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Structural basis for the nonlinear mechanics of fibrin networks under compression

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ABSTRACT

Fibrin is a protein polymer that forms a 3D filamentous network, a major structural component of protective physiological blood clots as well as life threatening pathological thrombi. It plays an important role in wound healing, tissue regeneration and is widely employed in surgery as a sealant and in tissue engineering as a scaffold. The goal of this study was to establish correlations between structural changes and mechanical responses of fibrin networks exposed to compressive loads. Rheological measurements revealed nonlinear changes of fibrin network viscoelastic properties under dynamic compression, resulting in network softening followed by its dramatic hardening. Repeated compression/decompression enhanced fibrin clot stiffening. Combining fibrin network rheology with simultaneous confocal microscopy provided direct evidence of structural modulations underlying nonlinear viscoelasticity of compressed fibrin networks. Fibrin clot softening in response to compression strongly correlated with fiber buckling and bending, while hardening was associated with fibrin network densification. Our results suggest a complex interplay of entropic and enthalpic mechanisms accompanying structural changes and accounting for the nonlinear mechanical response in fibrin networks undergoing compressive deformations. These findings provide new insight into the fibrin clot structural mechanics and can be useful for designing fibrin-based biomaterials with modulated viscoelastic properties.

Key points

1. Viscoelastic properties of fibrin networks under compression reveal dual softening–hardening transitions.
2. Structural modulations underlie nonlinear mechanical responses of compressed fibrin networks.

1. Introduction

The fibrin network is an end product of blood clotting and a major structural component of protective hemostatic clots and pathological obstructive thrombi that largely determines their mechanical stability [1]. Molecular mechanisms of fibrin formation and its basic structural characteristics have been extensively studied [2–5]. Normally, fibrin networks form at sites of vascular injuries and perform a mechanical task of stemming blood flow by forming a gel, which also incorporates platelets and red blood cells [6]. Fibrin networks have been also used for numerous purposes of surgical repairs and tissue engineering as a biodegradable tissue adhesive or sealant to stop or control bleeding [7–9] or to form a provisional fibrin matrix for growing blood vessels and tissue repair [10]. Additionally, fibrin has been utilized for drug delivery applications [11], when drug molecules or factors are loaded in the fibrin gel via impregnation and tethering to the gel through covalent linkages or affinity-based systems.

Fibrin clots must withstand deformations and stresses generated in the blood stream as they are exposed to different external forces including pulsatile hydrodynamic stresses induced by oscillating blood flow, forces resulting from fluctuations of the blood vessel wall or due to platelet contraction leading to clot retraction [1]. Understanding how the mechanical response of the fibrin network is related to the network structural topology can provide the structural basis for biomechanics of fibrin-based blood clots and thrombi as well as engineered biomaterials.

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The aggregate of forces that act on fibrin clots under various dynamic conditions in vivo can be segregated into shear, tensile, and compressive types with shear forces representing a complex combination of tension and compression [12,13]. Shear stresses acting on a clot originate from the velocity gradient of the blood flow across the vessel lumen and have been shown to affect fibrin network structure [14,15]. When exposed to shear or tensile stresses, fibrin networks display nonlinear mechanical responses [16–18] manifesting as a strain-stiffening behavior, i.e. an increase of the elastic modulus measured under shear or stretch as the magnitude of deformation increases.

Dynamic shear moduli of fibrin clots measured under moderate and large oscillatory deformations were systematically studied for clots with or without covalent ligation [19,20]. These studies showed that the differential shear storage modulus can increase by a factor of 20 when shear strain increases from 1% to 50%. Strain-stiffening of plasma clots was addressed in Ref. [21], where it was demonstrated that the presence of platelets in fibrin gels decreased the degree of strain stiffening although significantly increased the storage modulus at low strains. The phenomenon of strain-stiffening was demonstrated not only at the whole clot level, but also at the level of individual fibers [22,23]. It has been recently shown that nonlinear mechanical responses of networks formed from un-cross-linked fibrin continually change under repeated large-strain loading [12,24]. Remarkably, the imposed shear loading resulted not in weakening of the underlying matrices but rather in delayed occurrence of the strain stiffening. Another common feature of fibrin clots is their negative normal stress response when exposed to the shear stress [25].

Fibrin clots have a number of remarkable mechanical properties that make them very different from other proteinaceous biopolymers [1]. Tensile experiments have shown that fibrin clots are highly extensible and can be stretched more than four times their relaxed length before breaking [26]. Moreover, stretching of fibrin networks resulted in fiber densification and aggregation accompanied by expulsion of water. These properties were shown to result from protein unfolding [27], which was not observed in other matrix proteins such as collagen.

Despite the fact that a compressive deformation is inherent to (patho)physiological conditions, such as blood flow, vasoconstriction (contraction of the wall of the vessel), and clot retraction induced by platelets [28] and myofibroblasts [29], changes in clot elasticity and plasticity under compression and their structural origin are largely unknown. Currently, there are only few studies on how the structural and mechanical properties of blood clots are affected by compressive load. Litvinov et al. [30] recently demonstrated that compression as well as stretching of fibrin fibers was accompanied by transition of fibrin network microstructure and molecular unfolding followed by an increase in the fraction of β-sheets and a corresponding reduction of α-helices.

The goal of the present paper is to establish correlations between the mechanical response and corresponding structural changes of fibrin networks exposed to compression. We hypothesized that dynamically compressed polymeric fibrin, similar to shear- or stretch-induced deformations, have non-linear mechanical behavior caused by structural rearrangements of the entire fibrin network as well as alterations of individual fibers. This hypothesis was tested by using a custom-built experimental setup, which combines a confocal microscope and a rotational rheometer. This instrument enabled us to explore shear viscoelastic properties of dynamically compressed fibrin clots in parallel with simultaneous structural changes at the levels of the entire filamentous network as well as single fibrin fibers. This combined approach revealed complex non-linear mechanical properties of fibrin gels originating from their structural rearrangements in response to compressive deformations.

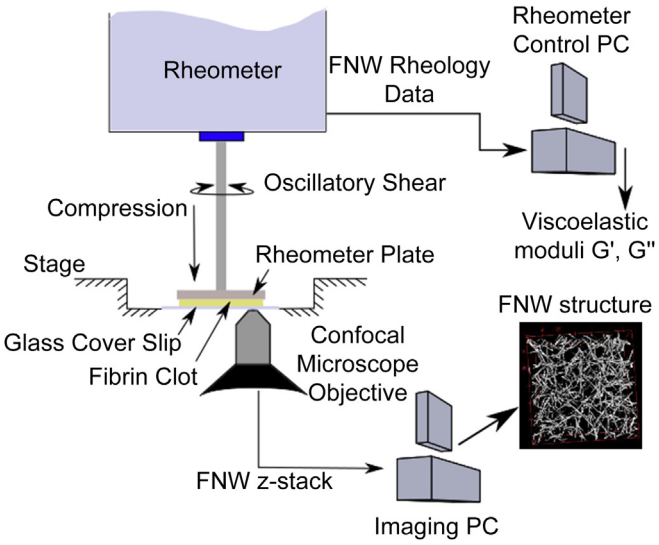

Fig. 1. Experimental setup combining rheometry and confocal microscopy to measure structural mechanics of human plasma fibrin clots. Rheological and structural data were simultaneously collected and stored independently by the coupled systems of data acquisition. Here, the abbreviation FNW stands for fibrin network.

2. Materials and methods

2.1. Formation of fibrin clots

Fibrin clots were prepared by mixing pooled human citrated platelet-poor plasma with calcium chloride (40 mM final concentration) and human thrombin (from 0.1 to 1 U/mL). Alexa-Fluor 488-labeled human fibrinogen (Molecular Probes, Grand Island, NY) was added to the plasma samples (5% of total volume, 0.08 mg/mL final concentration) before clotting to visualize fibrin structure in a fluorescence confocal microscope. Plasma clots were allowed to form at the room temperature for 30–100 min in a gap between horizontal rheometer plates separated by a distance of 500–750 μm. Sample volume varied from 300 to 375 μl. The upper rheometer plate was a 20-mm-diameter acrylic disc and the lower plate comprised a 22-mm-diameter microscope glass cover slip permitting confocal imaging of the network in combination with rheological measurements. During clotting and measurements, a piece of wet filter paper was placed around the rheometer plates and periodically (each 20–30 min) sprayed with water to prevent sample drying. Before each experiment the surfaces were thoroughly cleaned and dried to assure firm attachment of fibrin clots. 30 fibrin clot samples were prepared as described and analyzed at various experimental conditions.

2.2. Compressive deformations of fibrin clots

The 500–700-μm-thick fresh hydrated fibrin clots formed between horizontal plates of the rheometer were compressed vertically stepwise up to 1/10 of their initial thickness. Compression was performed in 5–25 μm steps at the rates of 10–30 μm/s, as the upper rheometer plate exerted an axial force on the upper surface of the clot. The degree of compression (compressive strain) was defined as the absolute fractional decrease in fibrin clot thickness, $\gamma = \left|\frac{\Delta L}{L_0}\right|$, where $\Delta L = L - L_0$, and L_0 and L are the initial and reduced thickness dimensions of the uncompressed and compressed clots, respectively.

2.3. Rotational rheometry of fibrin clots

Viscoelastic properties of fibrin clots were characterized using a Bohlin Gemini rheometer (Malvern Instruments, Westborough, MA). During a strain-controlled test, the rheometer imposed a sinusoidal oscillatory shear strain on the clot sample in 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 was 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 clot is characterized by the shear elastic modulus, G', corresponding to the part of shear stress, which 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 $G'' = (\sigma_0/\gamma_0)\sin(\delta)$.

To probe a clot's shear stress-strain response, the shear strain of 0.005 and oscillatory frequency of 1 Hz were used to produce a linear stress-strain response. The linearity was assured by running a shear strain and frequency sweep tests from 0.005 to 0.03 shear strain at 1 Hz and over the frequency range from 0.5 to 10 Hz.