence [2]. To study elasticity of a F-actin network and concentration behavior actin linkers were used [2]. These linkers are rigid enough so that they do not contribute to the elasticity of the gel. By varying the linker density, the elastic modulus can be increased by more than three decades [2]. At low concentrations of actin or linker, the elasticity of network remains linear for strains as large as unity. For a sufficient concentration elasticity becomes nonlinear; elastic modulus in this region is strain dependant. We can represent this behavior with state diagram in Figure 7. Viscose component was neglected, because of the too long relaxation times.
The actin cytoskeleton is a highly dynamic and complex network; therefore also proteins that cross-link it in living cells have to be similar in nature. To investigate only the elastic properties of F-actin network, protein scruin is used, because it is sufficiently rigid so that does not contribute to the elasticity of the network [2]. Scruin is found in the sperm cell of the horseshoe crab, which is an ancient sea creature [2]. In a presence of actin molecules it forms permanent cross-links between them, and solution therefore bundles as we can see in Figure 8.

![Figure 7: Concentration dependence of elastic modulus. $R$ is ratio between linker and actin concentration. Blue colour represents region of linear elasticity. In the red region elastic modulus are strain dependant [2].](image)

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We can define also the ratio between actin and scruin concentrations

$$R = \frac{c_s}{c_a}.$$  \hspace{1cm} (5.2)

If we want to measure the strain-stress relation, the material is placed between two parallel plates. When shear stress is applied, plates move and form an angle $\theta$ between them. Tension is proportional to $\theta$ as $\sigma = G'\theta$. We can also apply stress as oscillatory function and the shear modulus is frequency dependent, $G''(\omega)$. But not all materials are completely elastic so we have to add one additional term, which represents dissipation, $G''(\omega)$. In elastic networks, there is no dissipation, and the modulus reaches a frequency-independent plateau $G_0$ at frequencies less than 1 Hz [7]. $G_0$ is dependent strongly on the density of cross-link and bundle actin filaments. As we already showed in the previous section, the frequency independent elasticity modulus is proportional to a power of actin concentration at fixed cross-linker concentration

$$G_0 \sim c_A^{5/2}.$$ \hspace{1cm} (5.3)

The elastic modulus is almost linear up to a strain $\gamma_{crit}$, which is normally around 10%. Beyond this point the material becomes stiffer with applied force, in this regime $G_0$ is strain dependent. The material is nevertheless completely elastic to the maximum strain $\gamma_{max}$, and beyond this threshold it breaks irreversibly [2].
Elasticity of entangled F-actin solutions arises because applied strain reduces chain fluctuations. This means that we have to deal with entropic elasticity, even though filaments which form the network are not completely flexible. When length between cross-links $l_c$ is smaller than persistence length $l_p$, we have to use model for a semiflexible filament segments such as the wormlike chain model used to describe the behavior of the DNA. Thus concentration dependence of the elastic modulus is slightly different,

$$G_0 \sim c^{11/5},$$

which holds for both weakly cross-linked networks, $R = 0.03$ and for thickly bundled networks, $R = 0.3$.

To compare low filament and cross-link density with high filament and cross-link density, researchers use two different polymer networks enclosed with two parallel plates under shear force [2]. At low densities enthalpic filament bending prevails, which leads to a highly inhomogeneous distribution of strain indicated by red arrows (Figure 8) [2]. On the other side, mechanical properties of densely connected network follows stretching out filament fluctuations, which is an entropic elasticity. We can summarize data for different densities in Table 1.

![Network Microstructure and under stress](image)

Table 1: Comparison between low (A) and high (B) filament and cross-link densities [2].

The two different elastic regimes in one state diagram (Figure 9). The elastic modulus varies continuously over four orders of magnitude as both actin concentration and cross-linker ratio are varied. In the first regime (plus signs), $G_0$ is highly sensitive to both $c_A$, and $R$, and shows strain stiffening. Mechanical response is explained by model of entropic elasticity. At low $c_A$ and $R$ there is a second regime of elasticity (circle signs), where response is dominated by bending of individual filaments.
7 Nonlinear elasticity of other biological materials

In previous section we focused on elasticity of actin network, but also other biological materials show similar nonlinear behavior. They all stiffen with applied strain, which is very important to prevent large deformations inside organism. This can eventually leads to cell damage and macroscopic injuries. With applied stress, the number of all possible conformation reduces to the point, when the filament is fully stretched. Next step is stretching of the bonds of the filament or the bond of the cross-link. This goes until filament or cross-link breaks. What happens first is due to the strength of the cross-link. Different materials can tolerate different strain. Neurofilament networks can be deformed by over 400% before failing, whereas actin networks rupture at strains of 20% [8]. Like actin, other relevant biological filaments belong to a category of semiflexible filaments, meaning that the persistence length of filament is comparable to its contour length. Experimental results show similar strain stiffening behavior of different biological material as seen in Figure 9. To compare biological gels with normal gels, polyacrylamide was used. It exhibits no strain stiffening behavior; its elastic modulus remains constant.

Figure 9: Elastic modulus for a series of crosslinked biopolymer networks as a function of dimensionless strain [3].

To compare all data from Figure 9, we have to rescale their elastic modulus and strain. Rescaled experimental results are shown in Figure 10. The collapsed experimental results fit well with theoretical predictions of nonlinear elasticity. In figure 10 strain $\gamma$ was normalized by a strain $\gamma_4$, where the elastic modulus is four times larger than normal.
For future implementation of artificial materials in the biological systems it is very important that we consider also its nonlinear behavior. This means that materials must have similar physical properties to be used in arterial wall for example.

8 Conclusion

It is very important that we understand physical background for elasticity of the biological gels, because they are and will be more and more connected with us, mostly implemented in materials that we will use in daily basis. To implement artificial materials into biological systems, strain stiffening behavior should be taken into account. For example, artificial blood vessels have to stretch comparable to original biological vessels [3]. With measurements of the cell stiffness, and its somehow abnormal behavior, diseases can be predicted [1]. By varying filament or cross-link concentration, cells can make rapid and precise transitions to alter the elasticity of their cytoskeletal network.

9 Literature