Negative normal stress in semiflexible biopolymer gels.

Paul A. Janmey[1,2], Margaret E. McCormick[1], Sebastian Rammensee[1,3], Jennifer L. Leight[1],
Penelope C. Georges[1], Fred C. MacKintosh[4]

[1]Institute for Medicine and Engineering, University of Pennsylvania

3340 Smith Walk, Philadelphia, PA 19104, USA

[2]Department of Physics and Astronomy, University of Pennsylvania

209 S 33rd St, Philadelphia, PA 19104, USA

[3]Technische Universität, München, Physik-Department E22 Biophysik

James-Franck-Str. 1, 85747 Garching, Germany

[4]Department of Physics and Astronomy, Vrije Universiteit Amsterdam

De Boelelaan 1081, 1081HV Amsterdam, The Netherlands

When subject to stress or external loads, most materials resist deformation. Any stable material, for instance, resists compression--even liquids. Solids also resist simple shear deformations that conserve volume. Under simple shear, however, most materials also have a tendency to expand in the direction perpendicular to the applied shear stress, a response that is known as positive normal stress [1]. For example, wet sand tends to dilate when sheared, and therefore dries around our feet when we walk on the beach. In the case of simple solids, Poynting showed nearly one hundred years ago that elastic rods or wires tend to elongate when subject to torsion [2]. Here, we show that networks of semiflexible biopolymers such as those that make up both the cytoskeleton of cells and the extracellular matrix exhibit the opposite tendency: when sheared between two plates, they tend to pull the plates together. We show that these negative normal stresses can be as large as the shear stress and that this property is directly related to the non-linear strain-stiffening behavior of biopolymer gels [3].

When viscoelastic materials are deformed by a sinusoidal shear strain, their stiffness is quantified by the elastic storage and loss shear moduli calculated from the amplitude and phase shift of the resulting sinusoidal shear stress. In some solids like soft hydrogels at moderate to large deformations, an additional stress can be measured in the direction orthogonal to the shearing force. Gels formed by flexible polymers with a linear stress-strain relationship generate a small upward stress, but gels formed by semiflexible polymers that become stiffer the more they are deformed, generate normal forces that are both larger in magnitude and of the opposite sign. Figure 1A shows that during an oscillatory shear deformation of a semiflexible fibrin gel a large negative normal stress is measured with a period exactly half of the strain period. The frequency doubling of the normal stress compared to strain frequency occurs because the normal stress depends only on the magnitude of the strain and not its direction. In contrast, a gel of the flexible polymer polyacrylamide under shear deformation (Figure 1B) produces a positive normal stress that is smaller in magnitude, even though the shear modulus of this polyacrylamide gel is greater than that of the fibrin gel in Fib 1A. The difference in direction and magnitude is evident in Figure 1C, which shows the momentary normal stress as a function of shear strain during the course of an oscillatory deformation. Since both fibrin and polyacrylamide gels have very little mechanical loss at the frequency of measurement (G'>>G"), each oscillation can be viewed as two stress-strain curves in opposite strain directions.

The negative normal stress is not unique to fibrin, but is seen for all measured gels formed from strain-stiffening semiflexible biopolymers. This is not, however, seen for any flexible polymer hydrogel tested. Figure 2 shows that F-actin crosslinked by biotin-avidin (2A) collagen (2B) fibrin (2C) neurofilaments (2D) and Matrigel, a cell-derived matrix containing primarily collagen and the flexible macromolecule laminin (2E) all show a negative normal stress, whose magnitude increases with strain. This increase in magnitude occurs only in the range over which the systems stiffen under shear. When the maximal strain is reached for each system and the shear modulus begins to decrease, due to filament rupture or elongational compliance of the polymers or to network rearrangements, the normal stress returns to zero or slightly positive values (data not shown). In contrast, polyacrylamide gels (2F) exhibit a positive normal stress over the entire range of deformations and do not show any evidence of strain-stiffening, since the stress is proportional to strain over a wider range of strains. The stiffest filaments like actin and collagen exhibit negative normal stress only over small strains before the sample fails, but softer polymers like neurofilaments and Matrigel exhibit negative normal stress at much larger strains where the normal stress magnitude may become greater than the shear stress.

The consistently large negative normal stresses that we observe can be understood in terms of a simple model that has been successfully used previously to quantitatively model strain-stiffening in the same biopolymer systems studied here. The basis of this model is the force extension curve of a single semiflexible filament, which has been shown to be highly nonlinear [3-5]. Specifically, under tension, such a filament resists extension in a way that increases much more rapidly than, for comparison, a simple Hookeian spring. This non-linear relationship is shown in Fig. 3A. For a network of randomly oriented filaments, an equal number of filaments can be expected to be (equally) stretched (e.g., the *red* filament in Fig. 3B) and compressed (the *yellow* filament in 3B) under shear. But, since those stretched filaments exert a greater tensile force than the compressive force of those under compression, we expect a net tension that results in a negative force normal to the plane of shear. In contrast, for linear, Hookean springs or filaments, the positive and negative contributions to the normal stress precisely cancel for small strains.

Furthermore, the fact that the normal and shear stresses are observed to be comparable when samples strain-stiffen is also a natural consequence of the non-linear force-extension of semiflexible filaments. For highly strained networks the stress is dominated by the most highly stretched filaments that are oriented approximately 45 degrees to the direction of shear (as illustrated by the *red* filament in Fig. 3B). Thus, by virtue of simple geometry, these filaments contribute equally to both normal and shear stresses. For smaller strains, however, we expect that, as for other materials, the shear stress is linear in the strain, while the normal stress is quadratic, as illustrated in Fig. 2F for polyacrylamide. For the biopolymer systems, however, not only is the normal stress negative, but the expected quadratic regime is too small to be apparent in Figs. 2A-E.

The precise details of the force-extension curve are worked out in the supplementary materials [3, 5]. In Fig. 3A we show the result for the dimensionless force as a function of the relative extension. Here, we see both a linear regime (much like a simple spring, in which force is proportional to the degree of compression/extension), as well as the strongly increasing tension for extensions exceeding some threshold. Under compression, the effective spring constant actually decreases. This force extension curve can be used to calculate the macroscopic shear [3, 5-7] and normal stresses [Supplemental Material] of a network of filaments subject to a given shear strain. This can be done assuming that the network deforms affinely, i.e., that the strain is everywhere uniform.

The concentration-dependence and relation of normal stress to shear stress allow more quantitative comparisons of the experimental results with predictions of the molecular theory for elasticity of semiflexible polymer gels. Figure 4A shows that the maximal magnitudes of shear stress and normal stress are both proportional to the concentration of polymers, or equivalently to the inverse square of the polymer mesh size [8]. This is expected for a microscopic model in which the maximal stress is determined by network failure corresponding to a characteristic maximal tension in each filament, independent of concentration [7]. The relation of normal stress to shear stress is shown in Figure 4B for fibrin and Matrigel. At large stresses, there is a linear relation between normal and shear stress, with a rounding off in the limit of small stresses. The latter is evidence of the expected (small) quadratic relationship between normal stress and both strain and shear stress in the linear regime. In contrast, the same plot for polyacrylamide shows a positive normal stress relationship in which a quadratic dependence is evident over a large strain range (Figure 4C). For comparison, the theoretical results for a network of semiflexible polymers are shown in Figure 4D, in which the normal stress is plotted as a function of the shear stress. Here, the expected linear relationship between shear and normal stresses is shown, with more or less of the expected quadratic dependence at small stresses, depending on the proximity of the strain-stiffening threshold.

Negative normal stress has been shown to occur in some complex fluids at high flow rates [9, 10]. But, to our knowledge this has not been reported for small quasistatic deformations of elastic of viscoelastic solids. A wide range of elastic solids, from metals to rubbers exhibit the Poynting effect [2], for instance, in which torsion of a wire or elastic rod results in an axial elongation. Although the elastic equations permit stable solutions with negative normal stress [11], Mooney-Rivlin theory of rubber elasticity predicts positive normal stresses [12], as seen in classic experiments by Rivlin and Saunders [13]. Here, we demonstrate a class of elastic materials that exhibit the inverse of the usual Poynting effect, together with a microscopic model that accounts for this result. The relationship between strain-stiffening and negative normal stress reported here is a natural consequence of the mechanical properties of thermally undulating semiflexible polymers crosslinked in isotropic networks, but the two features are not inextricably linked. Other geometries and force-extension relations of elastic elements might produce one effect without necessarily the other.

The negative normal stress of semiflexible polymer gels might be exploited in biological and technological contexts. In some settings such as the movement of micron-sized mitochondria through the crowded cytoskeleton of a narrow axon, shear rates are similar to those used for the studies in Figure 3, and a negative normal stress coincident with the shear deformation may facilitate organelle motion without distending the axon diameter. Another possible example is shear flow in a blood vessel above a fibrin gel at a local wound site that may help compress the gel to the vessel wall rather than dislodging it into the flowing stream. In any case, these stresses that we have shown to be generally as large as the shear stresses can be expected to significantly affect the overall force balance in the cytoskeleton. This work also suggests a design principle for the reduction of normal stresses, which can pose severe limitations in materials processing.

Acknowledgements

This work was supported by the US National Institutes of Health, the NSF MRSEC program, the CompInt program of the Elitenetzwerk Bayern, the (Netherlands) Foundation for Fundamental Research on Matter (FOM), and the NSF through the Kavli Institute for Theoretical Physics at the University of California. We are grateful to Tom Lubensky and Robert Bucki for helpful discussions, and to an anonymous referee for the references to Poynting and Rivlin.

References

1. Ferry, J. Viscoelastic properties of polymers (John Wiley, New York, 1980).
2. Poynting, J. H. On pressure perpendicular to the shear planes in finite pure shears, and on the lengthening of loaded wirtes when twisted. Proc. R. Soc. Lond. A 82, 546. (1909).
3. Storm, C., Pastore, J. J., MacKintosh, F. C., Lubensky, T. C. & Janmey, P. A. Nonlinear elasticity in biological gels. Nature 435, 191-4 (2005).
4. Bustamante, C., Marko, J. F., Siggia, E. D. & Smith, S. Entropic elasticity of lambda-phage DNA. Science 265, 1599-600 (1994).
5. MacKintosh, F., Käs, J. & Janmey, P. Elasticity of semiflexible biopolymer networks. Physical Review Letters 75, 4425-4428 (1995).
6. Gittes, F. & MacKintosh, F. Dynamic shear modulus of a semiflexible polymer network. Phys. Rev. E 58, R1241-R1244 (1998).
7. Gardel, M. L. et al. Elastic behavior of cross-linked and bundled actin networks. Science 304, 1301-5 (2004).
8. Schmidt, C. F., Barmann, M., Isenberg, G. & Sackmann, E. Chain dynamics, mesh size, and diffusive transport in networks of polymerized actin. A quasielastic light scattering and microfluorescence study. Macromolecules 22, 3638-3649 (1989).
9. Lin-Gibson, S., Pathak, J. A., Grulke, E. A., Wang, H. & Hobbie, E. K. Elastic flow instability in nanotube suspensions. Phys Rev Lett 92, 048302 (2004).
10. Montesi, A., Pena, A. A. & Pasquali, M. Vorticity alignment and negative normal stresses in sheared attractive emulsions. Phys Rev Lett 92, 058303 (2004).
11. Rivlin, R. S. Large elastic deformations of isotropic materials.II. Some uniqueness theorems for pure, homogeneous deformation. Philos. Trans. R. Soc. London, Ser. A 240, 491 (1948).
12. Rivlin, R. S. Large elastic deformations of isotropic materials. IV. Further developments of the general theory. Philos. Trans. R. Soc. London, Ser. A 241, 379 (1948).
13. Rivlin, R. S. & Saunders, D. W. Large Elastic Deformations of Isotropic Materials. VII. Experiments on the Deformation of Rubber. Philos. Trans. R. Soc. London, Ser. A 243, 251 (1951).
14. Wang, L. Z. et al. Purification of salmon clotting factors and their use as tissue sealants. Thromb Res 100, 537-48 (2000).
15. Leterrier, J. F., Kas, J., Hartwig, J., Vegners, R. & Janmey, P. A. Mechanical effects of neurofilament cross-bridges. Modulation by phosphorylation, lipids, and interactions with F-actin. J Biol Chem 271, 15687-94 (1996).
16. Morse, D. Viscoelasticity of tightly entangled solutions of semiflexible polymers. Phys. Rev. E 58, R1237-40 (1998).

Figure legends

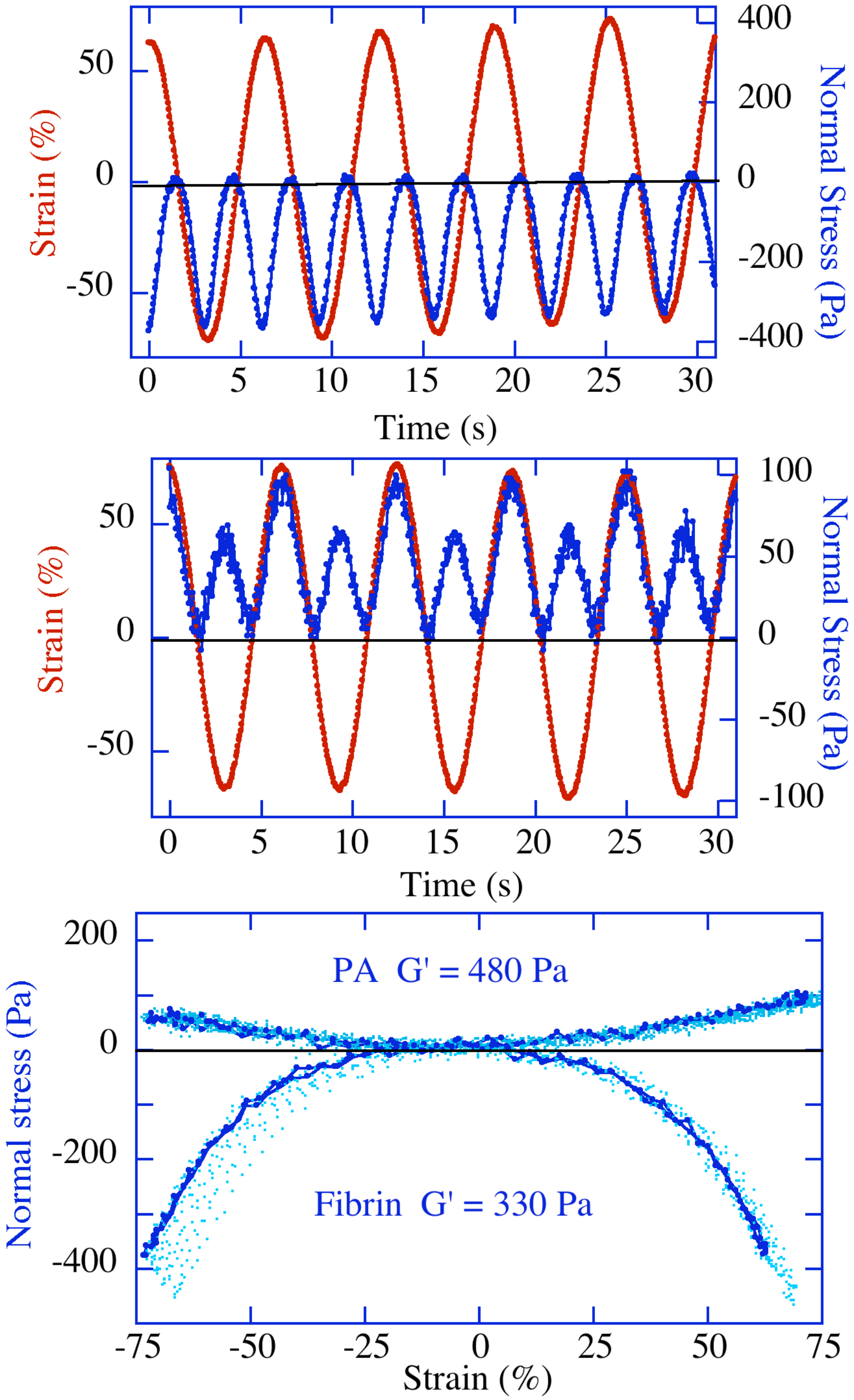
Figure 1 Relationship of normal stress to shear strain for semiflexible and flexible polymer hydrogels. Figure 1A shows the development of a negative normal stress (blue) during oscillatory shear strain (red) of a gel formed by the semiflexible polymer fibrin. The sign of the normal force in fibrin is opposite to that measured in a gel of the flexible polymer polyacrylamide (Figure 1B), and the magnitude of normal stress is greater for fibrin even though its shear modulus is smaller. Figure 1C shows the direct comparison of these two polymers made by overlaying a series of oscillations and plotting the normal stress vs. the shear strain. Measurements were made using a strain-controlled rheometer (RFS-III, Rheometrics). Analog data of shear strain, shear stress, and normal stress were collected from the rheometer using a Vernier analog/digital converter sampling the raw data from the rheometer transducers. The normal stress was determined from the measured rheometer thrust by dividing by the sample contact area. Sample preparation: Polyacrylamide (acrylamide (7.5%) and bis (0.02%)) polymerized with ammonium persulfate and TEMED by standard methods. Salmon fibrinogen [14] (10 mg ml^{-1}) was prepared according to [3].

Figure 2 Negative normal stress is a common feature of semiflexible biopolymer gels. Shear (red) and normal stresses (blue) are shown as the strain is gradually increased in gels made from actin crosslinked by biotin/avidin (A), collagen (B), fibrin (C), neurofilaments (D), Matrigel, a complex extracellular matrix containing mainly collagen and laminin (E) and polyacrylamide (F). Shear stress and normal stress values were continuously measured at a shear rate of 2.5% s^{-1}. Sample preparation: Bovine neurofilaments (2 mg ml^{-1}) were prepared as described [15] and polymerized with 5 mM $MgCl_2$; matrigel (BD Biosciences, Bedford, MA) in EMEM was polymerized at room temperature; type I Rat tail collagen (BD Biosciences, Bedford, MA) was diluted to 1 mg ml^{-1} at 4^{0}C in 1M NaOH, following by addition of 1:10 volume of 10X PBS to neutralize pH and heating to 37°C to trigger gelation; actin (4 mg/ml) , fibrin (8.75 mg ml^{-1}), and polyacrylamide (3%) gels were prepared as previously described [3].

Figure 3. Schematic diagram of the deformations leading to negative normal stress. The quantitative force-extension relation derived in refs [3, 5] is shown in 3A. Here, the force is measured in units of $\kappa\pi^2/L^2$, where κ is the bending stiffness and L is the end-to-end length of a filament strand, e.g., between crosslinks of the network. The extension is measured relative to the full extension. As shown in 3B, in simple shear of an isotropic network of semiflexible polymers, some filaments are elongated (red filament), while an equal number are compressed (yellow filament). Due to the non-linear force-extension relation for semiflexible filaments, stretched filaments exert more force than the compressed ones, which leads to a negative net tension in the direction orthogonal to the shear direction. In this case, the tension is along the vertical axis.

Figure 4 Concentration dependence of normal stress in fibrin gels and comparison with theoretical predictions. Figure 4A shows the relationship between fibrin concentration and the magnitudes of maximal shear stress and normal stress reached just before the sample fails. Figures 4B and 4C compare the relation of normal stress to shear stress for fibrin (10 mg ml^{-1} circles 4B) Matrigel (triangles 4B) and polyacrylamide (7.5%, 4C) gels developed during oscillatory deformation of 75% maximal strain amplitude. Error bars depict standard deviations from averages of 5-10 oscillations. Figure 4D shows the predicted relation between shear and normal stresses for a network formed from semiflexible polymers as depicted in Figure 3, based on the model of ref [3].

Figure 1.



Strain (%)
50
0
-50
400
200
0
-200
-400
Normal Stress (Pa)
0
5
10
15
20
25
30
Time (s)
Strain (%)
50
0
-50
100
50
0
-50
-100
Normal Stress (Pa)
0
5
10
15
20
25
30
Time (s)
Normal stress (Pa)
200
0
-200
-400
PA G' = 480 Pa
Fibrin G' = 330 Pa
-75
-50
-25
0
25
50
75
Strain (%)

Figure 2

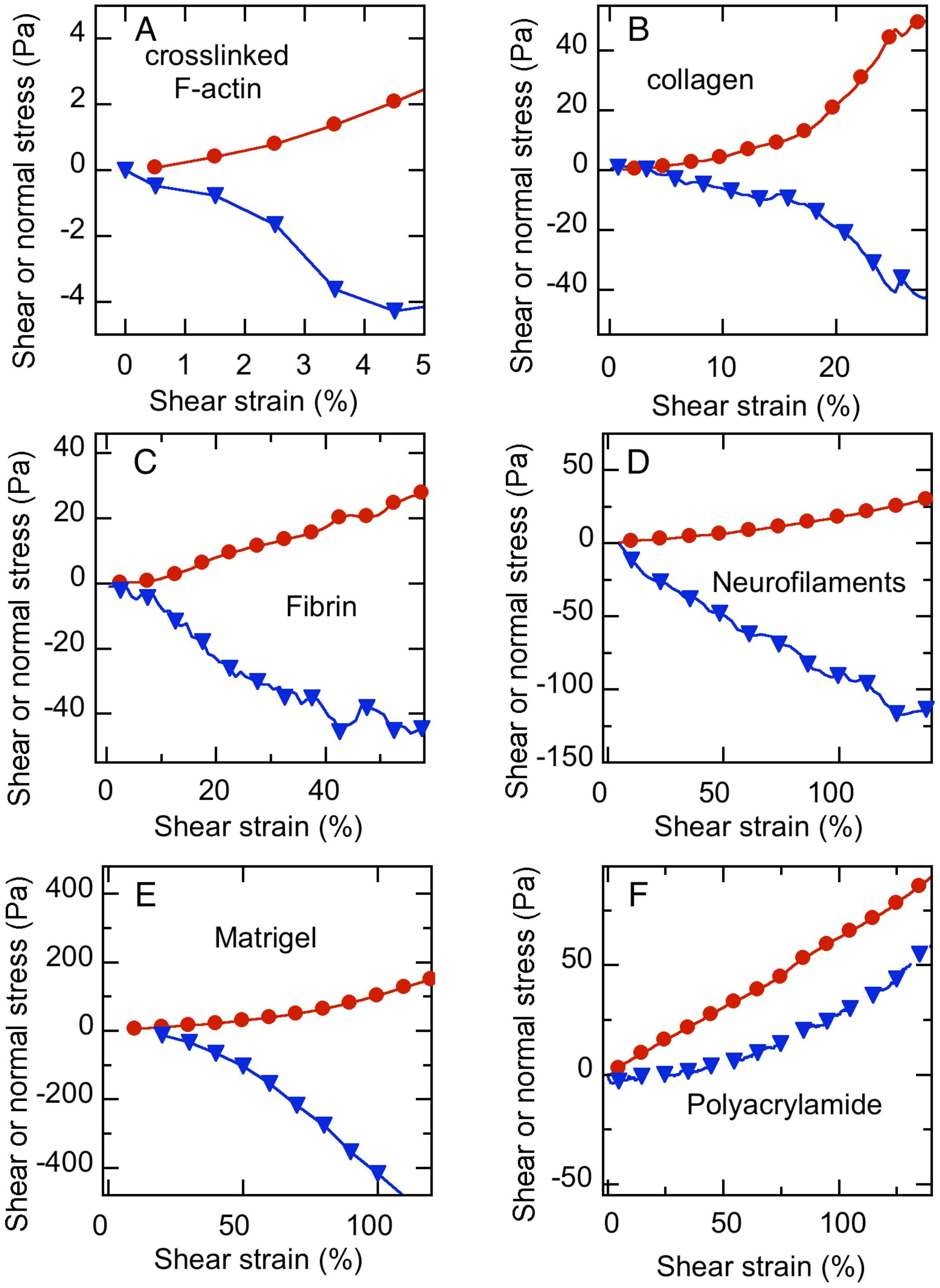

A
crosslinked F-actin
Shear or normal stress (Pa)
4
2
0
-2
-4
0
1
2
3
4
5
Shear strain (%)
B
collagen
Shear or normal stress (Pa)
40
20
0
-20
-40
0
10
20
Shear strain (%)
C
Fibrin
Shear or normal stress (Pa)
40
20
0
-20
-40
0
20
40
Shear strain (%)
D
Neurofilaments
Shear or normal stress (Pa)
50
0
-50
-100
-150
0
50
100
Shear strain (%)
E
Matrigel
Shear or normal stress (Pa)
400
200
0
-200
-400
0
50
100
Shear strain (%)
F
Polyacrylamide
Shear or normal stress (Pa)
50
0
-50
0
50
100
Shear strain (%)

Figure 3

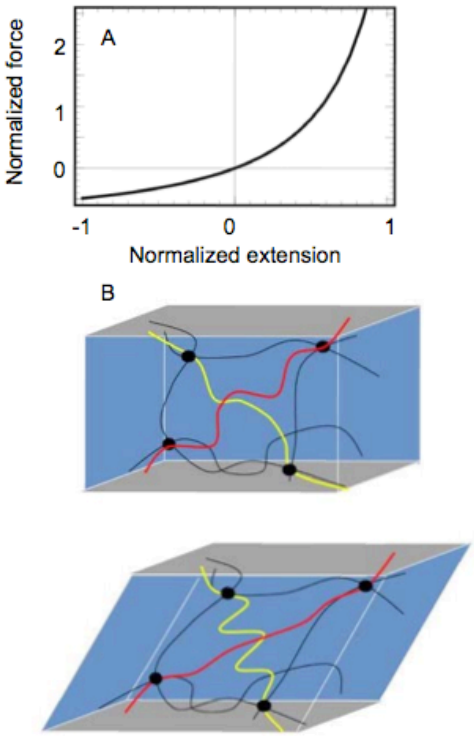
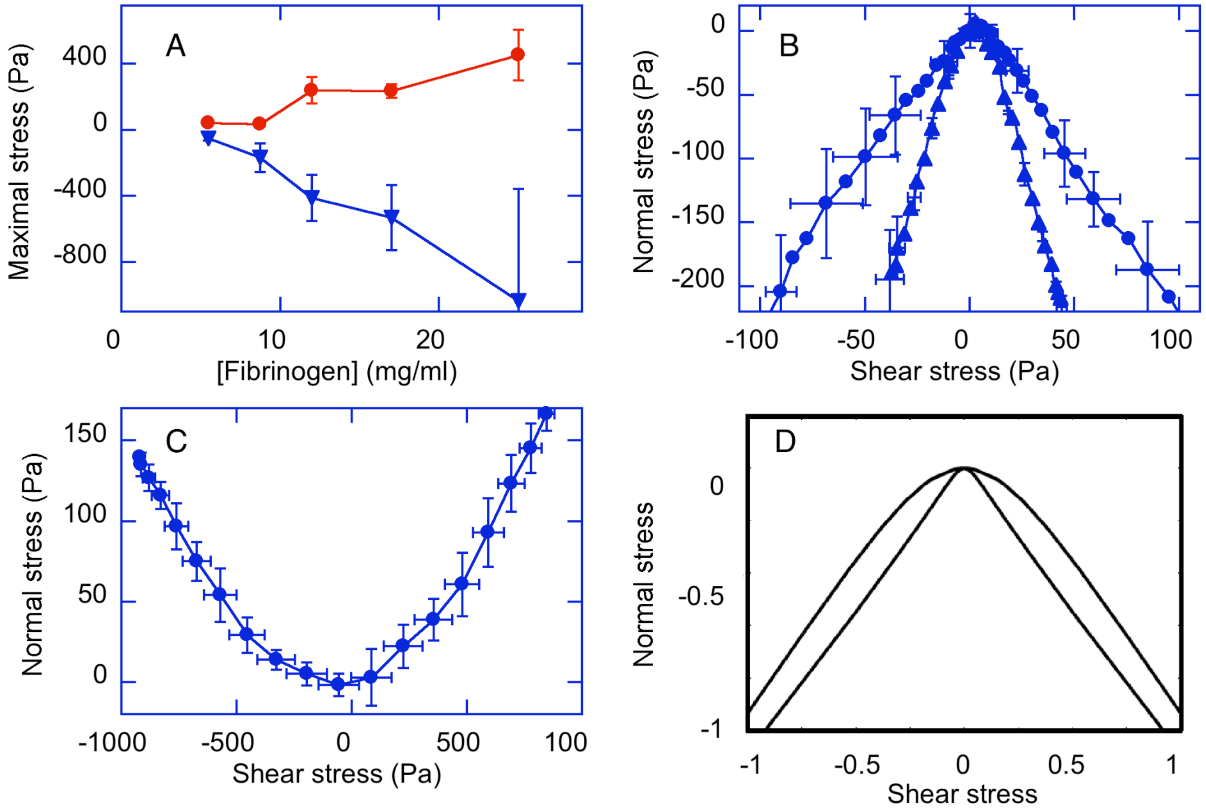


Figure 4


A
Maximal stress (Pa)
400
0
-400
-800
0
10
20
[Fibrinogen] (mg/ml)
B
Normal stress (Pa)
0
-50
-100
-150
-200
-100
-50
0
50
100
Shear stress (Pa)
C
Normal stress (Pa)
150
100
50
0
-1000
-500
0
500
100
Shear stress (Pa)
D
Normal stress
0
-0.5
-1
-1
-0.5
0
0.5
1
Shear stress

Supplemental Material

S1 – Theory

Following Ref. [5], we model a single semiflexible polymer segment of length L with an energy given by

$$E = \int_0^L \left(\tfrac{1}{2}\kappa \left|\nabla^2 u\right|^2 + \tfrac{1}{2}\tau \left|\nabla u\right|^2 \right) dx$$

where $\kappa = k_B T \ell_P$ is the bending rigidity, ℓ_P is the persistence length, and τ is the tension in the filament. Here, $u(x)$ represents the transverse displacement of the filament, *e.g.*, relative to the line between the endpoints. In fact, the filament can deflect in two independent directions relative to the end-to-end line. We assume that these transverse deflections are the only degrees of freedom. In the limit that L is much less than ℓ_P, the rod is nearly straight, and we will not distinguish between the contour length of the filament segment L_c and the end-to-end distance L, at least for purposes of the integral above. We can, however, calculate the (small) difference $\Delta\ell = L_c - L$ using the integral

$$\Delta\ell = \int_0^L \tfrac{1}{2} \left|\nabla u\right|^2 dx$$

The chain conformation can be decomposed into Fourier modes:

$$u(x) = \sum_q u_q \sin(qx),$$

where we include wavevectors $q = n\pi / L$, accounting for fixed ends of the chain segment. For the harmonic energy above, the mean-square amplitudes $\left\langle \left| u_q \right|^2 \right\rangle$ can be calculated using the equipartition theorem of statistical mechanics, the result of which is [5]

$$\Delta\ell = k_B T \sum_q \frac{1}{\kappa q^2 + \tau}$$

where we have accounted for the two independent directions for filament deflections relative to the line between the ends.

This is actually a calculation for the thermal average end-to-end distance as a function of tension $\langle \Delta L(\tau) \rangle$, about which there will be thermal fluctuations. This result can be calculated analytically. It is convenient to express this relative to the end-to-end distance at zero tension. This *extension* $\delta\ell(\tau) = \langle L(\tau) \rangle - \langle L(0) \rangle$ can be calculated analytically, and can be numerically inverted to yield the force/tension $\tau(\delta\ell)$ as a function of extension $\delta\ell$. This is shown in Fig. 3A.

From this force-extension relation, the shear stress can be obtained for a given strain γ assuming that the strain field is uniform (affine) [3, 5-7, 16]. If the shear is in the x-direction, for a filament with an orientation given by the usual polar and azimuthal angles θ and ϕ, the contribution to the shear stress for such a filament under tension is $\sin(\theta)\cos(\phi)\tau$. In a shear plane, however, there is a number density of these filaments given by $\cos(\theta)\rho$, where ρ is the density of chains measured in length per unit volume. The tension also depends on the orientation of the chain segment, since the extension of the chain is given by $\delta\ell = \gamma L\cos(\phi)\sin(\theta)\cos(\theta)$. Thus, the shear stress is given by the integral (over all angles $0 \le \theta \le \pi$ and $0 \le \phi \le 2\pi$)

$$\sigma_{xz} = \int \sin(\theta)\cos(\theta)\cos(\phi)\tau[\gamma L\sin(\theta)\cos(\theta)\cos(\phi)]\sin(\theta)d\theta d\phi .$$

The calculation for the thrust as measured by the rheometer is similar, except that it is the z-component of tension $\cos(\theta)\tau$, resulting in the integral

$$\sigma_{zz} = \int \cos(\theta)\cos(\theta)\tau[\gamma L\sin(\theta)\cos(\theta)\cos(\phi)]\sin(\theta)d\theta d\phi .$$

This is sufficient to calculate the thrust only in the quasi-static (zero frequency) limit, since there will also be a non-zero component of the tension in the x-direction, i.e., in the direction of shear. Ordinarily this also contributes to the measured thrust. This gives, for instance, hoop stresses in a cone-and plate rheometer. These hoop stresses are balanced by a radial pressure gradient in incompressible samples, resulting in a positive contribution to the thrust. The relative openness of biopolymer gels, however, means that these hoop stresses can relax more quickly than for denser flexible gels. Our calculations here are for the quasistatic or zero frequency limit.