Fibrin structure and mechanics

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7.1. SUMMARY

THE focus of this Thesis was on the mechanical and structural properties of fibrous materials found in nature. Specifically, we focused on fibrin, the main structural component of a blood clot: a biological material that is essential for survival, since it stops the flow of blood through a vascular breach. Its mechanical rigidity originates from a network of fibrin fibers, which assemble through an enzymatic cascade from monomers into protofibrils, fibers and finally the macroscopic network. Interestingly, this network performs its function at a low protein density, yet it is able to withstand large stresses and deformations without rupture. So far, the origin for this remarkable mechanical behaviour has been poorly understood. Until now, our understanding of the mechanical behaviour of fibrin gels was based on models of semiflexible filaments, ignoring the internal structure of the fibrin fibers and their ability to engage in (non-covalent) interactions, as well as ignoring the solvent that permeates the network.

In this Thesis, we asked the question how the complex structure of fibrin and its constituent fibers, protofibrils and monomers relate to the network's complex mechanical properties. To answer this question, we first implemented three existing light scattering models [51–53], to obtain quantitative information on the fiber radius and fiber mass density. We improved these models by including the contribution of wavelength dispersion of the refractive index of the solvent and the differential refractive index. We also implemented the use of a power spectral density analysis to obtain from confocal microscopy images the network's fractal dimension, which is an input parameter in an advanced light scattering model that takes the network structure into account. The light scattering analysis we developed allows us to quantify the structural properties of fibrin fibers, which is a prerequisite to interpret mechanical data. The analysis is also useful for a much broader class of fibrous networks.

We then considered three different, unaddressed mechanical aspects of the fibrin gel: (1) the role of the solvent and its coupling to the fiber network on the mechanical properties of the fibrin gel; (2) changes in structure at different length scales that occur when fibrin networks are exposed to mechanical deformation; and (3) remodeling of the fibrin network through fiber-fiber interactions.

Starting at the macroscopic length scale of the fibrin clot, we demonstrated that the fibrin network and the solvent are coupled on short time scales, set by the elastic modulus and the pore size of the fibrin gel, the viscosity of the solvent, and the macroscopic dimensions of the gel, rendering the network effectively incompressible. Yet on long time scales, the network and solvent are decoupled, such that deformation of the network can occur without solvent movement. Using a combination of experiments and theory, we demonstrated the two-fluid character of the fibrin gel during compression of the network, and also during shear, which is intrinsically a volume-conserving mode of deformation. Under shear deformation, we observed that the direction of the normal stress in response to applied shear stresses is either positive or negative, depending on the coupling between network and solvent. Our findings resolve an apparent paradox between biopolymer networks and synthetic networks, where the former exhibit a negative normal stress and the latter a positive normal stress in response to a shear stress. We demonstrated that both classes of materials are capable of exerting both positive and negative normal stresses, if probed on the right time scale.

So far, the fibrin network was still considered a uniform elastic material, without any hierarchical structure in its constituents. We demonstrated, using a combination of *in situ* microscopy and X-ray scattering, that to understand the full mechanical response of fibrin networks to shear deformation, the orienting effect that shear has on rod-like particles is not sufficient. At small strains of a few percent, before this orienting effect starts to play a role, the stretching of entropic undulations in the fibrin fibers causes network stiffening. At large strains, the mechanical response of the network is dominated by stretching of the fibers themselves, which we showed is facilitated by the stretching of the unstructured αC-regions of the monomer, rather than unfolding of the coiled-coil regions, an idea that dominates the current opinion in literature.

Next, we showed that the constituent fibers that form the network can form new bonds with each other when brought in close contact, in particular during compression of the network. We demonstrated that the αC-regions that are attached to both ends of the fibrin molecule, can interact with the αC-regions of monomers in adjacent fibers. The bonds that are formed this way require a high force to be disrupted, such that these bonds are stressed but not broken when an initially compressed network is brought back to its original configuration. This built-in stress is in turn responsible for an increase of the network stiffness, which can be finely tuned by controlling the amount of applied compression.

Finally, we presented the development of a fluorescence light sheet microscope in combination with a device that can stretch fibrin gels, to enable *in situ* imaging of the effect of mechanical deformation on the network structure, allowing both the fast, elastic response and the slow, inelastic response to be captured.

In conclusion, this research provides a better understanding of the structure-function relation of fibrin networks, which is essential for understanding fibrin's role in hemostasis, thrombosis, and wound healing. We have demonstrated that the mechanical behaviour of fibrin networks originates not only from an assembly of ideal, semiflexible polymers, but rather from a set of hierarchically ordered filaments that can stretch, but also interact with each other, and that is surrounded by an incompressible solvent that couples to the network and grants it high rigidity on short time scales. Moreover, this research also provides new handholds in the directed search and design of materials with specific mechanical properties. In a broader context, this Thesis illustrates how Nature adopts an extensive toolbox of physical principles to optimally perform in the challenging environment we call Life.