journal of the mechanical behavior of biomedical materials 154 (2024) 106508

Contents lists available at ScienceDirect

Journal of the Mechanical Behavior of Biomedical Materials

journal homepage: www.elsevier.com/locate/jmbbm

Research paper

Mechanical properties of clot made from human and bovine whole blood differ significantly

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ARTICLE INFO

Dataset link: https://dataverse.tdl.org/dataverse/STBML

Keywords:
Venous thrombus
Viscoelasticity
Fracture mechanics
Cyclic loading
Prestrain

ABSTRACT

Thromboembolism – that is, clot formation and the subsequent fragmentation of clot – is a leading cause of death worldwide. Clots' mechanical properties are critical determinants of both the embolization process and the pathophysiological consequences thereof. Thus, understanding and quantifying the mechanical properties of clots is important to our ability to treat and prevent thromboembolic disease. However, assessing these properties from in vivo clots is experimentally challenging. Therefore, we and others have turned to studying in vitro clot mimics instead. Unfortunately, there are significant discrepancies in the reported properties of these clot mimics, which have been hypothesized to arise from differences in experimental techniques and blood sources. The goal of our current work is therefore to compare the mechanical behavior of clots made from the two most common sources, human and bovine blood, using the same experimental techniques. To this end, we tested clots under pure shear with and without initial cracks, under cyclic loading, and under stress relaxation. Based on these data, we computed and compared stiffness, strength, work-to-rupture, fracture toughness, relaxation time constants, and prestrain. While clots from both sources behaved qualitatively similarly, they differed quantitatively in almost every metric. We also correlated each mechanical metric to measures of blood composition. Thereby, we traced this inter-species variability in clot mechanics back to significant differences in hematocrit, but not platelet count. Thus, our work suggests that the results of past studies that have used bovine blood to make in vitro mimics – without adjusting blood composition – should be interpreted carefully. Future studies about the mechanical properties of blood clots should focus on human blood alone.

1. Introduction

Thromboembolic disease is a leading cause of morbidity and mortality worldwide (Cushman, 2007). That is, when blood clots first form and then break off, emboli may travel downstream and occlude vital arteries and veins such as those of the heart, brain, or lungs. Respectively, these occlusions may lead to cardiac ischemia, ischemic stroke, and pulmonary embolism (Beckman et al., 2010). The specific clinical manifestation of thromboembolic disease depends on clots' mechanical properties (Rausch and Humphrey, 2017). For example, clots' fracture toughness determines whether and when they break off, while clots' stiffness determines how far they can travel and which arteries or veins they occlude (Fereidoonnezhad et al., 2021b; Rausch et al., 2021a). Thus, quantifying and understanding blood clots' mechanical properties is important to our ability to treat and prevent thromboembolic disease and its deadly sequelae.

In pursuit of quantifying and understanding blood clots' mechanical properties, we and many others have resorted to studying the properties of in-vitro clot mimics (Liu et al., 2021; Fereidoonnezhad et al., 2021a; van Kempen et al., 2016; Liang et al., 2017; Malone et al., 2018; Johnson et al., 2021; He et al., 2022; Varner et al., 2023; Riha et al., 1999; Gersh et al., 2009; Chueh et al., 2011; Krasokha et al., 2010; Huang et al., 2013; Litvinov and Weisel, 2022). However, there is lack of consensus in the existing literature. For example, reported stiffness values differ substantially (Varner et al., 2023). These discrepancies likely stem from at least two different sources: widely varying mechanical testing protocols and variability in sample preparation, including

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https://doi.org/10.1016/j.jmbbm.2024.106508
Received 18 August 2023; Received in revised form 24 February 2024; Accepted 13 March 2024
Available online 15 March 2024

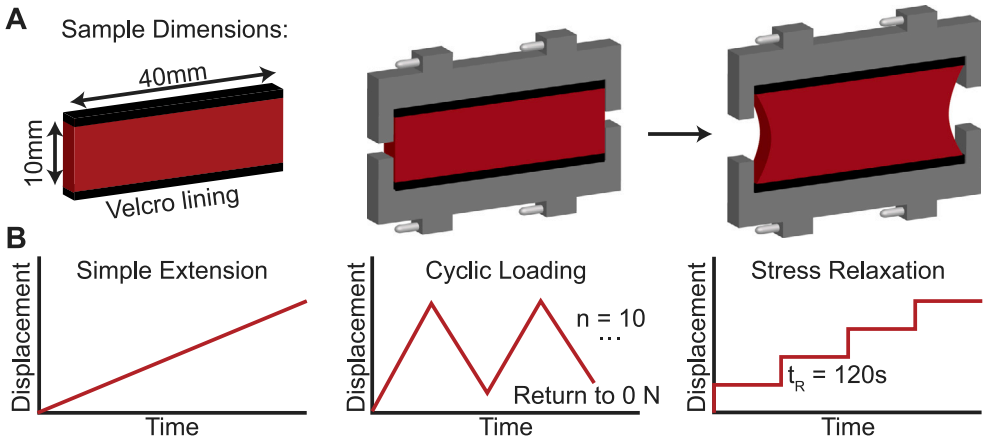

Fig. 1. Sample dimensions and mechanical testing protocols. (A) We cast our samples in custom molds lined with Velcro which securely hold the samples. We mounted them onto our uniaxial tensile tester and deformed them according to one of three protocols: (B) Pure shear and Mode-I samples underwent simple extension to failure. Cyclic loading samples were displaced to 40% clamp-to-clamp strain before returning to 0 N for a total of ten cycles. Stress relaxation samples were loaded to 10, 20, 30, and 40% clamp-to-clamp strain in subsequent steps with a 120 s hold after each step.
Source: Reproduced from Sugerman et al. (2020) with permission from the Royal Society of Chemistry.

blood source. In a step toward overcoming these challenges, we have previously developed and shared robust, repeatable, and highly controlled sample preparation and mechanical testing protocols for blood clot (Sugerman et al., 2020, 2021a,b, 2023; Lohr et al., 2022; Rausch et al., 2021b).

We developed and tested these sample preparation and mechanical testing protocols using bovine blood for practical reasons — it is widely available and inexpensive, and in addition to ovine and porcine blood it has historically been used to model human blood clots (Chueh et al., 2011; Malone et al., 2018; Ghezelbash et al., 2022; Varner et al., 2023). However, blood composition and coagulation chemistry vary across these species (Siller-Matula et al., 2008; Dibiasi et al., 2018). For example, hematocrit – or the volume percentage of blood occupied by red blood cells (RBCs) – varies between humans and cows. The healthy, non-pregnant reference range for human hematocrit is 37%–52% while that of the cow is 21%–38% (Dixon, 1997; Roland et al., 2014). Additionally, cows have smaller RBCs than humans. Human RBCs have a mean volume of 81–99 fL and a mean diameter of 7.5–8.7 μm (Dixon, 1997; Diez-Silva et al., 2010) while bovine RBCs have a mean volume of 36–50 fL and a mean diameter of 5–6 μm (Roland et al., 2014; Adili et al., 2016). Given that hematocrit has been shown to strongly affect clot mechanical properties, it is to be suspected that the mechanical properties of clots from bovine blood may fundamentally differ from those made of human blood (Tynngård et al., 2006; Thurston, 1978; Fereidoonnezhad et al., 2021a). However, to date, no comprehensive comparison between the elastic, viscoelastic, and fracture mechanical properties of clots from unmodified blood of both sources has been conducted. This knowledge gap should be filled to ensure that future efforts focus on a blood clot mimic that represents human blood clots well, which is the goal of this study.

2. Methods

2.1. Blood collection

We obtained bovine blood in CPDA-1 anticoagulant (86:14 blood:anticoagulant) from a commercial vendor (Lampire Biological Laboratories, Pipersville, PA, USA), while we drew human blood from healthy donors into ACD anticoagulant (85:15 blood:anticoagulant). The composition of the anticoagulants is compared in Table 1. Human research protocols were reviewed and approved by the Institutional Review Board at The University of Texas at Austin. For both the human and bovine subjects, we used blood samples from six distinct subjects. Bovine blood samples were tested within 4 h after a 48-hour shipping period. Human samples were stored for the same duration, i.e., 48 h, after the draw and then tested within 4 h to match the storage time of the bovine samples. During storage, both bovine and human blood samples were kept at 4 °C. Our human subjects include four male and two female participants, with a mean age of 25.3 years (range 22–29 years). Our bovine subjects include three male and three female donors.

Table 1
Contents of anticoagulants: ACD anticoagulant was used for human samples while CPDA-1 anticoagulant was used for bovine samples.

Contents (g/L)	ACD	CPDA-1
Trisodium citrate	22	26.3
Citric acid	8	2.98
Dextrose	24.5	31.8
Sodium phosphate	–	2.21
Adenine	–	0.28

2.2. Sample preparation

We prepared our samples according to previously published protocols (Sugerman et al., 2021a). Briefly, we mixed blood with calcium chloride (20 mM final concentration) to reverse the effects of the anticoagulants. We then cast the blood into custom molds according to the test modality. For prestrain assessment, we filled 30 × 30 mm square frames (3 mm thick) with blood and covered both sides with polymer sheets (Gel-Pak tack level 4, Gel-Pak, Hayward, CA, USA) to prevent evaporation. For all other tests, we added the blood to custom rectangular molds lined with Velcro to create a secure attachment, see Fig. 1 for an illustration. We incubated all the samples at 37 °C for 60 min prior to testing. We measured the incubation time from the addition of calcium chloride until the start of the given test modality. We conducted three technical replicates per subject per test modality.

2.3. Complete blood count

We obtained complete blood counts (CBC) from all human subjects and from four of the six bovine subjects. We replaced the missing two CBC data sets with sets from age- and sex-matched cows from the same vendor. All measurements were performed by commercial medical or veterinary labs.

2.4. Mechanical testing

To measure blood clot prestrain, we cut the squares free from their 30 x 30 mm frames and floated them on phosphate-buffered saline. We then photographed the samples on a calibrated grid and used a custom MATLAB program (Mathworks, Natick, MA, USA, Version 2022a) to measure the deformed size and compute the resulting Green–Lagrange strains in the x- and y-directions (The MathWorks Inc, 2022). Please note that due to sample breakage, only 5 bovine subjects are represented in the prestrain data along with the 6 human subjects.

To measure blood clot stiffness, strength, work to fracture, and fracture toughness, we conducted Pure Shear and Mode-I tests. For the latter, we precut samples along one-third of their width (approximately 13 mm). Then, we mounted the samples onto our uniaxial tensile tester (Instron, Norwood, MA, USA) using custom fixtures that avoid the need for clamping or gluing (Sugerman et al., 2021a). Next, we extended both sample types at a rate of 0.2 mm/s while measuring force with a 10 N load cell. During these tests, we captured force, displacement, and high-resolution images of the samples at a rate of 5 Hz synchronized using a custom LabVIEW program (National Instruments, Austin, TX, USA, Version 2021 SP1) (National Instruments, 2021).

We used the sequential images of the Mode-I tests to identify the stretch at which crack propagation began, which we call λ_c. Then, we calculated fracture toughness as

$$\Gamma_c = W(\lambda_c)H \tag{1}$$

where H is the initial height of the sample and $W(\lambda_c)$ is the strain energy density of the pure shear sample at the fracture stretch λ_c (Rivlin and Thomas, 1953).

Cyclic samples were mounted in the uniaxial tensile tester as described above but were instead subjected to cyclic loading to 40% clamp-to-clamp strain with a return to 0 N for ten cycles. A depiction of the loading scheme can be found in Fig. 1B. Similarly, to quantify the stress relaxation behavior of our samples, we mounted samples as with the other mechanical test. Then, we loaded them to 10, 20, 30, and 40% clamp-to-clamp strain consecutively with a 120 s hold following each step. For a depiction of the loading scheme, see Fig. 1B. As we have reported previously, the relaxation steps are well-represented by the following two-term exponential decay function (Sugerman et al., 2020):

$$\hat{\sigma} = 1 - \sum_{i}^{2} c_i\left(1 - \exp\left(\frac{t}{\tau_i}\right)\right) \tag{2}$$

where $\hat{\sigma}$ is the normalized stress for the individual strain level, t is time, c_i are scaling parameters, and τ_i are the time constants.

2.5. Statistics

To statistically compare bovine and human-derived blood clots, we used linear mixed models. Specifically, we used the "afex" library in R (The R Foundation, Vienna, Austria, Version 4.1.0) and conducted pairwise comparisons on significant effects using the "emmeans" library (R Core Team, 2021). For stress relaxation and cyclic tests, we statistically checked for the presence of interaction effects between species and strain step or cycle, respectively. We included interaction effects when a Chi-square test indicated that the model with interaction effects fit the data significantly better than the model without interaction effects. To conduct correlative studies, we used linear regression models to fit our scalar mechanical metrics to measures of blood composition using MATLAB (Mathworks, Natick, MA, USA, Version 2022a). We directly compared human and bovine CBCs using independent, two-sided Student's t-tests. Comparisons with p-values smaller than 0.05 were considered significant. All data are reported as mean ± standard deviation.

3. Results

3.1. Human clots are less resistant to fracture, more compliant, and weaker than bovine clots

We extended blood clots with and without pre-cuts to failure at a rate of 0.2 mm/s. In Fig. 2A, we show the Cauchy stress curves for the Pure Shear (left) and Mode-I (right) modes for human and bovine clots. Fig. 2B compares scalar metrics derived from these curves. We first assessed resistance to fracture with the metric fracture toughness, calculated using Eq. (1). Human clots have significantly lower fracture toughness than bovine clots (3.7 ± 0.88 vs. 10.2 ±2.2 J/m^2, $p < 0.0001$). We evaluated compliance using the tangent modulus, or stiffness, calculated at a stretch of 1.35, and we found that human clots have a significantly lower tangent modulus than bovine clots (5.1 ± 1.05 vs. 12 ± 2.3 kPa, $p < 0.0001$). We measured strength using the peak stress of un-notched samples. Human clots have significantly lower peak stress than bovine clots (2.7 ± 0.67 vs. 6.3 ± 1.11 kPa, $p < 0.0001$). Finally, we determined the amount of work required to rupture un-notched blood clots. We found that human clots have significantly lower work to rupture than bovine clots (0.5 ± 0.16 vs. 1.2 ±0.24 kJ/m^3, p = 0.0001). In summary, human clots require less energy to propagate cracks, are less stiff, fail at lower stress, and rupture more easily than bovine clots.

3.2. Human clots show less hysteresis and more set than bovine clots under cyclic loading

We deformed blood clots to 40% clamp-to-clamp strain and returned them to 0 N at a rate of 0.2 mm/s for ten consecutive cycles. Fig. 3A provides an example curve depicting the three scalar metrics we derive from each curve. Fig. 3B–D compare these scalar metrics between human and bovine clots, i.e., peak force, hysteresis, and set. Qualitatively, we found that these metrics equilibrate after ten cycles in both human and bovine clots. Quantitatively, we first compared force at 40% clamp-to-clamp strain between human and bovine clots across each cycle. Human clots have significantly lower peak force than bovine clots averaged across all cycles (130 ± 29 vs. 370 ± 54 mN, $p < 0.0001$). Next, we computed and compared the area between the loading and unloading curve to evaluate hysteresis, or the energy dissipated on each cycle. We showed that human clots have significantly lower hysteresis than bovine clots averaged across all samples (140 ± 36 vs. 430 ± 76 μJ, $p < 0.0001$). Finally, we measured and compared the amount of sample elongation after each loading step using the measure "set" across each cycle in both human and bovine clots. Human clots have a significantly higher set than bovine clots averaged across all cycles (16.6 ± 1.70 vs. 14.5 ± 1.78%, p = 0.0017). In summary, human clots have lower peak stress and energy lost to hysteresis than bovine clots but undergo more elongation than bovine clots during cyclic loading.

3.3. Human and bovine clots show similar relaxation behavior

We displaced blood clots to 10, 20, 30, and 40% clamp-to-clamp strain at a rate of 0.2 mm/s in four consecutive loading steps with a 3-minute hold following each step. Fig. 4A shows the mean ± standard deviation of force over time for each species. The same force data are presented in Fig. 4B relative to the applied displacement. We fit each stress relaxation step independently using Eq. (2). Fig. 4C presents the first time constant while Fig. 4D presents the second time constant. The first time constant, τ_1, does not differ significantly between human and bovine clots (3.0 ± 0.46 vs. 3.1 ± 0.35, p = 0.2222). The second time constant, τ_2, is significantly higher in human clots than bovine clots but is within the same order of magnitude (61 ± 7.4 vs. 56 ± 4.6, p = 0.0061). In short, human clots relax slightly slower than bovine clots under stepwise loading.

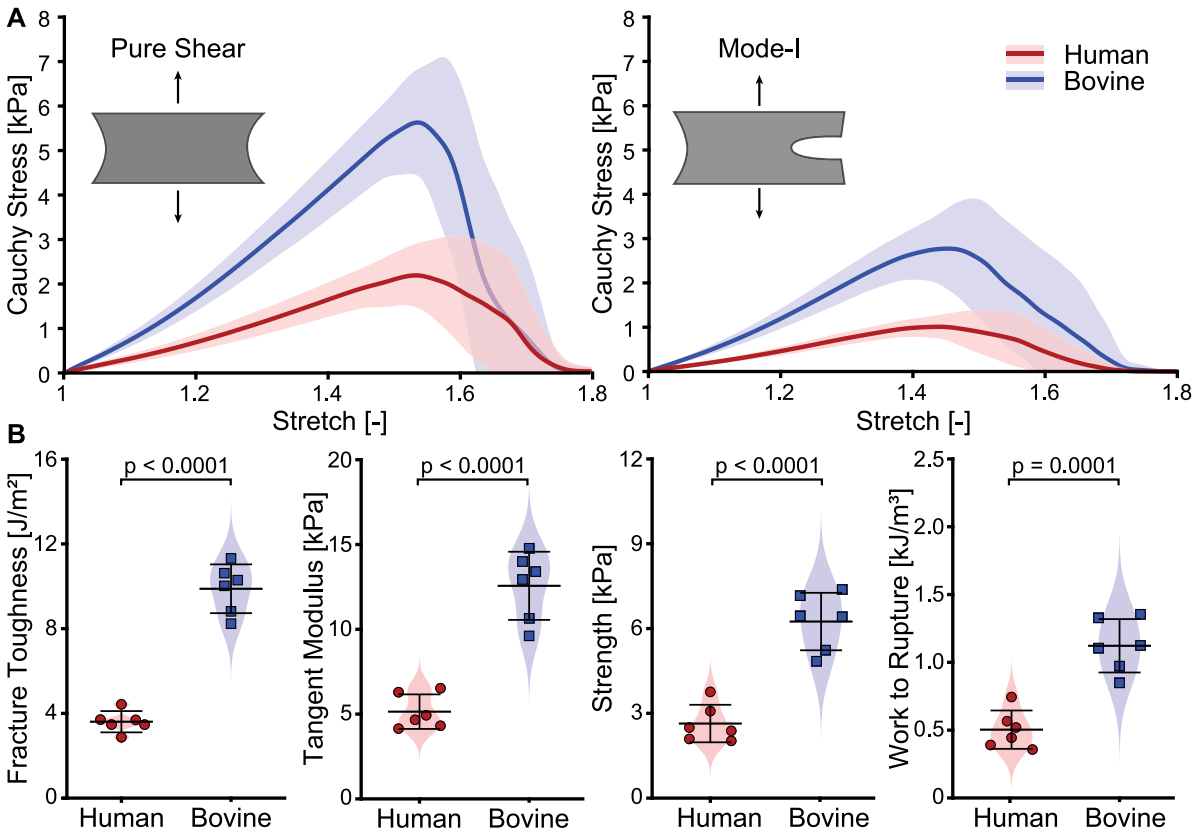

Fig. 2. Human clots are less resistant to fracture, more compliant, and weaker than bovine clots. (A) Cauchy stress–stretch curves for the Pure Shear (left) and Mode-I (right) modes show a substantial difference between species. Curves show mean ± 1 standard deviation. (B) Fracture toughness, tangent modulus at a stretch of 1.35, strength, and work to rupture of human clots are significantly lower than those made from bovine blood ($p \leq 0.0001$). In the violin plots, each point represents the average across three technical replicates from one subject.

3.4. Human and bovine clots exhibit similar, isotropic prestrain

We cut thin squares of blood clot out of their molds and measured the extent to which they contracted during coagulation by imaging them on a calibrated grid. Fig. 5A depicts the experimental protocol. Fig. 5B compares the strain in the *x*- and *y*-directions between human and bovine clots. We failed to detect a significant difference between the measurements in the *x*- and *y*-directions (0.88 ± 0.056 vs. 0.88 ± 0.054, respectively, p = 0.4435). We also failed to detect a significant difference in prestrain between human and bovine clots (0.875 ± 0.067 vs. 0.881 ± 0.037, p = 0.9823). Overall, prestrain is isotropic and similar between human and bovine clots.

3.5. Hematocrit is responsible for much, but not all, of the difference between species

Based on CBC analyses, we found a human hematocrit of 43 ± 1.9% and a bovine hematocrit of 33 ± 1.4% ($p < 0.0001$), while we found the human and bovine platelet counts to be 220,000 ± 27,000 per μL and 310,000 ± 89,000 per μL, respectively (p=0.0538). Next, we fit linear regression models to our scalar mechanical metrics relative to the hematocrit and platelet content of each sample. We found no statistically significant correlations with platelet count. However, we did find significant correlations with hematocrit. Fig. 6 presents the eight linear regression models that yielded significant fits (i.e., with $p < 0.05$). The eight metrics with significant fits included all of the metrics calculated from the Pure Shear and Mode-I curves (fracture toughness, tangent modulus, strength, and work to rupture), all of the metrics derived from the cyclic tests (peak force, hysteresis, and set) and one of the two time constants from the stress relaxation analysis (τ_2). Large R^2 values indicate that inter-species differences in hematocrit explain much of the difference in blood clot mechanics between human and bovine clots, but not all, as some R^2s were as low as 0.15.

4. Discussion

The goal of our current work was to isolate the effect of blood source on the mechanics of blood clots through a robust characterization of clots' viscoelastic, fracture, and prestrain behavior. To this end we compared human and bovine blood. We choose the latter for its extensive prior use and wide availability. In short, we found that most mechanical measures differed significantly between both species.

In detail, we found that human blood clots are less resistant to fracture, more compliant, and weaker than bovine blood clots. We also found that human blood clots exhibit less hysteresis, and undergo more set under cyclic loading. In contrast, we found that the stress relaxation behavior between human and bovine blood clot is quite similar and that prestrain does not differ.

Thus, our findings do support the notion that some contradictory findings in the literature may be due to inter-species differences. That being said, while our findings show significant differences based on species, scalar metrics are generally on the same order of magnitude. For example, fracture toughness is approximately 2.8 times lower in our human clots than in our bovine clots. It is, therefore, likely that inter-species differences do not explain all discrepancies in reported blood clot mechanics that can exceed orders of magnitude. Thus, mechanical testing modality likely remains a consequential contributor.

There are many potential origins for the differences in clot behavior between human and bovine blood. Predominantly, as mentioned

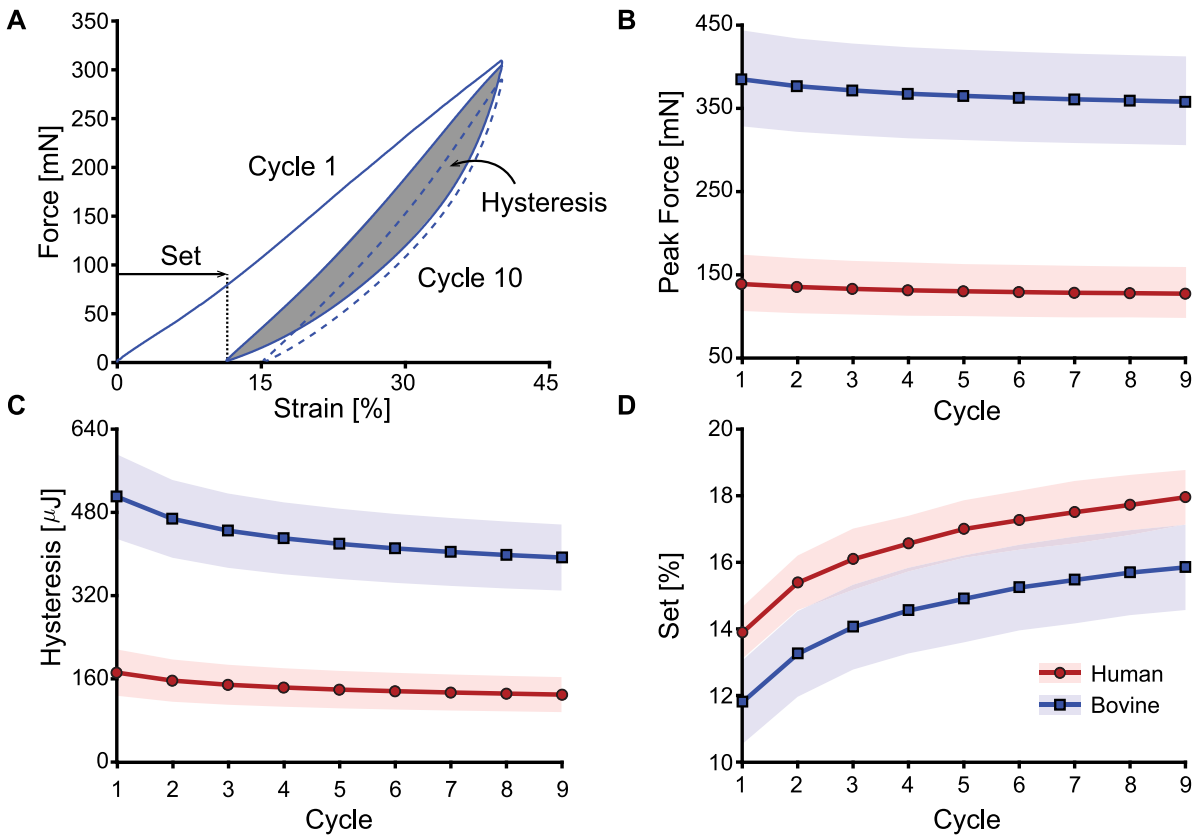

Fig. 3. Human clots show less hysteresis and more set than bovine clots under cyclic loading. (A) A representative test shows how scalar metrics are calculated: the peak force was recorded at every cycle, the area between loading and unloading determined work lost to hysteresis, and the effective strain samples reached when returning to 0 N is the "set". (B) Peak force equilibrates rapidly in both human and bovine clots. The peak force is significantly different between species at each cycle ($p < 0.0001$). (C) Hysteresis also equilibrates rapidly in both clot types, again to significantly different values, with human samples being lower ($p < 0.0001$). (D) The amount of set trends toward equilibrium within ten cycles. Human samples have a significantly higher set at every cycle ($p = 0.0017$). In B-D, points are the mean of three technical replicates per subject within the species, and the shaded region shows ± 1 standard deviation.

previously, the relative abundance of blood components in human and bovine blood differs. The relationship we see wherein human blood has higher hematocrit than bovine blood, and the resulting clots are weaker, more compliant, and less resistant to fracture is consistent with previous literature (Fereidoonnezhad et al., 2021a). In fact, through correlative analyses, we found that up to 89% of the variability in scalar mechanical metrics is attributable to the difference in hematocrit. We also found that differences in platelet count had no detectable effect on tested measures of blood clot mechanics.

There are likely other contributing factors to the difference in clot mechanical behavior, such as differences in fibrin density. Estimates of bovine plasma fibrinogen concentration range from 300–700 mg/dL (Jones and Allison, 2007) which is generally higher than the reference range for humans (160–400 mg/dL (Asselta et al., 2006)). In studies of fibrin gel mechanics, increased fibrinogen concentration has been shown to increase clot strength and resistance to fracture, and clots made from human fibrinogen were determined to be mechanically weaker than those prepared with bovine fibrinogen (Tutwiler et al., 2021, 2020; Zeng et al., 2020), which is consistent with the findings in this work. Additionally, there are notable differences in the prothrombin time (PT) and activated partial thromboplastin time (aPTT) reference ranges between humans and cows. Human PT has been reported as 9.9–13.5 s and aPTT has been reported as 21.7–29.3 s (Patil et al., 2022). Bovine times are noticeably longer, with PT reference ranges around 26–38 s and aPTT reference ranges around 30–58 s (Cornell University College of Veterinary Medicine, 2022). This difference may also play a role in the discrepancy between human and bovine clot mechanical behavior. In order to further isolate the mechanisms responsible for our findings, future studies should control for compositional differences among species such as hematocrit and fibrin(ogen) concentration.

Before us, others have investigated inter-species differences in blood clot properties. For example, Dibiasi et al. have compared the viscoelastic behavior of clots made from human, horse, rat, and camel and found that they differ significantly when measured by rheometry (Dibiasi et al., 2018). Additionally, Chueh et al. found significant differences in the stiffness of human, ovine, and bovine clot mimics when measured via dynamic mechanical analysis (Chueh et al., 2011). Our data adds to these previous studies through a direct and comprehensive comparison between bovine and human clot including elastic, viscoelastic, prestrain, and fracture mechanical properties.

Our study is subject to important limitations. For example, we chose to initiate clotting using calcium chloride alone (rather than also adding thrombin). This may result in clots that are less contracted and have expelled less plasma during coagulation, which, in turn, may impact mechanical properties (Chueh et al., 2011). This choice was made to maintain consistency with our and others' previously published work (Sugerman et al., 2021b, 2020; Fereidoonnezhad et al., 2021a; Liu et al., 2021; Ghezelbash et al., 2022; Dibiasi et al., 2018; Naseri et al., 2020; Gennisson et al., 2006; Weafer et al., 2019; Johnson et al., 2021). Additionally, we assessed clot contraction using a two-dimensional assay. That is, we did not quantify volume change but only area change. Moreover, we assessed clot contraction after coagulation under constrained boundaries rather than unconstrained conditions like others have done before (Cines et al., 2014; Tutwiler et al., 2016,

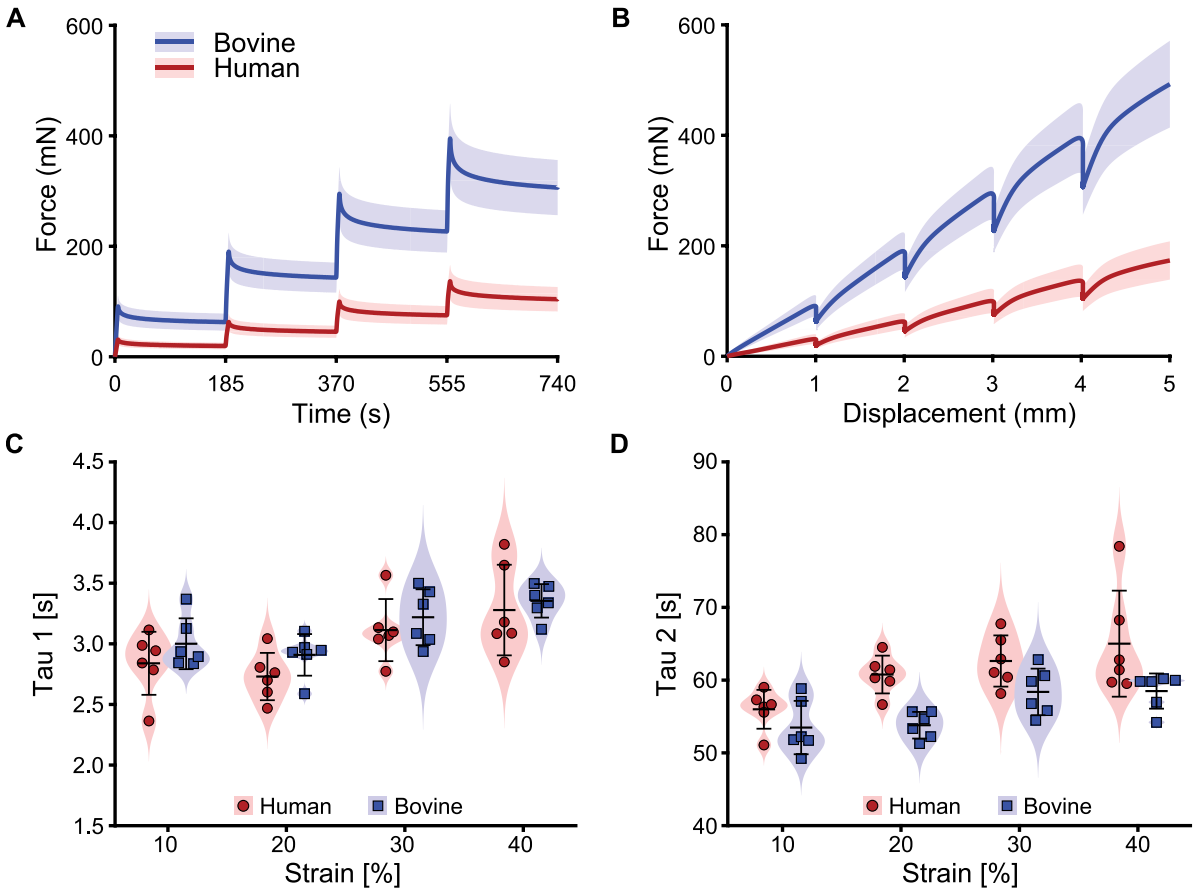

Fig. 4. Human and bovine clots show similar relaxation behavior. (A) Force-time and (B) force–displacement curves show substantial stress relaxation behavior at each displacement step. Curves show mean ± standard deviation. (C) The first relaxation time constant from Eq. (2), τ_1, does not demonstrate a significant difference between human and bovine samples but does show a significant effect of strain level ($p < 0.0001$). (D) The second relaxation time constant from Eq. (2), τ_2, is significantly higher in samples made from human blood (p = 0.0061). For both plots, samples are averaged over three technical replicates so each point represents one independent subject.

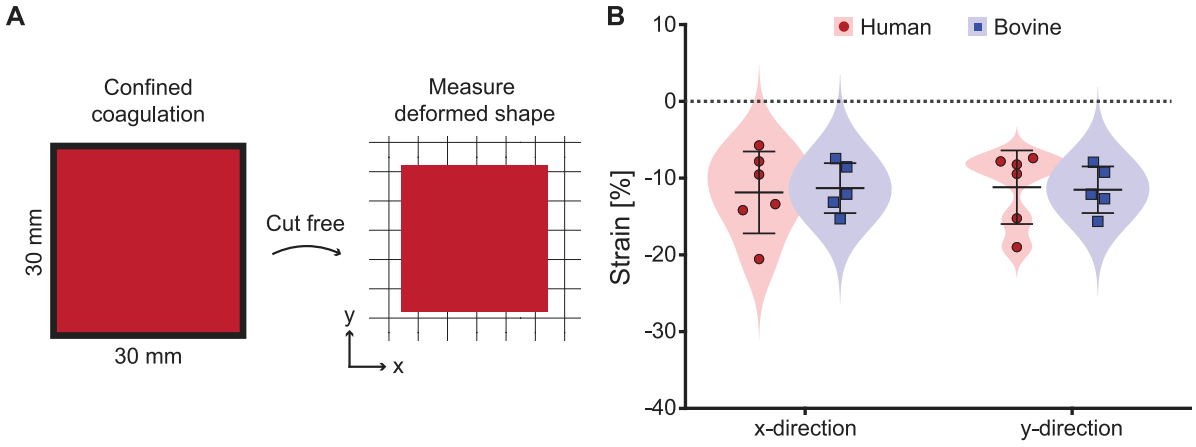

Fig. 5. Human and bovine clots exhibit similar, isotropic prestrain. (A) We coagulated a sample in a thin square frame for 60 min and then cut the sample free and floated it on PBS on a calibrated grid. We measured the deformed shape from images of the floating samples. (B) We did not detect a difference in prestrain between clots made from human and bovine blood (p = 0.9823), nor did we detect a difference across directions (p = 0.4435). Each point represents the average value per independent subject, with 3–4 technical replicates per subject.

2018). Those two differences may explain why our prestrain values are relatively low as compared to other reports (Tutwiler et al., 2016).

4.1. Conclusion

In our work, we directly compared the mechanics of clots made from native, i.e., unaltered, human and bovine blood. We found that – driven by differing blood composition – the mechanical behavior of both types of clots are qualitatively similar but substantially different quantitatively. Importantly, the mechanics of blood clots between both species differed in almost every measure, including stiffness, strength, work-to-fracture, amount of energy dissipation, and fracture toughness. Thus, future work should focus on using human blood to make blood clot

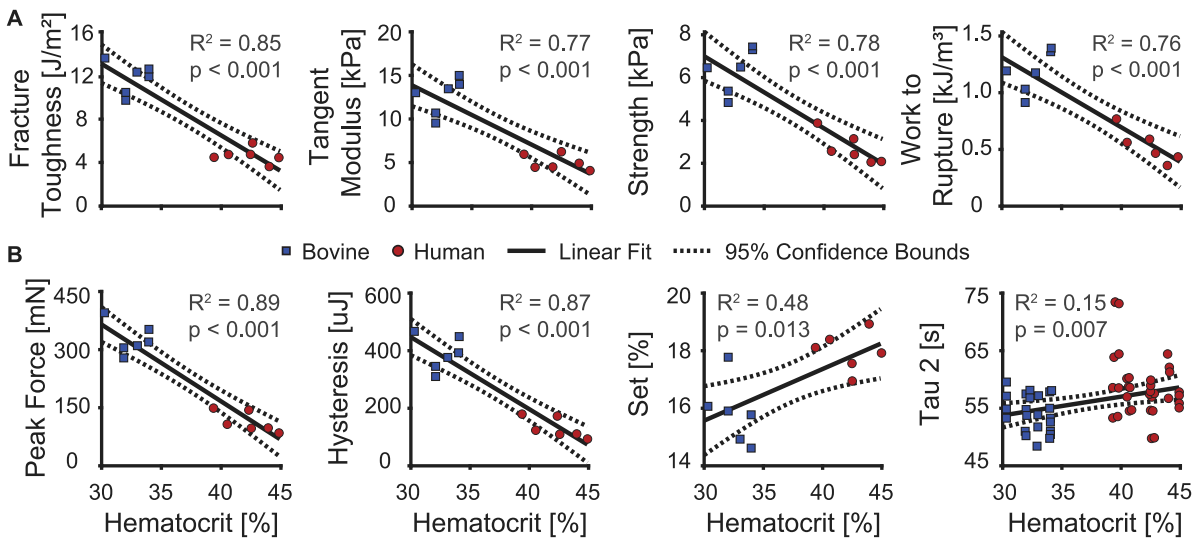

Fig. 6. Hematocrit content is a strong contributor to inter-species differences in blood clot mechanics. (A) Hematocrit is responsible for much of the variation in fracture toughness (R^2 = 0.85,p < 0.001), tangent modulus (R^2 = 0.77,p < 0.001), strength (R^2 = 0.78, p < 0.001), and work to rupture (R^2 = 0.76, p < 0.001) across human and bovine clots. (B) Hematocrit is also responsible for much of the variation in peak force (R^2 = 0.89, p < 0.001) and hysteresis (R^2 = 0.87, p < 0.001), as well as approximately half of the variation in set (R^2 = 0.48, p = 0.013). The contribution to τ_2, one of the stress relaxation time constants, is relatively minor but significant (R^2 = 0.15, p = 0.007).

mimics, and past work that used blood from other species (including our own) should be carefully interpreted, given these findings.

CRediT authorship contribution statement

Gabriella P. Sugerman: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Grace N. Bechtel:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **Zuzanna Malinowska:** Investigation, Formal analysis. **Sapun H. Parekh:** Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Manuel K. Rausch:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Manuel Rausch reports financial support was provided by National Science Foundation. Manuel Rausch reports financial support was provided by Office of Naval Research. Manuel Rausch reports a relationship with Edwards Lifesciences Corporation that includes: speaking and lecture fees.

Data availability

All data are openly available through the Texas Data Repository: https://dataverse.tdl.org/dataverse/STBML.

Acknowledgments

We acknowledge support from the Office of Naval Research, United States via grant N00014-22-1-2073 and from the National Science Foundation, United States via grants 2046148, 2105175, and 2127925.

Disclosures

Manuel K. Rausch has a speaking agreement with Edwards Lifesciences.

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