Ex-Vivo Human Clot Analog Fabrication for Mechanical Thrombectomy Device Validation

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Abstract

Introduction: This project aims to contribute towards improved treatment of ischemic stroke. Proper vessel recanalization is essential for ischemic stroke treatment and currently is often done using mechanical thrombectomy devices. To improve recanalization rates and achieve better patient outcomes with new device designs, proper device validation is required. Validation can be more easily, and cost effectively, completed using ex-vivo clot analogs. As such in this project novel ex-vivo clot analogs are fabricated. Various coagulation conditions are explored to form clot analogs that mimic real human thrombi properties so the ex-vivo analogs can be used for thrombectomy device validation.

Methods: During project work various blood products and biochemical ingredients were combined in different ratios for different analog fabrication trials. Blood products used included human red blood cells (RBCs) and platelet rich plasma (PRP), and porcine (pig) whole blood. Solutions of $CaCl_2$ were used as clotting initiators and type I collagen and thrombin solutions were used to enhance clot coagulation. Clot analogs were mixed and then allowed to coagulate for 1-2 hours. Coagulation occurred at room temperature or human body temperature of 37 degrees C, and under static or orbital shaking conditions. Fabricated analogs were characterized via dynamic mechanical analysis (DMA) testing and histology imaging.

Results: Clot analogs were made in multiple trials differing by ingredients used and coagulation conditions. All trials resulted in solid clot analogs after addition of $CaCl_2$ solution in a 1:10 ratio to blood product volume. Solid clots fully formed after coagulation for 1-2 hours, both at room and human body temperatures, and under static and orbital shaking conditions. For select analog trials DMA testing revealed stiffness properties, and histology imaging revealed microscopic structure properties. Human blood-based analogs were found to be significantly stiffer and coagulated into solid clots in only 10 seconds with thrombin added but were found to lack real clot-like networking in their structure. Porcine whole blood-based analogs coagulated into solid clots faster with greater concentrations of $CaCl_2$ added in the same 1:10 ratio to blood product volume and have yet to be characterized by DMA testing or histology.

Conclusions: Solid clot analogs based on human and porcine blood products were successfully fabricated. Addition of thrombin improved analog stiffness and higher concentrations of $CaCl_2$ led to faster coagulation of solid clots. Current analog results showed poor stiffness and microscopic structure. More work is needed to improve clot analog properties to better match those of real human thrombi and be suitable for thrombectomy device validation. Further trials and testing are also needed to determine the exact effects of coagulation temperature and motion conditions on clot analog properties.

1. Introduction

Stroke is a serious medical problem both in the United States (US) and throughout the world. In the US alone there are currently close to 800,000 stroke patients every year, and stroke acts as a leading cause for permanent adult disability and is the fifth leading cause of death [1]. As the population of senior citizens rises in the US, the number of stroke patients and associated deaths are expected to rise significantly in the next several years. At the same time healthcare costs and livelihood costs due to stroke events are currently enormous at over $30 billion, and likewise are also expected to rise significantly in the next several years [1]. Generally, a stroke is a medical condition where normal blood flow to the brain is interrupted and causes cell death in the affected area. This can lead to brain damage and other neurological complications if the stroke is left untreated or poorly addressed. Stroke events are categorized as either hemorrhagic stroke or ischemic stroke. Hemorrhagic strokes involve blood vessels around the brain rupturing, causing bleeding into the brain and loss of normal blood flow. Ischemic strokes involve a blockage within a blood vessel around the brain causing a loss of normal blood flow. The vast majority of strokes (near 80%) in patients are ischemic [1]. Ischemic strokes are typically caused by a blood clot or thrombus that formed elsewhere in the body travelling through larger blood vessels until becoming stuck in blood vessels surrounding the brain as vessel size decreases. This partially or fully occludes the blood vessel and halts normal blood flow, leading to a stroke.

Treatment of strokes is a highly complex problem. Treatment of ischemic strokes in particular is generally a time sensitive affair and dependent on available hospital resources [2]. For ischemic stroke treatment, proper vessel recanalization is essential to restore normal blood flow and prevent further cell death and brain damage. In ideal conditions, an ischemic stroke patient can be brought to a hospital and undergo primary treatment. This often involves endovascular treatment using a mechanical thrombectomy procedure [2]. Currently, mechanical thrombectomy procedures are the gold standard for treatment of ischemic stroke. Mechanical thrombectomy procedures rely on specialized mechanical thrombectomy devices including components such as stents, vacuums, and drilling heads. Current mechanical thrombectomy devices when used in ischemic stroke treatment procedures have a 60% to over 80% blood vessel recanalization rate depending on specific device types used [3]. Generally, the rates of successful recanalization have increased over the past serval years. In order to improve ischemic stroke patient treatment and yield better patient outcomes, further improved rates of blood vessel recanalization during mechanical thrombectomy procedures are desired. Additionally, current mechanical thrombectomy procedures and devices are highly expensive and require extensive supporting hospital infrastructure [3]. To improve recanalization rates, and reduce cost and required supporting infrastructure, further innovation to mechanical thrombectomy device designs is needed. This innovation can include generating new device designs that are more effective at thrombus removal, less expensive to operate, and more portable to deploy in broader hospital settings with less supporting infrastructure.

To support innovation in mechanical thrombectomy device designs, proper validation methods are required. Such validation is key to ensure mechanical thrombectomy devices and their newest designs are effective for real thrombus removal and stroke patient treatment. In particular, this device validation involves running tests to ensure the design being validated can effectively remove obstructing thrombi in a blood vessel via cutting, drilling, or suction. This validation

testing often requires real human thrombi. Access to real human thrombi is highly limited, as obtaining them can be expensive or difficult due to surgical procedures often required to access them [4]. Obtaining real thrombi from porcine (pig), bovine (cow), or other animal sources for device validation testing is likewise difficult for the same reasons. In recent years, the use of fabricated thrombi or blood clot analogs that mimic real thrombi properties for clot modeling and other testing procedures that rely on real thrombi behavior has become increasingly common. These clot analogs are generally created in an ex-vivo or lab bench environment. This allows for a much less expensive and faster production of clots that can be suitable for thrombectomy device validation. Clot analogs are often made using mixed human blood products or animal blood products [5]. These can all be relatively easily obtained from donated or commercial sources compared to real thrombi. Clot analogs have already been made in my lab, the University of Michigan Biomedical Manufacturing and Design Lab (BMDL), using simple mixtures of human red blood cells (RBCs) and plasma components [4]. These simple clot analogs can be seen as useful for simultaneous thrombectomy and stroke related research in the BMDL, specifically for clot cutting tests [6]. These same types of simple clot analogs have also been made and used elsewhere for similar thrombectomy testing purposes [7].

Current published experiments in ex-vivo clot analog fabrication show a wide variety of ingredients and techniques used, and at the same time some key similarities. Basic clot analogs have again been made simply by mixing raw human blood products [6], [7]. Raw animal blood products have also again been used in the same manner [5]. However, these experiments resulted in analogs that better modeled soft thrombi that represent easier to remove obstructions with poor coagulation strength. In real stroke scenarios, obstructing thrombi vary significantly in their properties, and often have far stiffer meachnical behavior with stronger fibrin-based or collagen-based internal networking [8]. Real thrombi can also evolve over time as they form and travel through the vascular system before becoming stuck and obstructing blood flow, and even evolve further after becoming stuck [8]. This variation in real thrombi composition and properties results in differences in behavior as they are removed during mechanical thrombectomy procedures [9]. As such there is also a need to create clot analogs of varying and predictable properties, and particularly with high internal networking and stiffness, for improved thrombectomy device validation. To produce clot analogs with better and real thrombi-like properties, better coagulation is required and can be achieved with additional ingredients. Solutions of calcium chloride ($CaCl_2$) and thrombin have been shown to improve clot analog coagulation [10]. Addition of collagen solution has also been shown to help activate platelets in utilized blood products and enhance clotting and coagulation [11]. This platelet activation by collagen solution is particularly useful when platelet rich plasma (PRP) is used [12]. Lastly, commercial whole blood from animal sources represents an even more inexpensive option than using separated human blood products for clot analog production. However, commercial whole blood often contains added anticoagulants such as sodium citrate [13]. Those anticoagulants typically must be deactivated with additional $CaCl_2$ in order to permit whole blood coagulation and use in clot analog experiments [14].

Given the need for validation of mechanical thrombectomy devices, the ability of ex-vivo clot analogs to fulfil this need, and current experimental knowledge on clot analog production, my work in this capstone project involves fabrication of novel ex-vivo clot analogs. Various clot analog ingredient combinations are explored, as well as various clot analog coagulation

conditions. Importantly, current clot analog experiments do not fully consider the specific effects of certain coagulation conditions on resultant clot analog properties. My work in this project specifically involves producing ex-vivo clot analogs using various commercial or donated blood products with added biochemical ingredients to induce coagulation. Based on previously reviewed clot analog experimental work and knowledge, $CaCl_2$, collagen, and thrombin are key coagulation agents. The effects of coagulation conditions of time, motion, and temperature can also be explored by varying these conditions during clot analog fabrication. The properties of my fabricated clot analogs can then importantly be characterized. This includes both characterization of mechanical properties and stiffness via dynamic mechanical analysis (DMA) testing, and characterization of microscopic structure properties via histology imaging. This then allows resultant properties for my clot analogs to be compared with those of real human thrombi. This will show whether my clot analogs are suitable for the end goal of thrombectomy device validation within my BMDL group. This will also show the effects of certain ingredients and coagulation conditions on analog properties. In the future this can finally allow for controlled reproduction of clot analogs to mimic specific human thrombus conditions as needed for thrombectomy device design testing and other clot-related experiments.

2. Methods

During project work multiple separate trials of clot analog fabrication were completed. This section specifically describes the materials and methods used in each fabrication trial. Each of the following subsections corresponds to an individual fabrication trial. For each trial the amounts of materials used are listed including blood product ingredients and added biochemical or coagulation ingredients. Material sources are also given. Meanwhile, the methods used to actually mix together clot analogs in each trial and the utilized coagulation conditions are described. Lastly, any additional methods used to yield mechanical or histology property results for certain trials are described. In general, each trial followed a similar workflow method of mixing together clot ingredients, allowing clots to coagulate under a prescribed condition, then characterizing clot properties in select trials.

2.1. Fabrication Trial #1 (7/21/2022)

For this initial trial a single clot analog sample was made. Blood products used included human type O+ RBCs donated from the Michigan Medicine blood bank, and human PRP from an online supplier. Table 1 below lists all clot ingredients used. All clot ingredients were mixed in a 15 mL conical tube. The tube was shaken by hand and the clot was left static and at room temperature to coagulate. DMA testing was completed for 2 cut sections of the clot sample through a 0-55% strain range with 1%/second strain rate.

Table 1. Clot sample ingredients for fabrication trial #1.

Clot Sample 1
10.00 mL – Human PRP
0.05 mL – Human type O+ RBCs
1.00 mL – 2.27 w/v% aqueous $CaCl_2$ solution
0.65 mL – solution of 0.50 mL of 3 mg/mL type 1 bovine collagen solution neutralized with 0.15 mL of 0.1 M aqueous NaOH

2.2. Fabrication Trial #2 (7/29/2022)

For this trial a single clot analog sample was made. Blood products used were human type O+ RBCs donated from the Michigan Medicine blood bank, and human PRP from an online supplier. Table 2 below lists all clot ingredients used. All clot ingredients were mixed in a 15 mL conical tube. The tube was shaken by hand and the clot was left on an active oscillating shaker table at room temperature to coagulate. DMA testing was completed for 2 cut sections of the clot sample through a 0-55% strain range with 1%/second strain rate.

Table 2. Clot sample ingredients for fabrication trial #2.

Clot Sample 1
5.00 mL – Human PRP
0.025 mL – Human type O+ RBCs
0.50 mL – 2.27 w/v% aqueous $CaCl_2$ solution
0.65 mL – solution of 0.50 mL of 3 mg/mL type 1 bovine collagen solution neutralized with 0.15 mL of 0.1 M aqueous NaOH

2.3. Fabrication Trial #3 (8/3/2022)

For this trial a single clot analog sample, sample 1, was made. The exact same ingredients and amounts were used as in fabrication trial #2 and mixed in a 15 mL conical tube. Table 2 above lists all the same clot ingredients used. The tube was shaken by hand and the clot was left static and incubating in a water bath held at human body temperature (37 degrees C) to coagulate. DMA testing was completed for 3 cut sections of the clot sample through a 0-55% strain range with 1%/second strain rate. Images of the DMA machine used for DMA testing and the testing setup are shown in figure 1 below.

Figure 1. DMA testing setup and machine used to characterize the mechanical properties of clot analog samples.

2.4. Fabrication Trial #4 (8/8/2022)

For this trial a single clot analog sample was made. Blood products used included human type O+ RBCs donated from the Michigan Medicine blood bank, and human PRP from an online

supplier. Table 3 below lists all clot ingredients used. All clot ingredients were mixed in a 15 mL conical tube. The tube was shaken by hand and the clot was left inside an air incubator held at human body temperature (37 degrees C) on top of an active orbital shaker tray set running at 100 rpm. An image of the dual incubator and shaker tray setup used while the clot sample coagulated is given below in figure 2. DMA testing was completed for 2 cut sections of the clot sample through a 0-55% or 0-35% strain range with 1%/second strain rate.

Table 3. Clot sample ingredients for fabrication trial #4.

Clot Sample 1
5.00 mL – Human PRP
0.025 mL – Human type O+ RBCs
0.50 mL – 2.27 w/v% aqueous $CaCl_2$ solution
0.65 mL – solution of 0.50 mL of 3 mg/mL type 1 bovine collagen solution neutralized with 0.15 mL of 0.1 M aqueous NaOH
0.50 mL – solution of 1x PBS with added 3.5 mg of raw thrombin powder (from bovine plasma) dissolved inside

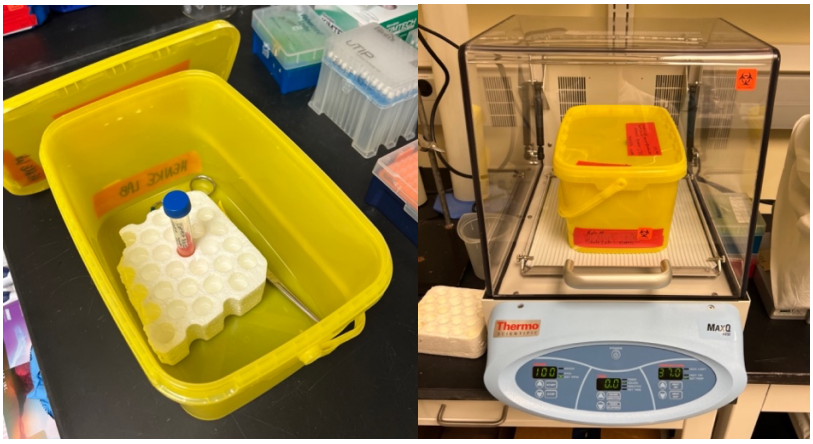

Figure 2. Dual air incubator and orbital shaker tray setup used to heat and vibrate clot analog samples during coagulation.

2.5. Fabrication Trial #5 (8/10/2022)

For this trial 2 separate clot analog samples were made. Blood products used included human type O+ RBCs donated from the Michigan Medicine blood bank, and human PRP from an online supplier. Table 4 below lists all clot ingredients used for each sample, with their key difference being a presence or absence of added thrombin. All clot ingredients for the samples were mixed in 15 mL conical tubes. The tubes were shaken by hand and the clots were left inside an air incubator held at human body temperature (37 degrees C) on top of an active orbital shaker tray set running at 100 rpm (same setup from figure 2 above). DMA testing was completed for 2 cut sections of clot sample 1 over a 0-35% strain range, and 1 cut section of clot sample 2 over a 0-60% strain range, both with a 1%/second strain rate.

Table 4. Clot sample ingredients for fabrication trial #5.

Clot Sample 1	**Clot Sample 2**
5.00 mL – Human PRP	5.00 mL – Human PRP
0.025 mL – Human type O+ RBCs	0.025 mL – Human type O+ RBCs
0.50 mL – 2.27 w/v% aqueous $CaCl_2$ solution	0.50 mL – 2.27 w/v% aqueous $CaCl_2$ solution

0.65 mL – solution of 0.50 mL of 3 mg/mL type 1 bovine collagen solution neutralized with 0.15 mL of 0.1 M aqueous NaOH	0.65 mL – solution of 0.50 mL of 3 mg/mL type 1 bovine collagen solution neutralized with 0.15 mL of 0.1 M aqueous NaOH
0.50 mL – solution of 1x PBS with added 3.5 mg of raw thrombin powder (from bovine plasma) dissolved inside	-

2.6. Fabrication Trial #6 (10/21/2022)

For this trial a single clot analog sample was made. Blood products used were human type O+ RBCs donated from the Michigan Medicine blood bank, and human PRP from an online supplier. Table 5 below lists all ingredients used. All clot ingredients were mixed in a 15 mL conical tube. The tube was shaken by hand and the clot was left inside an air incubator held at human body temperature (37 degrees C) on top of an active orbital shaker tray set running at 50 rpm. DMA testing was not completed. Histology imaging for the clot sample was completed by first fixing the sample in 10% formalin solution for 24 hours. After fixing the sample was placed in 70% ethanol and sent to the University of Michigan School of Dentistry histology core lab for cutting into cross-section slides with trichrome stain. The slides were then visualized under a light microscope with image capture software at 100x, 400x, and 1000x zoom.

Table 5. Clot sample ingredients for fabrication trial #6.

Clot Sample 1
1.00 mL – Human PRP
0.005 mL – Human type O+ RBCs
0.10 mL – 2.27 w/v% aqueous $CaCl_2$ solution
0.13 mL – solution of 0.10 mL of 3 mg/mL type 1 bovine collagen solution neutralized with 0.03 mL of 0.1 M aqueous NaOH
0.10 mL – solution of 1x PBS with added 1.5 mg of raw thrombin powder (from bovine plasma) dissolved inside

2.7. Fabrication Trial #7 (3/29/2023)

For this trial several clot samples were made. The only blood product used was now 100% porcine whole blood with added sodium citrate anticoagulant obtained from an online supplier. Clot ingredients were now mixed in cylindrical cavities inside of stereolithography (SLA) 3D printed clot molds. Clot ingredients were injected inside of the molds using a pipette. Examples of the newly used clot molds are shown in figure 3 below. For this trial different concentrations of $CaCl_2$ were used for coagulation to overcome the anticoagulant, with 2 clot samples each made for the ingredient conditions listed in table 6 below. After ingredients were injected into clot molds all samples were left static and at room temperature to coagulate. DMA testing was completed for 1 cut section of each clot sample through a 0-80% strain range with 1%/second strain rate. Histology imaging for selected clot samples was prepared by first fixing samples in 10% formalin solution for 24 hours. After fixing the samples were placed in 70% ethanol and sent to the University of Michigan School of Dentistry histology core lab for cutting into cross-section slides with trichrome stain. The samples are currently still being processed by the histology lab.

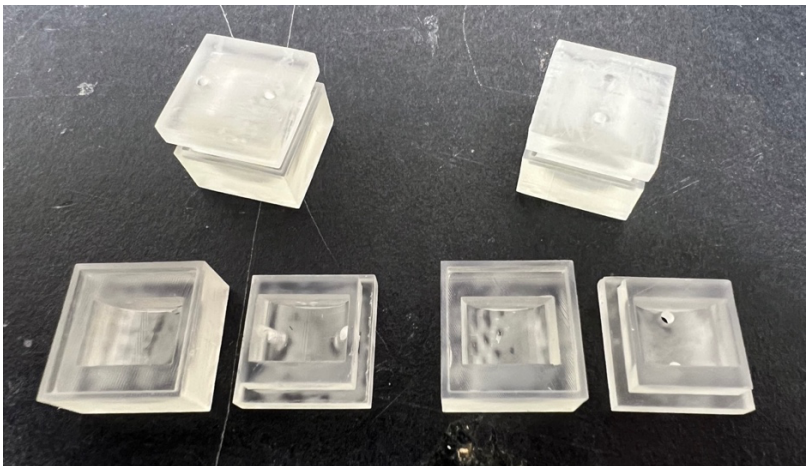

Figure 3. SLA 3D printed cylindrical clot molds with ports for injecting ingredients.

Table 6. Clot ingredient conditions for fabrication trial #7.

Clot Type 1	Clot Type 2	Clot Type 3	Clot Type 4
1.50 mL – 100% porcine whole blood with sodium citrate anticoagulant	1.50 mL – 100% porcine whole blood with sodium citrate anticoagulant	1.50 mL – 100% porcine whole blood with sodium citrate anticoagulant	1.50 mL – 100% porcine whole blood with sodium citrate anticoagulant
0.15 mL – 2.27 w/v% $CaCl_2$ solution in DI water or 1x PBS	0.15 mL – 3.00 w/v% $CaCl_2$ solution in DI water or 1x PBS	0.15 mL – 4.00 w/v% $CaCl_2$ solution in DI water or 1x PBS	0.15 mL – 5.00 w/v% $CaCl_2$ solution in DI water or 1x PBS

3. Results

In this section, results for all completed clot analog trials described in the methods section are given. Each of the following subsections corresponds to a single clot analog trial described previously and gives results for that trial. For each trial descriptive qualitative results for clot analog samples made are given. Clot analog images, mechanical results, and histology results, if made for the particular trial, are also given.

3.1. Fabrication Trial #1 (7/21/2022)

After 20-30 minutes of coagulation the clot analog remained liquid with low viscosity. After 1 full day of coagulation, a solid clot formed with a weakly solid and firm gel-like consistency. An image of the resulting clot sample 1 is shown below in figure 4 below. DMA testing results for the clot are shown below in figure 5.

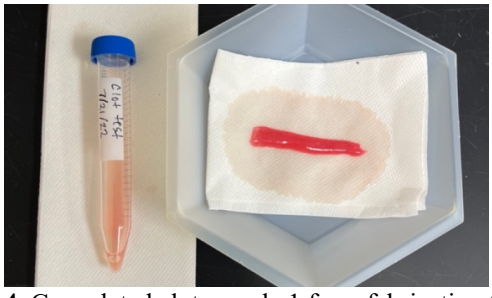

Figure 4. Coagulated clot sample 1 from fabrication trial #1.

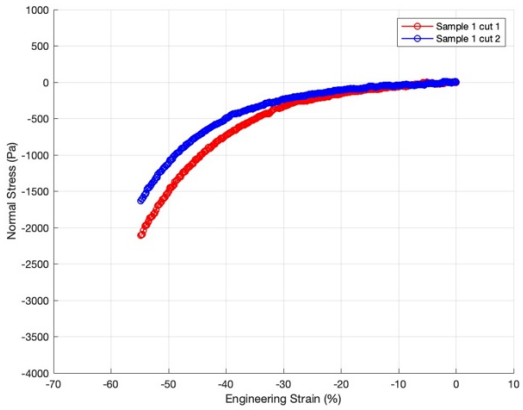

Figure 5. DMA results showing compressive stress versus strain for clot sample 1 from fabrication trial #1.

3.2. Fabrication Trial #2 (7/29/2022)

After 58 minutes of coagulation the clot analog remained overall liquid with small clumps of solid coagulated clots present. After 10 hours of sitting on the active rotator table, a full solid clot formed with a stretchy solid and firm gel-like consistency. An image of the resulting clot sample 1 is shown below in figure 6 below. DMA testing results are shown below in figure 7.

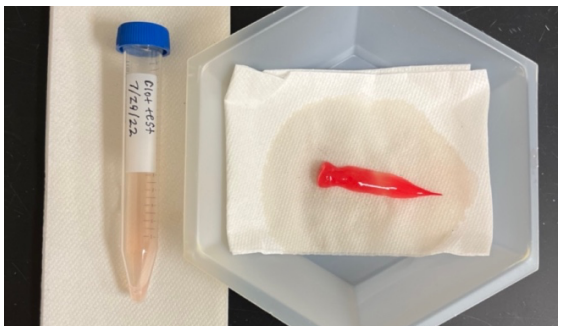

Figure 6. Coagulated clot sample 1 from fabrication trial #2.

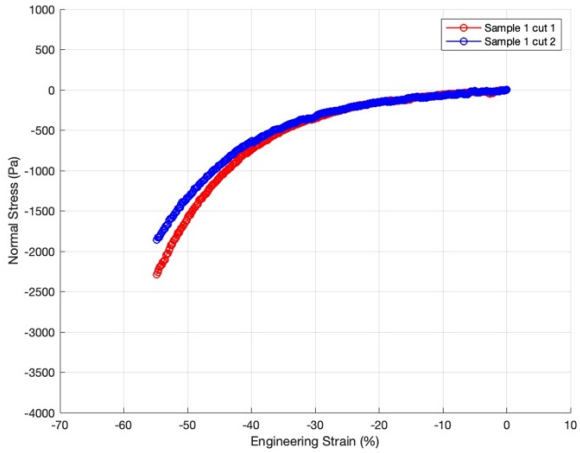

Figure 7. DMA results showing compressive stress versus strain for clot sample 1 from fabrication trial #2.

3.3. Fabrication Trial #3 (8/3/2022)

After 2.5 hours of coagulating a fully solid clot was found to have formed. The clot was found to have a stretchy solid and firm gel-like consistency. An image of cut sections suspended in 1x PBS from clot sample 1 prior to DMA testing is given in figure 8 below. DMA testing results are shown below in figure 9.

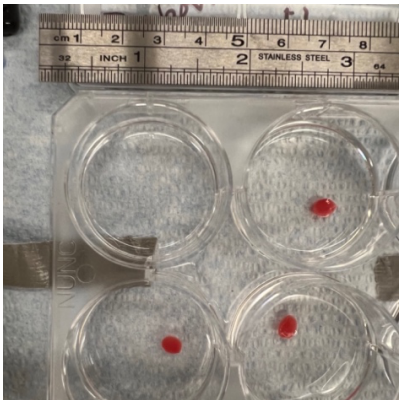

Figure 8. Cut sections of clot sample 1 from fabrication trial #3 ready for DMA testing.

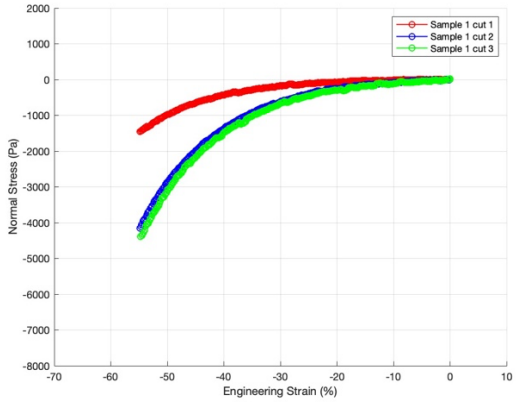

Figure 9. DMA results showing compressive stress versus strain for clot sample 1 from fabrication trial #3.

3.4. Fabrication Trial #4 (8/8/2022)

Immediately after all clot ingredients were mixed including thrombin-containing solution, a solid clot was observed to form within 10-20 seconds. After 5.5 hours of coagulation, the clot sample was removed and found to have a clumpy consistency and weakly stretchy feel, with tougher clumps interspersed throughout weaker sections prone to easy tearing or separation. An image of the resulting clot sample is shown in figure 10 below. DMA testing results are shown below in figure 11.

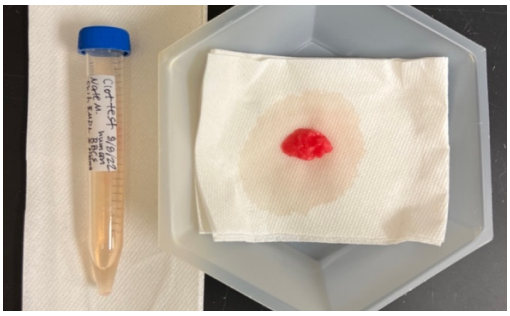

Figure 10. Coagulated clot sample 1 from fabrication trial #4.

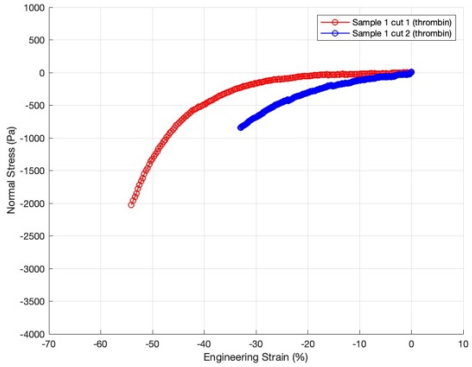

Figure 11. DMA results showing compressive stress versus strain for clot sample 1 from fabrication trial #4.

3.5. Fabrication Trial #5(8/10/2022)

Immediately after all clot ingredients were mixed, clot sample 1 with thrombin coagulated into a solid after 10-20 seconds, meanwhile clot sample 2 with no thrombin remained liquid initially. After 30 minutes of coagulation both samples were found to have coagulated into solid clots with tough gel-like consistency. An image of cut sections from both clot samples 1 and 2 suspended in 1x PBS prior to DMA testing is given in figure 12 below. DMA testing results for both samples are shown below in figure 13.

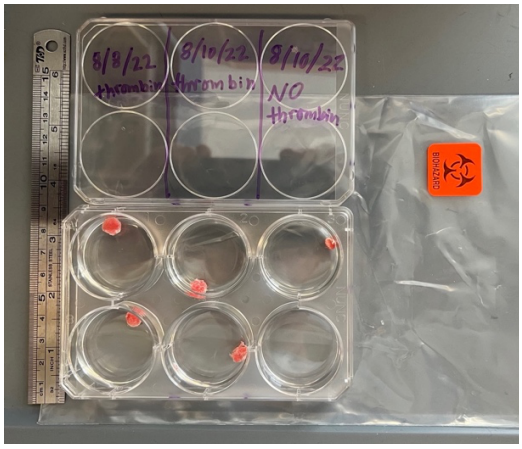

Figure 12. Cut sections of clot samples 1 and 2 from fabrication trial #5 ready for DMA testing, cut sections from fabrication trial #4 clot sample 1 are also seen as all were DMA tested on the same day.

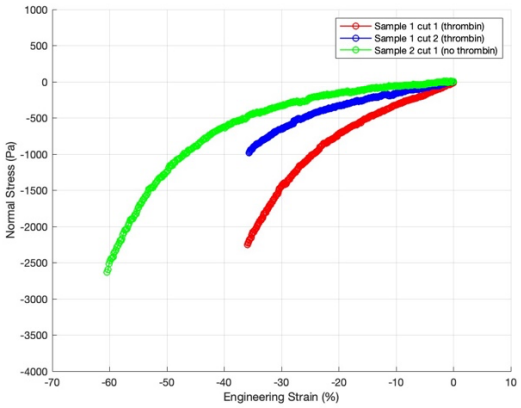

Figure 13. DMA results showing compressive stress versus strain for clot samples 1 and 2 from fabrication trial #5.

3.6. Fabrication Trial #6 (10/21/2022)

Immediately after all clot ingredients were mixed, a solid clot was observed to form within 10-20 seconds. After 1.5 hours of coagulation, the clot sample was found to have a soft and weakly-solid consistency, with the entire clot prone to easy tearing or separation. Results for the histology imaging of the clot sample with trichrome stain are shown below in figure 14 at multiple zoom levels.

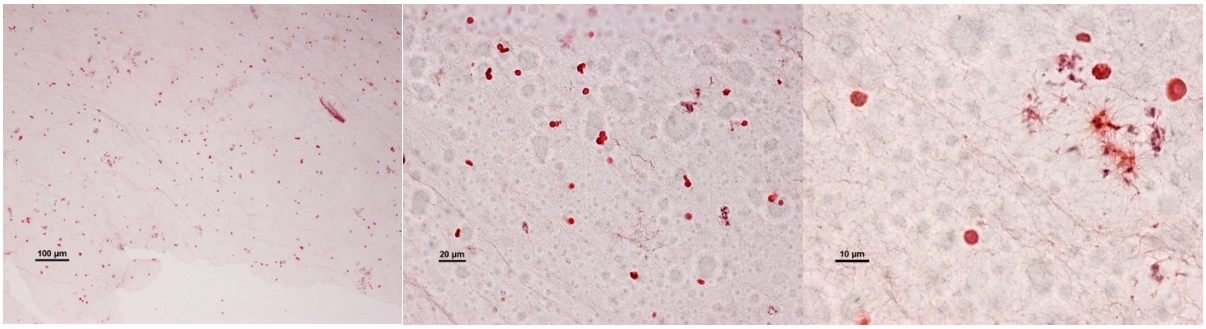

Figure 14. Histology imaging results for clot sample 1 from fabrication trial #6, zoom levels from left to right are 100x, 400x, and 1000x, all with trichrome stain.

3.7. Fabrication Trial #7 (3/29/2023)

After all clot ingredients were injected into their cylindrical molds and mixed, solid clots were found to have formed after 1-2 hours of coagulation. Clot samples were removed from their molds and all found to have weakly solid and gel-like consistencies. Clot samples were tougher and less runny as the concentration of $CaCl_2$ used increased. Images of resulting mixed clots in their molds and a removed clot ready for DMA testing are given in figure 15 below. DMA testing results currently have yet to be processed for clot samples from this trial. Histology results also have yet to be obtained as fixed clot samples are still being processed.

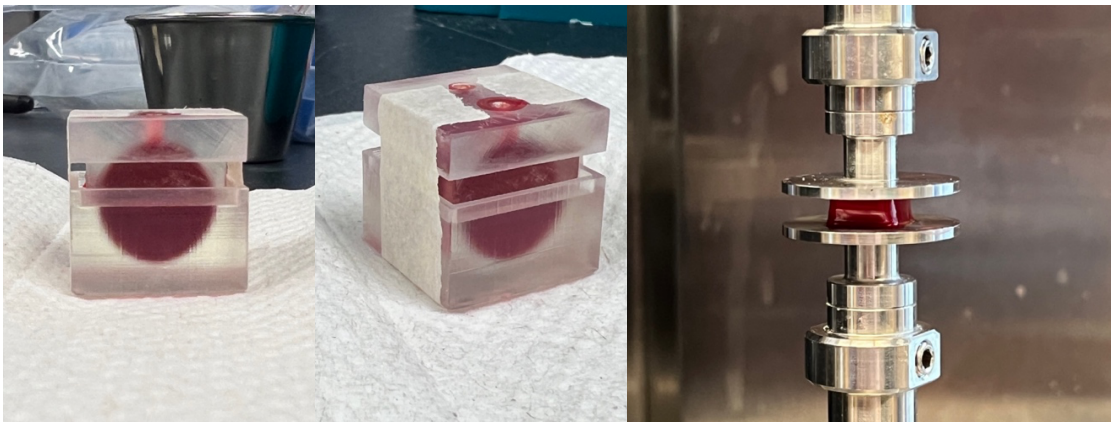

Figure 15. On the left, a pair of mixed clot samples from fabrication trial #7, and on the right, a clot sample from fabrication trial #7 removed from its mold and ready for DMA testing.

4. Discussion

As seen in the results for clot analog fabrication trials completed thus far, clot analogs for human thrombi were successfully made during this project. Multiple analog trials were completed with different ingredients and coagulation conditions to progressively yield better analogs. Analogs were made initially and primarily with human RBCs and PRP. Later trials used 100% porcine whole blood to explore a new blood source and further reduce analog costs. This was done since from commercial sources porcine whole blood was found to be available at far less expensive prices than human RBCs or PRP. In all analog fabrication trials $CaCl_2$ solutions that were added successfully acted to raise calcium ion concentration and initialize clotting. In certain trials some analog samples had thrombin and type I collagen added to improve clotting. From coagulation observations and DMA data this was found to be the case. Clots with thrombin, collagen, or both added tended to coagulate faster and stronger, and be stiffer, than clots without them. Most trials occurred at room temperature and some at 37 degrees C or human body temperature. Most trials also had static coagulation while some trials underwent orbital shaking while coagulating. Differences in observed clot coagulation and DMA results were found to generally be minimal across cases where temperature and motion conditions differed. Across all trials, full clot coagulation took 1-2 hours on average while analogs with thrombin added coagulated after just 10-20 seconds. This shows how thrombin addition rapidly leads to effective clot analog formation. This behavior can be taken advantage of to produce clot analogs more rapidly with fresher ingredients. Importantly, created analogs were able to be successfully quantified by their mechanical properties and histology. Mechanical quantification via DMA testing yielded stress versus strain data for samples from analog trials that thus showed clot analog stiffness. Lastly, the used histology techniques yielded clot analog section slides that allowed analog structure to be viewed under a microscope at the cellular level. This allows for cell-level clot analog structure to be compared with real human thrombi to ensure structural similarities and thus show whether the ex-vivo fabricated clots are suitable as analogs for real human thrombi.

5. Conclusions

Key findings and conclusions from current project work and completed analog trials include that clot analogs could be successfully made based on human and porcine blood products. However,

mechanical and histology results show that there is still significant room for improvement regarding analog properties to match real thrombi properties and be suitable for thrombectomy device validation. This is particularly the case for analog stiffness. Impacts of time, motion, and temperature on analog fabrication were explored but more trials are needed to determine their exact effects on analog stiffness and efficacy. Adding thrombin was found to yield stiffer clot analogs based on human blood products. This can be seen from stress versus strain data for human blood-based clot analogs with and without thrombin added. With thrombin present, higher stress occurs at lower strain indicating higher analog stiffness. Histology results showed that human blood-based clot analogs failed to form real thrombi-like fibrin or collagen rich networking from current fabrication techniques. This is evident in the given histology image results for selected analog samples. Under increasing zoom levels real thrombi-like networking is absent, it would show up as a rich purple or blue color under the utilized trichrome stain as is the case for real thrombi. Meanwhile, more recently completed analog trials based on porcine whole blood saw faster coagulation or clotting with higher concentrations of $CaCl_2$ solution added in the same 1:10 ratio to blood volume. Mechanical and histology results for porcine blood-based analogs have yet to be fully collected and analyzed, doing so is currently ongoing work. Given the success and shortcomings of clot analog work thus far, future work primarily focuses on yielding stiffer analogs that better match real thrombi. Future work also includes further exploring the effects of temperature and motion on analogs, and newly the effect of pressure. All of those effects may better mimic real blood vessel conditions to allow for better clot formation and coagulation. More regular trials are also planned with DMA and histology analysis to yield better baseline analog results. Lastly, new ingredients can be explored to determine their effects on analog properties and improve stiffness. This overall project remains ongoing as of now. Ultimately, the end goal of future work is to eventually use clot analogs in validation trials for new thrombectomy device designs.

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