8. English Summary

The cells inside our body do not sit alone. Each cell is surrounded by neighboring cells and a mesh work termed the extracellular matrix (ECM). The ECM provides chemical information in terms of ligands and growth factors, mechanical information in terms of elastic stiffness and viscous dissipation, and structural information in terms of dimensionality, mesh size, and local nanotopography. The cell actively senses the mechanical cues in a process referred to as *mechanosensing* and responds to them in a process referred to as *mechanoresponse*, as discussed in detail in Chapter 1.

Mechanobiology is a growing interdisciplinary field involving biologists, chemists, engineers, and physicists. Until now, cellular mechanosensing has mostly been studied using synthetic gels like polyacrylamide, which have much simpler mechanical and chemical properties than biological tissues. Synthetic gels are characterized by one stiffness value over a wide range of strain levels, and they tend to be mainly elastic. In contrast, ECM networks stiffen when subjected to a mechanical deformation (*strain-stiffening*) as a consequence of their fibrous and hierarchical structure. The physical origin of this strain-stiffening response and the consequences for cell-matrix interactions are not clear. The central goal of this thesis was to elucidate the origin of the mechanical properties of two important ECM networks: fibrin (Chapter 2-5) and collagen (Chapter 6 and 7). Both of these ECM networks are widely used as model systems for studying cell-matrix interactions in three dimensions *in vitro*. We use shear rheology to measure the viscoelastic properties of networks reconstituted from purified fibrinogen and collagen, and compare our data with theoretical models for semiflexible polymers. In addition, we embedded cells in the ECM networks and tested whether the cells are able to change the structural and mechanical properties of the matrices.

Fibrin is a temporary ECM network that forms at sites of vascular injury to stop the bleeding. Cells use this matrix to migrate to the injury site and rebuild the lost tissue. The fibrin network thus needs to be able to withstand substantial forces, both from blood flow and cellular traction forces. In Chapter 2, we showed that the strain-stiffening response of fibrin is directly related to the hierarchical architecture of the fibrin fibers. Fibrin fibers are built up of semiflexible protofibrils, which are coupled together by covalent crosslinking mediated by the enzyme FXIII. We discovered that the mechanical properties of fibrin networks polymerized under conditions that suppress protofibril bundling agree well with predictions of an entropic network model, where stretching out of fiber undulations gives rise to stiffening. By varying

the number of protofibrils inside a fibrin fiber, and by varying how tightly these protofibrils are coupled together, we showed that the stiffness of fibrin networks is well described by a model that treats the fibers as semiflexible polymer bundles with a rigidity that is controlled by the protofibril number and the degree of intrafiber crosslinking.

An important consequence of the strain-stiffening response of fibrin gels is that cells can actively stiffen the gels, as shown in Chapter 3. The cells generate traction forces using their actomyosin cytoskeleton, which drives the fibrin network into a stiffened state. The low-strain modulus of cell-seeded fibrin gels is a function of cell density, while the nonlinear mechanical properties remain unaltered. This work has important implications for studying cell-matrix interactions in a three dimensional ECM environment, since cellular traction can create a (local) stiffer environment that can act as a positive feedback loop for cellular mechanosensing.

The entropic network model presented in Chapter 2 describes fibrin mechanics accurately, except at very high strain levels. One central assumption of the model is a constant stretch modulus of the protofibrils. However, it was previously shown by micromanipulation studies that fibrin fibers are elastomeric and stiffen when stretched. Various mechanisms for this nonlinear force-extension behavior have been proposed, including forced unfolding of fibrin monomers. However, direct evidence for any of these mechanisms is still lacking. To investigate whether forced monomer unfolding contributes to the elastomeric and nonlinear mechanics of fibrin networks, we performed *in situ* small-angle X-ray scattering (SAXS) measurements on uniaxially stretched fibrin networks to probe changes in molecular packing structure. By comparing our results with SAXS spectra calculated from molecular dynamics simulations of protofibril stretching, we showed that network stretching indeed causes unfolding of the γ-nodule of the fibrin molecules starting at a strain level of 30%. Moreover, the alpha-helical coiled coils are transformed into beta-sheet structures, explaining the extensibility as well as stiffening of fibrin fibers upon stretching. Altogether, our results show that fibrinogen unfolding plays a dominant role in fibrin network extension at high strain levels.

Most work on ECM mechanics has focused on the network mechanics on a global length scale, as probed by macroscopic shear rheology or tensile tests. However, cells embedded in an open fibrous ECM network probe its mechanical properties at a scale comparable to their own size, i.e. on the micron scale. To gain insight into the local mechanical properties of ECM networks, we have developed an optical tweezer setup to track the thermal displacement fluctuations of micron-sized probe beads embedded in an ECM network as a probe of the local shear modulus, as described in Chapter 5. By using a quadrant photodiode for position detection, we could probe the shear modulus over a broad frequency range from 1 Hz to 20 kHz. From the high frequency response,

which originates from the fluctuations of the fibrin fibers, we could infer that fibrin fibers behave as thermally undulating, semiflexible polymers, consistent with the entropic network model presented in Chapter 2. At low frequencies, we could measure the local network modulus, which we found to be in the same range as measured with macrorheology (within a factor 3). However, the magnitude of the local modulus depended on the probe surface properties, which we could relate to changes in the local network microstructure around the probe particles.

In Chapter 6 we switch our attention to collagen ECM networks. Collagen type I is the most abundant protein in the mammalian body and is widely used as an ECM model system for studying cell-matrix interactions *in vitro*. Despite its widespread use, the mechanics of collagen remains poorly understood. In Chapter 6, we measured the nonlinear elastic properties of collagen networks and showed that these are in quantitative agreement with an athermal network model, where stiffening occurs as a consequence of the submarginal connectivity of these networks. The average number of fibers coming together at each network junction is between 3 and 4, which is less than the Maxwell criterion for isostaticity of 3D spring networks, which requires a critical connectivity of 6 for network stability. At low strain, collagen networks are stabilized by the bending rigidity of the fibers. With increasing strain, a negative normal stress builds up, which causes network stiffening. When the strain reaches values on the order of 30%, the network undergoes a transition to stretch-dominated elasticity. We show that the strain-stiffening response of collagen networks shows signatures of an underlying mechanical phase transition. By varying collagen architecture through the protein concentration and polymerization temperature, we showed that the local architecture, in terms of the connectivity, governs the linear modulus and the strain-stiffening response. We can now use the athermal network model to predict the stiffness of collagen networks with more complex compositions and to study cell-collagen interactions.

In Chapter 7 we set out to test whether cellular traction forces could actively stiffen collagen networks in a similar manner as for the fibrin gels studied in Chapter 3. However, we could not observe any measurable difference in the mechanical properties of cell-seeded gels and unseeded gels. By using microscopy, we could track the absence of cell-induced matrix stiffening down to limited cell spreading inside the networks. We investigated several factors known to influence cell spreading, but to no affect. We conclude that, while in principle the cell line we used (3T3 fibroblasts) does express integrins that bind collagen I, this cell line is not ideal for studying the effect of cellular traction forces on collagen mechanical properties.

In summary, in this thesis we showed the physical basis for the remarkable strain-stiffening behavior of two major components of the ECM, namely fibrin and collagen. A major new insight is that the molecular packing structure of

the fibers plays an important role in controlling the network mechanics. Even though collagen and fibrin fibers have similar diameters and build networks with a comparable architecture, fibrin networks exhibit entropic elasticity and elastomeric properties with rupture strains of up to 200%, whereas collagen networks behave as athermal fibrous networks that break at strains of only 40%. We attribute this remarkable difference to the difference in packing structure of the two systems: collagen fibers are rigid, tightly packed bundles of thousands of collagen molecules across, whereas fibrin fibers are flexible, open bundles containing only on the order of 100 fibrin molecules across. Also, the presence of molecular domains that can undergo forced unfolding plays a major role in the mechanics of fibrin networks.

The work in this thesis can provide new insights into the origin of mechanical deficiencies in human diseases related to mutations in collagen and fibrinogen. Also, the mechanical models presented in this work can in future be utilized to design either bio-based or synthetic materials of a desired stiffness, for instance for tissue engineering purposes. Finally, the difference in mechanical behavior of fibrin and collagen networks likely has consequences for cellular mechanobiology.