



Original Article

Investigating the Mechanical Behavior of Clot Analogues Through Experimental and Computational Analysis

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Abstract—With mechanical thrombectomy emerging as the new standard of care for stroke treatment, clot analogues provide an extremely useful tool in the testing and design of these treatment devices. The aim of this study is to characterise the mechanical behavior of thrombus analogues as a function of composition. Platelet-contracted clot analogues were prepared from blood mixtures of various hematocrits. Mechanical testing was performed whereby clots were subjected to unconfined compression between two rigid plates. Two loading protocols were imposed: cyclic compression for 10 cycles at a constant strain-rate magnitude; stress-relaxation at a constant applied compressive strain. A hyper-viscoelastic constitutive law was identified and calibrated based on the experimental mechanical test data. Scanning electron microscopy (SEM) investigated the clot microstructure at various time-points. Clot analogue composition was found to strongly affect the observed mechanical behavior. The SEM found that the microstructure of the clot analogues was affected by the storage solution and age of the clot. The proposed hyper-viscoelastic constitutive model was found to successfully capture the material test data. The results presented in this study are of key importance to the evaluation and future development mechanical thrombectomy devices and procedures.

Keywords—Acute ischemic stroke, Mechanical thrombectomy, Mechanical characterisation, Thrombus.

INTRODUCTION

Thrombus material is a critically important tissue component that has the essential role of preventing blood loss in the human organism. However thrombus material is associated with a range of life-threatening conditions, such as acute ischemic stroke (AIS), which accounts for 85% of all strokes.^{3,4} AIS is often caused by the embolisation of cardiac or vascular originating clots that cause an occlusion in the neuro-vasculature. Mechanical thrombectomy has recently been established as the new standard of care for the treatment of AIS.⁹ However, it is proposed that the success of the thrombectomy procedure can be significantly affected by the mechanical properties of the occluding thrombus.^{3,13}

Analysis of thrombus material from human sources can often be expensive and highly regulated, making specimens difficult to obtain. Therefore clinically relevant thrombus analogues that are fabricated *in-vitro* are an extremely useful for pre-clinical testing of thrombectomy devices^{3,6,19} and assessment of the effectiveness of a device for a range of clot compositions. Such clot analogues should be constructed from blood proteins and components present in the clotting cascade so that a similar chemical composition to native clots is achieved.

A variety of protocols have been proposed for fabrication of clot analogues involving numerous variables, which include donor species and concentration of thrombin.^{3,6,13,16,19} Other studies have also investigated the effect of barium sulphate on the clot material.^{3,16} The effects of these variables on the mechanical behavior of the clot analogues under

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compressive^{3,13,16,20,25} and tensile loading,^{16,20,25} as well as clot permeability and structure,^{13,14} have been investigated. Clot composition can also differ substantially with varying red blood cell (RBC) and fibrin content,^{18,21} and to-date, the effect of clot composition and hematocrit (%H), i.e., the volume percentage of RBCs in the mixture, on the rate-dependent mechanical behavior of the material has not been adequately investigated.

Previous studies have carried out a variety of mechanical testing on thrombus material, including compression testing,^{1,3,16,20,25} uniaxial and biaxial tensile testing,^{5,8,20,24,25,29,36} rheometry,^{15,20,32} nanoindentation^{28,39} and friction testing,¹⁰ with the earliest studies dating back to the 1940s and 1950s with the work of Ferry and co-workers.⁷ However, developing a reliable test method that is suitable to test the broad range of clot types and compositions can be challenging due to the fragile nature of the clot material.

Few studies have investigated the viscoelastic behavior of clot material and have proposed constitutive models to capture this behavior,^{20,26,28,33,35} yet the loading–unloading hysteresis and stress-relaxation due to rate-dependent viscoelasticity of thrombus material, as a function of clot composition, has been neglected.

We previously investigated the effect of platelet-driven clot contraction on the clot mechanical properties and microstructure.¹³ The current study provides a significant advance in the current understanding of the relationship between clot composition and rate-dependent non-linear mechanical behavior. Our experimental testing and constitutive law calibration provides the first detailed characterisation of cyclic loading–unloading hysteresis and stress-relaxation for a range of clot analogue compositions. Such mechanical characterisation is of key importance for the design and development of next-generation thrombectomy devices.

MATERIALS AND METHODS

Sample Preparation

Fresh venous blood was collected from the jugular vein of sheep, into receiving vessels that were pre-loaded with ACPD (Adenine citrate phosphate dextrose) anticoagulant solution (Ash Stream vets, Co. Mayo, Ireland), for the preparation of the analogue clot samples for testing, similar to.^{6,13} The blood was then transported to the lab and stored at room temperature before use. Ovine blood was chosen as it has been found to be a suitable substitute for human blood for coagulation studies²⁷ and the clot samples produced

using this methodology have been found to be histologically similar to human clots.⁶ All clots were prepared within 5 h of blood collection. The clot samples prepared for this study were produced from 5 separate blood collections.

To begin clot preparation, the blood was centrifuged at 180 g for 10 min. The platelet-rich plasma (PRP) was removed and collected into a separate container. The remaining blood mixture was centrifuged at 2200 g for a further 10 min to isolate the red blood cells (RBCs). Platelet-contracted clots were formed by mixing the PRP with the RBCs in controlled ratios, to represent clots with different levels of hematocrit (%H), i.e., the volume percentage of RBCs in the mixture – 0, 5 and 40%H.

Once the various clot mixtures were produced, coagulation was initiated by the addition of a 2.06% calcium chloride solution to the blood components in a 1:9 ratio. The samples were formed in cylindrical-shaped moulds (Figs. 1a and 1b) and were allowed to mature overnight at 37 °C. A whole blood (WB) clot was also formed by collecting whole blood into a syringe and allowing spontaneous coagulation to occur at room temperature overnight.⁶

After maturation overnight, the weights of the resultant clots and the expelled serum were measured separately on a gravimetric balance to determine the percentage of clot contraction and to ensure that the clot analogues had contracted fully. The volume reduction due to contraction was calculated by expressing the weight of each clot as a percentage of the weight of the original blood mixture (weight of resultant clot + weight of expelled serum). The platelet-contracted clot size has previously been shown to have a linear correlation with the hematocrit of the clot mixture.¹³ This correlation was used to ensure that clot analogues contracted appropriately and only clots that had contracted sufficiently (i.e., $H \pm 5\%$) were used for testing (Fig. 1c).

Test samples were then prepared by cutting the clots into cylindrical-shaped samples with an approximate diameter of 10 mm (Fig. 1b), and an approximate height of 5 mm. Unconfined compression was used to determine the material behavior of the analogue clot samples, the day after formation (minimum of $n \geq 15$ for each clot type). The 0, 5 and 40% H clot analogues were also aged, by storing the samples in serum at 4 °C or at 37 °C. These samples were then tested at various time-points; 1 day, 4 days, 7 days and 14 days after contraction ($n = 9$ at each time-point), to investigate the effect of aging and storage temperature on the mechanical behavior.

A group of 5 and 40% H clots were also aged at 4 °C for up to 7 days in saline. These samples were not

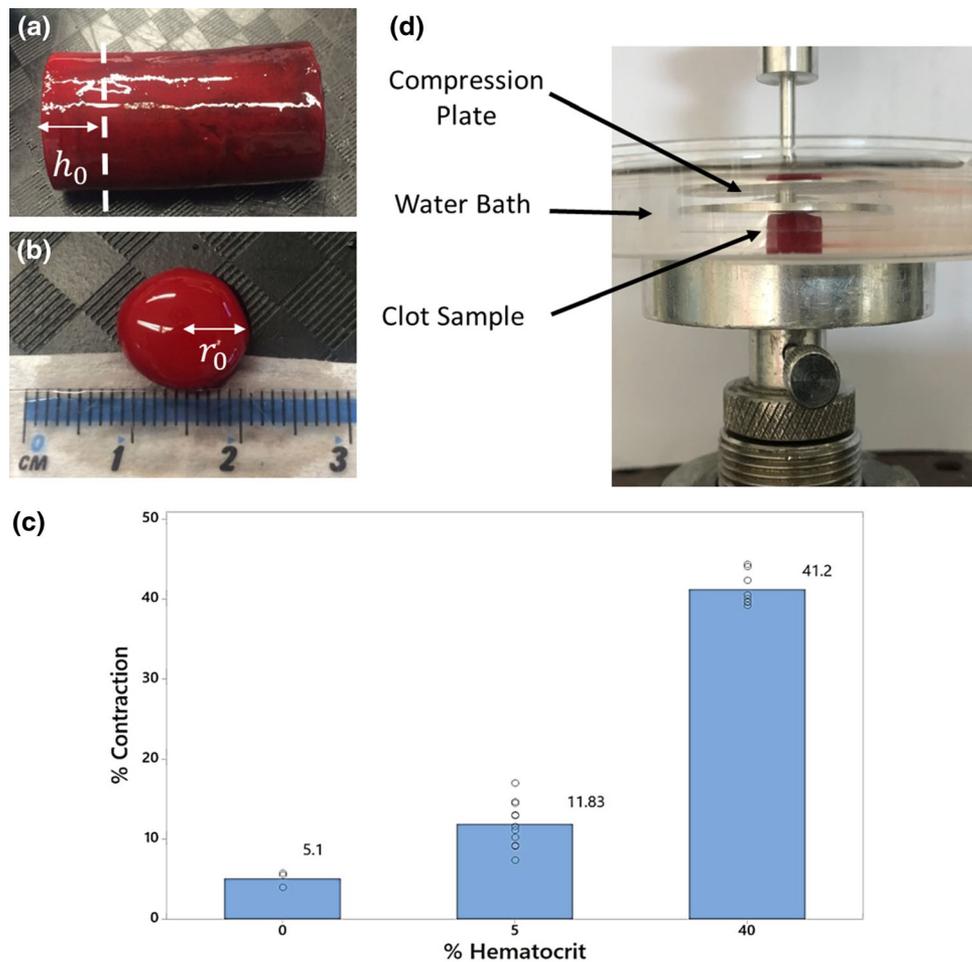


FIGURE 1. Images illustrating (a) the cylindrical shaped clot slug after formation, with the white dashed line indicating how the test samples were cut, with h_0 indicating the specimen height, and (b) an example of a test-sample (5% H), with r_0 indicating the specimen radius. (c) Bar chart illustrating the mean percentage contraction of the clot analogues vs the percentage hematocrit (% H) of the blood mixture, with the individual data points illustrated by the circular symbols. (d) The compression test set-up.

mechanically tested, although their microstructure was examined under SEM at time points of 1, 4 and 7 days.

Compression Testing

The compression test set-up is shown in Fig. 1d, with all samples tested saline. The testing was performed on a Zwick uniaxial tensile machine (Zwick Z2.5, Ulm, Germany), using a customised aluminium platen. The samples were placed between two platens and the crosshead position of the machine was adjusted so that the top platen was slightly touching the top of the sample at the beginning of the test. The clot specimens were loaded to a compressive nominal strain of 80% at a constant axial strain-rate magnitude of 10% per second and then unloaded to their initial configuration at the same axial strain-rate magnitude. This was performed for 10 cycles.

The compressive axial nominal stress is calculated as $\sigma = -F/(\pi r_0^2)$, where F was the measured loading force and r_0 is the radius of the undeformed cylindrical specimen. The nominal axial compressive strain (ϵ) under compression was calculated as $\epsilon = (h_0 - h)/h_0$, where h is the deformed height of the cylindrical sample and h_0 is the undeformed height prior to load application. Note that, for clarity of presentation of experimental results, this convention results in positive values of σ and ϵ in compression.

Stress-Relaxation

Unconfined compression stress-relaxation tests were also carried out on clot samples ($n \geq 15$ for each clot type). Samples were instantaneously loaded to a nominal compressive axial strain of $\epsilon = 60\%$. The applied strain is then held constant at this value for

1000 seconds. During this period the changes in nominal axial compressive stress, σ , are recorded.

Statistical Analysis

Statistical analysis of the experimental results was carried out using the general linear model ANOVA procedure in Minitab (ver. 18.1). The Bonferroni model ($\alpha = 0.05$) was used to compare the peak stress and tangent stiffnesses of each of the clot analogues. Similarly, the initial stiffness, calculated at 10% strain, of the clot analogues when stored in serum and tested 1 day, 4 days, 7 days and 14 days after formation were compared.

Scanning Electron Microscopy (SEM)

The analogue samples were fixed with 2.5% glutaraldehyde and dehydrated in a series of ethanol concentrations up to 100%. The samples were frozen in liquid nitrogen and fractured so that the interior surface of the clot analogues could be examined. The samples were then critical-point dried, mounted and sputter-coated with iridium.

Constitutive Model

A preliminary assessment of established hyperelastic material models reveals that the Yeoh provides a reasonably accurate fit to unconfined compression test results of clot analogue material. The Yeoh strain energy density function, W is given as

$$W = \sum_{i=1}^3 C_{i0} (\bar{I}_1 - 3)^i + \sum_{i=1}^3 \frac{K}{2} (J - 1)^{2i}. \quad (1)$$

The parameters C_{i0} describe the non-linear shear stiffness, while the parameter K is the bulk modulus. \bar{I}_1 the isochoric first strain invariant and J is the volume jacobian. In all cases near incompressible material behavior, based on previous assumptions for material models of clot material,^{8,11,33,37,38,40} was enforced by setting a value for the bulk modulus (K) that was three orders of magnitude greater than the effective shear moduli C_{i0} .

Rate-dependent viscoelasticity is implemented through the specification of a non-dimensional stress-relaxation curve, parameterised through a Prony series. The effective shear-relaxation modulus is given as

$$\bar{g}(t) = 1 - \sum_{i=1}^n g_i \left(1 - e^{-\frac{t}{\tau_i}}\right) \quad (2)$$

where n is the number of the terms in the Prony series, τ_i are the relaxation time constants for each term of the

series, while the parameters g_i sets the ratio of long-term to instantaneous effective shear modulus.

A series of finite element analyses were performed to identify a set of material law parameters for each clot type so that a good agreement was obtained between model predictions and experimental cyclic compression and stress-relaxation tests. Loading platens were modelled as rigid surfaces and frictionless contact was assumed.

RESULTS

Mechanical Testing

Representative curves showing nominal stress vs time for 10 loading–unloading cycles for a 0%, 5% and 40% H clot is shown in Fig. 2a. Each of the clot groups were found to exhibit dynamic stress relaxation, suggesting that the clot exhibits viscoelastic behavior. Corresponding stress–strain curves are shown in Fig. 2b, demonstrating non-linear hyperelastic strain stiffening, with loading–unloading hysteresis again demonstrating material viscoelasticity.

Stress-strain curves (mean \pm SD) are shown in Figs. 3a and 3b. All of the clot compositions exhibited loading–unloading hysteresis, suggesting that the analogue material is viscoelastic (Fig. 3a). The stress-strain curves (mean \pm SD) for the first loading half-cycle to 80% compression are shown in Fig. 3b. The 5% H clot had the greatest peak stress at 80% strain, followed by the 0% H clot and the WB clot respectively (Figs. 3a and 3b). The 40% H clot had the lowest peak stress at maximum strain and was significantly lower than that of the 5% H clot.

Although the 0% H clot appears to have the highest initial stiffness at low levels of strain, the 5% H clot was found to have the greatest stiffness at strains of $< 30\%$ (Fig. 3c). The 40% H clot was found to have the lowest stiffness of the three clot types at all strains and was found to be significantly lower than both the 0% and 5% H clots. There was no statistical difference when comparing the behavior of the 40% H clot and the WB clot.

0%, 5% and 40% H clot analogues were stored in their own serum at 4 °C and subjected to unconfined parallel plate compression testing at time-points of 1 day, 4 days, 7 days and 14 days after formation. No significant difference in mechanical behavior was observed between each time-point (Figs. 4a and 4d). Similarly, no significant difference in the mechanical behavior was observed between clots stored at 4 °C and 37 °C. However, the 40% H clots stored at 37 °C were found to disintegrate if stored for longer than one

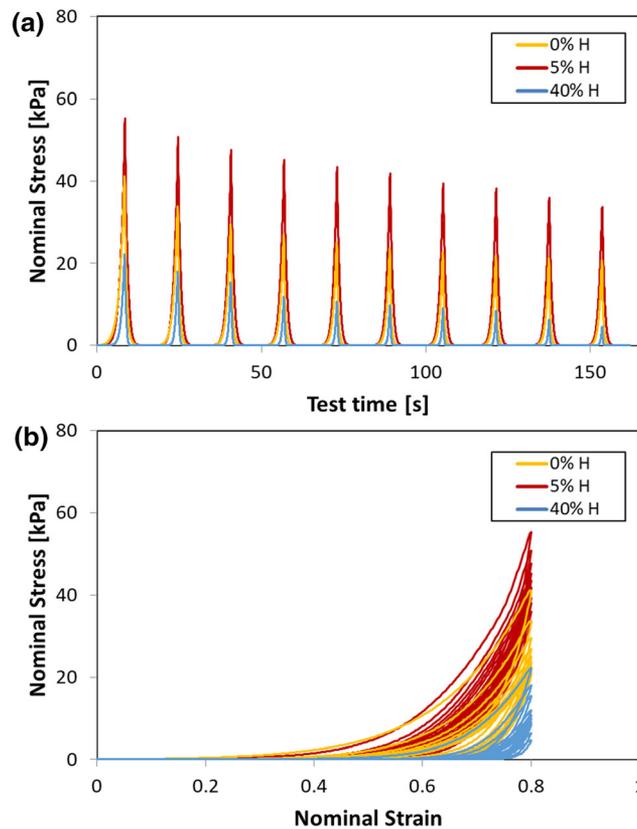


FIGURE 2. Representative curves showing the typical response of a 0% H, 5% H clot and a 40% H clot analogue during cyclic compression. Plot of (a) nominal stress vs time and (b) nominal stress vs nominal strain showing the typical loading–unloading curves for 10 cycles. From both curves, the viscoelastic nature of the material is apparent through the dynamic stress-relaxation and energy-dissipation after each cycle evident in (a) and (b) respectively.

day and therefore they were unable to be tested under compression for time-points of 4, 7 and 14 days.

Measured stress-relaxation curves under an applied compressive strain of 60% are shown in Fig. 5a for each clot composition. The percentage relaxation following 1000 s of constant applied strain is quantified (mean \pm SD) in Fig. 5b. While all clot compositions exhibit significant relaxation, 0% H clots exhibit the greatest stress relaxation ($97.35 \pm 4.76\%$), followed by the 5% H clot ($89.08 \pm 8.24\%$), and experienced significantly greater relaxation than both the WB clot ($61.83 \pm 19.24\%$) and the 40% H clot ($53.55 \pm 19.81\%$). There was no statistical difference between the 40% H clot and the WB clot.

SEM

Figure 6 shows SEM images of the interior of the clot analogues. The 0% H clot is composed of thick fibrin fibres and areas of very dense compressed fibrin (Fig. 6a). The 5% H clot consists of very dense fibrin compacted around compressed RBCs, indicated by the white arrows in Fig. 6b. The 40% H, shown in Fig. 6c,

clot consists mainly of compacted RBCs entrapped in a network of thick fibrin fibres. There is also evidence of a thin hyper-branched fibrin network that has been significantly compressed through the action of clot contraction, appearing as a very thin layer on the faces of closely packed polyhedron-shaped RBCs (also referred to as "polyhydrocytes"^{4,30}). All clots were found to have a fibrous outer layer.

Figures 6c and 6f also compares the microstructure of the 40% H clots stored in serum and saline for various time points. At day 1 both clot groups had a relatively similar microstructure with compacted fibrin compressing around RBCs as described previously. However, at day 4 and day 7, the clots stored in serum appear to have compressed further, with polyhydrocyte shapes becoming more apparent (Figs. 6c and 6d). However, this was not the case for the clots stored and aged in saline. Although the compressed fibrin network is still apparent, the RBCs appear to have returned to a spherical shape and to have become dislodged from the surrounding fibrin, leaving spherical cavities where the RBCs once were empty (Figs. 6e and 6f).

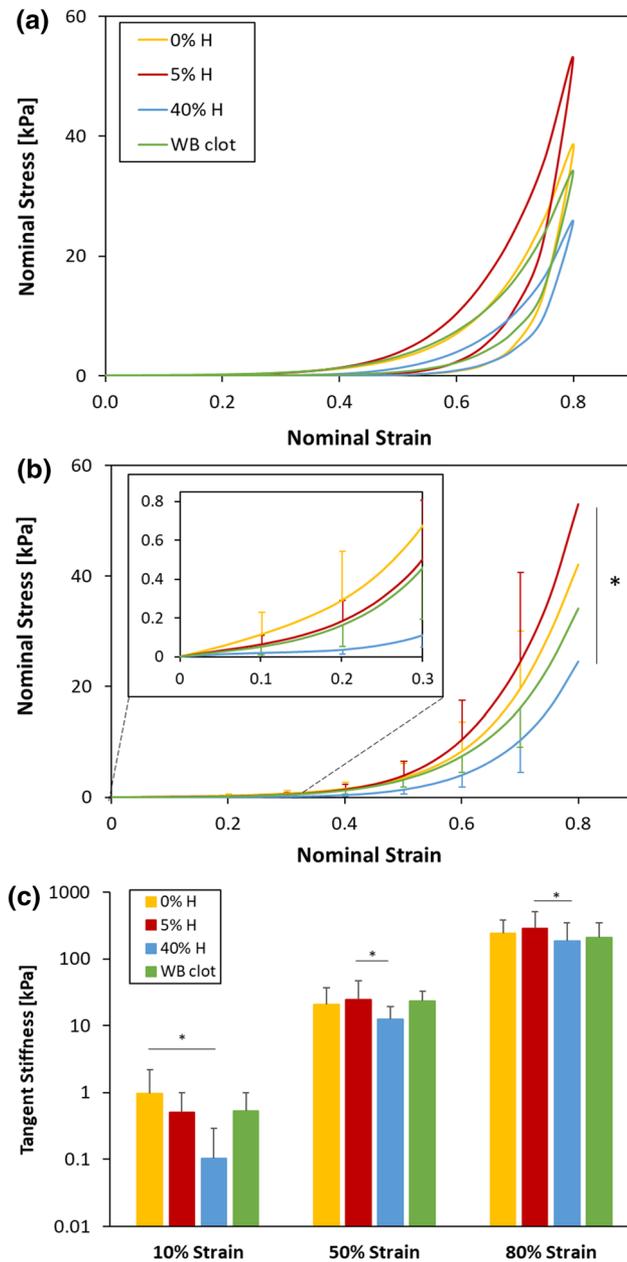


FIGURE 3. (a) Average nominal stress-strain curves ($n \geq 15$ for each clot type), shown as positive values for clarity, comparing the loading–unloading curves from the first cycle of the compression testing, for the 4 clot types with varying hematocrit. (b) Average stress-strain curves comparing the first loading half-cycle to 80% compression, including error bars representing standard deviation, for each clot group. Inset, is a zoomed-in image of the behavior at low levels of strain. * denotes statistical significance between the peak stresses of the 5% H and 40% H clots, where $p < 0.05$. (c) Comparison of tangent stiffness values (mean \pm SD) at various levels of strain. * denotes statistical significance where $p < 0.05$.

Material Law Calibration

As shown in Fig. 7, the hyper-viscoelastic material law outlined in the methods section, is found to provide a reasonable fit to the experimental cyclic compression and stress-relaxation tests (Fig. 7). The Yeoh hyperelastic formulation with three term-Prony series modulus-relaxation was found to provide reasonable

agreement with experimentally measured stress-relaxation data ($R^2 > 0.77$ in all cases) and compression data ($R^2 > 0.76$ in all cases). This suggests that the material behavior of each clot composition can be approximately captured using a hyper-viscoelastic material law. Calibrated model parameters for the clot analogue materials are shown in Table 1.

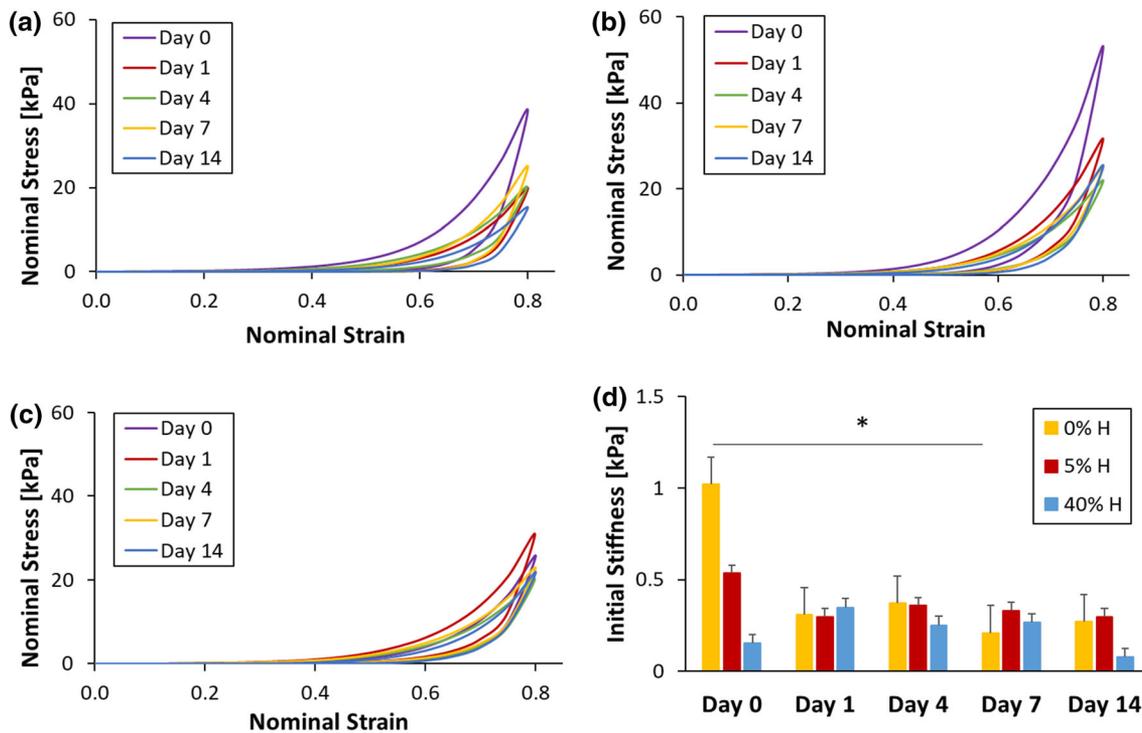


FIGURE 4. Plots of nominal stress vs nominal strain showing the average curves for (a) the 0% H clot, (b) 5% H clots and (c) 40% H clots, comparing the mechanical behavior of a fresh clots (Day 0), with clots aged for 1, 4, 7 and 14 days at 4 °C in serum. (d) Plot comparing the initial tangent stiffness calculated at 10% strain for the aged clots with the freshly formed clot analogue, with error bars indicating standard error. There was no statistical significance in the initial stiffness of the clots compared to the fresh clot analogue when aged over 14 days, with the exception of the 0% H clot at 7 days (statistical significance is denoted by *).

DISCUSSION

This study investigated the mechanical behavior of clot analogues, with varying composition, under compressive loading. Both cyclic compression tests and stress-relaxation tests were performed. The experimentally observed material behavior was shown to be accurately predicted by a hyper-viscoelastic material law for all clot compositions.

All of the clot groups were found to exhibit the typical behavior of a non-linear hyperelastic material, with low stiffness behavior occurring at low compressive strains ($< 30\%$), and significant strain stiffening occurring at higher strains ($> 30\%$), similar to previously published data for clot material.^{1,3,13,16,20} The 0% H clot was found to have the largest initial stiffness, followed by the 5% H and the WB clot, which had very a very similar stiffness as low levels of strain ($< 30\%$ strain). However, the 5% H clot appears to stiffen significantly for strains greater than 30%, and was found to have the greatest large strain stiffness, followed by the 0% H and the WB clot groups. Whole blood has a hematocrit of approximately 45%, which is similar to that of the 40% H clot. No statistical difference was observed between the behavior of the 40% H clot and the WB clot when comparing the

tangent stiffnesses at various levels of strain. Similarly, there was no significant difference when comparing peak stress and % relaxation of the 40% H and WB clots.

The behavior observed from the results of the unconfined compression and stress-relaxation experiments suggest that the material is viscoelastic.² This is supported by previous studies in the literature which have also reported on the viscoelastic behavior of clot material.^{1,3,12,20,25,32} However, the rate-dependent viscoelasticity has not previously been characterised for clot analogue material or for haemorrhagic stroke clots removed by mechanical thrombectomy. The characterisation of rate-dependent viscoelasticity is important for design of aspiration and MT devices.

Similarly, the effect of clot composition on the viscoelastic behavior of clot material has not been investigated to date. The results from the mechanical testing show the behavior of the clot analogues varied with hematocrit for both test types. Clots with a greater hematocrit were found to have a lower stiffness than clots with a lower hematocrit. In contrast the lower hematocrit clots, the 0% H and 5% H clot groups, experienced greater stress-relaxation. This is assumed to be due to the high RBC content and low fibrin content in these analogues. The larger fibrin

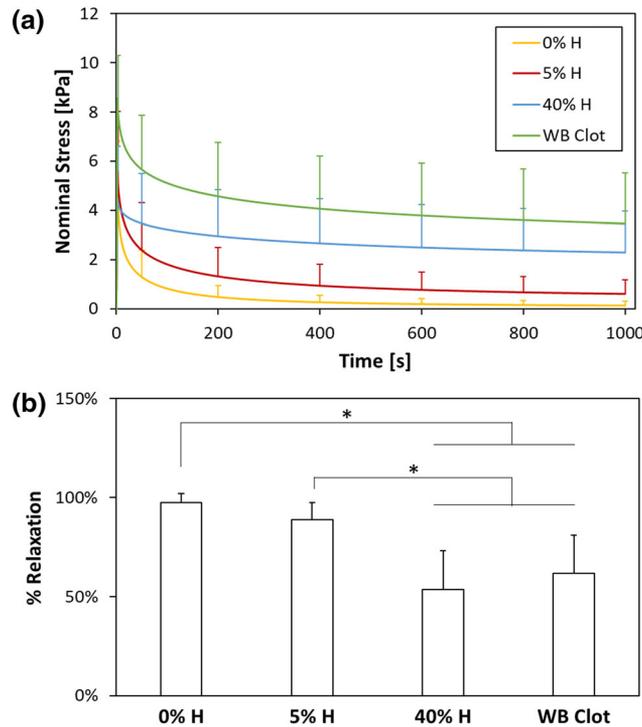


FIGURE 5. (a) Average stress-relaxation curves ($n \geq 15$ for each clot type), including error bars representing the standard deviation. (b) Plot comparing the average percentage stress-relaxation at 1000 seconds for each of the clot analogues. * denotes statistical significance where $p < 0.05$.

network in the other two groups can allow for further fibre rearrangement and in turn, greater relaxation.

The variation in mechanical behavior can also be explained by examining the material microstructure. The lower hematocrit clot analogues have a very dense fibrin network, due to the contracting platelets pulling on the fibrin fibres causing them to become compacted around the few RBCs present.^{4,13,31} In contrast, the 40% H clot consists of closely packed RBCs with little fibrin present. Fibrin therefore appears to make a strong contribution to the stiffness and relaxation of the material.

Aging and Storage

Although there are few reports in the literature on the effects of storage conditions on clot material,^{16,17} the impact on clot mechanical behavior is not fully understood. Previous studies have reported an decrease in clot stiffness and stability due to aging.^{16,17} However, in this study, aging was not found to have a significant impact on the mechanical behavior of the clot analogues stored in serum at 4 °C when tested under compression after 14 days. Although this is not consistent with the previously reported findings, it may be explained by the difference in protocols for the

formation of the clot analogues, as both of the previous studies included thrombin.

Likewise, storage temperature was found to have little impact on clot mechanical behavior of the lower haematocrit clot analogues, with little difference between the initial tangent stiffness of the clots stored at 37 °C vs 4 °C. However, the 40% H clot was found to completely disintegrate when stored at 37 °C for longer than one day. Similarly, the 0 and 5% H clots were found to disintegrate when stored at 37 °C for longer than four days. Although aging appeared to have little effect on the clot mechanical behavior, it is likely that the viability of components within the clot, such as the RBCs, is affected after storing for long periods of time. Therefore, it is recommended that clots are tested as soon as possible after formation/collection to ensure cell viability.

Although the impact of the storage solution on the mechanical behavior of the clot analogues was not investigated in this study, there were obvious effects on the clot microstructure. The clots stored in serum were found to have further compaction of the RBCs to a polyhedron shape between day 1 and day 7, than the clots stored in saline, where the RBCs appeared to swell and return to their native shape. This is likely due to the saline solution being hypotonic (i.e., has a lower solute concentration to the RBCs), and therefore

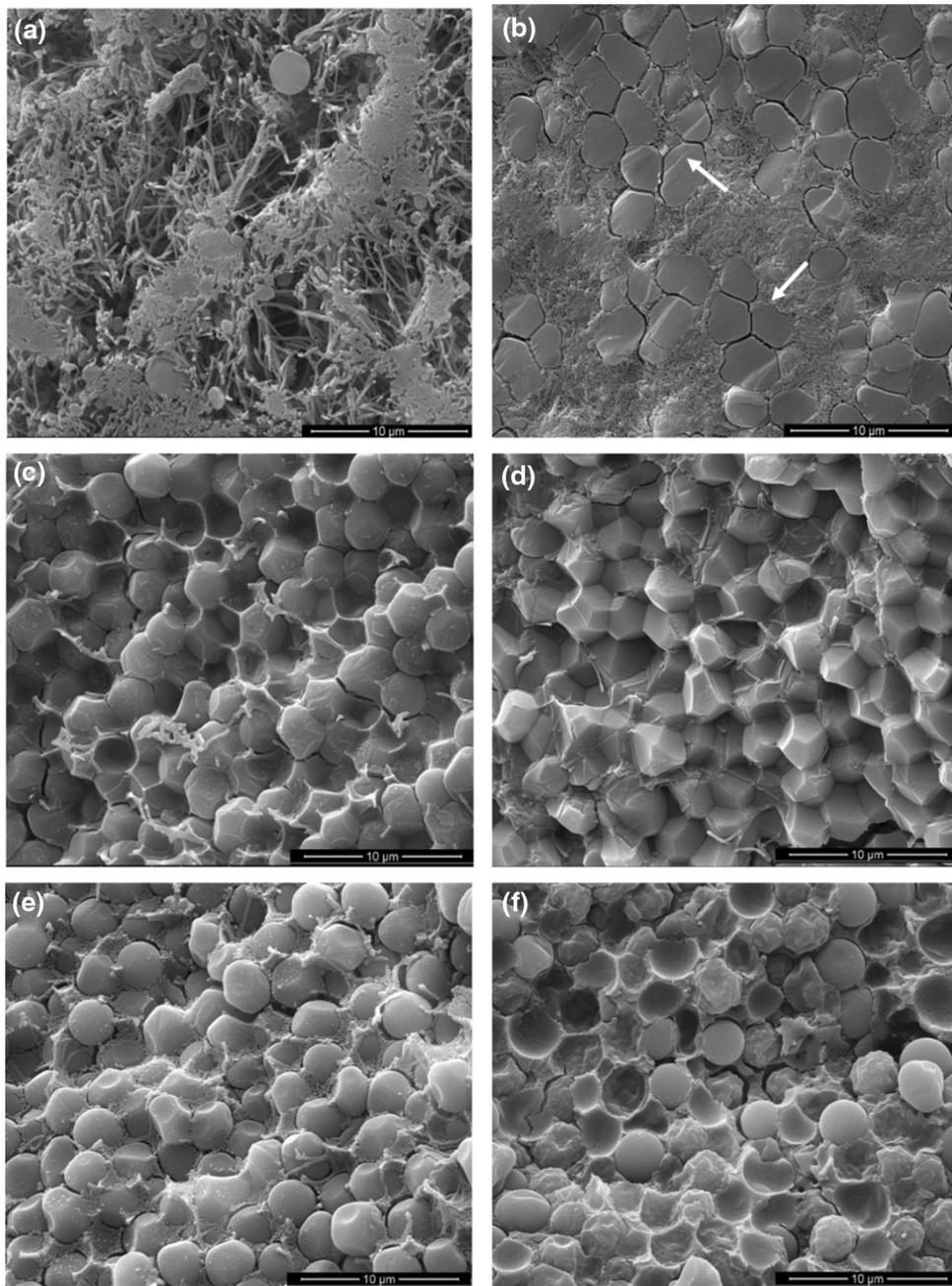


FIGURE 6. SEM images of the interior of clot analogues. (a) Interior of a 0% H clot, consisting of areas of loose thick fibrin strands and highly compacted fibrin. (b) Interior of a 5% H clot, with the white arrows indicating the red blood cells (RBCs), surrounded by a compacted fibrin network. These observations have also been reported in our previous study.¹³ (c) Interior of a 40% H clot stored in serum at 4 °C for 1 day and (d) 7 days. The closely packed polyhedrocytes are surrounded by a network of thick fibrin fibres, with evidence of a thin hyper-branched fibrin network appearing as a very thin layer on the faces of closely packed RBCs. (e) Interior of a 40% H clot stored in saline at 4 °C for 1 and (f) 7 days. The RBCs appear to have retained their natural spherical shape.

causes water to move into the RBCs, causing them to swell and burst. The serum appears to be an isotonic solution for the RBCs (i.e., has the same solute concentration as the RBCs) and therefore the RBCs maintain their polyhedron shape.

There is little information in the literature regarding optimal storage conditions for analogue clot material.

However, from the observations in this study, it is recommended that clot analogues are stored in their own serum where possible and stored at 4 °C to ensure that the mechanical properties and microstructure are preserved. It is also recommended that clots are tested as soon as possible after formation to ensure accurate results.

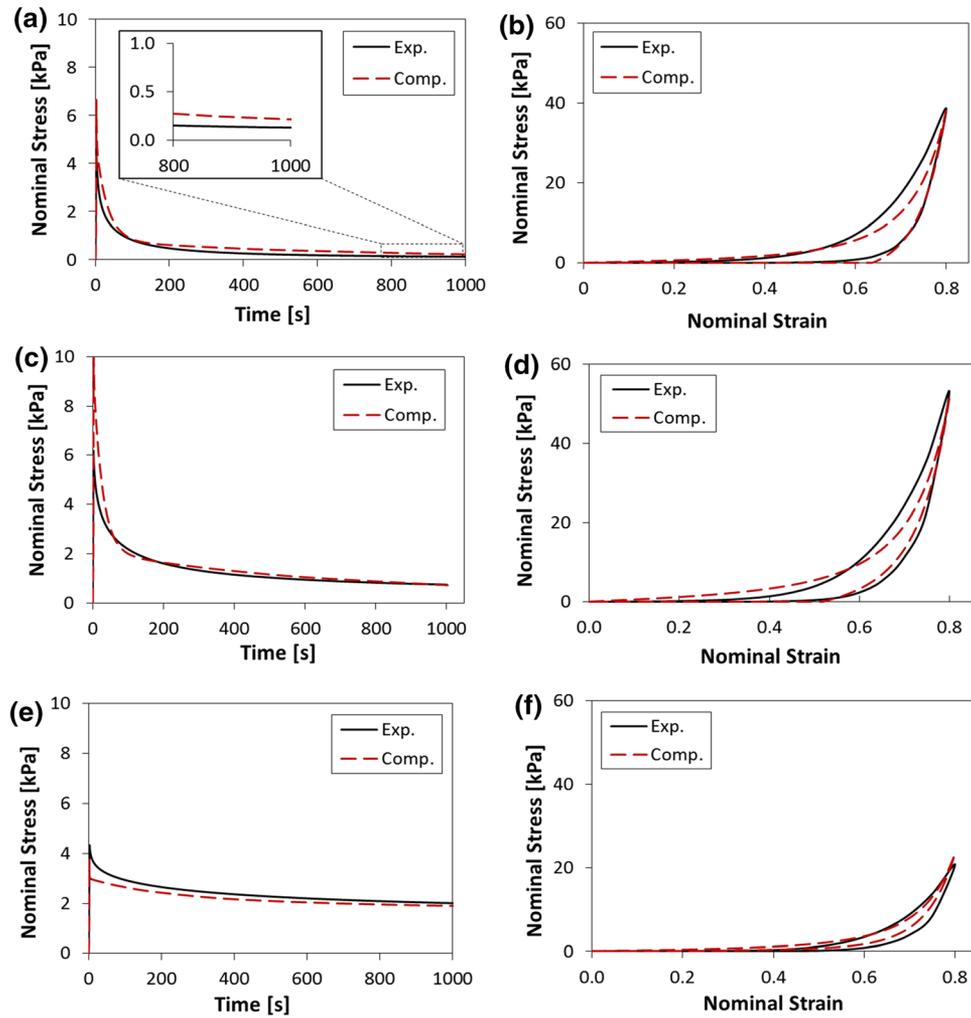


FIGURE 7. Graphs comparing the experimental results with the computational fit for the stress-relaxation experiment (left), and the first cycle of the load–unload compression experiment (right) for the 0% H clot (a and b), the 5% H clot (c and d) and the 40% H clot (e and f). Inset of (a), is a zoomed-in image of the nominal stress at a time of 1000 s.

TABLE 1. Fitted material parameters for the material law calibration to the compression and stress-relaxation experiments.

	0% H clot	5% H clot	40% H clot
C10 (Pa)	520	880	250
C20 (Pa)	40	30	28
C30 (Pa)	− 0.1	− 0.1	− 0.4476
K (Pa)	100000	100000	100000
g_1	0.4	0.2	0.25
τ_1	1	1	1
g_2	0.5	0.62	0.2
τ_2	30	25	200
g_3	0.09	0.15	0.2
τ_3	600	650	2000

Material Law Calibration

The hyper-viscoelastic model was calibrated to the experimental results reported in this study. The model was fitted to the mean experimental curves for each clot type to provide one set of parameters to model the clot behavior at each %H. The calibrated hyper-viscoelastic constitutive model was found to give a good fit for the experimental data from both loading conditions. The model adopted in this study is similar to models described previously in the literature for clot material, where similar hyperelastic models, such as the Neo–Hookean and Mooney–Rivlin models,^{20,33} and viscoelastic models, using the generalised Maxwell model,^{26,33,35} have been proposed. However, this paper reports the first model to successfully capture the loading–unloading hysteresis and stress-relaxation of the thrombus material, and provides a significant advance in the current understanding of the relationship

between clot composition and rate-dependent non-linear mechanical behavior.

LIMITATIONS

The authors acknowledge that there are some limitations to this study. In particular, the clot analogues produced are homogeneous. This is due to the preparation of the analogue material in a controlled environment and may make the analogue material slightly less clinically relevant as clots retrieved from patients have been found to be heterogeneous.³ However, for the purpose of mechanical testing, it is useful to use homogeneous samples as this allows for repeatable and comparable results. These protocols could be adapted to be formed under flow conditions to form more physiologically representative clot analogues as this has been shown to induce heterogeneity.⁶

Additionally, although all samples were formed using platelet-rich plasma, the platelet count for the samples was not measured. Platelet-rich plasma (PRP) is, by definition, a volume of autologous plasma that has a platelet concentration well above baseline. While the normal platelet counts in whole blood average 200,000/mL, future work could monitor the platelet count of each blood sample to ensure that it is consistent across each blood collection.

CONCLUSION

This study presents an experimental characterisation of analogue clot material with varying composition under two loading procedures; cyclic compression and stress-relaxation. Protocols for tensile,²² biaxial²² and confined compression²³ testing should be developed in follow-on studies. The experimental testing and constitutive law calibration presented in this study provides the first detailed characterisation of cyclic loading–unloading hysteresis and stress-relaxation for a range of clot analogue compositions, which are essential for the future development and evaluation of mechanical thrombectomy devices. This study also provides some recommendations for the storage of clot analogues before testing.

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CONFLICT OF INTEREST

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