Supplementary Materials:

Passive high-frequency microrheology of blood

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S1. Brief survey of hemorheological assays

Common coagulation assays include measurements of activated clotting time (ACT), activated partial thromboplastin time (aPTT), prothrombin time (PT), thromboelastography (TEG), rotational thromboelastometry (ROTEM), platelet count (PLT), and heparin concentration (HC), among others [1-3]. Importantly, the majority of these traditional coagulation assays are performed *in vitro*, and they all share two distinct features.

The first critical aspect is that in the existing techniques, the propensity of blood to coagulate is inferred from processes initiated by chemical triggers. In other words, these are end-point measurements, provocative, and non-physiologic in nature because the coagulation is induced by strong stimuli as kaolin, glass beads, or calcium [4]. In addition, some of them are contact-based methods that are likely to strain the blood sample beyond the linear viscoelastic regime, delay clot formation, and modify fibrin network structure, which ultimately leads to significant intraand inter-subject variability. Moreover, the time associated with the sample collection and treatment as well as the need to reach the endpoint of clot formation are serious limitations in situations where the status of blood changes rapidly and needs to be managed continuously.

The second common characteristic is more subtle. Like most rheological measurements, these assays probe bulk properties. This means that the rheological properties are considered scale-independent and the fluid under test is regarded as continuum matter. Its detailed molecular structure and dynamics are all included in effective, macroscopic properties of viscoelasticity. This usually suffices for common homogeneous fluids but misses a number of details in the case of complex, multi-component fluids such as blood.

Microrheology, on the other hand, is concerned with how viscoelastic materials flow as a function of length scales [5]. As such, physical details at submicron and even molecular scales are probed directly and over a large spectrum of frequencies based only on thermal forces [6]. In special cases, when the different physical scales are appropriately considered, the macro- and micro-rheological properties can be directly compared [7, 8]. In the general case of complex heterogeneous fluids, however, the homogenization of the mechanical properties is intrinsically scale-dependent [9].

As such, the issue is not whether the microscopic measurements correctly reflect the macroscopic elasticity and viscosity of a fluid but rather if microrheology can provide information beyond what is accessible at macroscales. In the case of blood, microscale measurements could examine, for example, the viscoelastic properties of molecular components, e.g. fibrin and other proteins [10, 11], while isolating the influence of influence of the much larger erythrocytes.

S2. Optical microrheology

In our experiments, both in artificial hemo-systems and on whole blood, we performed optical microrheology measurements by using a spatio-temporal coherence-gated dynamic light scattering (DLS) technique, whose implementation is optical fiber-based and can be used to measure microrheological properties of complex fluids passively based on the fluctuations of the scattered light [7, 12, 13], as shown in Figure S1. This technique allows accessing the temporal scales of interest where the proposed rheological model is valid.

To perform the measurements, the MMF is held by using a fiber holder (Newport model FPH-J) that allows holding the fiber straight. The fiber holder, in turn, is mounted on a fiber chuck holder (Newport model 561-FC), and the whole assembly is mounted on a 3D translation stage. In this way, we can control the position of the fiber tip three-dimensionally (transverse position and depth within the sample), to keep the fiber tip far from the walls and well within the sample, while always holding it straight. The general purpose, disposable 1 mL syringe is also held straight (vertically) by using a home-made holder (not shown in Fig. S1), and it has an internal diameter of 4.5 mm.



Figure S1. Schematic description of the coherence-gated DLS concept and how the measurements were performed (see text for details). The graphical illustration in the inset represents the region accessible by coherence-gated DLS: a picoliter-sized coherence volume where the RBCs probe their environment at small spatial and temporal scales.

A unique feature of our measurement is that the effects of multiple scattering and light absorption can be safely neglected. Specifically, the measurement relies on single-scattering: the optical signal measured comes from a picoliter-sized coherence volume, as determined by the size of the core of the optical fiber and the coherence length of the broadband light source, where single scattering amply dominates independently of the macroscopic scattering characteristics of the medium [12, 14]. Thus, interpreting the measured signal does not require any assumption regarding scattering regimes or models of diffusion, while the dynamics of complex systems can be measured over broad ranges of optical and temporal regimes using the same instrument [7, 13]. In this regime of single scattering, the effect of absorption is simply an attenuation of the optical signal and does not affect its dynamics and the subsequent temporal analysis of the fluctuations [7, 13].

Compared to other optical microrheology approaches, coherence-gated DLS is implemented in an all-optical-fiber photonic platform. It provides a compact and robust testing environment and, thanks to the endoscopic-type probe available in the setup, it also allows an easy integration with medical machine as demonstrated in [15] for passive, real-time and continuous monitoring of blood condition in clinical settings.

We would like to emphasize that the microrheology outcomes complement the information provided by bulk rheology because it examines the role of viscoelastic properties of plasma while effectively isolating the influence of erythrocytes. Blood is primarily a mixture of erythrocytes and plasma, and *macroscopically* its rheological properties are largely determined by the mechanical properties of the erythrocytes. *Microscopically*, on the other hand, the erythrocytes probe their own environment over much smaller spatial scales in much shorter times. In other words, over the spatial and temporal scales typical to a microrheology measurement, the complex fluid tests itself based solely on intrinsic thermal forces: the particulate component of blood (erythrocytes) tests the viscoelasticity of the fluid component (plasma + macromolecules)

S3. Maxwell parameters extraction from coherence-gated DLS spectra

The recorded coherence-gated DLS power spectra, P(f), were acquired in the frequency range from 1 Hz to 10 kHz, with 1 Hz resolution, and integration time of 30s. The spectral analysis of the dynamic signal is based on a multi-Lorentzian approach [8, 13]. Basically, the measured P(f) was decomposed into a collection of discrete contributions, $P(f) = (2/\pi) \sum_{i=1}^{N} (a_i/v_i) [f^2 + (1/v_i)^2]^{-1}$ with f being the frequency, a_i the relative amplitude, $\sum_{i=1}^{N} a_i = 1$, and v_i the representative relaxation time of the i-th population, respectively. It was found that two Lorentzian components (N=2) were sufficient to describe the overall shape of

found that two Lorentzian components (N=2) were sufficient to describe the overall shape of P(f) in all cases. A typical example of this signal analysis is illustrated in Figure S2, where we present P(f) measured from whole blood, right after drawing the sample (Figure S2(a)) and after 30 min (Figure S2(b)), together with the full Lorentzian reconstruction, as indicated. As can be seen, our approach fully describes these spectra with significantly different shapes.

The spectral decomposition of P(f) was then used to evaluate the mean squared displacement

$$\langle \Delta r^2(t) \rangle = -\frac{6}{q^2} ln \left[\sum_{i=1}^{N} a_i exp\left(-\frac{2\pi t}{v_i} \right) \right], \text{ where}$$

(MSD) of the diffusing particles (erythrocytes),

 $q = 2k_0 nsin(\theta/2)$ is the magnitude of the scattering vector, with $k_0 = \frac{1}{\lambda_0}$ and $\theta = \pi rad$ in our reflection geometry, and n is the refractive index of the medium. The results are illustrated in the inset panels in Figure S2. Note that the implicit normalization of the spectral decomposition allows treating P(f) as a probability density function. Then, the MSD, which is determined by the shape of P(f), was used to estimate the complex viscoelastic moduli for tracers of known size.

According to the approach described in [16, 17], the magnitude of the viscoelastic modulus is $\frac{L}{T}$

$$|G^*(\omega)| \approx \frac{\kappa_B^{1}}{\pi a \left(\Delta r^2 \left(\frac{1}{\omega} \right) \right) \Gamma[1 + \alpha(\omega)]}, \text{ where } \alpha(\omega) = \frac{\partial ln \left(\Delta r^2(t) \right)}{\partial ln(t)} \Big]_{t = \frac{1}{\omega} \text{ is the}}$$

determined as $(\pi \alpha (\omega))^{-1} (1 + \alpha (\omega))^{-1}$, where $O(n(t))^{-1} (1 - \alpha (\omega))^{-1}$, where $O(n(t))^{-1} (1 - \alpha (\omega))^{-1}$, where $O(n(t))^{-1} (1 - \alpha (\omega))^{-1}$, $G'(\omega) = \log (1 - \alpha (\omega))^{-1}$, $G'(\omega) = \log (1 - \alpha (\omega))^{-1}$, $G'(\omega) = |G^*(\omega)| \sin(\pi \alpha (\omega)/2)$, and also (iii) the loss tangent, $\tan \delta(\omega) = G''(\omega)/G'(\omega)$. The parameter $\alpha(\omega)$, which is calculated from the time-dependent logarithmic slope of the MSD, lies between the limits of elastic confinement ($\alpha = 0$) and viscous diffusion ($\alpha = 1$), and describes the transport properties of the diffusing particles in the intermediate viscoelastic regimes of complex fluids.



Figure S2. Power spectra with significantly different shapes corresponding to whole blood (a) right after drawing the sample and (b) after 30min of unperturbed evolution. The continuous red lines represent the fit with the sum of weighted Lorentzians while the blue dashed line, f^{-2} , indicates the frequency behavior corresponding to a normally diffusive particle in a Newtonian fluid. The insets show the associated mean-square-displacements (MSD) of the diffusing probes (erythrocytes); the dotted red lines indicate a linear MSD evolution for a random walker in a Newtonian fluid. Deviations of the MSD from linear dependence are indicative of a viscoelastic environment.

In a complex fluid such as blood, detecting a dynamic signal in the high frequency window of our measurements does not necessarily imply that the RBCs are free to move over length scales of the order of, or larger than, their size. Nevertheless, this condition where the RBCs remain mobile exists during the entire measurement duration as verified by the fact that a dynamic signal is detected at all times, from where the microrheological properties of plasma are retrieved. If the RBCs form a solid clot and their movement ceases in the coherence volume, the signal measured would report no fluctuations and the power spectrum would be flat the level of the noise floor; however, this never happens in our experiments.

Additional experimental facts and observations are:

- (i) All the measurements were performed without externally promoting clotting and without adding chemical triggers that initiate the coagulation cascade. During the measurement, blood rests unperturbed in the vial without external mechanical stimuli.
- (ii) Visual inspection of the samples after the measurements indicates that blood did not form a rigid clot and it flows inside the syringe if gently agitated.
- (iii) The optical fiber is placed halfway the column of liquid such that the coherence volume is constantly replenished with RBCs during the measurement, far from the bottom of the vial where aggregation effects would be more appreciable due to the increasing concentration resulting from sedimentation.

Such factors do not eliminate the fact that the status of blood changes and RBCs aggregate over time in the perspective of the bulk scope. However, the blood in the spatial and temporal scale of the DLS measurement would look like "liquid" as long as the RBC probes are still mobile over tens to hundreds of nanometers (high frequency regime of our experiments).

Furthermore, if sedimentation effects contribute to our measurement, they could be easily detected and even measured because DLS measurements can discriminate between the diffusive and advective motion of the scattering centers [18-22]. Sedimentation is an example of the later. Depending on the specific Péclet number, the advective motion associated to directional flow or sedimentation affects gradually the low frequencies and, eventually, leads to a Gaussian shape of the power spectrum as opposed to the Lorentzian shape typical to diffusive motion [18-22]. Thus, the fact that the measured power spectra have pure Lorentzian shapes, as shown in Fig. S2, is a direct experimental demonstration that the dynamic signal is solely determined by diffusive motion in the frequency range of interest.

Finally, as explained above, the measured optical signal originates from an optically isolated picolitre-sized coherence volume, where single scattering amply dominates. In this regime, changes in the concentration of scattering particles in the coherence volume result in variations of the magnitude of the scattered signal but not on the temporal characteristics of the fluctuations; therefore, the shape of the power spectrum, which contains the useful information, does not depend on the density of scattering particles. This feature of the coherence-gated DLS technique has proved advantageous to retrieve simultaneously the diffusion coefficient (from the shape of the spectrum) and the sedimentation speed (from the time evolution of the magnitude of the spectrum) of scattering particles subjected to significant sedimentation effects [13, 23].

The parametric description in terms of our model was realized by fitting simultaneously the three curves $G'(\omega)$, $G''(\omega)$, and $tan\delta(\omega)$ to the Maxwell rheological model described above to obtain a single set of fitting parameters (η_0, η_1, τ_1) that can describe the three curves over a broad frequency range. Even though the measurement is limited at high frequencies by the detection noise and, at low frequencies, by potential effects of sedimentation and aggregation, the analysis is sufficiently robust to reliably perform an optical micro-rheology measurement over a broad

frequency range from 1 to 10^4 Hz where our continuous rheological model is valid. The results are summarized in Figure S3. As can be seen, the proposed Maxwell model is sufficient to describe the microrheological functions. The fact that the measured properties are well described over the frequency range of interest is in itself a validation for our physics-based model, which goes beyond a simple description of generic viscoelastic elements [24, 25].

$$r^{2} = 1 - \frac{\sum_{i}^{i} (y_{i} - \hat{y}_{i})^{2}}{\sum_{i}^{i} (y_{i} - \mu)^{2}}$$

The Pearson coefficient i, where $\hat{y}_{i}\mu$ are the experimental values and average, and y_i are the fitted values for $G'(\omega)$, $G''(\omega)$, and $tan\delta(\omega)$ was larger than 0.91 for the data shown in Figure S3. Moreover, the results clearly capture the main characteristics of the physical condition of blood viscoelasticity after 30min. As seen in Figure S3(b), a significant reduction of the viscous component, and therefore of the loss tangent, has occurred (note the logarithmic scale) in comparison to the initial conditions shown in Figure S3(a).



Figure S3. Frequency-dependent micro-rheological functions $G(\omega)$, $G(\omega)$, and the associated $tan\delta(\omega)$, corresponding to P(f) shown in Figure 2(a) and 2(b), respectively. The continuous lines denote the micro-rheological functions estimated over an extended frequency range, while the empty markers indicate the fitting to the rheological model within the frequency range of interest, which is indicated by the two vertical dotted-dashed lines.

S4. Incipient whole-blood coagulation histogram distributions

The results in Section 3.3.3 in the main text are from a population of patients (See Materials and Methods). Figure S4 shows a more detailed analysis of the results presented in Figure 4 of the main text. Specifically, in Figure S4 we show the evolution of the histograms of the viscoelastic parameters across the entire population of patients, for the incipient coagulation whole blood in unperturbed samples under natural conditions. Basically, in Figure 4 of the main text only the first two moments of the distribution are plotted (mean values with their corresponding standard deviation) while in Figure S4 we show how the entire distribution evolves.



Figure S4. Distribution of viscoelastic parameters: 'long-term' evolution within a coagulation state (whole blood). The Maxwell parameters are extracted from fitting the complex viscoelastic moduli, calculated from P(f), simultaneously to Eq. (1-3) in the main text.

S5. Early-stage discrimination of coagulation propensity.

In this section, we demonstrate how our microrheological model can capture the different evolution of viscoelasticity when the blood coagulability is purposely inhibited through a pharmaceutical intervention. Figure S5(a) compares typical the early stages evolutions of the rheological parameters corresponding to normal and heparinized blood (note the logarithmic scale on the vertical axis). The effect of the anticoagulant is evident. The viscoelastic parameters η_1 and τ_1 of heparinized blood are significantly smaller in comparison to the case of normal blood because heparin inhibits the gradual increase of the viscoelastic component that progressively that appears naturally during blood coagulation. It can also be noted that, on average, the set of parameters vary less in the case of heparinized blood; note that η_1 for normal blood starts to ramp up after the first 5 min indicating the gradual increase in the influence of the viscoelastic element.



Figure S5. (a) Short-term temporal evolution of the Maxwell parameters after full systemic anticoagulation (dashed line and empty markers) for a representative case; the normal blood measurement before the administration of the anticoagulant is plotted for reference (solid line and markers). Panels (b)-(d) show the short-term evolution of the Maxwell model parameters for the entire population of patients, for the two different states of blood, normal and fully heparinized; the error bars illustrate the inherent interpatient variability: (b) the viscous components η_o and η_1 , (c) the relaxation time of the viscoelastic element, τ_1 , and (d) the purely elastic k_1 calculated based on the ratio viscous coefficient η_1 and the relaxation time τ_1 . In panel d) the significant reduction of the variability across the population of patients due to the effect of the anticoagulant is indicated by the shaded region. Time t= 0 represents the starting of the DLS measurement immediately after the blood draw before and after the heparin administration. See text for details. The interpatient variability is represented by the error bars that indicate the standard deviation across the 23 subjects; time t=0 is the starting of the DLS measurement immediately after the blood draw.

To capture the anticoagulant action immediately after administration, we conducted a comparison over 5 min with respect to the normal condition for the entire population. The results of this comparison are summarized in Figure S5(b)-(d). Notably, a similar background viscosity η_0 was obtained for both normal and heparinized blood, which indicates that the main difference between these two states of coagulation relies primarily in the viscoelastic component. Something that can also be noted is that our measurement captures the ongoing effect of heparin, as indicated by the decreasing trend of η_1 .

The significant effects of the anticoagulant can be clearly observed in Figure S5(c): aside from a smaller average value of τ_1 (lower elasticity), the lesser extent of the error bars demonstrates a general feature that extends over the entire population. Even though the elastic components differ significantly at the initial stages, this variability reduces after heparinization and converges towards a narrower distribution corresponding to a more Newtonian fluid.

Finally, the ongoing effect of heparin can also be seen in the decreasing trend of the elastic component k_1 , which is plotted in Figure S5(d). One can safely conclude that the effect of heparin is effectively traced by the changes in the values of the viscoelastic element while η_0 reflects the relatively constant background viscosity. These changes are significant taking into consideration that the changes occurred at early time as well as that both normal and heparinized blood were tested with any stimulus to accelerate the coagulation process.

In addition to the above results, one can perform some statistical analysis to verify the difference in the evolution of the Maxell parameters between the two states. Statistical analysis can be either 1) descriptive analysis or 2) inferential analysis. The descriptive analysis helps in describing basic features of the data in a study. They provide simple summaries about the sample and the measures. The inferential analysis can be conducted using different approaches. We performed an unpaired T-test to determine if the means of two data sets differ significantly from each other. We ran the test independently for the datasets corresponding to the three Maxwell parameters (η_0 , η_1 , τ_1) for normal blood versus heparinized blood (see Figure 5 in the main manuscript). Moreover, we ran the T-tests at different times as the measurement evolves. The results are shown in the table below and show that:

- 1. The background viscosity η_0 is not significantly different for normal blood and heparinized blood. But, as time passes, they can be discriminated more clearly.
- 2. The viscoelastic element (η_1, τ_1) is significantly different for normal blood and heparinized blood. At a level of 0.05 significance, the two sets are statistically different from the first minute of the measurement. The difference between the two sets gets even larger over time.

η_o	Prob > t	η_1	Prob > t	τ_1	Prob > t
1min	0.31932	1min	0.03373	1min	0.00376
3min	0.18156	3min	0.00409	3min	4.7146E-4
5min	0.15752	5min	1.0394E-4	5min	6.65002E-5

In addition to the T-test, Figure S6 shows the evolution of the histograms of the viscoelastic parameters across the entire population of patients, for the early discrimination of coagulation propensity between whole blood and after heparin. This visual presentation also shows that as time evolves the distribution for η_0 is still similar for the whole blood and heparinized blood. However, the viscoelastic element (η_1 and τ_1) for whole blood starts to evolve with time compared to the heparinized ones.



Figure S6. Distribution of viscoelastic parameters: 'short term' evolution within two different states (baseline from whole blood & heparinized whole blood).

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