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Nonlinear, dissipative phenomena in whole blood clot mechanics

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When a thrombus breaks off and embolizes it can occlude vital vessels such as those of the heart, lung, or brain. These thromboembolic conditions are responsible for 1 in 4 deaths worldwide. Thrombus resistance to embolization is driven by its intrinsic fracture toughness as well as other, non-surface-creating dissipative mechanisms. In our current work, we identify and quantify these latter mechanisms toward future studies that aim to delineate fracture from other forms of dissipation. To this end, we use an *in vitro* thrombus mimic system to produce whole blood clots and explore their dissipative mechanics under simple uniaxial extension, cyclic loading, and stress-relaxation. We found that whole blood clots exhibit Mullins-like effect, hysteresis, permanent set, strain-rate dependence, and nonlinear stress-relaxation. Interestingly, we found that performing these tests under dry or submerged conditions did not change our results. However, performing these tests under room temperature or body temperature conditions yielded differences. Importantly, because we use venous blood our work is most closely related to venous *in vivo* blood clots. Overall, we have demonstrated that whole blood clots show several dissipative phenomena – similarly to hydrogels – that will be critical to our understanding of thrombus embolization.

1 Introduction

In vivo blood clots, or thrombi, play diametric roles in our body. Viscoelastic blood clots prevent hemorrhage after vascular injury and are thus vital to our well-being.[1] On the other hand, they are also the source of devastating thromboembolic conditions such as strokes, heart attacks, and pulmonary thromboembolism. In fact, 1 in 4 deaths worldwide are attributed to thromboembolic conditions.[2] Thus, thromboembolic conditions are the number one cause of death. Additionally, 31% of patients under intensive care for COVID-19 have exhibited thromboembolic complications.[3]

The mechanical properties of thrombi are important to both their physiological and pathological roles. For instance, the high extensibility and resistance to fracture of blood clots are vital to their ability to seal the injured vasculature and resist the pulsatile, circulatory load while the host tissue heals.[4] On the other hand, it is when external forces overcome thrombus extensibility and resistance to fracture that they break off and embolization occurs, which precipitates the aforementioned thromboembolic conditions.[5]

The mechanical properties of blood clots result from the hierarchical interplay of their fibrin backbone, platelets, red blood cells, and pore-filling, interstitial plasma.[6,7] During clot formation, fibrinogen is cleaved and activated to form a semi-flexible fibrin polymer.[8] Fibrin is a remarkable polymer with extensibility up to 300% and significant fatigue resistance.[9–11] Thus, it lends blood clots much of their high deformability. This backbone is stabilized and prestrained by activated or "sticky" platelets. These formed bodies attach to fibrin and actively pull on its fibers during the initial clot formation, thus stiffening and densifying the material.[12,13] Finally, red blood cells contribute to clot mechanics by filling space and re-distributing loads. Although their role is passive, recent work has demonstrated that they drastically transform the behavior of their host material.[14] Finally, driven by gradients in pore pressure, interstitial plasma permeates through the clot matrix and ostensibly contributes to its time-dependent behavior as it exchanges momentum with the surrounding matrix.[15]

Because the mechanical properties of blood clots are critical in health and disease, they have been studied extensively. For a detailed review, we refer the reader to excellent work previously published by others.[16,17] However, relatively few studies have focused specifically on thrombus properties that give rise to its ability (or inability) to resist external forces, *i.e.* embolize, with few exceptions.[18] The study of thrombus embolization is complicated by its complex and time-variant composition and the resulting myriad nonlinear mechanical phenomena

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contributing to its behavior.[19,20] Generally speaking, resistance to fracture is driven by dissipative mechanisms.[21] We categorize them as those that result in surface creation and those that are non-surface creating. The former arise from the energy required to create new surfaces as the continuity of the material is disrupted. The latter mechanisms arise from dissipation before and during surface creation such as damage, plasticity, and frictional losses. In an effort to understand thrombus fracture as the biophysical phenomenon underlying thrombus embolization, we set out to characterize the non-surface creating dissipative mechanisms of whole blood clots. To this end, we use an *in vitro* system in which we produce whole blood clots to mimic fresh thrombi. We tested these thrombus mimics with uniaxial tensile tests, cyclic tensile tests, and stress-relaxation tests to give a detailed account of the time-dependent mechanical properties that give rise to these non-surface-creating, dissipative mechanisms of thrombi.

2 Methods

2.1 Sample preparation

We generated blood clots from bovine blood. Samples were obtained from a commercial service (Lampire Biological Laboratories, PA, USA) that collected blood by directly bleeding into an anticoagulant (CPDA-1 anticoagulant at 14% volume:volume) and stored samples at 4 °C prior to overnight shipping. In turn, the samples were then stored for 12–72 hours, also at 4 °C, in our laboratory before mechanical testing. We note that bovine blood has comparable cell counts to human blood.[22,23] To coagulate blood, we added calcium chloride to a final concentration of 20 mM to reverse the anticoagulant.[12,24] To support coagulation, we gently mixed the blood with a wide-mouth pipette before injecting it into a custom, 2-piece mold that was lined with hook-and-loop fabric on two sides (see Fig. 1A).[25] The mold, in turn, has sliding attachments for easy mounting to our table top Instron uniaxial tensile testing machine (Instron, Norwood, MA, USA). Thus, no direct clamping or gluing of the sample is necessary, avoiding the danger of altering or damaging the material. We covered all samples during the 60 minute coagulation period to prevent dehydration.[26]

2.2 Mechanical testing

After mounting the samples to the tensile testing machine, we displaced samples to 0.5 mm to disengage the two-part molds. Subsequently, we conducted the tests following three types of uniaxial loading protocols: simple extension to 40% clamp-to-clamp strain, cyclic loading for ten cycles with a peak strain of 40%, and stress relaxation tests with four 120 s holds at 10%, 20%, 30%, and 40% strains. We conducted the simple extension and cyclic tests at three displacement rates: 0.2 mm s^{-1}, 1.0 mm s^{-1}, and 5.0 mm s^{-1}, or equivalently at three strain rates: 2% s^{-1}, 10% s^{-1}, and 50% s^{-1}, respectively. These rates were motivated by reports of thrombus *in vivo* strains on the order of ten percent and heart rates of approximately 1 Hz.[20,27,28] During the stress-relaxation tests, we consecutively ramped the tissue to the four respective strains at a rate of 1 mm s^{-1}. Additionally, we conducted all experiments under three different environmental conditions: at room temperature and without being submerged in phosphate buffered saline (PBS), being submerged in PBS at room temperature, and being submerged in PBS at 37 °C. Note that, while testing the samples under "dry" conditions is non-physiological, we felt the inclusion of this condition may justify future, simpler experimental setups that would not require fluid baths. Finally, we prepared samples and conducted each experiment on Day 1, Day 2, and Day 3 after receipt of the blood.

2.3 Statistics

As described above, we designed a balanced study with each test type being performed for each condition on each day. Thus, we had one independent factor (blood batch, *i.e.*, subject) and three dependent factors (strain rate, environmental condition,

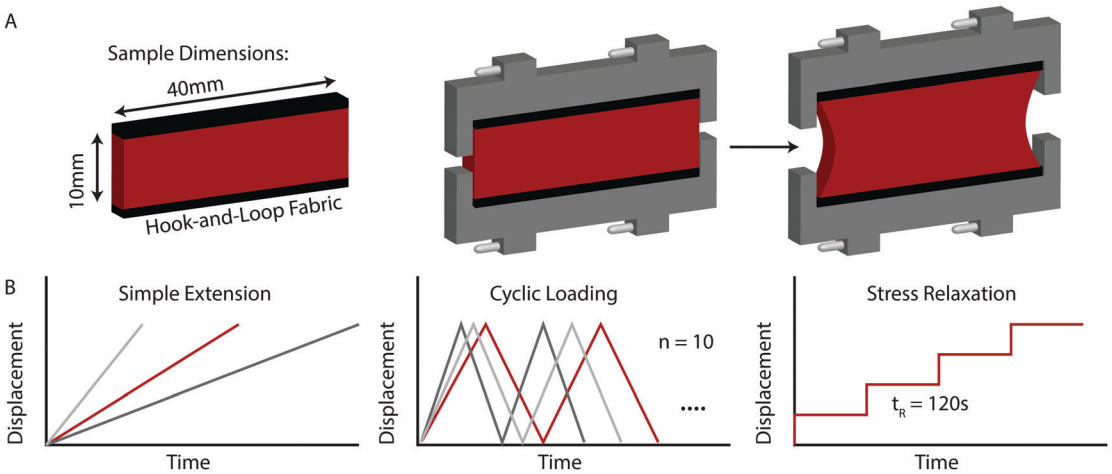

Fig. 1 Sample dimensions and loading protocols. (A) We coagulated whole blood into a custom, 2-piece mold that was lined with hook-and-loop fabric. (B) During testing, we used an Instron tensile testing machine to displace the upper mold piece according to three standard protocols: simple extension, cyclic testing, and stress relaxation tests. We conducted the simple extension and cyclic tests at three different strain rates.

and storage time for the tensile and cyclic protocol, as well as strain, environmental condition, and storage time for the stress-relaxation protocol). We used the "afex" library as implemented in R to fit a linear mixed model to our data and the "emmeans" library to conduct multicomparisons between groups.[29–31] For a direct comparison between two groups we conducted (paired) Student's *t*-tests. All data are reported as mean curves with standard error curves. The sample numbers for each graph are provided in the figure captions.

3 Results

Our methodology of coagulating blood clots into hook-and-loop fabric-lined molds successfully yielded homogeneous, consistent, and well-secured samples. We tested these samples under uniaxial extension to answer fundamental questions about the nonlinear, time-dependent mechanics of whole blood clots.

3.1 Whole blood clots show a linear constitutive behavior and strain-rate dependence during initial loading

We first performed simple uniaxial tensile tests on unconditioned samples. Importantly, during pilot experiments, we tested samples to failure to determine the maximum strains before whole blood clots demonstrated signs of damage. Based on these tests, we evaluated the "elastic" behavior of whole blood clots for extensions up to 40%. Fig. 2 shows extension curves for blood clot samples at 0.2, 1.0, and 5.0 mm s^{-1} or equivalent strain rates of 2, 10, and 50% s^{-1}. These curves demonstrate that under uniaxial extension the response of whole blood clots was approximately linear. Furthermore, the stiffness of these samples depended on strain rate with their material response becoming increasingly stiff with increasing strain rate ($p = 0.016$), *i.e.*, the mechanical behavior of whole blood clots is strain-rate dependent.

3.2 Whole blood clots show hysteresis, Mullins-like effect, and permanent set

In Fig. 3 we illustrate two distinct experiments to investigate the effect of the history-dependence of whole blood clots. First, we loaded the samples to 40% strain and then unloaded them to their starting configuration at zero displacement, before reloading to 40% strain (see Fig. 3A). In a second experiment, we unloaded the samples to zero force after the initial loading before returning to 40% strain (see Fig. 3B). In the first experiment we observe that whole blood clots did not follow the initial loading curve during unloading. Instead, their response fell below the initial loading curve and entered a compressive state when approaching its reference configuration. This implies that (i) whole blood clots dissipated energy during the initial loading cycle, and (ii) whole blood clots elongated during the original loading. The secondary loading to 40% strain followed neither the initial loading curve, nor the unloading curve. In other words, whole blood clots experienced stress-softening akin to Mullins effect in elastomers, *i.e.*, a Mullins-like effect, in addition to hysteresis and permanent set.[32] Interestingly, we found that the amount of hysteresis depended on the strain rate, with increasing strain rate increasing hysteresis ($p < 0.001$). In the second experiment we observe the same qualitative and quantitative behavior. However, because we reversed the test direction once the samples reached a zero-force configuration (which is different from the reference configuration) we could estimate the permanent set accrued during the initial loading as approximately 12%.

3.3 Whole blood clot mechanics equilibrate after ten cycles during which it transforms into a nonlinear material

In vivo thrombi are exposed to cyclic forces due to shear and wall motion. Here, we performed cyclic loading in ten cycles to investigate whether whole blood clots approach an equilibrium state during those ten cycles. Specifically, we noted peak force, hysteresis, and permanent set for those ten cycles. In Fig. 4A, we observed that blood clots showed preconditioning in that peak force, hysteresis, and permanent set dropped after the first cycles, but then trended toward an equilibrium response. While neither metric fully equilibrated by ten cycles, changes between the last and second-to-last cycles were small in comparison to the initial change between cycles one and two. Thus, ten cycles of loading may represent a practical compromise

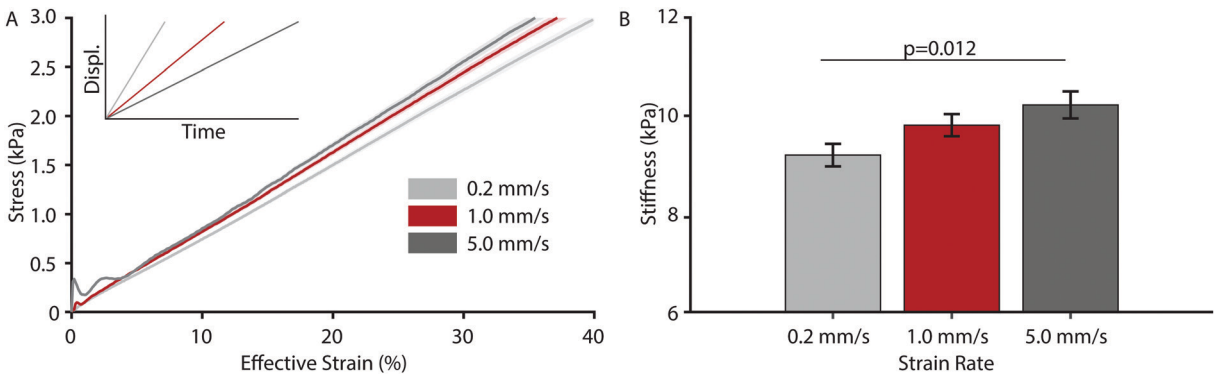

Fig. 2 During initial loading whole blood clots behave linearly and show strain-rate dependence. (A) Stress–strain behavior of initially loaded whole blood clots at three strain rates ($n = 18$ per group). (B) Stiffness of whole blood clots at three different strain rates ($n = 18$ per group). All data are mean ± standard error. Note that the initial spike in the 5.0 mm s^{-1} curve is due to motor acceleration and was not included in the stiffness calculation.

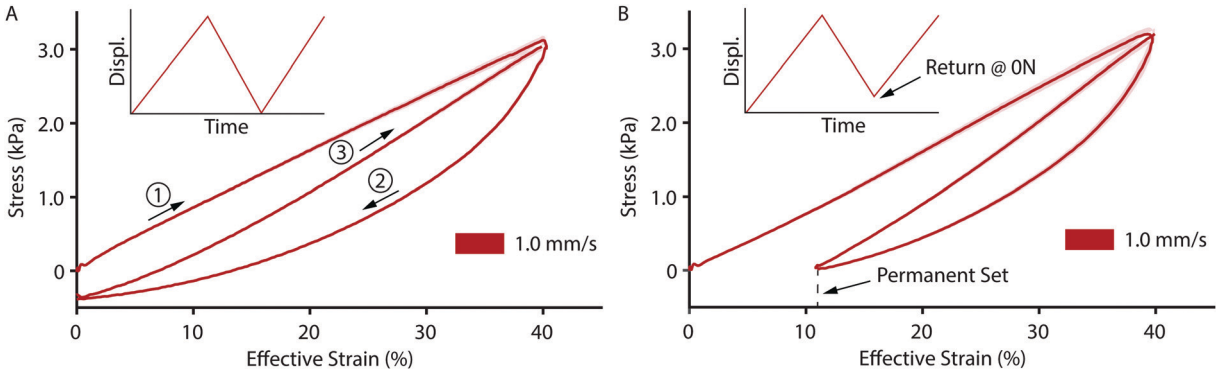

Fig. 3 Whole blood clots show hysteresis, Mullins-like effect, and permanent set. (A) A loading–unloading cycle with return to the original reference configuration (n = 9 per group). (B) A loading–unloading cycle with return to zero force, which allowed us to estimate the permanent set to be approximately 12% (n = 9 per group).

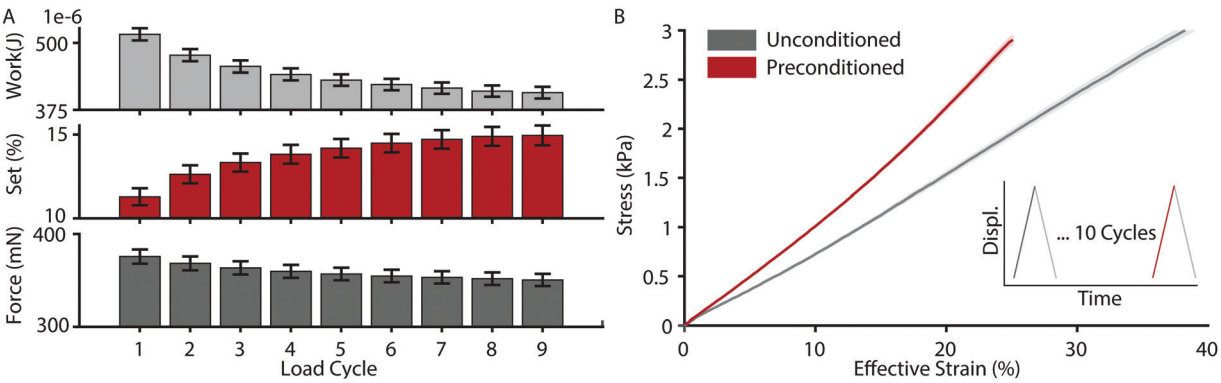

Fig. 4 The mechanical response of whole blood clots equilibrates after ten loading cycles. (A) Work lost in hysteresis, permanent set, and peak force trended toward an equilibrium state after ten loading cycles (n = 9). (B) After ten loading cycles, the stiffness of whole blood clots increased significantly (n = 9).

when simulating the equilibrated response of whole blood clots to repetitive mechanical stimuli in the body. Importantly, the equilibrated material response was significantly stiffer than that of the untested samples ($p < 0.001$ *via* paired Student's t-test between the first and last cycles). Fig. 4B compares the first and the tenth loading cycle illustrating the material stiffening. Note that, for illustration purposes, we removed the permanent set after the tenth cycle.

3.4 Whole blood clots undergo strain-dependent stress relaxation

To further investigate the time-dependent behavior of whole blood clots, we additionally performed sequential stress-relaxation experiments at 10%, 20%, 30%, and 40% strains and allowed the material to relax for 120 s in between. We observed that blood clots relaxed at each strain (see Fig. 5A). To quantify the relaxation behavior, we fit exponential decay functions to the data at each step, *viz.*

$$\hat{\sigma} = 1 - \sum_{i}^{n} c_i(1 - \exp(t/\tau_i)), \quad (1)$$

where $\hat{\sigma}$ is the normalized stress at each given strain level, t is the time, c_i are scaling parameters and τ_i are time constants. We compared the goodness of fit of 1-term (n = 1), 2-term (n = 2), 3-term (n = 3), and 4-term (n = 4) functions. We found that the goodness of fit measured as the normalized mean squared error (NMSE) significantly improved between one (NMSE = 0.79) and two terms (NMSE = 0.98), but did not improve for three terms (NMSE = 0.98) or four terms (NMSE = 0.98). Thus, we decided to report fits to a two-term function and identified two time constants, τ_1 and τ_2. Interestingly, our least squares fits consistently identified short-timescale (around 1 s) and long-timescale constants (around 30 s) (see Fig. 5B). Both time constants increased with strain ($p < 0.001$, $p < 0.001$), thus implying that whole blood clots show signs of nonlinear viscoelasticity similar to that reported by Nam *et al.*[33]

3.5 Whole blood clots recover their unloaded constitutive behavior after each relaxation step

When plotting stress against strain for the sequential stress-relaxation loading and comparing these curves to the unconditioned loading of the blood clots, we found that stress relaxation at lower strains does not affect the constitutive behavior of clots at larger strains (see Fig. 6). Specifically, with increasing strain after each stress relaxation period, clots recovered their unconditioned uniaxial loading behavior. This is only

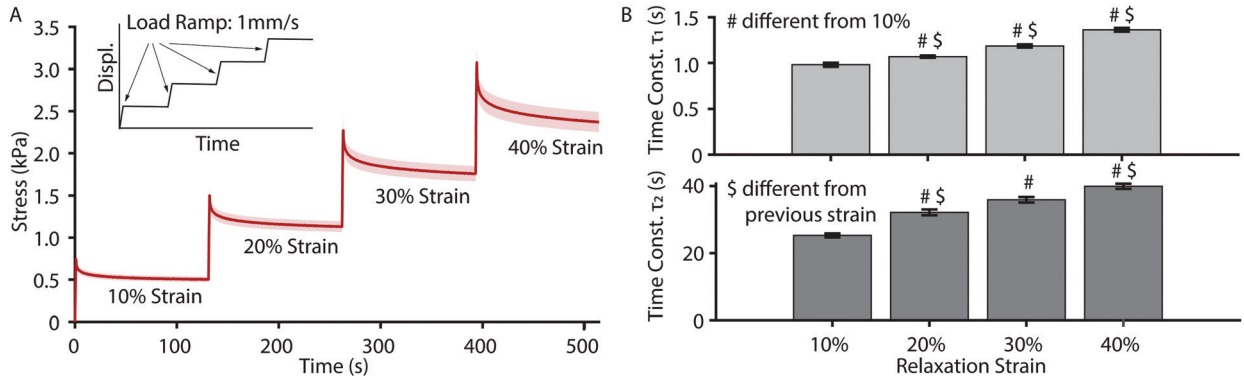

Fig. 5 Whole blood clots are nonlinear viscoelastic materials. (A) Whole blood clots showed stress-relaxation (n = 6). (B) The relaxation response is well represented by an exponential decay function with two time constants, both of which increased with strain (n = 6).

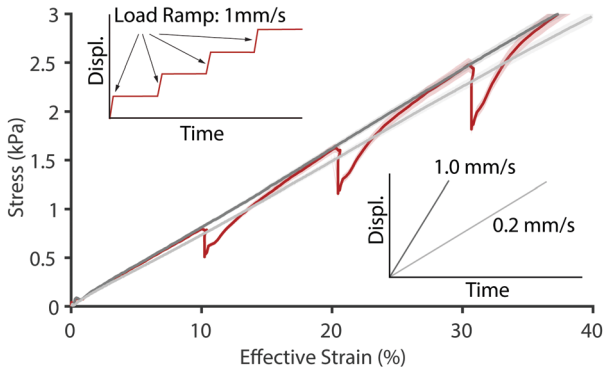

Fig. 6 Whole blood clots recover their unloaded constitutive behavior after each stress-relaxation test. We show the stress–strain behavior extracted from the sequential stress-relaxation tests (red, as shown in Fig. 5A, n = 18) and compared it to the unloaded behavior under simple extension at both 0.2 mm s^{-1} (light gray, n = 18) and 1.0 mm s^{-1} (dark gray, n = 18) (as shown in Fig. 2A).

Table 1 Time constants τ_1 and τ_2 as a function of sample size and strain. Data are shown as mean ± standard error (n = 6 per group)

Size	10%	20%	30%	40%
τ_1				
3 mm	1.09 ± 0.02	1.18 ± 0.04	1.31 ± 0.04	1.42 ± 0.02
4 mm	1.08 ± 0.01	1.18 ± 0.01	1.30 ± 0.01	1.41 ± 0.01
5 mm	0.97 ± 0.03	1.14 ± 0.02	1.26 ± 0.01	1.43 ± 0.02
τ_2				
3 mm	28.7 ± 0.6	29.9 ± 1.4	32.4 ± 0.6	33.6 ± 0.2
4 mm	25.7 ± 0.9	29.5 ± 0.3	31.2 ± 0.3	33.2 ± 0.5
5 mm	23.6 ± 0.6	28.2 ± 0.4	31.3 ± 0.3	33.5 ± 0.2

true when comparing the stress-relaxation data to the unconditioned loading curve at 1 mm s^{-1}. When we compare the stress–strain behavior during the stress-relaxation tests against the unconditioned uniaxial behavior at 0.2 mm s^{-1}, we find that it falls above the unconditioned uniaxial loading curve. These findings also imply that 0.2 mm s^{-1} does not represent the quasi-static behavior of blood clots, which we predict would represent the lower bound of the stress–strain curve during stress-relaxation.

3.6 The stress-relaxation of whole blood clots not strongly dependent on geometry

The identification of two time constants may imply that two distinct physical processes contribute to stress relaxation. Given the highly hydrated state of whole blood clots and the polymeric nature of the fibrous backbone of blood clots, it is possible that diffusion-based and solid viscoelasticity-based processes may collaboratively determine the time-dependent behavior of blood clots. While the former would be expected to be a function of the sample cross-section, the latter would not. To investigate the degree to which these time constants and thus the underlying physical processes depend on geometry, we repeated the above experiments on samples with 4 mm and 5 mm cross-sections (as opposed to all other samples that had 3 mm cross-sections). Table 1 shows a comparison of the time constants between those three sample types. Neither the short- nor the long-term response appeared to significantly increase with increasing cross-section (p = 0.287 and p = 0.289). This may support the hypothesis that time-dependence resulted from solid viscoelasticity rather than diffusion-based processes. However, future studies with a larger geometric range may be able to shed more confident light on this question.

3.7 Whole blood clot mechanics do not depend on humidity conditions, but on temperature

Given that thrombi form within vessels, their natural environment is within a physiological fluid bath and at body temperature. We have previously tested whole blood clots only under those conditions. As testing blood clots under non-submerged, room-temperature conditions would ease experimental protocols, we wanted to test the effect of both temperature and humidity on the mechanics of whole blood clots. Thus, we compared the mechanics of blood clots when tested at room temperature without a bath, in a room-temperature PBS bath, and in a 37 °C PBS bath, both under simple extension and during stress-relaxation. Fig. 7A shows the results for the simple uniaxial extension tests, while Fig. 7B shows the results for the stress-relaxation tests as a function of those conditions. In the former figure, variations appear

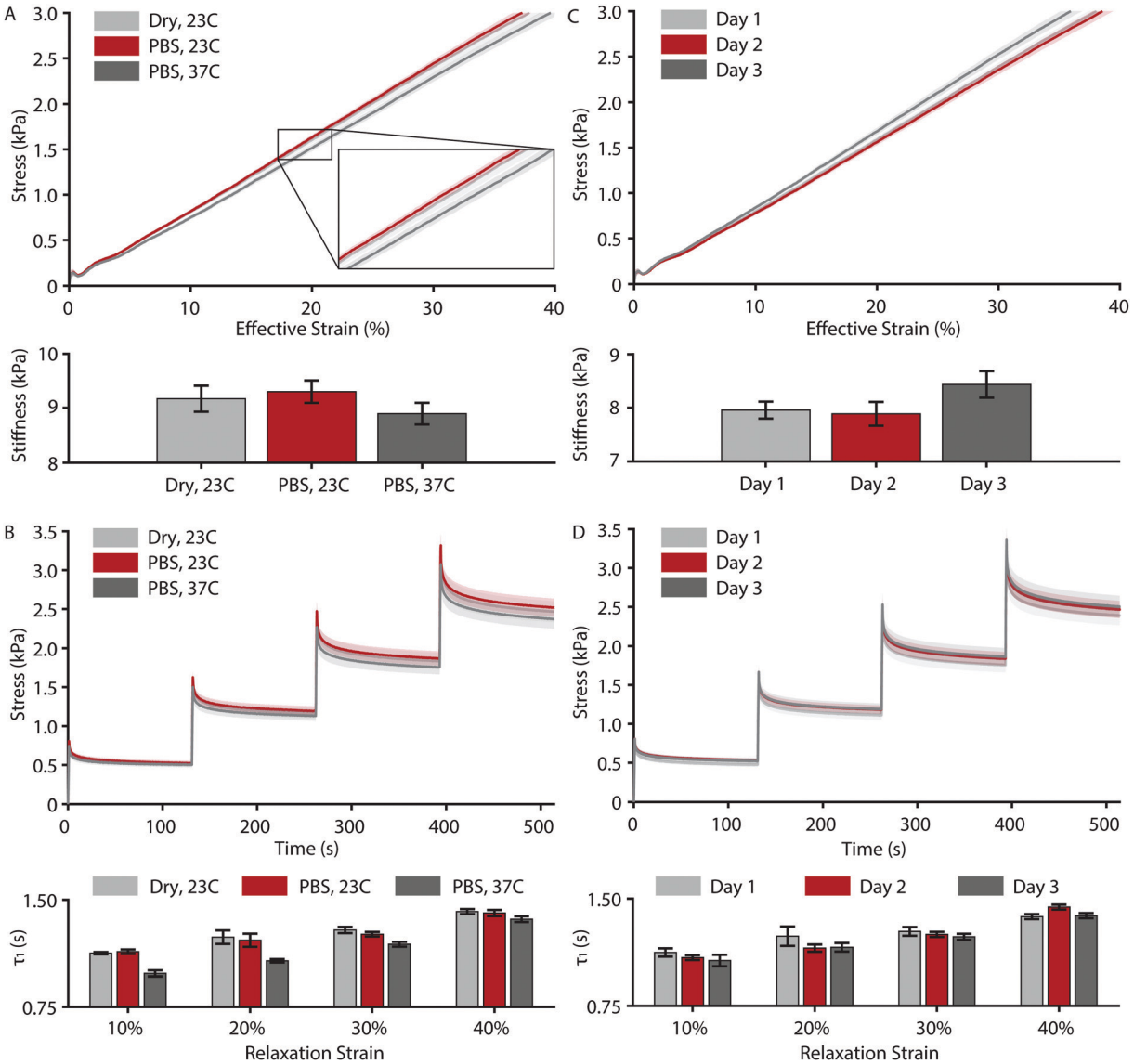

Fig. 7 The nonlinear, time-dependent behavior of whole blood clots depends on test temperature, but not on hydration conditions during testing or on blood storage time. (A) Simple uniaxial extension of whole blood clots as a function of environmental conditions (n = 18 per group). (B) Stress-relaxation of whole blood clots as a function of environmental conditions (n = 18 per group). (C) Simple uniaxial extension of whole blood clots as a function of blood storage time (n = 6 per group). (D) Stress-relaxation of whole blood clots as a function of blood storage time (n = 6 per group).

small suggesting that stiffness was not affected by testing conditions (p = 0.717). However, differences seem to exist in the stress-relaxation data. A closer examination revealed that the constant τ_1 was shorter in the 37 °C bath than under the other two conditions ($p < 0.001$). Interestingly, we did not find that τ_2 depended on temperature (p = 0.680).

3.8 The relaxation behavior of whole blood clots, but not their stiffness, depends on storage time

Similarly to our test of the dependence of the mechanical properties of whole blood clots on environmental conditions, we also wanted to investigate the effect of blood storage time. While we have previously performed a similar analysis under simple shear, during which we found that the stiffness of whole blood clots did not depend on blood storage time during days one through three, we only did so under one loading rate and did not consider the time-dependent behavior.[26] Fig. 7C and D show the uniaxial loading behavior and stress-relaxation behavior of whole blood clots as a function of blood age, comparing one day, two days, and three days of blood storage before coagulation and testing. We found slight differences between days one and three in the first time constant, which were not statistically significant τ_1 (p = 0.068). We suspect that an increased sample number would provide the necessary power to statistically support this difference. However, we did not see any differences in the stiffness of whole blood clots, which is in agreement with our previous findings (p = 0.121).[26]

4 Discussion

While the mechanics of blood clots and thrombi have been extensively investigated, comprehensive mechanical studies focusing on their time-dependent properties are sparse; moreover, studies investigating their fracture behavior are all but absent. This gap should be overcome as thrombus fracture leads to thromboembolic conditions, which is a substantial national and global health problem. Generally speaking, energy dissipating mechanisms, such as those studied here, may lower the energy available for surface creation required for fracture, thus increasing the apparent toughness of the material.[34] This non-surface-creating dissipation of energy is the basis for toughening strategies of both classic engineering materials such as steel and non-classic engineering materials such as hydrogels.[35,36] For example, in double network hydrogels, one network family, the sacrificial network, yields at relatively small loads and thereby dissipates energy that protects the non-sacrificial network from fracture.[37]

With an ultimate goal of identifying the surface-creating dissipative mechanisms during the fracture of whole blood clots, we felt it was prudent to first identify the non-surface-creating dissipative mechanisms. We anticipated that we would observe damage, hysteresis, stress-relaxation, and strain-rate dependence.[38] Each of these phenomena contributes to (or is an example of) energy dissipation before fracture. It is only after identifying and quantifying these phenomena that we can deconvolute the mechanisms that lead to the ability (or inability) of a thrombus to resist external forces. Additionally, we tested the effect of storage and environmental conditions on these mechanisms for future reference.

As we expected, we observed that whole blood clots were strain-rate dependent. Albeit, across the 25-fold increase in strain rate, the stiffness of the material increased only marginally. Additionally, we found that whole blood clots dissipate energy during the first loading–unloading cycle *via* a Mullins-like effect,[39] which is possibly enhanced compared to pure fibrin by the inclusion of RBCs as classical filler particles.[14,40] This initial energy dissipation due to the Mullins-like effect is compounded through hysteresis, which we continued to observe during subsequent loading cycles, not just the first. In the absence of direct evidence for material damage, it is also possible that alternative mechanisms such as viscoelastic-plasticity may be responsible for the initial energy dissipation, and we hope future studies will disentangle these effects. Interestingly, we found that the response of whole blood clots to cyclic loading equilibrated after around ten cycles. Thus, repeated or cyclic loading as seen *in vivo* likely alters the ability of a thrombus to resist fracture only during the first cardiac cycle and stabilizes afterwards. Note that fatigue, which we have not investigated here, may weaken the material over time at cycle numbers larger than ten.[41] We also found that whole blood clots relax under strain. This response changes with increasing strain indicating that whole blood clots behave like a nonlinear viscoelastic material.[33,42]

In our stress-relaxation experiments, we found two relaxation timescales, possibly suggesting a diffusion- and solid viscoelasticity-based relaxation. With our additional experiments showing the geometry-independence of the relaxation constants, our data indicate that the time-dependent behavior of whole blood clots may be driven by solid viscoelasticity, rather than diffusion-based mechanisms. Alternatively, the two time scales could be found in processes on different scales, *e.g.*, network-scale, fiber-scale, protofibrillar-scale, or molecular-scale. For instance, the strain stiffening of fibrin gels is driven by such hierarchical phenomena.[43]

In our work, we also found that the nonlinear, time-dependent behavior of whole blood clots was insensitive to whether tests were conducted in a fluid bath or under dry conditions. This finding may additionally support the hypothesis that the time-dependent mechanical properties of blood clots are driven by solid viscoelasticity rather than diffusion-based viscoelasticity. However, we did find that these properties were slightly different when conducted at room temperature or at body temperature. Similarly, we found marginal effects of blood storage conditions on these properties. Dependence on storage conditions is likely driven by the death of red blood cells and platelets, which we found to occur within days of storage.[26] Our data provide an incentive to test whole blood clots and thrombi at body temperature and with short blood storage. If future studies should ignore these recommendations, our data provide the means to estimate the error accrued by doing so.

We propose that future studies on the fracture of thrombi – or whole blood clots as thrombus mimics – may follow an approach as outlined by Zhang *et al.* in their study of hydrogel fracture.[34] Specifically, they combined a nonlinear finite element model with mode I fracture experiments to delineate the intrinsic and effective toughness of a hydrogel. Our current work provides the information and data to do so. Additionally, we are currently lacking an established constitutive model that may cast our data into a mathematical and numerically-amiable form. Future studies may also wish to explore the best constitutive frameworks and specific forms.

From an experimental perspective, we see a need for future work to explore the role of blood constituents in the nonlinear, dissipative phenomena described here. Fibrin is unarguably a critical contributor to blood clot mechanics as it is ostensibly the primary load-bearing constituent of clots. Thus, it is not entirely surprising that many of the phenomena described above have previously been observed in fibrin gels. For example, fibrin, similarly to our findings, demonstrates a nonlinear stress–strain behavior, nonlinear viscoelasticity, and hysteresis.[43–46] Recent work has also demonstrated the importance of constituents, other than fibrin, on the mechanics of blood clots. Specifically, van Oosten *et al.* demonstrated that red blood cells may act as space filling, passive inclusions that fundamentally alter the mechanics of fibrin gels.[14] Consequently, we expect that red blood cells affect nonlinear-dissipative phenomena by redistributing loads among the structural constituents of clots. Similarly, it is likely that platelets through their active contractile state contribute to the viscoelastic nature of blood clots and to their nonlinearity.[13] This is particularly true as there will

likely be a load at which they detach from the fibrin network, which would alter the inherent structural organization of a clot. Additionally, cells and formed elements such as red blood cells and platelets are inherently viscoelastic.[47] Thus, they also likely add to the viscoelasticity of their host material through their sheer presence. Understanding the importance of fibrin, platelets, and red blood cells on the herein described non-linear, dissipative mechanisms may also expand the relevance of future findings to arterial blood clots that are composed differently to the venous blood clots studied here.

As to the clinical relevance of our work, our findings add to the current knowledge about blood clots. Specifically, the identification of non-surface creating, dissipative mechanisms adds to our understanding of the resistance of blood clots to fracture. With these data, future fracture experiments will be able to identify the inherent fracture toughness of blood clots. By testing blood clots generated from the blood of at-risk patients, this metric may provide an insight into the underlying causes of embolization in those patient populations.

5 Conclusions

Whole blood clots show a number of non-surface-creating, dissipative phenomena. These materials show evidence of Mullins-like effect, hysteresis, permanent set, and nonlinear stress-relaxation. Quantifying these phenomena is critical to our understanding of thrombus fracture as the biophysical phenomenon underlying embolization. With support of our work and our data, future studies will be able to focus on explicit fracture, *i.e.*, the creation of surfaces *via* crack propagation while accounting for other dissipation behavior.

Conflicts of interest

Manuel K. Rausch has a speaking agreement with Edwards Lifesciences. None of the other authors have conflicts to declare.

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