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The mechanical properties of fibrin for basic scientists and clinicians

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Abstract

In this review, I set forth some basic information about the mechanical properties of fibrin clots and attempt to identify the big questions remaining. The intent is to make this topic understandable to both basic scientists who are interested in blood clotting and to hematologists or cardiologists, since I believe that this is something everyone working in these fields should know. The viscoelastic properties of fibrin are remarkable and unique among polymers. Moreover, these properties are essential to the physiology of blood clotting and are important for understanding and therefore preventing and treating thrombosis.
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1. Introduction

In my experience, most biologists, biochemists, hematologists or cardiologists do not know much about the mechanical properties of fibrin. Perhaps this topic is the least understood aspect of fibrin. Why is this? Maybe it is because this subject comprises very biophysical aspects, such that the language and techniques are foreign to most scientists outside of polymer chemistry. Structural biology may be almost as alien, with the difficulty of understanding reciprocal space in X-ray crystallography or the methods of specimen preservation for electron microscopy, but in this case the end result, i.e. the images, are more immediately comprehensible.

I think that some of the results described here will be surprising, even to experts on other aspects of fibrin. Probably the extent of what we do not yet know about this topic will also be unexpected. We do not know the answers to some of the most fundamental questions in this field. On the basic science side, we do not even know the origin of the viscoelastic properties of fibrin. On the clinical side, we know almost nothing about the mechanical properties of in vivo clots or thrombi, even though these properties are critical for the physiological function of fibrin and for the treatment of pathological conditions, such as bleeding and thrombotic disorders, including angioplasty and thrombolytic therapy. I will begin by describing briefly reasons why I think both scientists and clinicians should have a rudimentary understanding of the mechanical properties of fibrin.

2. Why are the mechanical properties of fibrin important?

The mechanical properties of fibrin are essential for its functions. In hemostasis, the clot must form a plug to stop bleeding and this structure must be strong enough to withstand the pressure of arterial blood flow. In the case of thrombus formation, the mechanical properties are also important. If a vessel is partially occluded, the viscoelastic properties of the thrombus will determine whether the flowing blood will cause it to deform reversibly or irreversibly, rupture, or embolize. Furthermore, the mechanical properties of a thrombus, determined by all the components including fibrin, will

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determine how it responds to treatments, such as coronary artery angioplasty.

Epidemiological studies have demonstrated a relationship between myocardial infarction and clot mechanical properties. In vitro formation of fibrin clots from patients with myocardial infarction show tight and rigid fibrin network structures compared to controls [1,2]. Clots that are very stiff could be more friable or clots that are more viscous or plastic could have a greater tendency to embolize, although almost nothing is known about the relationship between mechanical properties of fibrin and these pathological properties. There is little known of the relationships between fibrin's mechanical properties and rates of fibrinolysis, although this is an important issue for thrombolytic therapy.

Embolization of thrombi is one of the most important issues facing clinicians today, especially because of the consequences of stroke and pulmonary damage. It is not known why some individuals with deep vein thrombosis or atrial fibrillation tend to embolize and others do not. The answers to these questions may lie in understanding the mechanical properties of fibrin.

Some clinicians have used thromboelastography or similar methods for many years to evaluate the coagulation status of patients. This rapid method yields parameters that are related to the viscoelastic properties of whole blood clots so that corrections to therapy can be made. A distinct advantage of the use of thromboelastography is that it begins to gather information at the point that more commonly used clinical tests, such as thrombin time, PT and PTT, end. The time course of development of the physical character of the clot, rather than some kind of initiation time measured by these other methods, can be more useful clinically. Furthermore, the mechanical properties measured by thromboelastography, the strength and integrity of the clot, are what matters most in effective hemostasis. On the other hand, many of the parameters measured by thromboelastography have not been related to the normal viscoelastic properties used to characterize materials and have no physical meaning except in comparison with other such thromboelastography measurements.

Finally, I think that investigation of the viscoelastic properties of fibrin is a subject that requires interactions between basic scientists and clinicians. There is as yet little understanding of the clinical significance of the mechanical properties of fibrin that are known. At the same time, there is even less known of the basic mechanisms related to important clinical phenomena such as the mechanical properties of clots necessary for hemostasis, fibrinolysis, the deformation of thrombi in flow, embolization, and many others.

3. What kind of polymeric structure is a fibrin clot?

I assume that readers know major aspects of fibrinogen structure and fibrin polymerization. If not, there are good reviews available. Here I only summarize some basics. Fibrinogen is an elongated protein, 45 nm in length, and is made up of globular domains at each end connected by α-helical coiled-coils to a globular region in the middle. During clotting, thrombin converts fibrinogen to fibrin by cleaving fibrinopeptides from the central domain, exposing knobs that can then interact with holes that are always exposed at the ends of the molecule, giving rise to a half-staggered structure called the protofibril, which has a periodicity of 22.5 nm. Cleavage of the A fibrinopeptides exposes the glycine-proline-arginine sequence, while cleavage of the B fibrinopeptides exposes the glycine-histidine-arginine sequence. When protofibrils grow sufficiently long, they aggregate laterally to form fibers.

Fibers are twisted structures [3] (Fig. 1). Protofibrils are twisted, and when they aggregate in a specific manner with each other to make a fiber, they twist around each other [4]. Because the periodicity of 22.5 nm is maintained, as new protofibrils are added to the fiber, they must be stretched as the path length increases with fiber diameter. Thus, protofibrils on the exterior are under tension. Fibers stop growing when the energy required to stretch an added protofibril exceeds the energy of bonding [3]. The fact that fibers are under tension accounts for the observation that fibers making up a clot are very straight under most circumstances, as observed by confocal microscopy of native, hydrated clots (Fig. 2A). Furthermore, fibers being under tension is likely to have important consequences for the mechanical properties of the clot, although these consequences are still largely unknown.

Fibrinogen is a protein present at high abundance in the blood, with an average concentration of about 2.5 g/l. Under appropriate conditions, plasma will rapidly clot to form quite a stable structure mechanically. In fact, a stable gel can be formed at fibrinogen concentrations at least 100 fold lower. As a result, it may be startling to realize that in a clot made from fibrinogen at a concentration of 2.5 g/l, the fibrin is only 0.25 g out of 100 ml (or g) of volume. In other words, the fibrin constitutes 0.25% of the volume in a clot, so 99.75% of the clot is liquid occupying the space between the protein polymers. There are large spaces between the protofibrils in the fibers and much larger spaces between the fibers. The properties of this polymer are even more remarkable considering the small volume of material involved.

Fig. 1. Scanning electron micrograph of fibers in a fibrin clot. The surface appearance of the fibers shows that they are twisted structures. Note the appearance of twisting in the vicinity of the branch point. Magnification bar=1 μm.

4. Branching of fibers makes the clot a network

Some proteins polymerize to make fibers, but the fibers do not form a network. For example, type I collagen and actin assemble into fibrous structures but only form a network through the intervention of other proteins. On the other hand, other proteins, such as gelatin and elastin, yield networks through intermolecular linkages but without making fibers. Ferry proposed that an interspersed array of individual fibers form the basis of the fine fibrin gel [5]. However, subsequently he and other investigators showed that fibrin fibers branch in both fine and coarse clots [6–8]. Fiber branching is what produces a three-dimensional network. In fact, fiber ends are seldom seen in an undamaged, normal fibrin clot [8]. It is this three-dimensional network that gives fibrin its character as a gel that we are discussing here.

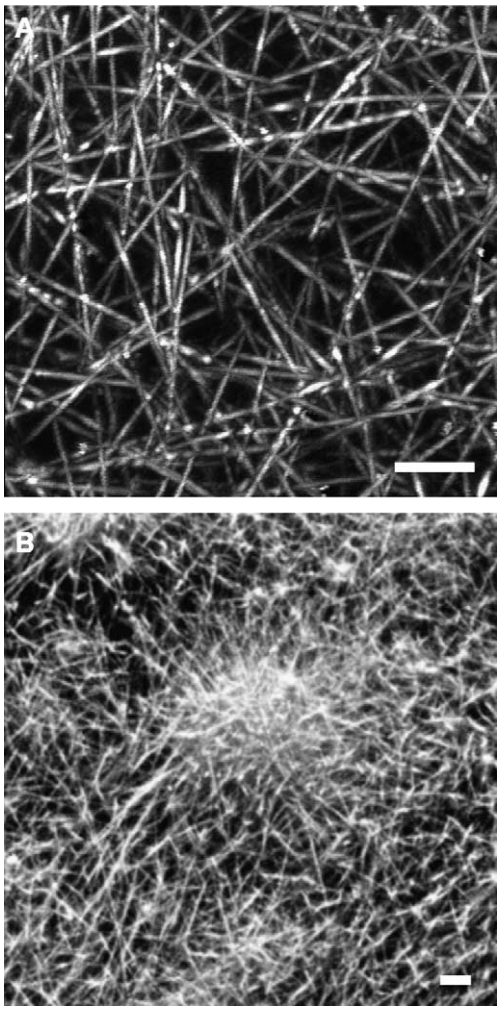

Fig. 2. Confocal light micrographs of fibrin clots labeled with colloidal gold. These are three-dimensional reconstructions of several optical sections, so it is difficult to distinguish branch points from fibers passing each other at different depths in the clot. Note that the fibers making up the clot are nearly all straight with very little or no curvature. (A) Clot made by addition of thrombin to platelet poor plasma. Magnification bar=5 μm. (B) Clot made from addition of thrombin to platelet rich plasma. Fibers are very dense around the platelet aggregates, with fibers radiating out from the platelets. Magnification bar=10 μm (Micrographs courtesy of Dr. Jean-Philippe Collet, Institut de Cardiologie, Pitié-Salpêtrière Hospital, Paris, France).

To interpret the results of viscoelastic measurements, it is important to understand what a branch point in a clot is, since this feature is what makes the fibers into a stable network. A quantitative study of clot structure demonstrated that nearly all branch points in clots are made up of three fibers at a junction [9]. The distances between branch points and the total number of branch points can vary dramatically, depending on many variables of fibrinogen structure and polymerization conditions. Although published electron and light micrographs clearly show branching fibers, the substructure of the branch point has not been much documented. Electron microscopy of fibers in a fine clot, together with mass measurements, demonstrated the existence of tetramolecular branch points, in which two protofibrils diverge, and trimolecular branch points, in which each of the three strands is the width of a single protofibril [10,11]. It was shown that trimolecular junctions predominate in fine clots made up of very thin fibers, but these structures, as well as tetramolecular junctions, may also arise at early stages of coarse clot formation.

Negatively contrasted fibrin reveals the substructure of the fibers since the stain distribution is directly related to protein density [12]. Stain is excluded from regions of high protein density, making them appear bright, while more stain penetrates regions of lower protein density, making them appear darker, such that fibrin fibers have a 22.5 nm periodicity with a typical band pattern [12]. The results of a new investigation of negatively contrasted thick fibrin fibers reported here reveals the structure of branch points in a coarse clot (Fig. 3). Nearly all of the branch points observed consisted of the junction of three fiber segments, as expected [9]. Even the more complex structures (Fig. 3C) can usually be analyzed as a combination of such trifunctional junctions. The angle between two diverging fibers at a branch point was usually small, with the other two angles being large. The band patterns of the diverging fibers were commonly aligned. All of these results are consistent with the structural origin of branch points as the divergence of fibrils that were associated with each other to make up a fiber. The diameters of the three segments making up the branch point were generally about the same, but there were differences sometimes. These results suggest that the branch points are formed early in polymerization and additional protofibrils are added to all parts of the network, rather than being made from the association of fully formed fibers.

From these results and extensive studies of many different clot structures, we can generalize concerning the nature of branching. Protofibrils aggregate laterally to form fibers, but sometimes they diverge, producing a branch

point. Thus, lateral aggregation and branching are basically opposite from each other. In other words, conditions that favor lateral aggregation tend to produce clots made of thick fibers with few branch points, while conditions that inhibit lateral aggregation tend to yield clots made up of thin fibers with many branch points (Fig. 4).

5. Fibrin is a viscoelastic polymer

Elastic solids are characterized by Hooke's law, which states that the strain, or deformation, is proportional to the stress, or force applied per area, but stress is independent of the *rate* of strain. On the other hand, in the classical theory of hydrodynamics, viscous materials are characterized by Newton's law, which says that the stress is proportional to the rate of strain but independent of the strain itself. Viscoelastic materials, such as fibrin, like rubber, plastic, and a great many polymers, have different degrees of both elastic and viscous properties [13]. Thus, viscoelastic polymers are often characterized by two different parameters, one representing the elastic properties and the other representing the viscous properties. For example, an elastic or storage modulus may be used to characterize the stiffness of the polymer, while a loss modulus or creep compliance may be used to characterize the inelastic or irreversible component.

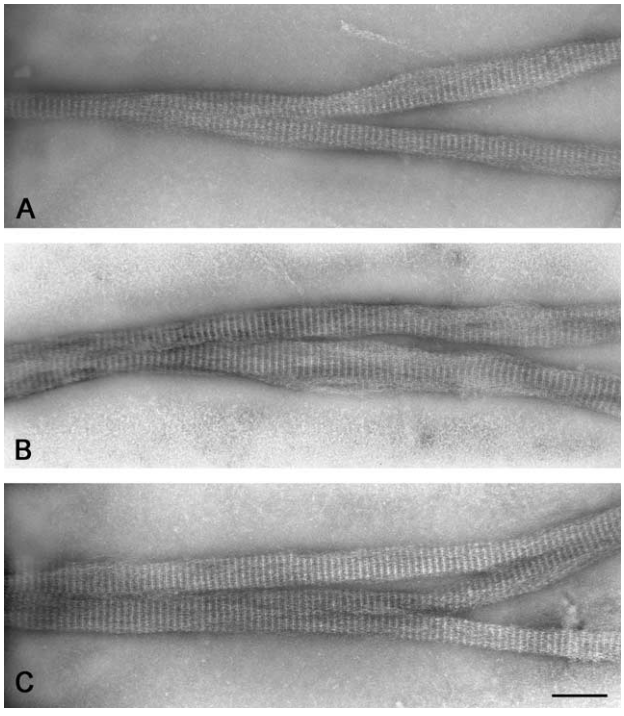

Fig. 3. Transmission electron micrographs of negatively contrasted fibrin fibers that show the substructure of branch points. Most branch points consist of three fiber segments of nearly equal diameters that join at a small acute angle with band patterns aligned. The band pattern with a repeat of 22.5 nm is characteristic of fibrin. Magnification bar=0.2 μm.

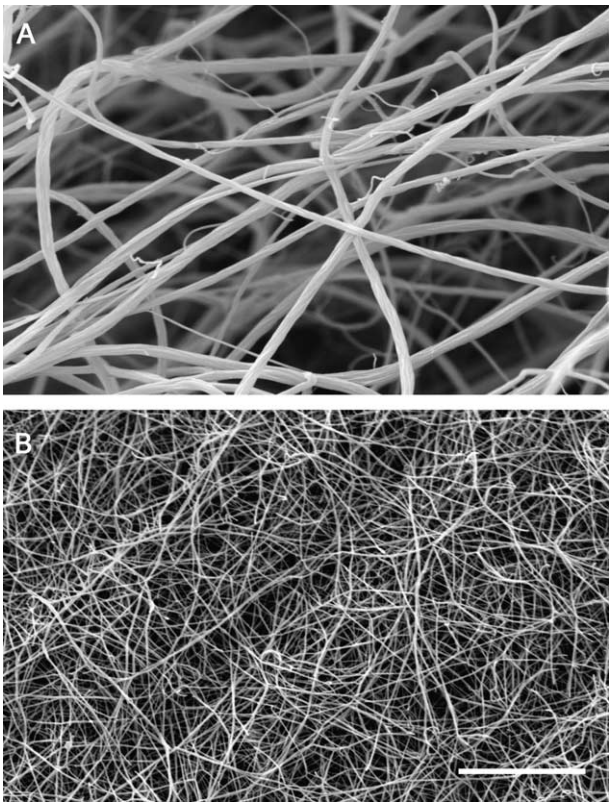

Fig. 4. Scanning electron micrographs of fibrin clots. (A) Clot with thick fibers and few branch points made from recalcified plasma with low thrombin concentration. (B) Clot with thin fibers and many branch points made from recalcified plasma at high thrombin concentration. Magnification bar=5 μm.

These parameters will determine how the clot or thrombus responds to the forces to which it is subjected, particularly flowing blood. For example, a very stiff clot will not deform as much as a less stiff one with the same applied stress. A clot with a greater inelastic component will incur more permanent deformation in response to flowing blood than a clot with a greater elastic component, which will return to its original shape when the stress is relieved.

Viscoelastic properties are commonly measured by applying a stress, or force per unit area, to the polymer and determining the resulting strain, which is the stretching or distortion of the polymer normalized with respect to total length. The resulting stress-strain curve is used to determine the ratio of the stress required to produce a certain strain, which is called the elastic modulus, a parameter independent of the object's shape and size that is used to characterize materials [13,14] (Fig. 5). A large modulus means that a large stress is necessary to produce displacement, so that object is stiff, while a small modulus means that less stress is required, so this other object is less stiff. These measurements can be made either statically (without movement) or dynamically (e.g., oscillatory motion), and different geometries can be employed [13].

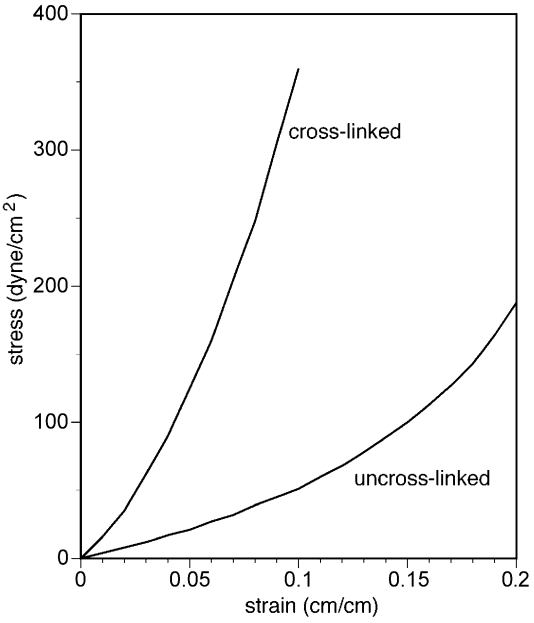

Fig. 5. Stress-strain curves for cross-linked and uncross-linked fibrin. Stress is plotted versus strain at peak strain in sinusoidal shear strain measured for cross-linked and uncross-linked fibrin clots in 0.05 M Tris–HCl, pH 7.5, 2.4 mM $CaCl_2$, 0.165 M NaCl, 3.8 mg/ml fibrinogen. Elastic moduli can be calculated as the slopes of such curves. Some strain hardening is evident for uncross-linked fibrin at higher strains. In other words, the elastic modulus increases with strain (from Ref. [14]).

A common configuration is to form the clot between two circular disks or a cone and circular plate, with the bottom plate being stationary and the top plate being suspended as a pendulum so that it can rotate in a torsional manner. In this case, the stress is said to be a shear stress, where shear is defined as the tendency for one part of an object to slide with respect to another. Static measurements would then be made by rotation of the top plate to apply a shear stress and measuring the strain. Dynamic measurements would be made by either free or forced oscillatory movements of the pendulum. The inelastic or viscous component can be measured in the static system by applying a strain and then measuring the decay in stress over time as creep occurs, i.e. rearrangements of the structure that relieve the stress. In dynamic measurements, the irrecoverable component is determined from the decay of the amplitude of the oscillations, which occurs because of these irreversible processes, or from the difference in phase between stress and strain in forced oscillations.

6. What are the elastic properties of fibrin?

A finite elastic modulus or stiffness appears at about the gel point or clotting time, when the network is first established [14–16] (Fig. 6). The gel point comes early in clotting, perhaps when only about 10% of the protein has been incorporated, depending on the environmental conditions. The network that is formed at the gel point is a scaffold that sets the plan for the clot structure formed by addition of further material [17]. Then, the stiffness increases as a function of time, much as the turbidity of the clot increases. However, it appears that the stiffness may be slower to reach a maximum, since it can take hours to reach a maximum, well after the gel point. It appears that a slow re-arrangement may sometimes take place in fibrin, since under certain conditions the rigidity may change slowly over more than 24 h [5].

The elastic moduli or stiffness of clots vary greatly depending on clot structure, and can be modulated by physiological conditions. The reported moduli range from less than 1 dyne/cm^2 to about 15,000 dyne/cm^2. The elastic moduli are about 2–4× greater for clots formed from thrombin than for those formed with cleavage of only the A fibrinopeptides [18].

Many experiments have been done using special types of clots because the results are somewhat easier to interpret. The most common of these are called fine clots, made at pH 8.5 and ionic strength 0.45 and consisting of very thin fibers. Fine clots are simpler structures because they are made up of very thin fibers, so that the inelastic component is small compared to the elastic component. Another model system, fibrin film, made by compression of a clot to form a thin, nearly planar structure, is easier to study because it is two-dimensional.

For small strains or deformations of fine clots, over a time scale of 1–100 s, Hooke's law is strictly obeyed and there is no inelastic component [5]. Experiments with shearing deformations at different frequencies allow the characterization of the viscoelastic behavior over different time scales of deformation, i.e. slow or fast. The storage modulus (G'), or stiffness, of fine clots is nearly constant over almost eight decades of frequency [19,20] (Fig. 7), which is remarkable and probably unmatched by any other material with such a low modulus of elasticity. For most polymers, the elastic modulus varies considerably with the

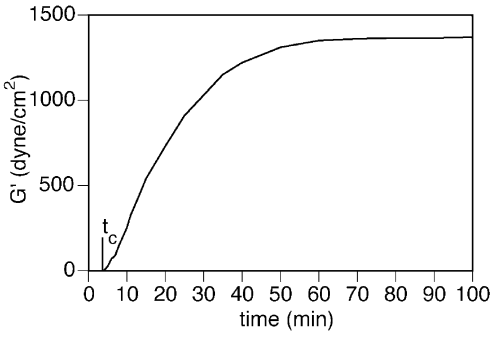

Fig. 6. Plot of elastic modulus versus time for fibrin clot formation with Factor XIIIa cross-linking. Increase in storage modulus with time for a clotting mixture containing 3.8 mg/ml fibrinogen, 0.05 M Tris–HCl, pH 7.5, 0.125 NaCl, 2.4 mM $CaCl_2$, and Factor XIIIa. t_c is the clotting or gelation time. A finite stiffness appears at about the gelation point and increases over time, somewhat like the better known increase of turbidity, but this is not a sigmoidal curve (replotted with a linear rather than log time scale from Ref. [14]).

frequency [13]. The stiffness of coarse clots changes somewhat with frequency (Fig. 7).

The fact that fibrin's modulus is constant with frequency means that the stiffness of fibrin is the same no matter what the rate of application of stress. In other words, the stiffness of the clot remains constant under different physiological and pathological conditions that result in changes of flow rate. In the body, the rate of application of stress is likely to vary tremendously, depending on whether the clot or thrombus is in the arterial or venous system, conditions at the site of injury and the presence of any pathology. I think that the fact that the stiffness is constant over such a wide range of frequencies indicates that the value of the clot stiffness must be important physiologically, although we do not know why.

7. The origin of clot stiffness is unknown

Superficially, the measurements of fibrin elastic and inelastic components resemble those of some types of rubber, except for the magnitudes and frequency range [13]. However, clot elasticity cannot be rubber-like. Electron microcopy shows thick branching fibers (Fig. 4), instead of a random-coil network with highly flexible strands required for rubber-like elasticity. Furthermore, the average mass between branch points can be calculated, using the formula for a rubber-like polymer, $Mc=cRT/G$, where Mc is the average molecular weight between branch points in a network and c is the concentration of fibrin [21]. For a typical coarse, uncross-linked clot with c=0.003 g/ml and G=2500 dyne/cm^2, Mc=30,000 Daltons, or only about 1/10 of the molecular mass of the monomer unit, one fibrin molecule. In other words, based on the stiffness of fibrin, we would expect about 10 branch points per fibrin molecule. In contrast to this rubber-like model, knowing the structure of fibrin we can determine that such a clot would have perhaps a half million fibrin molecules between branch points. Thus, the calculation based on rubber-like elasticity is off by about seven orders of magnitude, illustrating the ridiculousness of claims of rubber-like elasticity of fibrin.

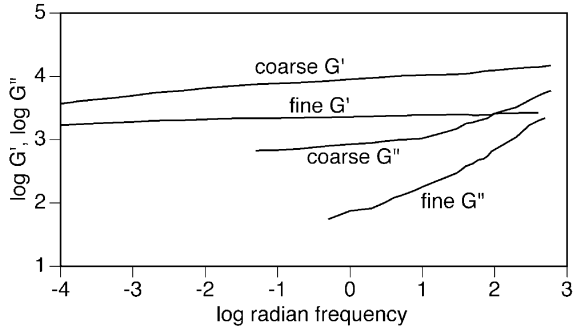

Fig. 7. Storage shear moduli (G') and loss shear moduli (G''), plotted logarithmically against radian frequency for fine and coarse uncross-linked clots. Storage moduli represent stiffness, whereas loss moduli correspond to the inelastic component of deformation. At low frequencies, loss moduli were calculated from creep measurements. Fibrinogen concentration was 9.4 mg/ml for all experiments. Fine clots were prepared at pH 8.5, 0.45 ionic strength, 4 U/ml thrombin; coarse clots were prepared at pH 7.5, 0.15 M ionic strength, 0.8 U/ml thrombin. Both storage and loss moduli are greater for coarse clots than for fine clots. The constancy of stiffness over many decades of frequency (i.e. slow and fast changes of deformation) is remarkable. The stiffness of fine clots is nearly invariant with frequency, while there is a slow change for coarse clots. The magnitude of inelastic deformation is small at low frequencies (slow changes) but increases at high frequencies (faster processes). (replotted, combining figures from Refs. [19,20]).

It has been suggested that the elasticity of fibrin may arise from the bending of fibers [22]. Part of the basis for this proposal is that fibrin fibers are likely to be stiffer with respect to stretching than bending. This bending hypothesis needs to be critically tested.

Some of the relationships between clot structure and mechanical properties have been investigated. Network morphology was manipulated by varying the concentrations of fibrinogen, thrombin and calcium ion, and cross-linking was controlled by a synthetic, active-center inhibitor of Factor XIIIa (see below) [9]. Quantitative measurements of network features (fiber lengths, fiber diameters, and fiber branching densities) were made by analyzing computerized, three-dimensional models constructed from stereo pairs of electron micrographs [23]. Large fiber diameters and lengths were established only when branching was minimal, and increases in fiber length were generally associated with increases in fiber diameter. Viscoelastic properties of the clots were measured and correlated with structural features of the networks. At constant fibrinogen, but varying thrombin and calcium concentrations, maximal rigidities were established in samples (both cross-linked and uncross-linked) that displayed a balance between large fiber sizes and great branching. Clot rigidity was also enhanced by increasing fiber and branch point densities at greater fibrinogen concentrations. Such studies allow us to begin to understand the structural basis of the mechanical properties of clots, but much remains unknown.

8. What are the inelastic properties of fibrin?

An elastic material with no inelasticity will deform quickly with applied stress, maintain constant deformation for long periods of stress and immediately regain its initial shape when the stress is removed. In contrast, an inelastic material with viscous energy loss will show delays in deforming with applied stress and regaining its shape afterward and will undergo continued deformation, or creep, during sustained application of constant stress.

At low frequency of oscillation (or slow changes), the inelastic component of deformation (G'') is small and clots show only elastic deformation [19,20] (Fig. 7). At higher frequencies, irreversible processes appear and increase in magnitude with frequency until they become nearly equal in magnitude to reversible deformation (i.e., $G'' \approx G'$). The

inelastic component of deformation can also be measured by creep experiments, in which continued changes in strain are measured over time after application of shearing stress. In fibrin, creep occurs over very long times and substantial irreversible deformation takes place.

If the time during which the clot is stressed is of the order of an hour or less, the clots made of thicker fibers incurred more irreversible deformation than clots made of thinner fibers [19,20]. This creep has been tentatively attributed to slippage of protofibrils past each other.

On the other hand, for stressing times of up to a day, fine clots had more irrecoverable deformation than coarse clots and behaved like a high viscosity viscolastic liquid with a long relaxation time. It has been speculated that this slower viscous deformation arises from slow dissociation of the complementary binding sites holding protofibrils together and re-formation of these junctions in unstressed configurations [18].

From these results, it might be assumed that there is considerable damage to the clot in such experiments, since by definition plastic deformation is irreversible. However, surprisingly the recovery of the original stiffness after removal of the stress was nearly complete [24]. More remarkably, the differential modulus of elasticity measured during the course of a creep experiment by applying a brief on-off step stress remained constant throughout the entire time. This result shows that there are no net changes in structure during whatever rearrangements are responsible for the creep. In other words, fibrin is a "self-repairing" structure.

Although the physiological consequences of these inelastic properties of fibrin are unknown, I will speculate. Irrecoverable deformation means that the polymer can be reshaped as a result of stresses applied. For example, a thrombus might be compressed against the vessel wall as a result of blood flow, so that is becomes less occlusive than it would be if there were no such irreversible deformation.

It is especially remarkable for any polymer that the clot is not damaged by such inelastic deformation, but instead its mechanical properties remain unchanged. This characteristic suggests that it must be important that the clot can maintain its mechanical stability over a long period of time, no matter what stresses to which it is subject. Without such self-repair, the clot might degenerate over time and no longer be able to fulfill its functions.

9. What is the mechanism of inelastic or irreversible deformation?

As described above, in fibrin there is irreversible deformation but no structural damage. The mechanisms of irreversible deformation, or the inelastic component, are unknown, but two possibilities have been suggested in the previous section for different time scales. For fine clots over long times, the knobs-into-holes bonds that hold fibrin together may occasionally break and then be re-formed in a different location [21,24,25]. This may be plausible for a fine clot where only a few such bonds in each thin fiber would need to be broken for rearrangements to occur, but seems unlikely for a coarse clot, where many such bonds would need to be broken simultaneously across the width of a thick fiber. The most likely explanation for the irrecoverable deformation of coarse clots is that protofibrils slip past one another, since the forces between protofibrils are weaker than those that hold the protofibril together. Since fibrin molecules are aligned or half-staggered across the fiber, maintenance of stiffness or self-repair would occur by the establishment of new specific interactions across protofibrils. A longitudinal shift of 22.5 nm between protofibrils would bring a new set of binding sites into alignment.

10. Effects of factor XIIIa on viscoelastic properties

The plasma transglutaminase, Factor XIIIa, introduces a pair of ε-amino-(γ-glutamyl)lysine isopeptide bridges between the C-terminal γ chains of two fibrin molecules and numerous other bridges between specific donor and acceptor sites in the C-terminal two-thirds of the α chains [26]. The introduction of these cross-links has dramatic effects on the viscoelastic properties of fibrin [14,27]. The stiffness of the clot is increased substantially and the creep or irreversible deformation is nearly eliminated.

It appears that α chain cross-linking is primarily responsible for the increase in stiffness and the lack of inelastic deformation [28–30]. However, these conclusions are not rigorous, since all experiments showed a mixture of γ and α chain cross-linking.

The effects of cross-linking on the mechanical stability of clots are an important part of blood clotting. The rare patients lacking Factor XIII generally have serious bleeding problems [31]. It is not known whether the lower stiffness or higher degree of inelastic deformation of clots without cross-links or both is responsible for the bleeding problems.

11. Clot stiffness increases at high deformation

At low strains or deformations, stress is directly proportional to strain and the slope of the curve, or the elastic modulus, is constant [5]. At large strains, the slope increases dramatically, so that the modulus or stiffness of the clot increases up to a factor of 20 fold [32]. This phenomenon, which is called strain hardening or stiffening in materials science, is opposite to what happens for rubber. Strain hardening may be important biologically because it allows fibrin clots to be compliant at normal strain levels and then become stiffer at larger deformations that could otherwise threaten clot integrity. The mechanism of strain hardening in fibrin is unknown. There must be another phenomenon

coming into play. It has been proposed that the strain hardening may arise from increasing number of contacts between the rigid fibers at large deformations [32]. On the hand, other mechanisms are also possible. For example, if the primary mechanism giving rise to clot elasticity is bending of the fibers, stretching of the fibers may come into play at larger strains.

12 .Viscoelastic properties are sensitive to small changes in polymerization and clot structure

From studies of dysfibrinogenemias, in which a single base change results in an amino acid substitution or truncation, it may be concluded that viscoelastic properties are one of the most sensitive measures of the effects of such modifications on fibrin polymerization and clot structure. An extreme case is the clot formed from fibrinogen Dusart, with a substitution at Aα554 and accompanying disulfide attachment of albumin [33]. In this case, lateral aggregation is severely impaired because of the modification to the αC domains with the effect that the clots are made up of very thin fibers with many branch points, so that these clots are about six fold stiffer than normal clots and the subjects with this substitution have severe problems with thrombosis and thromboembolism [34]. Fibrinogen Caracas II has a substitution at Aα554 with extra glycosylation, also affecting lateral aggregation so that clots have very thin fibers, but in this case the abnormal molecules appear to cap the ends of fibers, inhibiting further growth, with the result that the clots are nearly normal in viscoelastic properties and the subjects with the defect do not have serious problems of thrombosis or bleeding [35].

Viscoelastic measurements of clots can also be used to sort out the effects of numerous factors on clot properties. For example, a monoclonal antibody that binds specifically to the ends of fibrinogen modulates polymerization such that clots are made up of very thin fibers and many branch points but with numerous fiber ends, such that the stiffness is decreased and the inelastic component is increased [36]. In contrast, Fab fragments of this same antibody yield clots also with thin, highly branched fibers but without the capping to produce fiber ends, so that these clots are stiffer than controls. Clots made from the γ' spice variant of fibrinogen have increased stiffness, most likely from greater cross-linking as a result of increased Factor XIII binding [37].

13. Effects of deformation on clot structure

Uniaxial stretching of fibrin films caused extensive orientation of the fibers in the direction of pull [38]. The initial random orientation returned on return of the fibrin film to its original dimensions. The notion that clot elasticity arises from bending of fibers was reinforced by observations of bent fibers in the stretched fibrin films.

A small-angle X-ray study of fibrin films showed a prominent peak that appeared to be in a location consistent with a repeat of 23 nm, based on a simplified theoretical model of the fibrin structure [39]. There appeared to be a change of periodicity of about 25% with stretching. Since there are some methodological difficulties with these experiments because of the use of fibrin film and the theoretical scattering model, the interpretation of the results is not clear. If these results are confirmed, it will be necessary to interpret them in terms of structural changes that could occur. If so, there must be significant flexible regions of fibrinogen that would allow molecular stretching of about 25%.

14. Dissociation of network strands by competing reagents

Peptides with the sequence of the amino terminal end of the α chain of fibrin, glycine-proline-arginine, the knobs that are exposed by removal of the A fibrinopeptides and then fit into the holes in the γC domains at the ends of the molecule, bind strongly to fibrin(ogen) and inhibit fibrin polymerization [40]. Peptides that mimic the amino terminal end of the β chain, glycine-histidine-arginine, also inhibit polymerization but to a lesser extent. When glycine-proline-arginine-proline was introduced into a fine uncross-linked clot at millimolar concentrations, there were striking effects on viscoelastic properties [25, 41]. Clots were less stiff and the creep or irrecoverable deformation was enormously increased. In these experiments, it appeared that the peptide competed with the amino terminal α chain for the holes at the ends of the molecules. Slippage would occur when breaks cause severance of a network strand. Since the differential modulus remained constant throughout such experiments, there was no damage to the clot as severed junctions re-joined with new configurations. In effect, it appears that the peptide catalyzed the exchange of the knob-hole junctions. When the clots were cross-linked by Factor XIIIa, there was no effect of the peptides, since cross-linking prevented severance of the network strands. It is not known if there is any physiological significance of these findings, but it is possible that such competition might occur in disseminated intravascular coagulation, where there can be considerable quantities of fibrin degradation products present. Moreover, these results demonstrate the dynamic nature of clot structure, which is not generally appreciated from other types of studies.

15. Plasma clots, platelet-rich plasma clots and whole blood clots

Clots made from purified proteins, used for nearly all of the studies in the literature, are very different than in

vivo clots or thrombi. Other proteins present in plasma have large effects on the structure and mechanical properties of plasma clots [42], but little is known about the mechanisms involved. Platelets have even more dramatic effects on clot structure, organizing the fibrin around the platelet aggregates (Fig. 2B) [43,44]. However, little is known about the mechanical properties of these more complex structures. Similarly, few studies have been carried out to characterize the viscoelastic properties of whole blood in the terms of materials science. Instruments such as the Thromboelastograph, or Hemodyne, which measure viscoelastic parameters, have used by clinicians to determine the coagulation status of patients. These instruments often use whole blood, but the parameters measured have not generally been related to the structure and other properties of the clot. Thrombi have unique structural properties depending on blood flow and can be quite anisotropic or non-uniform in spatial distribution of components, so their viscoelastic properties would be difficult to determine.

Angioplasty is a common clinical procedure and its success is dependent on the viscoelastic properties of thrombi, yet little is known about the mechanical properties of thrombi. Embolization is a relatively common and serious clinical problem, which remains difficult even with new technology. However, little is known about the mechanical properties of thrombi that may make them more or less susceptible to embolization. These are areas for further research that could benefit from interactions between basic scientists and clinicians.

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Dedication

John Ferry was a gentleman, humble in spite of being a pioneer and having founded a whole area of polymer research. He was one of the first scientists to study purified fibrinogen, having been part of Edwin Cohn's group, which isolated the plasma proteins. His papers from the 1940's are remarkable for the amount of information and for their insight into fibrinogen and fibrin. When he came back to fibrin late in his career and published another set of outstanding papers, I carried out a correspondence with him and occasionally sent him manuscripts that I thought would interest him. After I sent him the last such package more recently, Mike Mosesson informed me of John's death. A few days later, I received a letter from his colleague and friend, John Schrag, saying that John Ferry had read the paper that I had sent him but apologized that he would not be able to comment in detail because he was in the hospital and that he had asked his friend to write to me. I was amazed by John Ferry's intellectual curiosity that he was still interested in science and even more touched by his kindness that he wanted me to know why I had not heard from him. Over the years, I had tried to convince John to write a review on the viscoelastic properties of fibrin. Although he declined because of the large gaps in our knowledge, I thought that the need remained because too many scientists are ignorant of his important research. I did not know it at that time, but that set in motion my idea to attempt this review article emphasizing what we do and do not know. I would like to think that he would have enjoyed this review.

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