Review

Fibrin mechanical properties and their structural origins

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Abstract

Fibrin is a protein polymer that is essential for hemostasis and thrombosis, wound healing, and several other biological functions and pathological conditions that involve extracellular matrix. In addition to molecular and cellular interactions, fibrin mechanics has been recently shown to underlie clot behavior in the highly dynamic intra- and extravascular environments. Fibrin has both elastic and viscous properties. Perhaps the most remarkable rheological feature of the fibrin network is an extremely high elasticity and stability despite very low protein content. Another important mechanical property that is common to many filamentous protein polymers but not other polymers is stiffening occurring in response to shear, tension, or compression. New data has begun to provide a structural basis for the unique mechanical behavior of fibrin that originates from its complex multi-scale hierarchical structure. The mechanical behavior of the whole fibrin gel is governed largely by the properties of single fibers and their ensembles, including changes in fiber orientation, stretching, bending, and buckling. The properties of individual fibrin fibers are determined by the number and packing arrangements of double-stranded half-staggered protofibrils, which still remain poorly understood. It has also been proposed that forced unfolding of sub-molecular structures, including elongation of flexible and relatively unstructured portions of fibrin molecules, can contribute to fibrin deformations. In spite of a great increase in our knowledge of the structural mechanics of fibrin, much about the mechanisms of fibrin's biological functions remains unknown. Fibrin deformability is not only an essential part of the biomechanics of hemostasis and thrombosis, but also a rapidly developing field of bioengineering that uses fibrin as a versatile biomaterial with exceptional and tunable biochemical and mechanical properties.

Introduction

Fibrin is a major component of intra- or extravascular blood clots that form at the sites of vessel wall damage and also of the extracellular matrix. Fibrin provides clots and thrombi with elasticity that is important for their hemostatic function, obstructiveness and stability; it also determines the functionality of various cellular processes, including adhesion, migration, proliferation and differentiation, wound healing, angiogenesis, inflammation, etc.

Since pioneering systematic studies of the structural biomechanics of fibrin performed by John Ferry [1–4], this problem has evolved into a rapidly developing area of interdisciplinary research. First of all, fibrin mechanics is an important part of the field of hemostasis and thrombosis because blood clots and thrombi that contain the fibrin scaffold undergo dramatic deformations under (patho)physiological conditions such as hydrodynamic blood shear [5–7], contraction of platelets [8,9], and aortic aneurisms [10–13]. Therefore, the outcomes of many bleeding and thrombotic disorders, including thromboembolisms, are largely determined by mechanical behavior of the fibrin network [14]. At the same time, fibrin mechanics has become increasingly important in view of extensive new applications of fibrin as a biomaterial, e.g., in tissue engineering, cell culturing, drug delivery, wound sealing, etc., where the mechanical support provided by a fibrin network, in combination with other

properties, makes it a unique, versatile, and quite useful hydrogel [15].

Viscoelastic properties of fibrin (fibrin rheology)

Fibrin is a viscoelastic polymer, which means that it has both elastic and viscous properties. The elasticity (or stiffness) is characterized by reversible mechanical deformation, while viscosity (or plasticity) is characterized by irreversible deformation induced by force. Viscoelastic biomaterials differ in the relative degrees of both elastic and viscous properties, which are quantified by measuring the responses to deformation, referred to as rheometry. For fibrin clots, the elastic component is generally about an order of magnitude higher than the viscous component, although the viscous component increases rapidly at higher rates of deformation. Remarkably, during creep experiments, in which continued changes in strain are measured over time after application of stress, some clots do not change in stiffness [16], which means that fibrin is a "self-repairing" structure, most likely because the knob-hole bonds holding the structure together are reversible [17].

Devices used to measure viscoelasticity (rheometers), vary by geometry and protocol of the induced deformation. The most commonly used are shear rheometers that impose a controlled shear strain (or a controlled shear force) on the fibrin clot sample placed between two surfaces, one of them being moved in an oscillatory manner to deform fibrin in the plane of shear. A sinusoidal oscillation has the form of $\gamma = \gamma_0 * \sin(\omega t)$, where ω is the frequency and γ_0 is the strain amplitude, and the shear stress σ required to impose such a deformation is measured. For a linear viscoelastic material, shear stress is a sinusoidal function with some phase shift δ, i.e. $\sigma = \sigma_0 * \sin(\omega t + \delta)$, where σ_0 is the shear stress amplitude. The elastic response of the fibrin clot is characterized by the shear storage modulus, G', corresponding to the part of shear stress that is in phase with strain and is calculated as $G' = (\sigma_0/\gamma_0) * \cos(\delta)$. The viscous response of the clot to applied shear is measured by the shear loss modulus, G'', calculated as the out-of-phase part of the stress as $G'' = (\sigma_0/\gamma_0) * \sin(\delta)$. The terms "storage" and "loss" refer to the energy stored or lost during the deformation, respectively. The storage and loss moduli determine how the clot responds to the forces to which it is subjected. For example, a stiffer clot (higher G') will not deform as much as a less stiff one with the same applied stress.

In a tensile rheometer, the elastic properties are measured by applying a longitudinal or stretching stress (i.e., force per unit area) to a cylindrical or bar-like sample and determining the resulting strain (S), which is the stretching or distortion of the polymer normalized with respect to total length ($S = L/L_0 - 1$, where L is the stretched length and L_0 is the initial length). The resulting stress–strain curve is used to determine its slope or the ratio of the stress required to produce a certain strain (tensile elastic modulus). If a large stress is necessary to produce displacement, then that object is stiff, while a small modulus means that less stress is required, so this other object is less stiff. Similarly, compressive properties can be measured by applying a pressure on the sample with the degree of compression defined as a negative strain and the (compressive) elastic modulus determined by the slope of a stress–strain curve obtained in response to strain- or force-controlled compression. The rheometry-based quantification of fibrin viscoelasticity is described in detail elsewhere [18]. Table 1 compares the elasticity of some proteinaceous filaments.

Simplified systems that are not rheometers yet record changes in clot stiffness over time, named thromboelastography (or thromboelastometry), have been widely used in clinical medicine to monitor the formation of whole blood or plasma clots based on their elasticity [19–22]. In thromboelastography a small cylindrical anchor (pin) is immersed into the activated blood or plasma sample placed inside a round cuvette, which is slowly rotated at a small angle around its initial position. In the absence of a clot, the rotation of the cuvette is not transmitted to the anchor, while after the beginning of clot formation the anchor becomes tethered to the walls of the cuvette and starts to rotate synchronously with the cuvette. The circular amplitude of the anchor increases as the clot is getting stiffer. A curve that circumscribes the amplitudes of the anchor over time

Table 1. Comparative stiffness and mechanical stability of various proteinaceous filaments.[a]

Protein	Young's modulus (MPa)	Fracture strain (%)
Fibrin fiber, uncrosslinked	1.7 ± 1.3[b]	226
Fibrin fiber, crosslinked	14.5 ± 3.5[b]	332
Elastin	1	150
Myofibrils	1	200
Resilin	1–2	190, 313
Fibronectin[c]	0.1–3.5	700
Spider silk (Araneus Flag)	3	270
Fibrillin	0.2–100	>185
Intermediate filament	6–300	160–220
Mussel byssus	10–500	109
Collagen, tendon	160–7500	12
Microtubules	1000–1500	≤20
α-Keratin wet	2000	45
Actin	1800–2500	≤15
Collagen, crosslinked	5000–7000	12–16
Spider silk (Araneus MA)	10,000	27

[a] Based on [43].
[b] [49].
[c] [133].