Effects of ex vivo aging and storage temperature on blood viscosity

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Abstract.
BACKGROUND: Research on hemorheology is driven in part by its significance in blood diseases and the possible use of hemorheology as a diagnostic tool. However, existing data on blood rheology are limited largely to measurements of steady shear behavior often with varying measurement protocols and insufficient characterization of the physiology.
OBJECTIVE: The effects of ex vivo aging and environmental conditions on blood viscosity are investigated to improve standards for hemorheology measurements.
METHODS: Measurements on the viscosity of blood from nine healthy donors are obtained and the physiological state of the blood determined. Steady and transient shear measurements are reported as a function of time from withdrawal. The effect of transportation temperature is also assessed.
RESULTS: Blood transported at 4 °C may exhibit anomalous viscosity variations for short to intermediate times, as opposed to blood transported at room temperature. A time of approximately 3.0 hours was identified as the maximum time after the initial test that accurate rheological tests can be conducted on blood samples.
CONCLUSIONS: Measurement protocol and time limit guidelines are established for conducting accurate rheological measurements on blood.

Keywords: Hemorheology, aging, thixotropy, measurement protocol, hyperviscosity

1. Introduction

Heorheology is currently an active area of research due to the significant number of diseases which have been shown to affect the viscosity of blood [1–7]. However, the exact connection between the viscosity of blood and the physiology remains a mystery partially due to the complex rheological behavior that blood demonstrates. Blood exhibits, in addition to the shear thinning nature under steady shear, viscoelasticity [8], a nonzero yield stress [9], and thixotropy [10]. The Casson model

$$\tau^{\frac{1}{2}} = (\mu\dot{\gamma})^{\frac{1}{2}} + \tau_0^{\frac{1}{2}} \tag{1.1}$$

offers a suitable representation of the steady shear stress response (τ) for blood over a wide range of shear rates [9,11,12], and one way to characterize the steady shear behavior of blood is in terms of a Casson yield stress, τ_0, and viscosity, μ. Recent work has been able to identify connections between these two parameters and the blood physiology [11,13].

The development of advanced constitutive models for blood rheology requires rheological data on physiologically well-characterized samples obtained under protocols that ensure reproducible and accurate measurements. To prevent changes due to hemostasis resulting in clot formation, an anticoagulant or blood thinner, such as EDTA or heparin is added [14]. However, even with the addition of an anticoagulant, the platelets morphology and functionality have been shown to change in times as short as 4 hours from withdrawal [15]. Ex vivo aging also affects the deformability and aggregation state of the red blood cells, and clinical studies have been performed to document changes in the red blood cells with extended storage. Results show that red blood cells become less deformable with increased storage time, due in part to deprivation of ATP, which is critical in maintaining the cell shape and membrane properties [16]. Furthermore, the aggregation state of red blood cells has been shown to change with extended storage. This effect is due to several factors, including the depletion of sialic acid from surface membranes, which results in a reduction in the surface charge of the red blood cells and increased aggregation [17]. This effect can be offset by the reduction in fibrinogen level within the plasma, which can occur over extended storage time, reducing aggregation [17]. Measurement conditions and storage temperature have also been shown to affect both the red blood cell properties [18] and the platelet activation [19,20]. Moreover, all of the aforementioned changes have been shown to be species dependent and may change over different time scales for different animals [21]. To mitigate such effects, blood viscosity measurements must be conducted with protocols for storage time and temperature as well as measurement conditions [22].

In addition to concerns surrounding the biological effects occurring within the aging blood sample, physical and measurement-induced effects must also be considered when making rheological measurements. As blood is a suspension, the red blood cells in the sample will sediment leading to concentration gradients along the vertical orientation of the measurement apparatus. Furthermore, in flow within small capillaries or channels, blood exhibits the well-known Fahraeus effect [23]. This effect is characterized by the formation of a plasma enriched layer near the capillary walls which arises as a result of excluded volume effects. A similar phenomenon, known as red blood cell syneresis, can occur even within larger devices at low shear rates in which a variable concentration profile will develop across the measurement gap [24]. This effect is characterized by an initial rise to a peak stress followed by a decay to a lower final value. Guidelines from the International Society for Clinical Hemorheology advise that the final stress value measured at low shear rates often corresponds to a sample which has undergone full syneresis, and therefore, it is instead preferable to use the maximum stress

measurement [22]. Failure to do so will lead to lower measured stresses at steady state. Moreover, this syneresis effect can also influence interpretation of transient measurements [25].

Of primary interest in this work is the aging process that blood samples undergo upon withdrawal. To ensure accurate measurements, previous guidelines set forth by the International Committee for Standardization in Haematology recommend a maximum time of 4 hours for rheological measurements on blood [26]. This maximum time is also supported by studies performed on the deformability and aggregability of red blood cells [18]. However, other authors have suggested that consistent rheological measurements on human blood samples can be obtained for up to 12 hours [27]. One environmental factor of particular interest for hemorheology measurements is the temperature at which the blood sample is transported. Recent works suggest that 4 ℃ is the optimal temperature to store blood samples at if rheological measurements cannot be immediately obtained [18,22]. However, other guidelines advise against cooling the sample [26].

Of equal importance in making accurate and reproducible measurements is the need to properly precondition the sample. Protocols for thixotropic suspensions can be looked to for guidance on developing protocols for hemorheology that produce a reproducible state of the sample prior to each measurement [28]. Although many of the aforementioned measurement challenges will have a relatively small effect on the viscosity at high shear rates, the changes that can occur in the blood throughout the duration of the experiments can have a significant effect on the rheology at low shear rates. This is particularly worrisome because the low shear region typically exhibits complex rheological phenomena due to the rouleaux formation [29]. Accurate measurements of the rheology at low shear rates is critical for obtaining constitutive equation modeling parameters such as the yield stress, and complementary transient measurements can provide insight into the mechanics behind red blood cell rouleaux formation and breakup.

This paper builds on prior work to develop protocols for the accurate measurement of blood viscosity by investigating the effects of ex vivo aging. Viscosity measurements are presented from nine healthy human donors, along with a broad range of physiological properties and the aging monitored at both high and low shear rates, for both steady and dynamic flows. The effects of transportation temperature on the rheological data are also analyzed and are presented as a means of clarifying the proper handling conditions. We show that the viscosity is sensitive to biological changes that occur within the blood over time from withdrawal. Moreover, we demonstrate how susceptible blood viscosity measurements are to the handling procedure and environmental conditions. A statistical analysis of the data is performed to develop guidelines for accurate blood viscosity measurements and to resolve some discrepancies evident in literature.

2. Methodology

Human blood samples were collected by a licensed nurse practitioner at the Nurse Managed Primary Care Center located at the University of Delaware STAR campus in compliance with UD's Institutional Review Board (Study Number 767478-2). In total, nine healthy donors were sampled ranging in age from 19 to 28. Once each donor was placed in a seated position, blood was drawn from the antecubital vein through a 21 G needle after the application of a tourniquet into a 6 mL Vacutainer tube containing 1.8 mg/mL of ethylenediaminetetraacetic acid (EDTA). All donors fasted for 8 to 10 hours. This protocol is consistent with guidelines established by the International Society for Clinical Hemorheology [22]. Three additional samples were obtained from each donor and sent to Quest Diagnostics for

Complete Blood Count, Lipid Panel, and Fibrinogen Activity testing. The results of the physiological tests for each donor are summarized in Table 1.

Promptly after withdrawal, each blood sample was transported to the rheometer and loaded within 45 to 60 minutes after withdrawal. The effect of transportation temperature will be discussed in a later section of this paper. All rheological tests were conducted using an ARES-G2 strain control rheometer from TA Instruments equipped with a double wall Couette geometry. (Fig. 1). This rotational rheometer has high sensitivity with a minimum torque and angular frequency of 0.1 μN.m and 1 μrad/s respectively, and the double walled-gap maximizes the stress sensitivity over the relevant range of shear rates to yield minimum stresses of 0.6335 mPa and minimum shear rates of 3.34×10^{-5} s^{-1}. The cup is composed of stainless steel and the bob is titanium. A solvent trap is used to minimize the effects of evaporation. The measurement gap is 0.5 mm for the outer gap and 0.43 mm for the inner gap. The loading volume for the geometry was 5 mL. This geometry was selected due to the limited free surface area, relatively long vertical component to reduce effects of sedimentation, large measurement gap relative to the red blood cell, and large measurement area to provide accurate stress measurements. Additionally, the materials of construction have been shown to not interact with blood significantly through either electrostatic repulsion of red blood cells or through protein adsorption [30].

All rheological tests were conducted at the standard human body temperature of 37 °C which was maintained using a Peltier temperature controller to within 0.1 °C. 5 mL of sample was inserted into the measurement geometry using a 5 mL syringe equipped with a 21 G needle and allowed 5 minutes to soak to ensure temperature equilibration. Between tests, the sample was sheared at 300 s^{-1} for 30 s to remove any effects of the previous test. A complete measurement of the steady shear viscosity over the range 0.1 s^{-1} to 700 s^{-1} was obtained at the start of the test sequence as well as after 4 hours from withdrawal to identify changes that may have occurred within the sample over the measurement period. For measurements below 2 s^{-1} on these steady state flow curves, the points were obtained by taking the maximum of the time dependent data sampled over an interval of 1 s at the constant shear rate. This methodology was carried out to minimize the effects of syneresis. Transient stress measurements are reported in Fig. 2 for startup flow for relatively low shear rates as a function of the strain after being subjected to a previously high shear rate of 300 s^{-1}. The stress decays substantially over the measurement duration, which is thought to be a consequence in part due to syneresis. As for stress measurements above 2 s^{-1}, the steady shear data points were obtained by averaging the measured points after 15 s of constant shear.

Over the course of the 4 hours a variety of steady and dynamic measurements were performed, with the standard preshear monitored to detect any changes in the state of the sample. To prevent damage to the red blood cells, shear rates in the measurement device never exceeded 1000 s^{-1} [31]. The effects of storage/transportation temperature were analyzed by obtaining two different blood samples from the same donor (Donor A) three weeks apart. The first sample was cooled to 4 °C for the 45 minute transportation period then heated up to 37 °C for rheological testing (denoted at A_{cooled} in Tables 1 & 2). The second sample was transported at room temperature (23 °C) before being heated to 37 °C for the rheological tests. This experiment was also repeated for blood from Donor C which was cooled for transportation and remeasured months after the original room temperature blood sample (denoted at C_{cooled} in Tables 1 & 2).

Although the measurement geometry has a solvent trap, any effects of solvent evaporation must be established when considering sample aging. This was performed by conducting the same protocol on an aqueous 0.04 wt. % xanthan gum (MP Biomedicals, LLC;

Lot No. Q1844), glycerol (Fisher Chemical; Lot No. 153401) blood simulant [32], which resulted in an insignificant change in the measured stresses over 3 hours, as shown in Figs. 3 and 4. Although the stress change for the 10 s^{-1} measurements exhibits a statistically significant decrease, this decrease is similar in magnitude to the limitations of the machine and can mostly be attributed to experimental noise.

3. Results

For the comparison of the samples transported at room temperature and cooled for transportation, steady shear measurements were performed throughout the duration of the experiments at 10 s^{-1} and 300 s^{-1}, and the results are reported in Fig. 5 as a function of time from withdrawal. Examination of Fig. 5 shows that the cooled blood had a more significant change in viscosity over the course of the experiment. This sample exhibited a high initial viscosity that decayed over the duration of the experiments to a value similar to that obtained for the sample that remained at room temperature. For blood from Donor C, the steady shear viscosity measurements at 10 s^{-1} and 300 s^{-1} as a function of time from withdrawal are compared for both samples in Fig. 6, where differences in the viscosity at 300 s^{-1} are likely due to differences in blood physiology for the two separate withdrawals (see Table 1). Interestingly, the cooled blood sample from Donor C did not exhibit any anomalies as compared to blood transported at room temperature, suggesting that any effects of cooling may be sample specific.

The results of the initial measurements of the steady flow curve for each donor can be seen in Fig. 7. The data in Fig. 7 align well at high shear rates which would be expected for healthy donors with similar hematocrits. However, these data deviate at low shear rates indicating that the unique physiological properties in each blood sample dictate the bulk behavior in this region. Casson model fits for the various donors are shown in Fig. 8 with the model parameters listed in Table 2. Relatively large variation is observed for Casson yield stress, while the Casson viscosity exhibits less variation between donors. Recent attempts have been made to develop phenomenological relations between the differences in these parameters and the physiological properties of the blood, such as cholesterol levels, fibrinogen content, and hematocrit [13,33]. However, such relations are limited due largely to the lack of reliable, well characterized data. Although the present work does not attempt to identify correlations, these observable differences demonstrate the potential future use of rheology in evaluating blood samples.

Steady shear measurements were conducted on the sample periodically at 10 s^{-1} and 300 s^{-1} over a 3 hour period for Donors A, C, D, G, H, & I, each of which followed the exact same acquisition and measurement protocol. The magnitude of the change in the measured stress from the initial measured value (Fig. 7) as a function of time from the initial measurement is presented in Figs. 9 and 10, respectively. For high shear rates (300 s^{-1}), a systematic increase in measured stress of order 10 % is observed, while for moderate shear rates (10 s^{-1}), the stress change can be much more significant. This error should be addressed in experimental works that are unable to perform rheological measurements immediately. Furthermore, for high shear rates, the measured stress values increase monotonically by similar magnitudes for each sample, while at moderate shear rates, the measured stresses undergo more random changes.

The temporal variations in individual samples over the course of hours reported in Figs. 9 and 10 are much more significant than the limiting machine measurement accuracy (0.6 mPa). The time variation of individual measurements over 40 s is on the order of the machine's accuracy as shown in Fig. 11, suggesting that the longer temporal variations are a consequence

of actual changes in the blood itself possibly coupled to memory effects associated with prior measurements, although each reported test is preceded by the conditioning step detailed in the measurement protocol. What is evident, however, is that the stress at high shear rates shows a clearly increasing trend with time from withdrawal.

The long-time aging behavior over a 24 hour period is reported for Donors G in Fig. 12, where a clear aging trend toward increasing viscosity is observed. Additional strain amplitude sweeps at a fixed frequency of 2 Hz were conducted at the beginning and end of the experiment to better characterize the aging and are presented in Fig. 13. Both the storage and loss modulus increase over the time period. However, the onset of nonlinearity decreases only slightly with aging. These tests were also conducted on blood from Donor H & I with similar results, although these results are not shown here. The relatively long-time aging studies on Donors G, H, & I provide guidance for interpreting the trends observed more broadly for shorter measurement times.

4. Discussion

Aging is evident for all samples as a monotonic increase in the measured stress at the high shear rate of 300 s^{-1} (Fig. 9). However, there is less evidence for aging at the moderate shear rate of 10 s^{-1} (Fig. 10) for short times from withdrawal. A statistical analysis of the data is performed, and the linear trend lines with 95 % confidence bands are shown in Figs. 9 and 10. The analysis shows that the viscosity at 300 s^{-1} shows a statistically significant positive trend. A statistically significant positive trend is also observed for the 10 s^{-1} data. However, this trend is not significant for a 99 % confidence level.

As noted in the methods section, this trend for the high shear data is not a consequence of evaporation, which is ruled out by calibration experiments, and is not a consequence of sedimentation, which should occur over longer time periods given the length of the vertical axis of the measurement device as well as the fact that the sample was continuously undergoing shear. Rather, this aging at higher shear rates is thought to be a consequence of stiffening of the red blood cells over time ex vivo. Such stiffening has been reported as a consequence of the reduction in ATP ex vivo [16]. Theory shows that such stiffening of the red blood cell membrane would result in increased stress measurements at high shear rates [34]. Furthermore, this effect would be less significant at low shear rates as the stress measurements in this region are primarily dependent on the rouleaux. Some of the observed trends for the viscosity at lower shear rates may result from a reported decrease in fibrinogen concentration following withdrawal [17]. Because fibrinogen drives rouleaux formation [35], rheological aging at the lower shear rates to lower stresses would be a consequence.

Assuming that the red blood cells stiffen over the measurement time and neglecting other changes that may be occurring in the blood sample, we can use the high shear rheology measurements to gain insight into the rigidity of the red blood cells at a specific time during the experiment. A measure of rigidity proposed by Dintenfass, *Tk*, is related to the hematocrit, *H*, and relative viscosity, η_r, of the blood sample according to:

$$Tk = \frac{\eta_r^{0.4} - 1}{\eta_r^{0.4} H} \tag{4.1}$$

This relation applies at high enough shear rates where any structure in the sample is almost entirely broken up [34]. Using this equation with a standard value of 1.3 mPa.s for the bulk plasma viscosity at 37 °C, the percent change in rigidity over the duration of the experiments ranges from 4.4 % to 10 %.

The blood sample taken from Donor A which was stored at 4 °C for transportation exhibits the opposite trend for the steady shear stress response as a function of time. The results of the linear regression slope parameters of both cooled blood samples can be found in Table 3. For Donor A, the stress measurements taken at both 10 s^{-1} and at 300 s^{-1} show a statistically significant negative trend with respect to time from withdrawal. However, depending on the shear rate, the data from Donor C show either the opposite trend or no discernible trend. This observation could be explained by a necessary rewarming period on the order of 30-60 minutes that may be required to return the blood sample to physiological conditions. For Donor A, after this time, the viscosity of the sample returned to normal levels as shown in Fig. 5 and exhibited a trend that is comparable to trends exhibited by data from other donor blood samples that were not cooled. For Donor C, the rewarming period before starting measurements was slightly longer and may have been sufficient to return the sample to normal conditions or reduce the effects of cooling to a level that could be overshadowed by other fluctuations occurring within the sample. It should be noted that the viscosity at 300 s^{-1} for cooled blood from Donor C was statistically different than the viscosity of the blood that remained at room temperature. However, as the measurements were performed several months apart, this is likely due to the physiological changes such as an increase in the triglyceride level of the blood sample from 36 mg/dL to 47 mg/dL.

When measuring blood rheology, it is necessary to establish a maximum time over which reliable measurements can be obtained. In this work, this time was evaluated by measuring blood from Donors G, H, & I continuously for more than 24 hours from withdrawal. Throughout this time, steady shear measurements were taken periodically at 300 s^{-1} and 10 s^{-1}. These data are shown in Figs. 14 and 15, respectively. Several factors enter into defining a maximum measurement time, including the transportation/storage time, time in the rheometer, as well as various environmental factors including shearing history. For this work, we have only examined the maximum duration that the sample may remain in the rheometer before significant changes occur while keeping the transportation/storage time fixed at 45-60 minutes at room temperature, and restricting shearing to shear rates below 1000 s^{-1} and at 37 °C. The maximum duration for experimental measurements is defined to be a deviation outside of the 95 % prediction interval for the linear evolution of the steady shear stress measurements with respect to time from initial measurement. This corresponds to the time at which the prediction interval no encompasses a zero stress difference from the initial measurement. This analysis is shown in Figs. 14 and 15 at fixed shear rates of 300 s^{-1} and 10 s^{-1}, respectively. Using this methodology, we obtain a maximum time of 3.0 hours for both the 300 s^{-1} and the 10 s^{-1} data. When factoring in the 1 hour transportation time, we obtain a maximum time of 4.0 hours from withdrawal. This time agrees with previous guidelines which recommend a maximum time of 4 hours for blood viscosity measurements [18,26].

Any error associated with previous improper measurement techniques or handling protocol can be perpetuated when attempting to model inaccurate experimental data. Two parameters which govern the steady shear behavior of blood rheology are the yield stress and model viscosity. Since there have been several recent works attempting to relate these rheological model parameters to the physiological properties of the blood samples [11,13,33], it is critical to ensure that the protocol that is used for measuring the blood samples does not affect the modeling parameters. To first test the effect of cooling the blood before measuring, a t-test is performed for the Casson yield stress and model viscosity of the two data sets from Donor A

with the null hypothesis being that the blood cooled to 4 °C and the blood transported at room temperature exhibit the same model parameters. The t-value can be computed by:

$$t = \frac{x_1 - x_2}{\sqrt{s_{x_1}^2 + s_{x_2}^2}} \tag{4.2}$$

in which x represents the modeling parameter being analyzed for the specific data set and s_x represents the standard error for the modeling parameter as determined by the linear regression fit [36]. This t-value may then be translated to a p-value.

Since the data from Donor C were taken months apart, the physiology of the donor may have changed significantly over this period, which will be responsible for some of the observed differences. However, data from Donor A were taken just weeks apart and may be compared. The results are summarized in Table 4. From this analysis, it is apparent that transporting the blood at 4 °C may result in an initial increase in the yield stress if not given ample time to rewarm. Despite this initial increase, the modeling parameters for the cooled blood sample at the end of the experiment did not show a significant difference from the modeling parameters obtained for the sample transported at room temperature. This observation indicates that any change in the rheology that may occur upon cooling the blood sample is not permanent such that the rheology is expected to return to physiologically normal conditions if given ample time to rewarm.

The changes in the modeling parameters from the beginning of the experiments to the end of the experiments may be assessed for the various donors as presented in Table 5. For several of the donors listed in Table 5, both the yield stress and model viscosity changed significantly over the duration of the experiments. However, this significant change was not observed for Donor A and the yield stress for Donor I. It should be noted that although the yield stress changes more with time than the model viscosity, the significance of the change in the yield stress is less due to the high uncertainty associated with the yield stress estimation. Since blood from some donors exhibited a change while others did not, it is likely that the duration of the experiments is approaching the maximum time to conduct measurements without experiencing significant changes in the fitted model parameters. This further supports the value for the maximum time of 3.0 hours from the initial measurement that was determined previously.

5. Conclusions

In this paper, the effects of transportation temperature on bulk blood viscosity measurements were assessed by comparing blood transported at 4 °C and 23 °C for the same donors. Blood from Donor A demonstrated a statistically significant negative trend for the stress evolution as a function of time from withdrawal while blood from Donor C demonstrated either no statistically significant trend or an increase in stress depending on the shear rate. When compared to the blood sample which remained at room temperature, the cooled blood sample from Donor A exhibited high initial stresses that returned to normal conditions approximately one hour after the initial measurement. This result was further validated through a statistical analysis of the Casson modeling parameters. By comparing the results obtained for both blood transported at 4 °C and 23 °C, we can conclude that if not given ample time to rewarm back to the measurement temperature, an initially heightened viscosity may be observed that is not representative of the blood flow under physiological conditions. This time required for rewarming is donor dependent and may be as much as 30 to 60 minutes. Future measurements should ensure ample rewarming time is provided by measuring the blood throughout the

experiment to ensure that the viscosity is not changing more than would be expected for normal ex vivo aging.

For blood that was transported at room temperature, no trend for steady shear measurements at moderate shear rates within 4 hours of withdrawal could be identified. However, a statistically significant increase in the steady shear stress response at high shear rates within 4 hours from withdrawal was observed. This result signifies that two different mechanisms may be governing the bulk behavior in these regimes. At the high shear rate of 300 s^{-1}, the bulk behavior is highly dependent on the deformability of the red blood cells. In ex vivo conditions the red blood cells will be deprived of ATP which will lead to decreased deformability [16] and an increased viscosity at high shear rates [34]. In addition to an increase in viscosity after 4 hours from withdrawal for the high shear measurements, within the 4 hours donor dependent fluctuations were observed that were greater in magnitude than the instrument sensitivity. These fluctuations demonstrate the complexity of the system and show how the time evolution of the sample may be nonlinear and donor specific. As for the Casson modeling parameters, for some blood samples a significant change was observed from the initial measurement to the final measurement. This result demonstrates the sensitivity of the Casson model parameters to the measurement protocol.

Over longer periods (approximately 24 hours), a continual, monotonic increase in the viscosity was observed for high shear rates. At moderate shear rates an increase in viscosity was also observed after a possible induction time. This increase in viscosity can be quite significant, resulting in changes of more than 100 %. Additionally, the change in the bulk rheology is complex and cannot be easily adjusted as shown by the amplitude sweep data presented in Fig. 13. Due to the complex and perpetual changes that the sample will undergo in ex vivo conditions, a maximum measurement time is dependent on the specific error tolerance of the experiment. Defining the maximum time as the point where the prediction interval no longer encompasses a zero change in stress as a function of time from initial measurement, we obtain a maximum measurement time of 3.0 hours. When the 1 hour transportation time is factored in, this time corresponds to a maximum time of 4.0 hours from withdrawal, which agrees well with previous works [18,26]. Nevertheless, when measuring blood viscosity, measurements should be obtained as soon following withdrawal as possible to mitigate these ex vivo changes.

Recently, blood rheology has been a growing area of active research and offers several important challenges. Due to inconsistencies between handling protocols in some previous works, modeling efforts have been limited and are often over simplified and fail to take into account the various physiological parameters that contribute to the bulk rheological behavior. For future experimental works in blood rheology, it is critical to properly handle and measure the blood sample, fully characterize the physiological profile, and investigate not only the steady shear behavior but also the transient behavior. There also remain several aspects of blood rheology that are currently not well understood. The effects associated with the migration of red blood cells away from vessel walls continues to be an area of active research and has leads to numerous measurement issues as demonstrated by Fig. 2. Another facet of blood rheology that remains relatively uninvestigated is the effect of components such as platelets or cholesterol levels on the bulk flow behavior. With an established handling and measurement protocol, the more subtle effects of such physicochemical properties on blood rheology can be more accurately determined in future work.

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Tables

Table 1
Relevant physiological properties for blood from all donors currently sampled.

Donor	Hematocrit (%)	Fibrinogen (mg/dL)	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL Cholesterol (mg/dL)	LDL Cholesterol (mg/dL)
A	42.6	186	126	51	48	68
A_{cooled}	44.5	--	129	41	49	72
B	41.7	223	178	30	62	110
C	38.6	249	128	36	56	65
C_{cooled}	39.3	261	133	47	59	65
D	38.9	282	199	63	53	133
E	45.9	209	168	32	85	77
F	35.8	223	153	33	99	47
G	36.9	271	162	85	73	72
H	40.8	287	205	94	71	115
I	41.6	272	137	88	62	58

Table 2
Model parameters and 95 % confidence intervals for Casson model fit to experimental steady shear data.

Donor	Yield Stress (mPa)	Yield Stress 95 % Confidence Interval (mPa)	Model Viscosity (mPa.s)	Model Viscosity 95 % Confidence Interval (mPa.s)
A	5.09	[3.32, 7.24]	3.02	[2.87, 3.18]
A_{cooled}	12.4	[9.71, 15.5]	3.31	[3.15, 3.49]
B	5.82	[5.09, 6.60]	3.23	[3.17, 3.29]
C	8.94	[8.19, 9.71]	2.83	[2.79, 2.87]
C_{cooled}	4.17	[2.81, 5.81]	3.65	[3.51, 3.80]
D	9.71	[8.83, 10.6]	3.04	[2.98, 3.09]
E	6.78	[5.70, 7.95]	2.92	[2.88, 2.97]
F	4.15	[3.09, 5.36]	2.54	[2.45, 2.64]
G	4.05	[2.38, 6.16]	2.93	[2.77, 3.10]
H	8.42	[6.44, 10.7]	3.33	[3.19, 3.46]
I	7.47	[5.78, 9.37]	3.20	[3.08, 3.33]

Table 3
Cooled blood linear regression slope statistics for the time evolution of the stress at constant shear rates.

Donor	Shear Rate (s^{-1})	Linear Regression Slope (mPa/hr)	95 % Confidence Interval (mPa/hr)
A	10	-9.39	[-11.5, -7.25]
	300	-77.0	[-95.6, -58.5]
C	10	1.48	[-4.46, 7.42]
	300	14.0	[7.94, 20.1]

Table 4

Casson model parameters for steady shear curves taken on blood from Donor A. For the naming of the blood sample, the number indicates the temperature in ℃ at which the blood sample was transported. I denotes initial steady shear measurements, and F denotes the final steady shear measurements. The ranges for parameter values denote 95 % confidence intervals. All bolded p-values are significant on a 95 % confidence level.

Blood Sample	Parameter	Value (mPa or mPa.s)	p-Values		
			4I	4F	23I
4I	τ_0	12.4 [9.71, 15.5]			
	μ	3.31 [3.15, 3.49]			
4F	τ_0	4.40 [2.66, 6.56]	**5.0E-5**		
	μ	3.17 [2.98, 3.36]	0.21		
23I	τ_0	5.09 [3.32, 7.24]	**5.8E-5**	0.59	
	μ	3.02 [2.87, 3.18]	**0.010**	0.20	
23F	τ_0	5.31 [3.71, 7.19]	**7.0E-5**	0.45	0.86
	μ	3.17 [3.04, 3.31]	0.17	0.96	0.13

Table 5
Casson model parameters for steady shear curves from various donors taken at the beginning and end of experiments. The ranges for parameter values denote 95 % confidence intervals. All bolded p-values are significant on a 95 % confidence level.

Donor	Time from Initial (hr)	Yield Stress (mPa)	Model Viscosity (mPa.s)	Yield Stress p-Value	Model Viscosity p-Value
A	0	5.09 [3.32, 7.24]	3.02 [2.87, 3.18]	0.86	0.13
	2.9	5.31 [3.71, 7.19]	3.17 [3.04, 3.31]		
C	0	8.94 [8.20, 9.72]	2.83 [2.79, 2.87]	**0.029**	**1.3E-4**
	2.4	7.31 [6.09, 8.63]	3.02 [2.94, 3.11]		
D	0	9.71 [8.83, 10.6]	3.04 [2.98, 3.09]	**1.9E-5**	**2.4E-7**
	2.3	6.25 [5.23, 7.35]	3.34 [3.26, 3.42]		
G	0	4.05 [2.38, 6.16]	2.93 [2.77, 3.10]	**0.047**	**0.046**
	3.1	6.89 [4.85, 9.30]	3.15 [3.00, 3.31]		
H	0	8.42 [6.44, 10.7]	3.33 [3.19, 3.46]	**0.017**	**1.2E-4**
	3.9	12.4 [9.86, 15.2]	3.75 [3.60, 3.90]		
I	0	7.47 [5.78, 9.37]	3.20 [3.08, 3.33]	0.148	**0.00148**
	2.8	9.27 [7.50, 11.2]	3.48 [3.36, 3.60]		

Figure Captions

Fig. 1. Schematic of the double wall Couette geometry used for the rheology measurements. The bob is composed of titanium, and the cup is stainless steel.

Fig. 2. Constant stress measurements at low shear rates as a function of strain for blood from Donor E. The stress values are scaled for each shear rate by the maximum obtained stress measurement at the specific shear rate. The decreasing stress measurement observed at high strains demonstrates the significance of syneresis.

Fig. 3. Change in measured stress from the initial measurement for an aqueous 0.04 wt. % xanthan gum, 35 wt. % glycerol blood simulant. All measurements were obtained at 300 s^{-1}. The 95 % confidence bands and prediction bands for the linear regression fit to the combined data are also shown. No statistically significant trend is observed indicating that the effects of evaporation are negligible.

Fig. 4. Change in measured stress from the initial measurement for an aqueous 0.04 wt. % xanthan gum, 35 wt. % glycerol blood simulant. All measurements were obtained at 10 s^{-1}. The 95 % confidence bands and prediction bands for the linear regression fit to the combined data are also shown. A statistically significant decrease in stress as a function of time from initial measurement is identified. However, the magnitude of this decrease is similar to the machine sensitivity.

Fig. 5. Viscosity as a function of time from withdrawal for both samples taken from Donor A. Open symbols represent steady shear measurements taken at 10 s^{-1}. Filled symbols represent steady shear measurements taken at 300 s^{-1}. Prediction bands are shown for the viscosity of the sample transported at room temperature. Comparison shows a heightened initial viscosity for the cooled blood which subsequently returns to normal conditions.

Fig. 6. Viscosity as a function of time from withdrawal for both samples taken from Donor C. Open symbols represent steady shear measurements taken at 10 s^{-1}. Filled symbols represent steady shear measurements taken at 300 s^{-1}. Prediction bands are shown for the viscosity of the sample transported at room temperature. Comparison shows no discernible changes in trend between the two samples.

Fig. 7. Steady shear flow curves for all donors. A similar stress response is observed at high shear rates while differences are observed at low shear rates.

Fig. 8. Casson plot and linear fit to experimental steady shear data for all donors. The unique linear trends for each donor can be extrapolated to determine the yield stress and model viscosity.

Fig. 9. Change in measured stress from the initial measurement for an applied shear rate of 300 s^{-1}. The 95 % confidence bands and prediction bands for the linear regression fit to the combined data are also shown. A statistically significant increase in stress as a function of time from initial measurement is identified.

Fig. 10. Change in measured stress from the initial measurement for an applied shear rate of 10 s^{-1}. The 95 % confidence bands and prediction bands for the linear regression fit to the combined data are also shown. A statistically significant increase in stress as a function of time from initial measurement is identified. However, this trend is not statistically significant for a 99 % confidence interval.

Fig. 11. Transient data for Donor C at 10 s^{-1} used to obtain steady shear points at different times from initial measurement demonstrate the instrument precision in transient stress measurements.

Fig. 12. Steady shear experimental results for Donor G at different times from withdrawal. A significant increase in the steady shear stress curve is observed for extended time from withdrawal.

Fig. 13. Amplitude sweep experimental results for Donor G at 2 hours and 24.5 hours from withdrawal. Open symbols represent the loss modulus. Filled symbols represent the storage modulus. The frequency was held constant at 2 Hz. A nonlinear change is observed for both moduli over the duration of the experiments.

Fig. 14. Change in measured stress from the initial measurement for Donors G, H, & I data taken over 24 hours. Measurements shown were taken at 300 s^{-1}. Use of prediction bands enables determination of guidelines for maximum time from withdrawal for accurate measurements at high shear rates.

Fig. 15. Change in measured stress from the initial measurement for Donors G, H, & I data taken over 24 hours. Measurements shown were taken at 10 s^{-1}. Use of prediction bands enables determination of guidelines for maximum time from withdrawal for accurate measurements at low to moderate shear rates.

Figures

Fig. 1

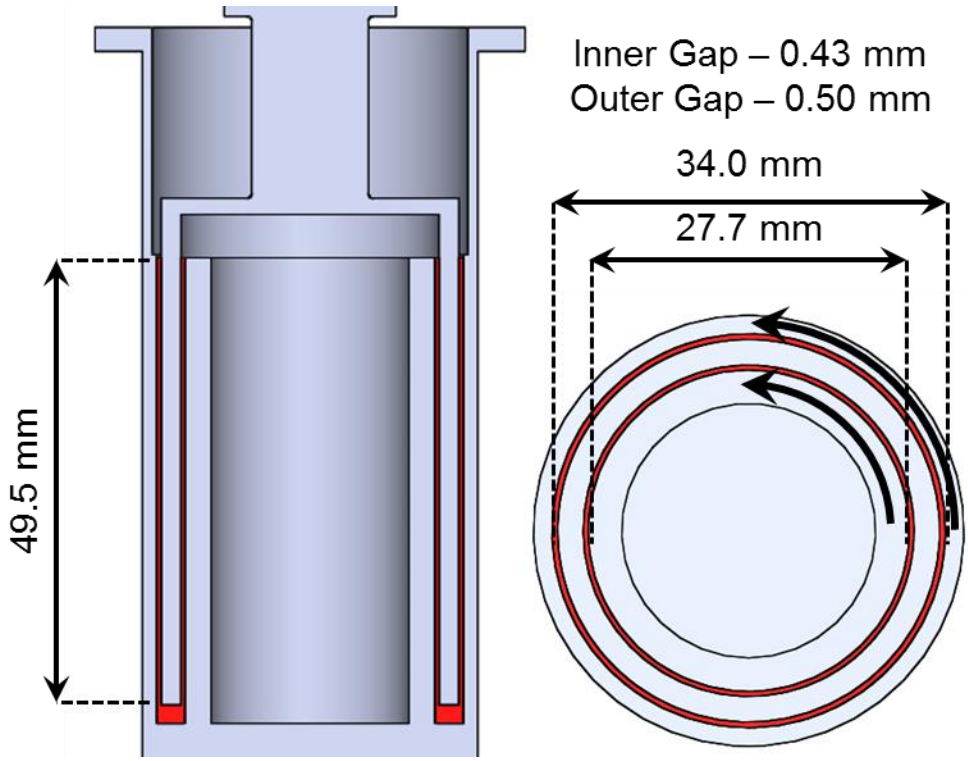


Fig. 2

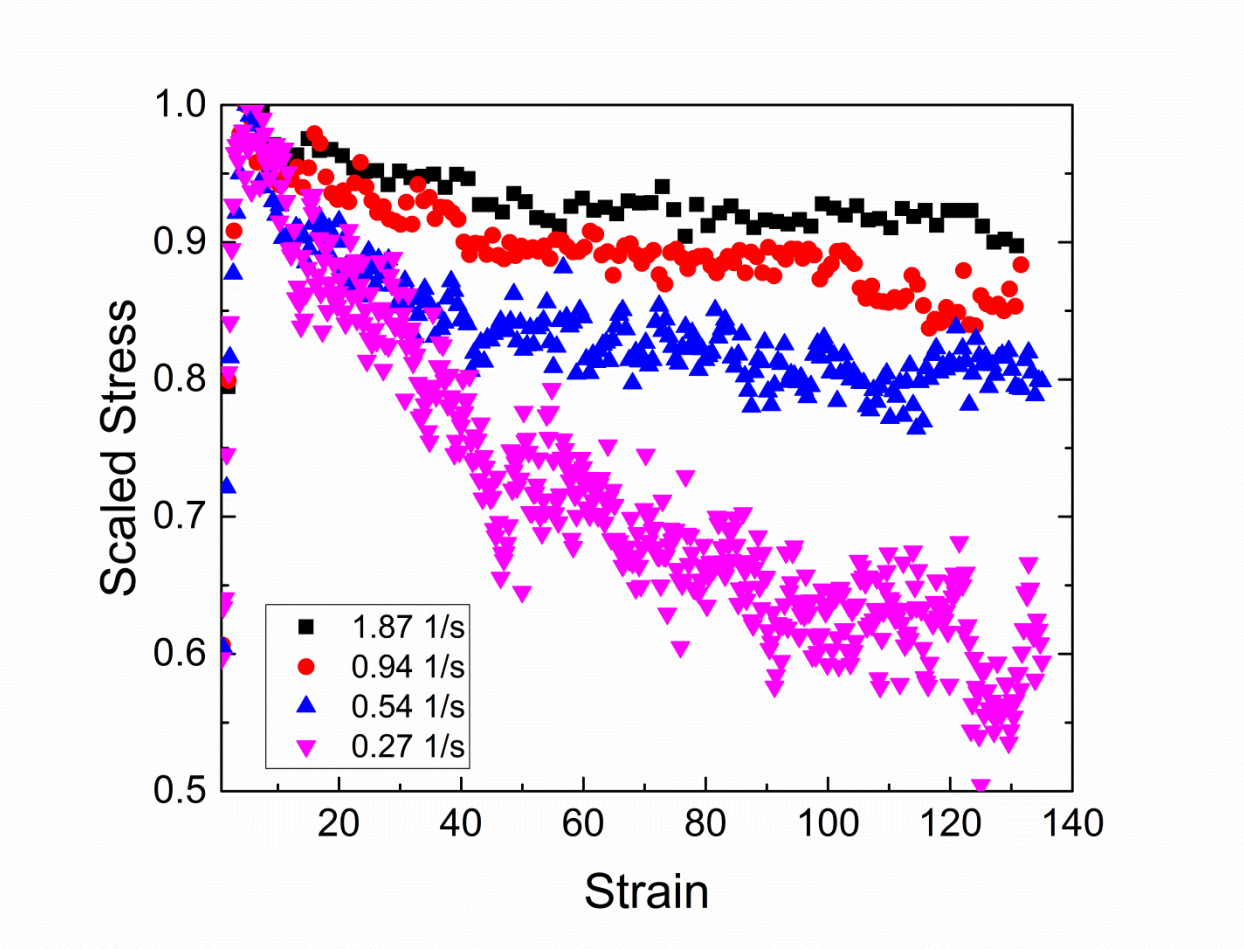

Scaled Stress
Strain
1.0
0.9
0.8
0.7
0.6
0.5
20
40
60
80
100
120
140
1.87 1/s
0.94 1/s
0.54 1/s
0.27 1/s

Fig. 3

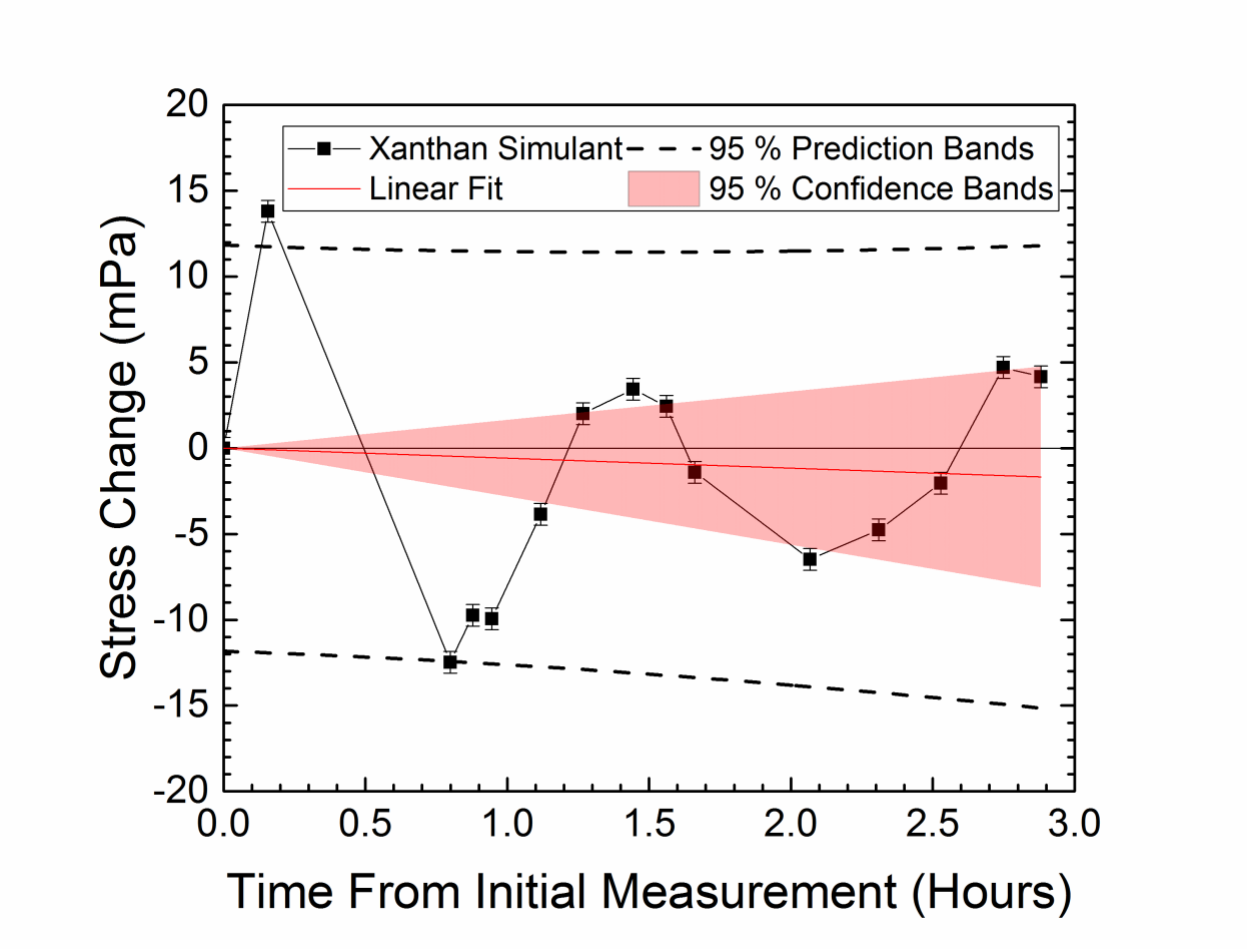

Xanthan Simulant
95 % Prediction Bands
Linear Fit
95 % Confidence Bands
Stress Change (mPa)
Time From Initial Measurement (Hours)
20
15
10
5
0
-5
-10
-15
-20
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Fig. 4

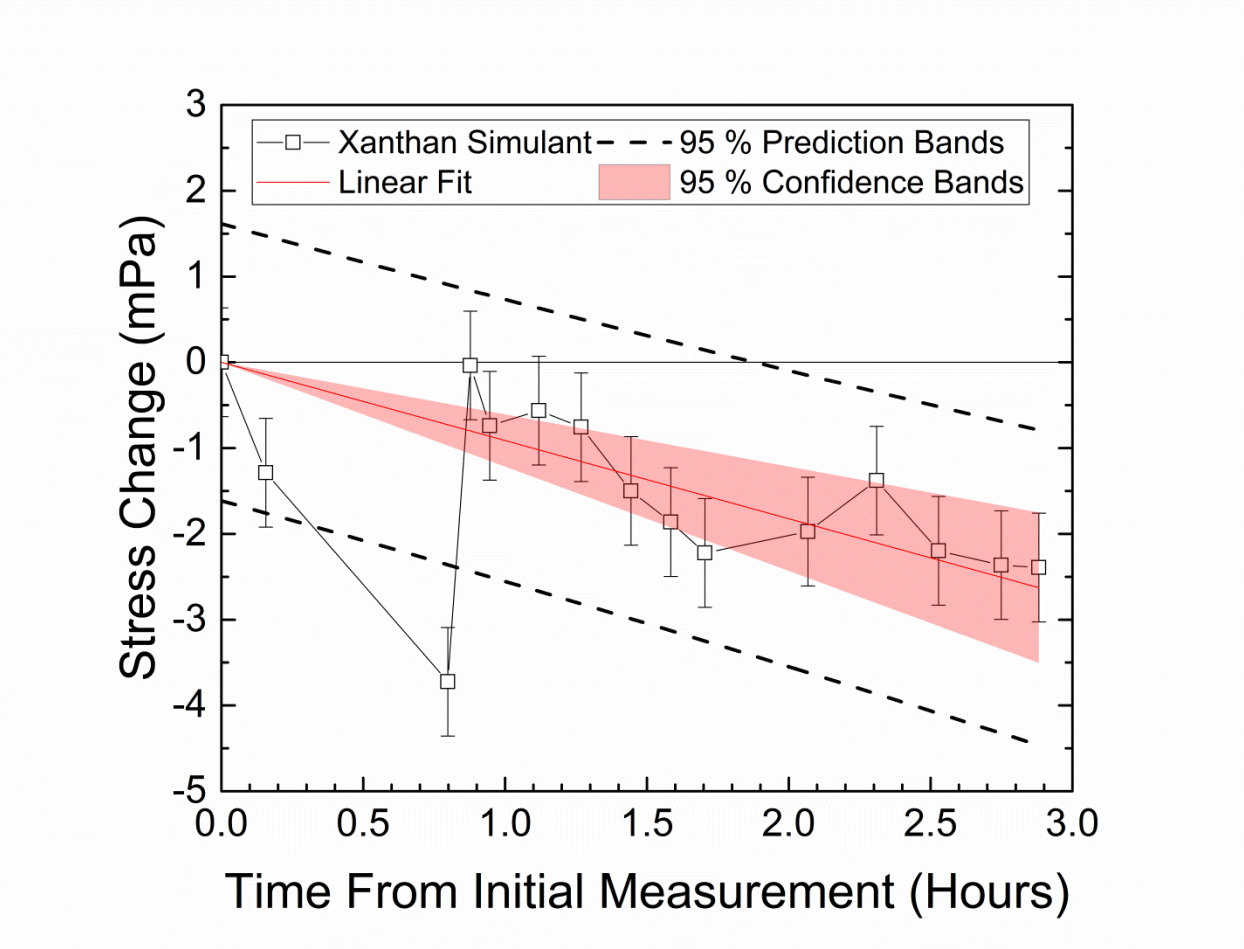

Xanthan Simulant
95 % Prediction Bands
Linear Fit
95 % Confidence Bands
Stress Change (mPa)
Time From Initial Measurement (Hours)
3
2
1
0
-1
-2
-3
-4
-5
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Fig. 5

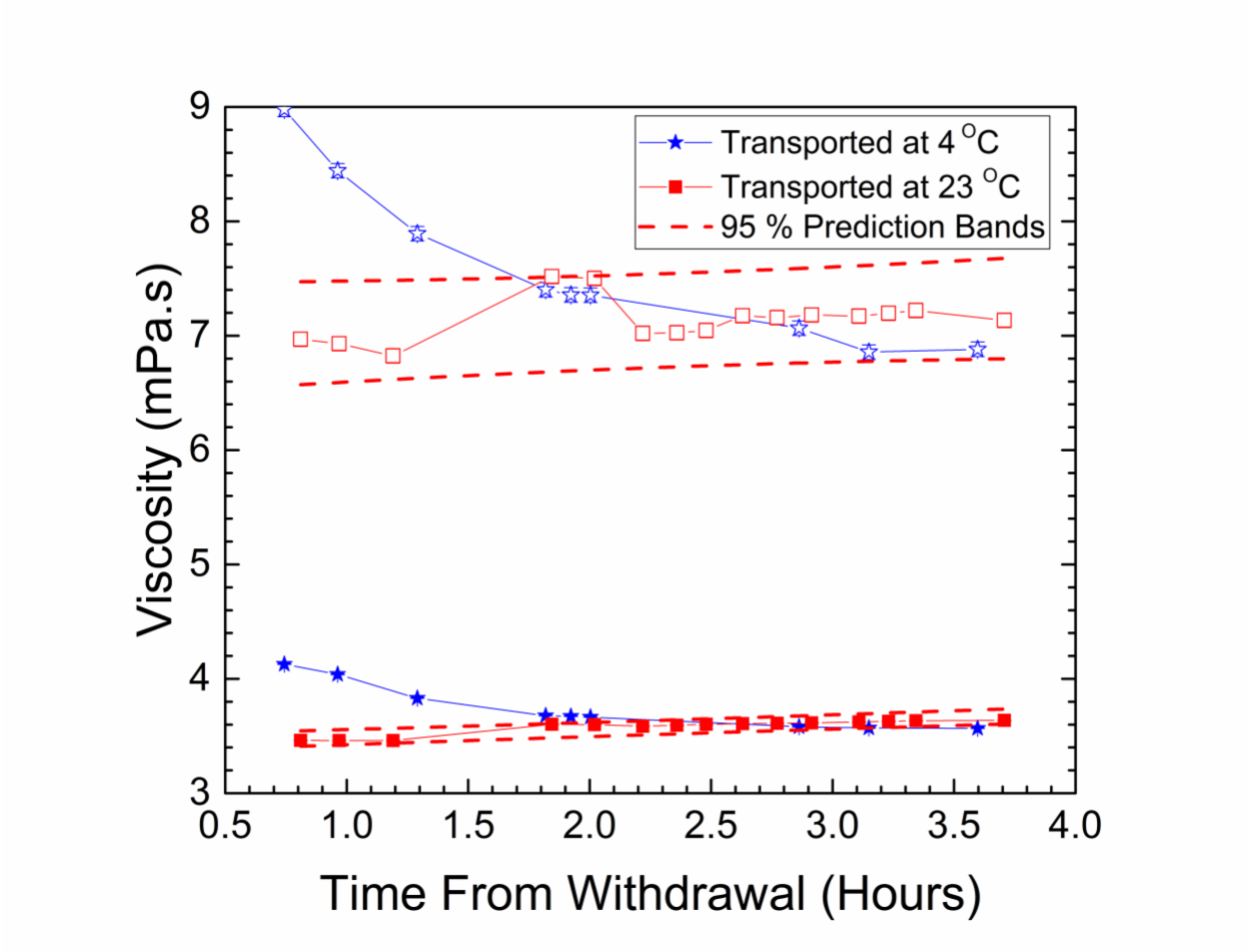

Transported at 4 °C
Transported at 23 °C
95 % Prediction Bands
Viscosity (mPa.s)
Time From Withdrawal (Hours)
9
8
7
6
5
4
3
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

Fig. 6

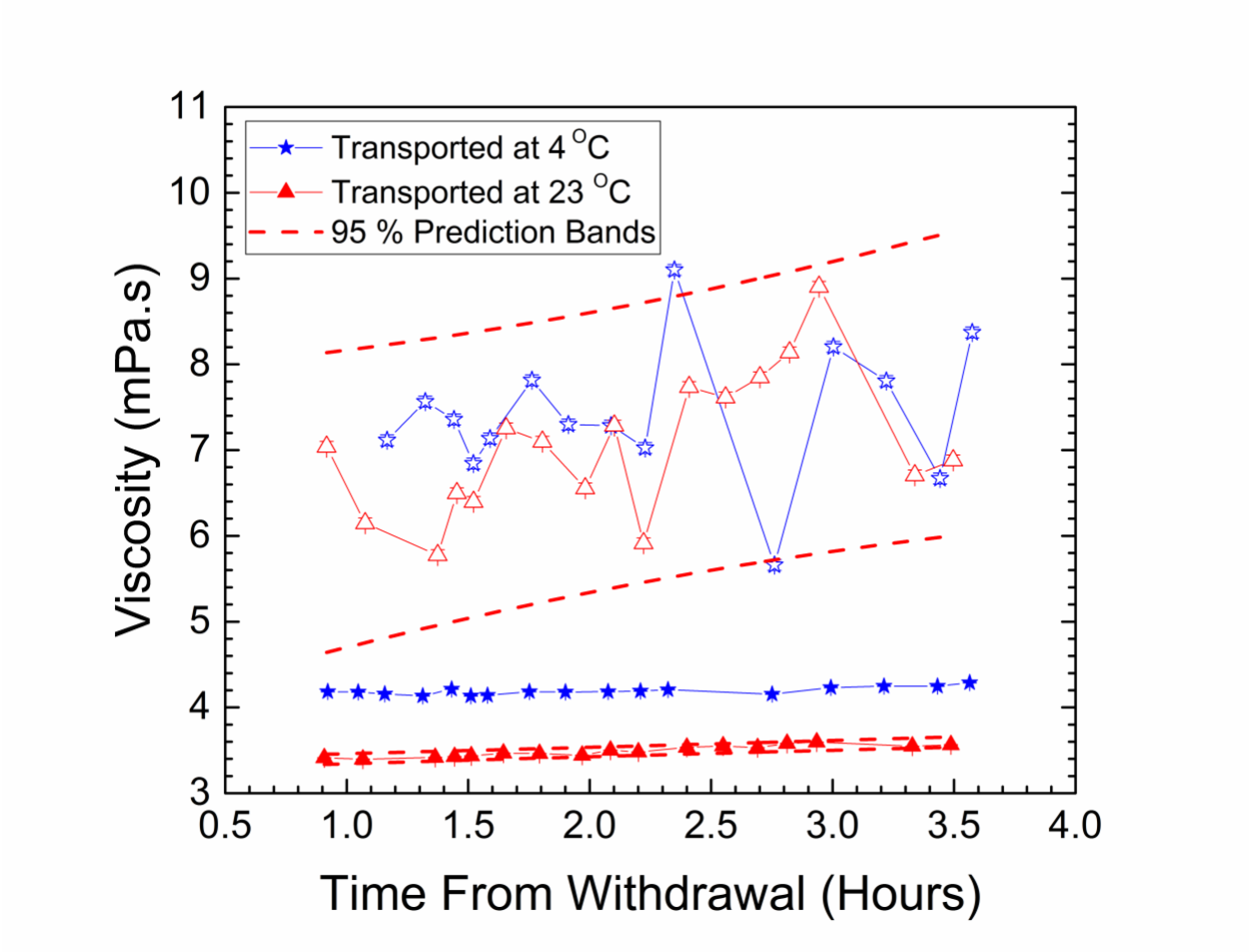

Transported at 4 °C
Transported at 23 °C
95 % Prediction Bands
Viscosity (mPa.s)
Time From Withdrawal (Hours)
11
10
9
8
7
6
5
4
3
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0

Fig. 7

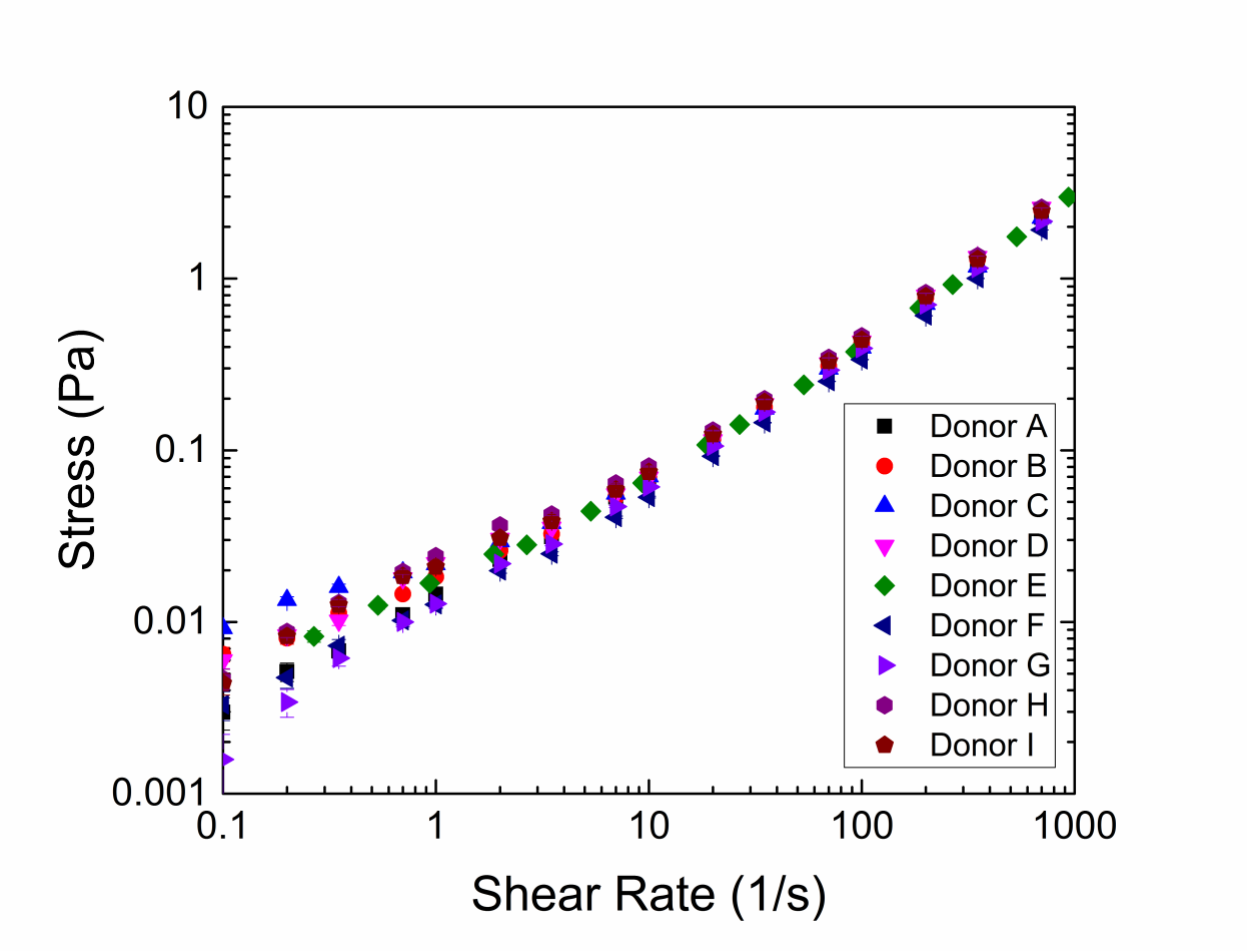

Stress (Pa)
Shear Rate (1/s)
10
1
0.1
0.01
0.001
0.1
1
10
100
1000
Donor A
Donor B
Donor C
Donor D
Donor E
Donor F
Donor G
Donor H
Donor I

Fig. 8

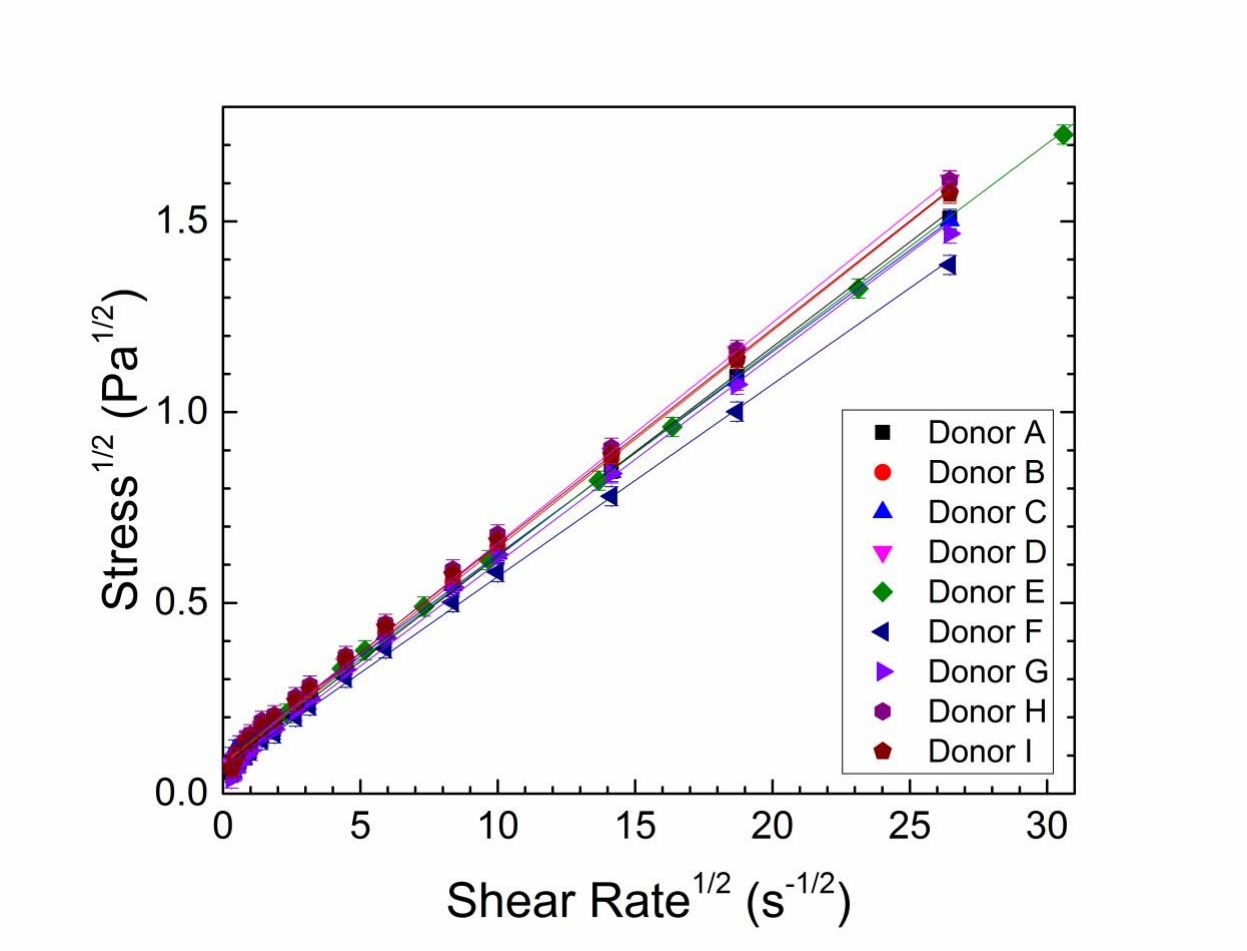

Stress$^{1/2}$ (Pa$^{1/2}$)
Shear Rate$^{1/2}$ (s$^{-1/2}$)
0.0
0.5
1.0
1.5
0
5
10
15
20
25
30
Donor A
Donor B
Donor C
Donor D
Donor E
Donor F
Donor G
Donor H
Donor I

Fig. 9

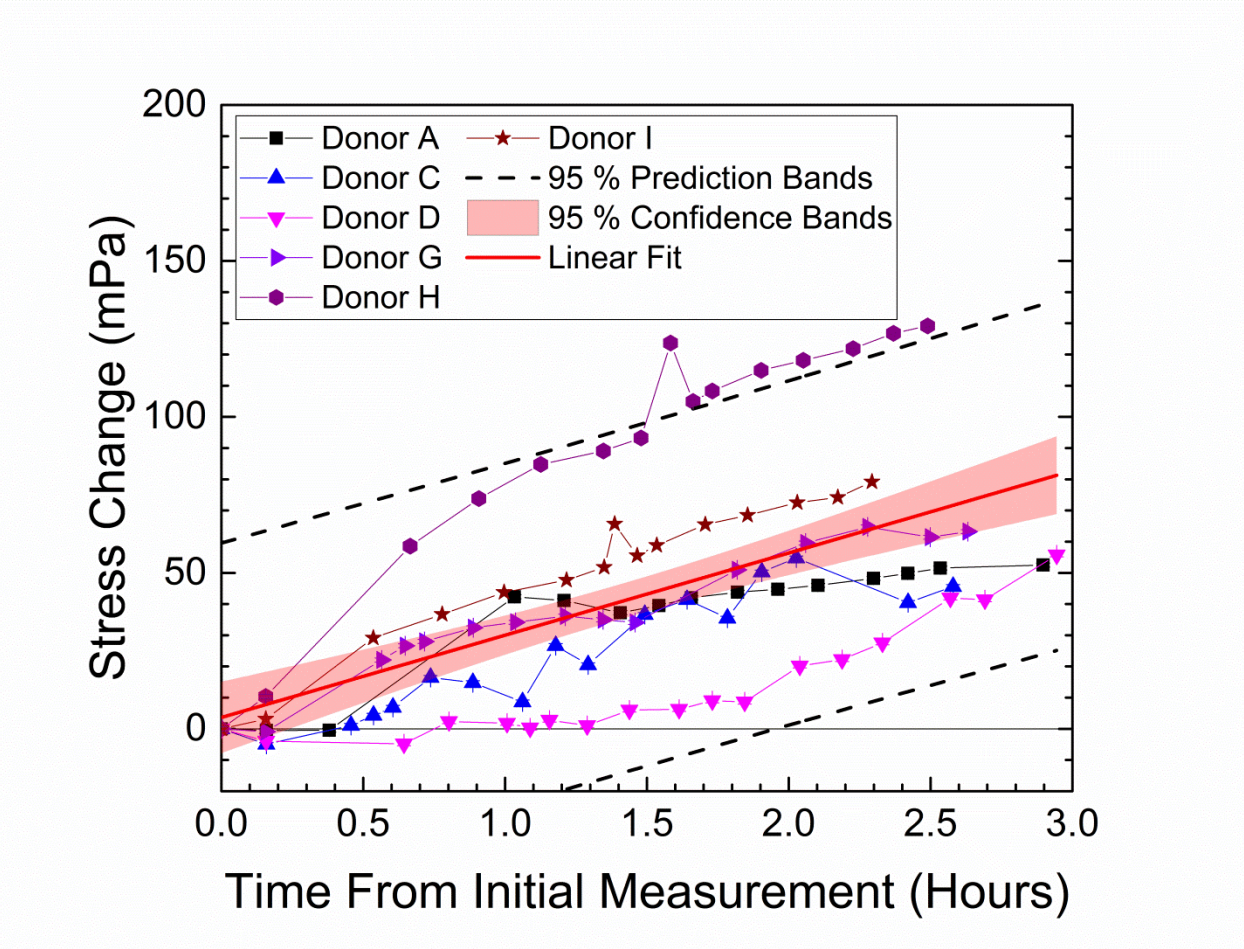

Donor A
Donor C
Donor D
Donor G
Donor H
Donor I
95 % Prediction Bands
95 % Confidence Bands
Linear Fit
Stress Change (mPa)
Time From Initial Measurement (Hours)
200
150
100
50
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Fig. 10

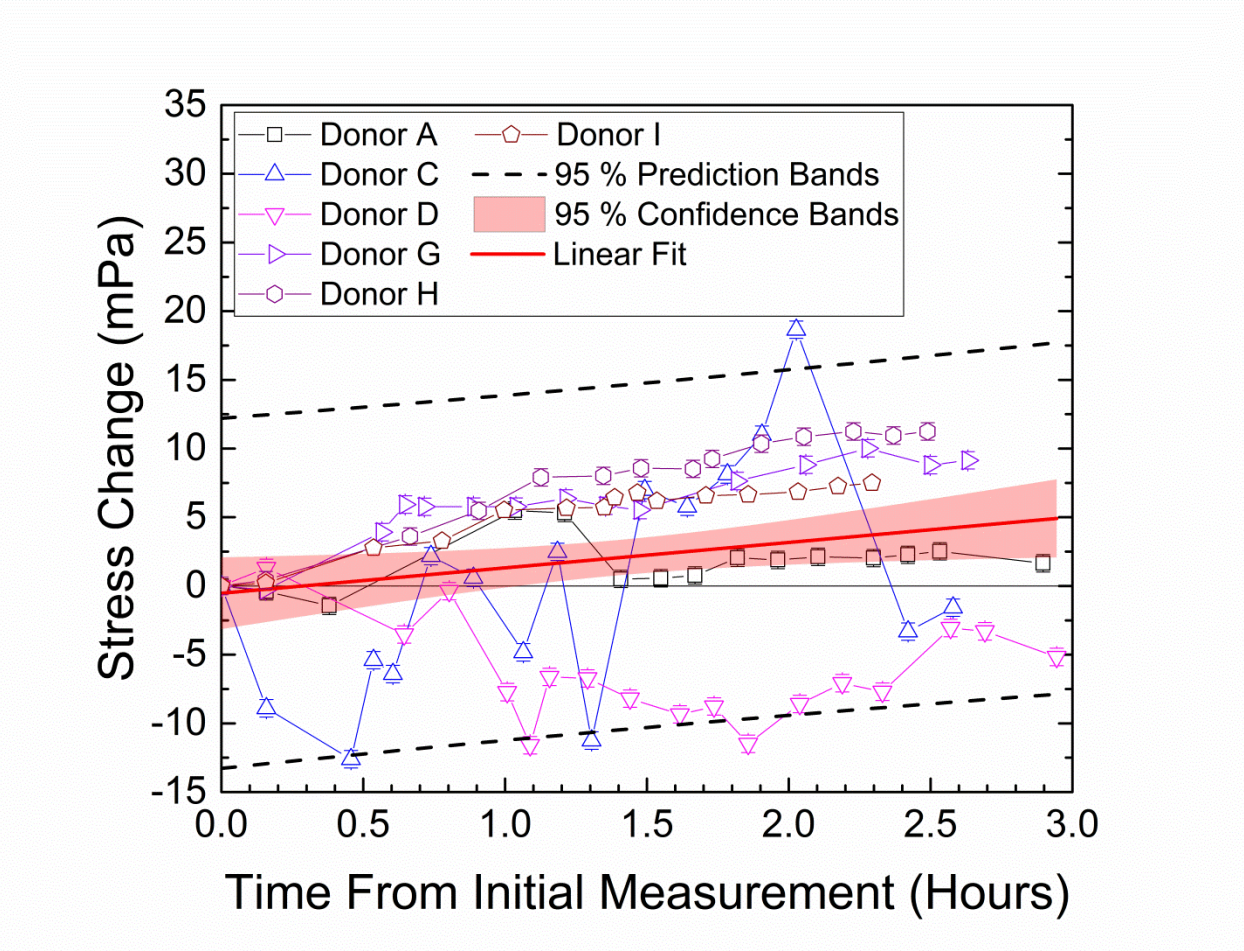

Donor A
Donor C
Donor D
Donor G
Donor H
Donor I
95 % Prediction Bands
95 % Confidence Bands
Linear Fit
Stress Change (mPa)
Time From Initial Measurement (Hours)
35
30
25
20
15
10
5
0
-5
-10
-15
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Fig. 11

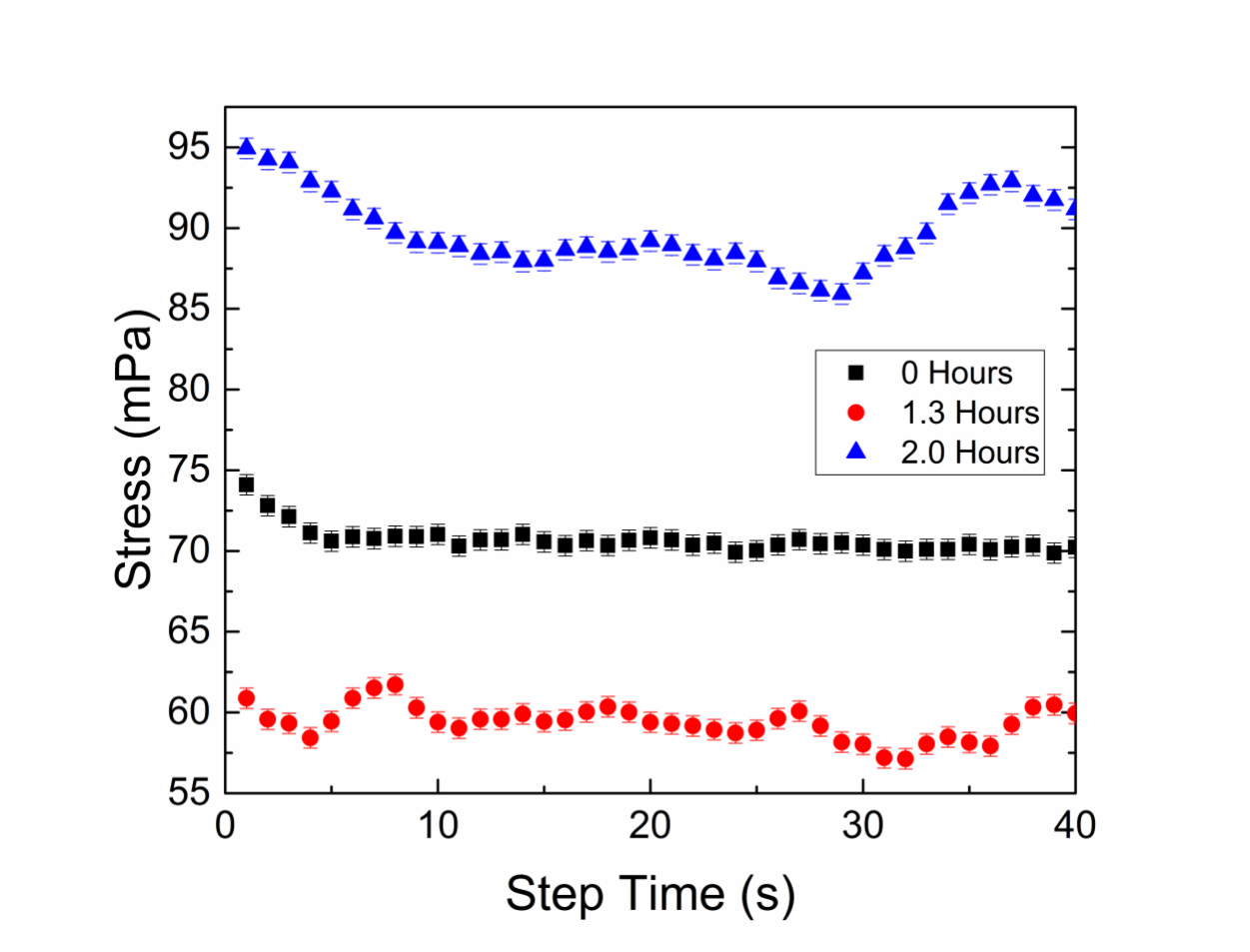

Stress (mPa)
Step Time (s)
0 Hours
1.3 Hours
2.0 Hours
95
90
85
80
75
70
65
60
55
0
10
20
30
40

Fig. 12

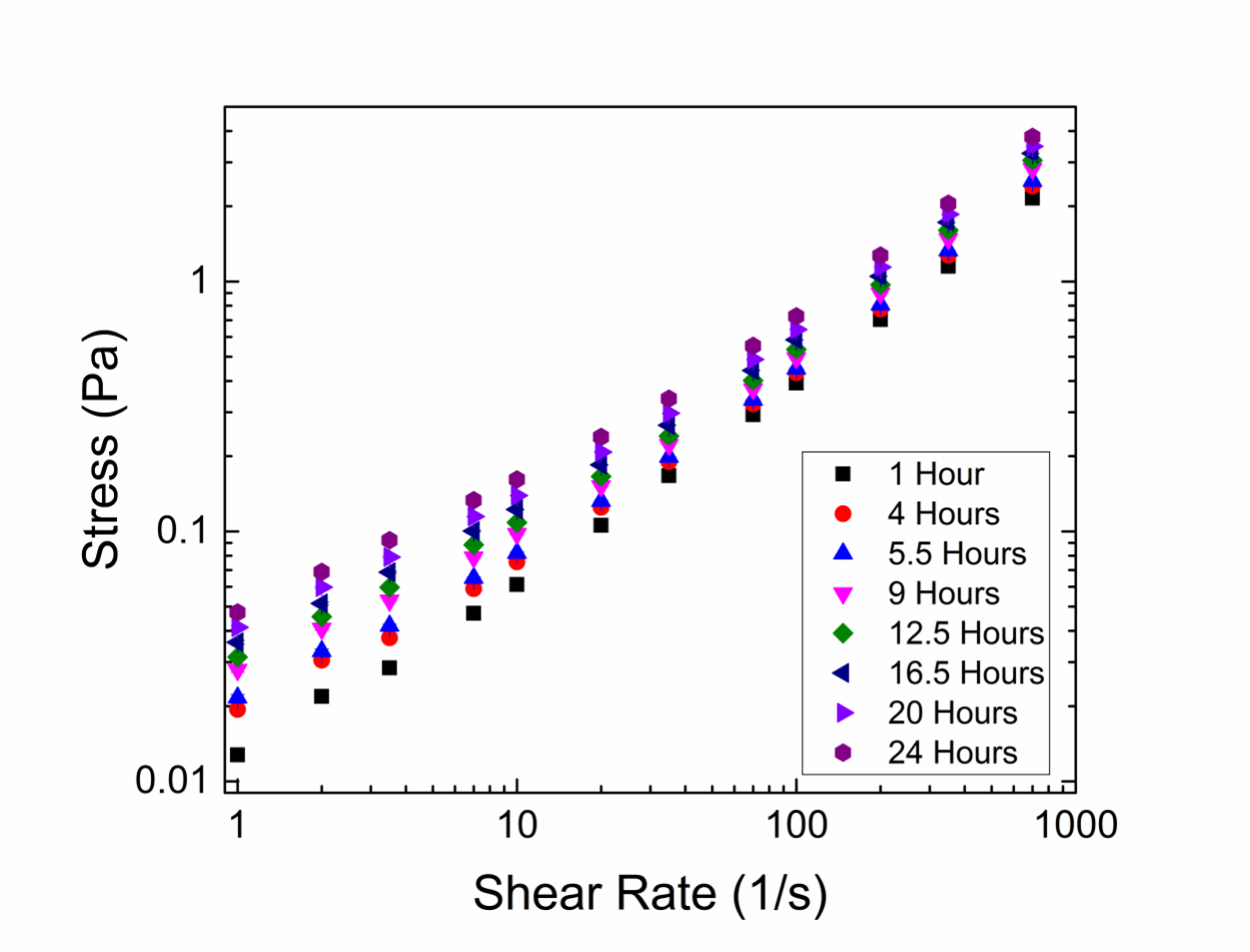

Stress (Pa)
1
0.1
0.01
1
10
100
1000
Shear Rate (1/s)
1 Hour
4 Hours
5.5 Hours
9 Hours
12.5 Hours
16.5 Hours
20 Hours
24 Hours

Fig. 13

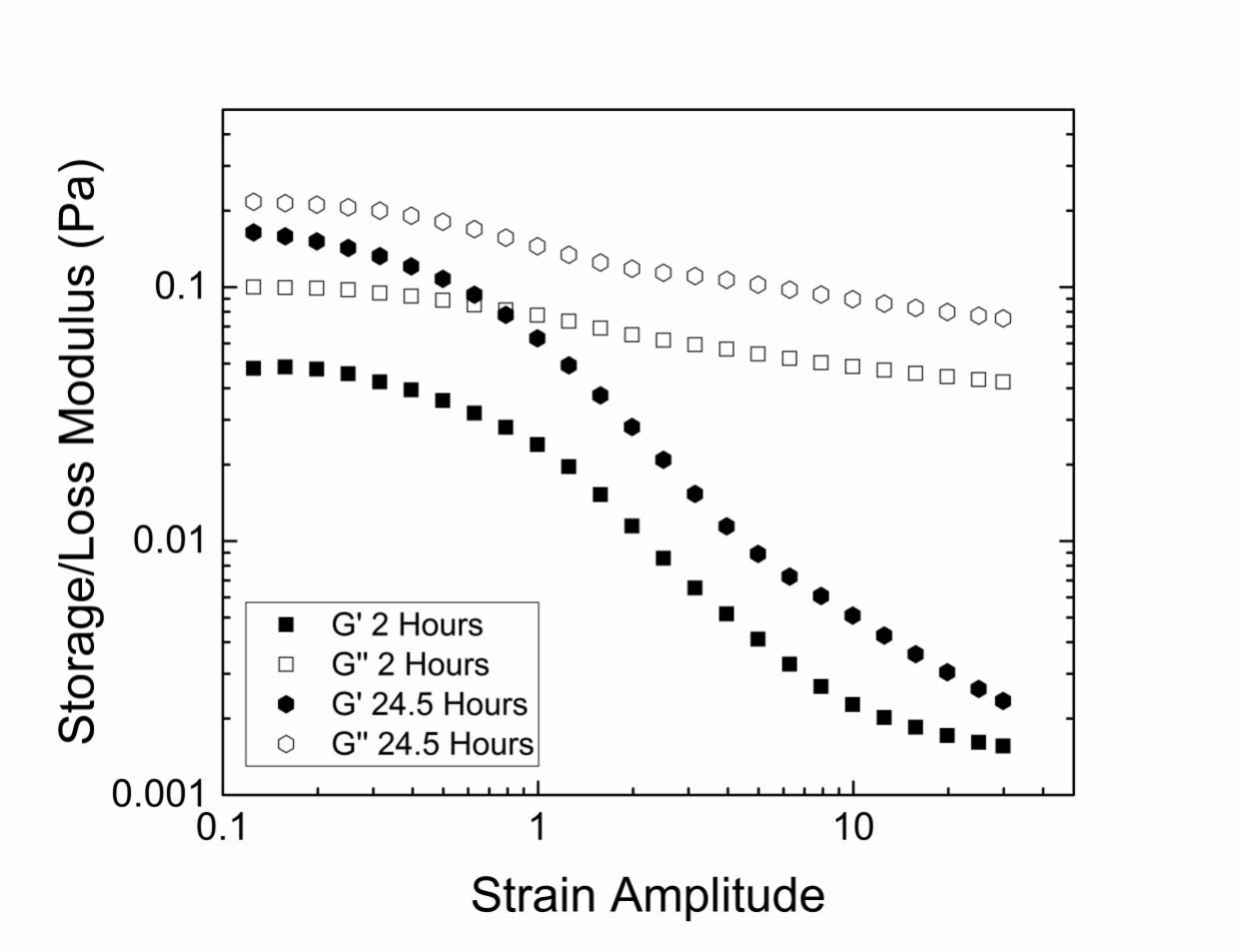

Storage/Loss Modulus (Pa)
0.1
0.01
0.001
0.1
1
10
Strain Amplitude
G' 2 Hours
G" 2 Hours
G' 24.5 Hours
G" 24.5 Hours

Fig. 14

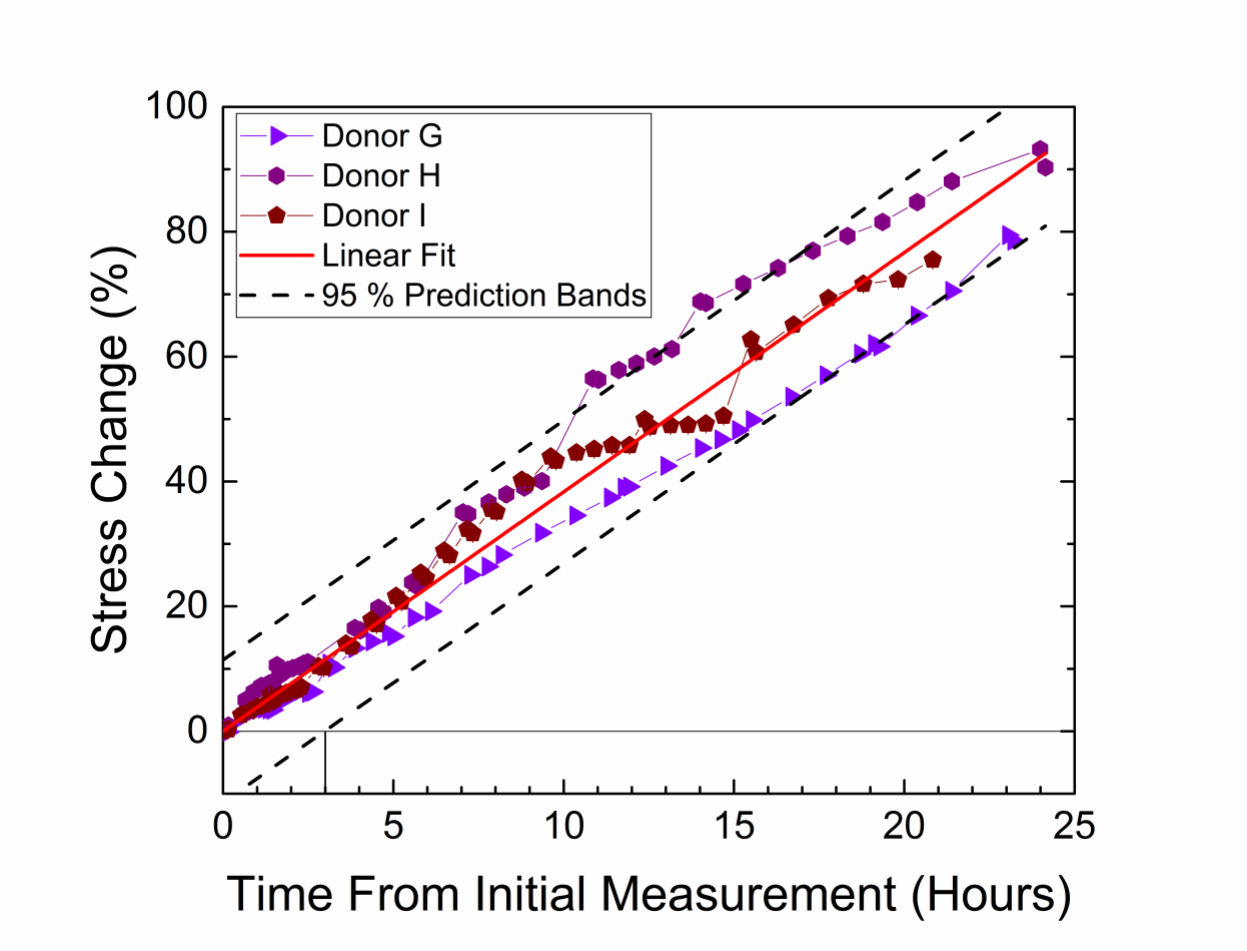
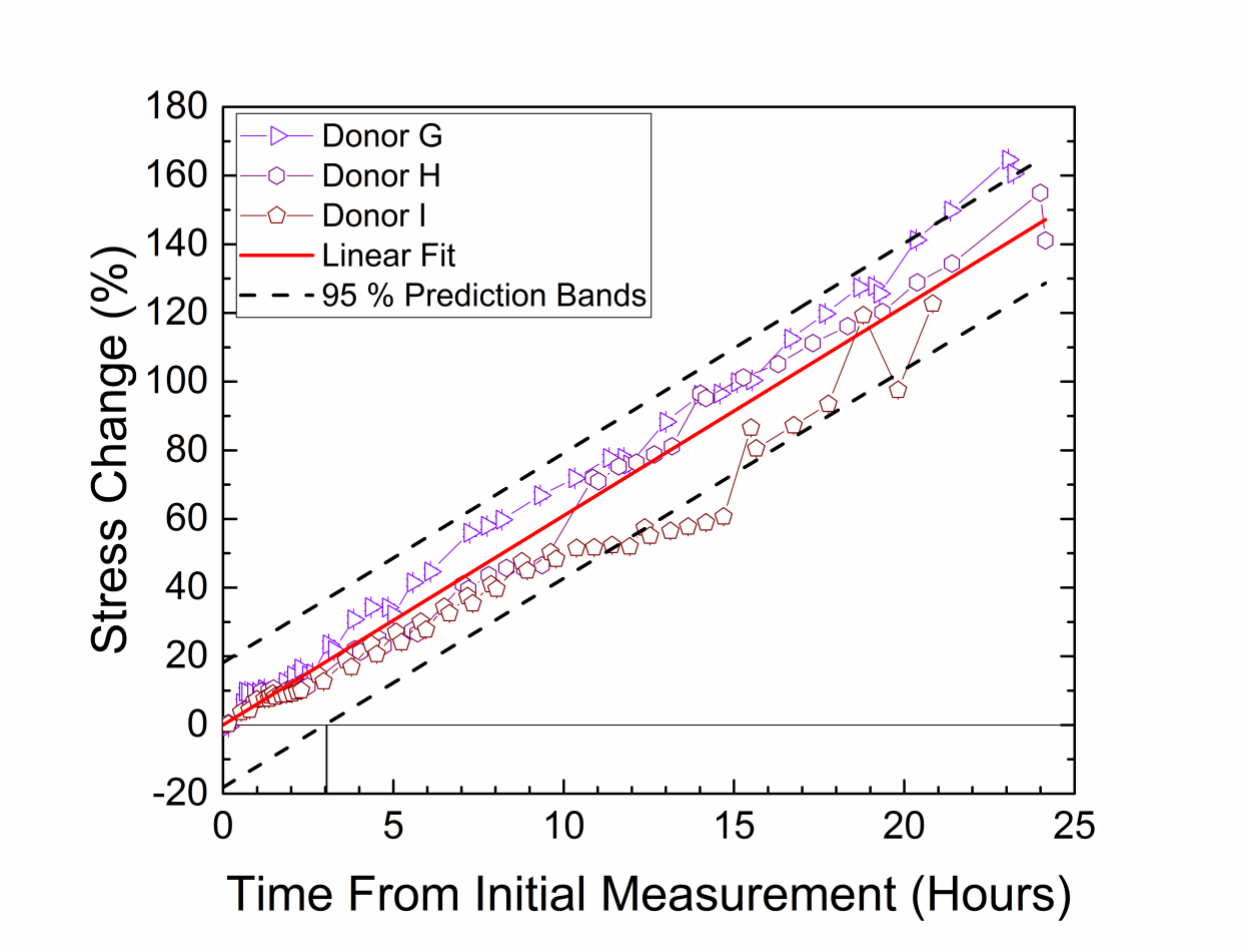

Donor G
Donor H
Donor I
Linear Fit
95 % Prediction Bands
Stress Change (%)
Time From Initial Measurement (Hours)
0
20
40
60
80
100
0
5
10
15
20
25

Fig. 15


Donor G
Donor H
Donor I
Linear Fit
95 % Prediction Bands
Stress Change (%)
Time From Initial Measurement (Hours)
180
160
140
120
100
80
60
40
20
0
-20
0
5
10
15
20
25