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A physical model for a fibrous network and its application to the shear modulus and other data of the fibrin gel

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Abstract

A physical model for a fibrous network is developed and used to calculate its shear modulus. The model is applied to the shear modulus data of the fibrin gel and compared with other data related to the fibrin gel to elucidate the physical origins for some of the interesting properties of the gel such as the concentration dependence of the shear modulus and the difference between fine and course gels.

Keywords: Fibrinogen; Network; Fibrin gel; Model

1. Introduction

Numerous investigators [1–5] over the years have measured the mechanical and other properties of fibrin gels, the result of the enzyme thrombin acting on fibrinogen, to elucidate the microstructure of the gel as well as the mechanisms for energy storage and loss. One of the problems has been the lack of a model within which to interpret the results of the various unique properties of the fibrin gel. For example, the concentration dependence or the difference in mechanical strength between coarse and fine gels (networks or clots). Within this context, the model presented here is based on some simple assumptions regarding the structure of the network which results in a fairly reasonable explanation for some of the properties of the fibrin gel.

2. Model

As in a previous paper [6], the network consisting of varying length strands of fibrin joined at functional junctions is modeled as a collection of fixed length cylindrical fibers of uniform random angular orientation in space. All junctions have been removed and the degree of functionality of the junctions is not included in the model (Figs. 1 and 2). Starting with the definitions for the shear modulus and Young's modulus, the shear strain of the network is related to the strand extension of Young's modulus for a strand oriented at angles θ and ϕ. An average shear force for a single fiber is calculated by averaging over all angles, and the total shear force is arrived at by multiplying the average shear force for one fiber by the number of fibers. Strands (fibers) whose orientation is such that they should compress rather the stretch are excluded from the model because, presumably, the strands will bend and provide little or no energy storage, i.e., in Fig. 2, fiber "*i*" will stretch and fiber "*j*" is excluded.

From the definition of Young's modulus E, the restoring force for a single fiber i of length l under extension Δl is

$$f_i = \frac{EA_c\Delta l}{l} \tag{1}$$

with cross sectional area A_c and diameter d.

$$A_c = \frac{\pi d^2}{4} \tag{2}$$

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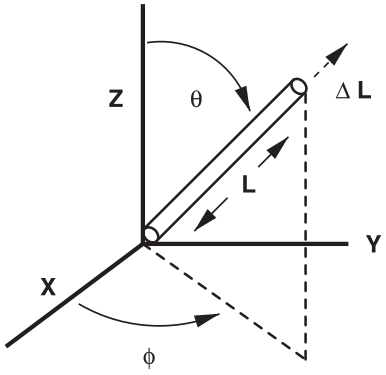

Fig. 1. A single fiber of length l with coordinate system.

The definition of the shear modulus in a typical shear experiment (Fig. 2) is

$$G = \frac{F g_w}{As} \tag{3}$$

where F is the total shear force per unit area A with gap width g_w and shear strain s.

Scaling the total shear s across a gap width of g_w to that of the ith fiber at angles θ and ϕ (Fig. 1) gives the extension experienced by a single fiber.

$$\Delta l = \frac{sl\cos\phi\sin\phi\sin\theta}{g_w} \tag{4}$$

Combining the above equation with the definition of Young's modulus for a single fiber, Eq. (1) gives the retractive force for a single fiber as a function of the angles θ and ϕ.

$$f_i(\theta,\phi) = \frac{\pi d^2 sE}{4g_w}\cos\phi\sin\phi\sin\theta \tag{5}$$

The integral of f_i over the angles divided by the same integral over the surface area (S_o) of the unit sphere will then give the retractive force for an average fiber. In Eq. (6), the range of integration has been restricted to set the energy storage for fibers undergoing compression to zero; however, these fibers are included in the averaging process by incorporating a 1/2 in the final result.

$$f_{i,\mathrm{avg}} = \frac{\pi d^2 sE}{4S_o g_w}\int_0^{\frac{\pi}{2}}\cos\phi\sin^2\phi d\phi\int_0^{\frac{\pi}{2}}\sin\theta d\theta \tag{6}$$

Carrying out the integration gives

$$f_{i,\mathrm{avg}} = \frac{sd^2E}{12g_w}. \tag{7}$$

The number of strands n in a volume V of the network is [6]

$$n = \frac{4cV}{\pi d^2 l\rho_s} \tag{8}$$

where c is the concentration of material in the network and ρ_s is the density of material in the strands. The ratio c/ρ_s is the volume fraction of the network.

The total shear force F is then just n times the shear force for one average fiber

$$F = nf_{i,\mathrm{avg}} = \frac{csVE}{3\pi g_w l\rho_s}, \tag{9}$$

and substituting for F from the above equation (Eq. (9)) into the definition of the shear modulus (Eq. (3)) gives

$$G = \frac{cVE}{3\pi Al\rho_s}. \tag{10}$$

Using a volume of $V=Al$ results in an equation for the shear modulus of the gel in terms of the Young's modulus for a fiber (gel strand) and the material concentration to the first power.

$$G = \frac{Ec}{3\pi\rho_s} \tag{11}$$

3. Concentration dependence

Experimental data from various papers [1–5] have shown the concentration dependence of the shear modulus to be generally in the range of c to the power of 1.5 to 2.0. For example, Ferry and Morrison [1] many years ago published a paper giving an experimental value for the concentration dependence of c to the power of 1.56. That and several other examples including those of Ferry et al. [2], Kaibara and Fukuda [3], Roberts et al. [4] and Carr et al. [5] leave the question as to why, in light of the model above (Eq. (11)), the concentration dependence is greater than a power of one.

The following is an approach to account for the additional concentration dependence with the point of view that the above model (Eq. (11)) is essentially correct and that the additional concentration dependence is a second-order correction to the basic model.

The premise for the second-order dependence is that gels formed at very low concentrations are incomplete, where not all of the strands, or fibrin, are incorporated into the gel and thereby contributing to the shear modulus, i.e., either isolated strands or groups of semi-aggregated strands and strands with a single free end. The groups of semi-aggregated strands would lead to inhomogeneous gels because of insufficient material to form a complete network. Strand branching, presumably, would contribute to this

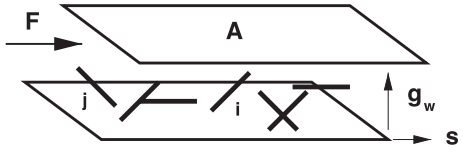

Fig. 2. Shear modulus experiment with network strands modeled as fibers. Fiber "i" contributes to the shear modulus whereas fiber "j", included in the average, contributes zero energy storage.

localization of fibrous material. Within this context, the following is a model for the second-order concentration dependence.

The number of strands n_g actually contributing to a homogeneous network and thereby the shear modulus can be approximated as some fraction f_n ($0 \leq f_n \geq 1$) of the total number of strands n, where n is the value from Eq. (8).

$$n_g = f_n n \tag{12}$$

Let the fraction equal a probability (P_s) to the fourth power where P_s is the chance of one strand end making contact. For a single isolated strand, four contacts need to be made to incorporate it into the network.

$$fn = P_s^4 \tag{13}$$

Define P_s as

$$P_s \equiv 1 - \frac{l}{l_o}, \tag{14}$$

where l is the average length of the strands at the particular working concentration and l_o is, on average, the maximum length that a strand can obtain under the experimental conditions of gelation. In essence, as the fibrin concentration is decreased to the point where the length equals l_o, a homogeneous network no longer forms and one has a "gel point". As the concentration is increased, the length of the strands decreases and the number of strands increases, which results in a probability of strand incorporation approaching one.

Then

$$n_g = \left[1 - \frac{l}{l_o}\right]^4 n. \tag{15}$$

Using the above equation (Eq. (8)) for the number of strands, one can write the following two equations for l and l_o.

$$\pi l n r^2 \rho_s = cV \qquad \text{and} \qquad \pi l_o n_o r_o^2 \rho_s = c_o V_o. \tag{16}$$

Along with this, assume that the diameter of the strands is constant with concentration. This assumption is justified by the gel permeation data of Blombäck et al. [8] (also of Nestler [6]), which has the diameter (or radius r) constant with concentration for a coarse fibrin network.

$$r = r_o \tag{17}$$

V and V_o are the sample volumes and can be approximated by $n/3$ or $n_o/3$ times the cubic lattice volume formed by l or l_o on each edge.

$$V = \frac{n}{3} l^3 \qquad \text{and} \qquad V_o = \frac{n_o}{3} l_o^3 \tag{18}$$

Combining the above equations and solving for l and l_o gives

$$l = \sqrt{3\pi} r_o \sqrt{\frac{\rho_s}{c}} \qquad \text{and} \qquad l_o = \sqrt{3\pi} r_o \sqrt{\frac{\rho_s}{c_o}}, \tag{19}$$

which when substituted into Eq. (15) for n_g gives

$$n_g = n\left[1 - \sqrt{\frac{c_o}{c}}\right]^4. \tag{20}$$

Incorporating this into the original derivation of G leads to the final equation for the shear modulus with second-order concentration dependence.

$$G = \frac{Ec}{3\pi\rho_s}\left[1 - \sqrt{\frac{c_o}{c}}\right]^4 \tag{21}$$

4. Results

Of the sets of data available, two will be used to evaluate the model in Eq. (21). The dynamic viscoelastic measurements of Roberts et al. [4] using the Birnboim transducer provides a convenient starting point. For a coarse gel with a concentration of about 1%, they measured a dynamic storage modulus, G', of about 1×10^4 dynes/cm^2. Because the loss modulus, G'', is roughly 10% of the storage, one can use the value of G' as a good approximation for the shear modulus G.

$$c = 0.0094 \text{ g/ml} \qquad \text{and} \qquad G = 1.0 \times 10^4 \ dynes/cm^2 \tag{22}$$

Using a value for the density of protein (fibrin) in a strand (ρ_s=0.4 g/ml) from a previous paper [6] and assuming that c_o is small (~1% of c, see below), one can calculate (Eq. (21)) a value for Young's modulus of

$$E = 4.0 \times 10^6 \ dynes/cm^2. \tag{23}$$

The second set of data is from a paper by Carr et al. [5] where the shear modulus, G, of various fibrin gels was measured using an elastometer of their own construction. Using the data in their Fig. 5, which have an extensive concentration range, a value for c_o can be approximated. The data, lifted from the figure, have been approximately reploted in Fig. 3 except for two modifications. One, a small constant value (G_o=1.0 dynes/cm^2) has been subtracted from the values of G to account for the roughly constant value of G at concentrations less than 0.1×10^{-3} g/ml. The two regions, above and below c=0.1×10^{-3} g/ml, are assumed to represent two different networks where the region above c=0.1×10^{-3} g/ml, of interest here, has a "gel point" at c=0.1×10^{-3} g/ml. The second modification is the shifting up of the data (Δ log G=0.2) on the log scale to

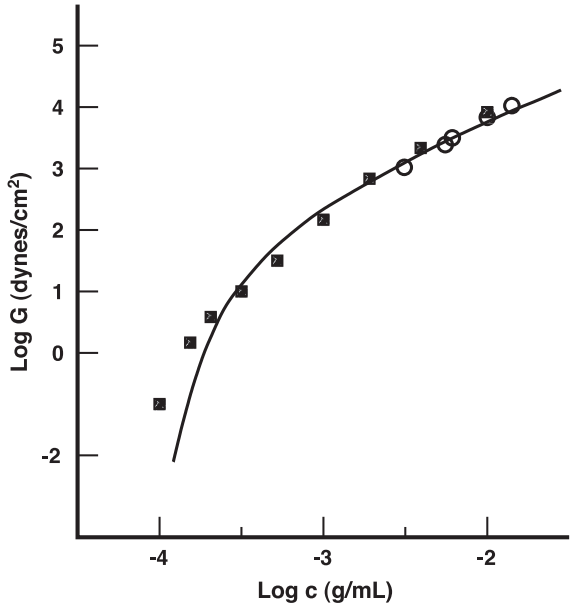

Fig. 3. Logarithmic plot of G or G' against fibrin concentration in g/ml. (○), human, pH 7.5, μ 0.15, from Roberts et al. [4], Table 1; (■), human, pH 7.4, μ 0.15, from Carr et al. [5] (Fig. 5; see text); (—), Eq. (21) with c_o=0.1×10^{-3} g/ml and E=4.0×10^6 dynes/cm^2.

provide visual magnitude agreement with the data of Roberts et al. [4]. The curve in Fig. 3 above is a plot of Eq. (21), the model, with

$$c_o = 0.1 \times 10^{-3}\ \text{g/ml} \quad \text{and}$$
$$E = 4.0 \times 10^6\ dynes/cm^2. \tag{24}$$

5. Discussion

5.1. Coarse gels

Considering the simplicity of the model and the number of assumptions, the agreement between the experimental data and theoretical equation in Fig. 3 is remarkably good. There is the obvious disagreement at low fibrin concentrations close to the "gel point", but the trend is in agreement. This disagreement may be due to inhomogeneous gel formation where portions of the gel stick to the sides of the cell and not form uniformly throughout the cell gap.

An independent approximation of Young's modulus for a strand can be determined by using the fibrin film data of Roska and Ferry [7]. Here, a fibrin gel is formed at approximately 5 g/l and then slowly compressed and plasticized with glycerol to form a film with a fibrin weight fraction, w_2, of 15–20%. The film will then have some alignment of the strands and when stretched, further alignment of the strands will occur. Under these conditions, it is assumed that the film represents a collection of strands oriented parallel to the film long axis, and ideally, a direct measurement of Young's modulus. From the paper, for an unligated film with w_2=0.17

$$E = 1.7 \times 10^6\ dynes/cm^2. \tag{25}$$

Because the value of the weight fraction (w_2=0.17) is the approximate density of the film (within ~5%) and the value of E is based on the unstretched cross-sectional area of the film, one can scale E to a film density of 0.4 g/ml, which corresponds to the fiber protein density used in the results section above.

$$E_f = E\left[\frac{0.4}{0.17}\right] = 1.7 \times 10^6[2.36] = 4 \times 10^6\ dynes/cm^2 \tag{26}$$

In a similar manner, the value for Young's modulus for a ligated film can be calculated. With a weight fraction of 15% and E=4.6×10^6 dynes/cm^2

$$E_f = 12 \times 10^6\ \text{dynes/cm}^2 \tag{27}$$

The value of Young's modulus previously calculated from the shear modulus data of Roberts et al. [4]. Eq. (23) is in agreement with Young's modulus derived from the fibrin film data for an unligated film.

5.2. Fine gels

The second interesting aspect of fibrin gels is the difference in shear modulus between "fine" (strand diameter of ~100–200 Å, Nestler [6]) and "coarse" (strand diameter of ~1000–2000 Å, Nestler [6]) gels. From the data of Roberts et al. [6], (Fig. 3), Gerth e t al. [10] and Nelb et al. [11,12], the difference in shear modulus between fine and coarse unligated gels at high concentration (~10 g/l) is a factor of 3 to 4, where the fine gel has the lower modulus. Within the context of the model above, this can be explained as a change in the protein density of the strands. For the coarse strand, the density of protein used was ρ_s=0.4 g/ml, which when compared with the dry weight density of fibrinogen ρ=1.38 g/ml is rather low, although in agreement with proposed structures for the coarse gel strand (Hermans [9]). For strands of the fine gel with their small diameter, little opportunity is afforded for forming the tertiary structure that exists in the coarse gel strands. This implies that the strand protein density should approach the dry weight density which is 3.5 times the value used for coarse gels and gives the required factor of 3 to 4 decrease in the fine gel shear modulus.

Nomenclature

F	Force
L	Length of the fiber
E	Young's Modulus
A_c	Cross-sectional area of fiber
V	Volume
f_i	Force per strand
Δl	Extension of the fiber under shear s
A	Area of the clot

r	Radius
n	Number of fibers in the network
ρ_s	Density of protein in a fiber
c	Fibrinogen concentration in the clot
S_o	Surface area of unit sphere
t	Time
g_w	Gap width
d	Strand diameter
s	Total shear
G	Shear modulus
c_o	Concentration at “gel point”
l_o	Length at “gel point”
P_s	Probability of strand incorporation

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