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in-vitro Hydrogel Blood Clots

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ENTITLED

in-vitro HYDROGEL BLOOD CLOTS

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in-vitro HYDROGEL BLOOD CLOTS

By

Ethan Evans, Khoi-Austin Ngo

SENIOR DESIGN PROJECT REPORT

Submitted to
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Abstract

Creating a sustainable, ethical, and affordable substitute for animal blood in balloon catheter testing for Deep Vein Thrombosis (DVT) is important to take into consideration. This research utilizes agarose and dextran to develop hydrogel-based artificial blood clots. These hydrogels replicate the mechanical characteristics of human thrombi across acute, subacute, and chronic stages through their elastic and viscous moduli (G' and G") and phase angles. This project addresses extant testing protocols, including temperature regulation, agarose processing, and well plate size, and suggests efficient approaches to encounter these problems.

The hydrogels were characterized by means of a thorough literature review and rigorous mechanical testing with rheometers, allowing their properties to be aligned with those of human clots at different stages of hardening. The outcomes showed that the mechanical characteristics of the hydrogel may be precisely adjusted to mimic the viscoelastic characteristics of actual thrombi. Future testing will have a solid result thanks to the vital insights about the gels' behavior under varying situations that the data gathered from frequency and amplitude sweeps offered.

By reducing the dependency on animal blood, this technique addresses ethical issues and enhances scalability and cost-efficiency. In order to test medical devices, synthetic hydrogels provide a stable, reusable, and biodegradable resource that encourages sustainability in biomedical research. Subsequent research endeavors will center on optimizing phase angle measurements and classifying hydrogels into distinct clotting phases. This will be done by hands-on catheter manipulation assessments to ascertain the catheters' effectiveness and dependability in replicating authentic medical situations. In addition to advancing the creation of better medical procedures, this research suggests moral and environmentally friendly research methods in the industry.

Acknowledgments

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List of Abbreviations

Abbreviation	Meaning
σ	Compressive Stress
ε	Compressive Strain
A#	# of Percent Agarose
D#	# of Percent Dextran
DMA	Dynamic Mechanical Analysis
E'/G'	Elastic Modulus
E"/G"	Viscous Modulus
G*	Complex Shear Modulus

CHAPTER 1

Introduction

1.1 Background Information

The human body is composed of veins that deliver oxygen-poor blood back to the heart in order to become oxygenated[1]. Almost every organ in the human body requires this blood in order to function properly, so blood is constantly cycled and filtered over 300 times per day[2]. There are two types of veins: superficial and deep veins. Superficial veins are located near the surface of the skin. These veins are responsible for carrying blood from tissue closer to the skin back to the deep veins[3]. Deep veins are located deep within this tissue and forcibly push blood back to the heart. These veins have a wider diameter than arteries due to their thinner walls[4].

1.2 Deep Vein Thrombosis

Deep Vein Thrombosis (DVT) occurs when a thrombus, more commonly known as a blood clot, forms on these walls. A thrombus is a collection of fibrin and platelets that form in response to an injury[5]. Fibrin, the protein responsible for the adherence of blood, is released into the blood once an injury occurs to initiate the clotting process[6]. Platelets begin to stick to the injury site due to the release of this protein and related enzymes. These platelets also stick to red blood cells, helping build the thrombus once it has initially formed[7].

1.3 Mechanical Properties of Thrombus

The composition of these molecules affects the mechanical properties of the thrombus, with there being an observed increase in stiffness over time[8]. This corresponds to an increase in elastic and viscous moduli due to the thrombus being a viscoelastic material, which is a material that exhibits both elasticity and viscosity characteristics when going under deformation[9]. The elastic modulus measures the resistance of a material to elasticity, with a higher value representing a greater resistance to deformation[10]. Similarly, the viscous modulus measures the resistance of a material to viscosity, with a higher value representing a greater resistance to flow[11]. The moduli can be measured by compression, which corresponds to Young's modulus (E), and elastic and viscous moduli being represented by E' and E" respectively[12]. They can also

be measured by shear, which corresponds to shear modulus (G) and the moduli being depicted by G' and G".

The hardening stages for thrombus can be defined as acute, subacute, and chronic[13,14]. Acute clots are clots within the first two days of formation and are the softest stage[15]. Subacute clots are present from 2 days to roughly 2 weeks after formation and are noticeably stiffer than acute clots[16]. Finally, chronic clots are thrombus older than 2 weeks and are significantly stiffer than the previous two stages[17]. For thrombus, their E' can be estimated based on published literature and previous testing[18]. E", G', and G" are relatively unknown and still need to be found consistently through testing. Table 1 below depicts the estimates for G' and G" for each clotting stage based on previous testing.

Table 1. Blood Clotting Mechanical Properties

	G' (Elastic Modulus) (kPa)	**G" (Viscous Modulus) (Pa)***
Acute	1.68 - 6.71	0.01 - 0.04
Subacute	3.36 - 67.11	0.04 - 0.08
Chronic	> 167.79	0.08 - 0.14

1.4 Current Treatments

To currently treat DVT and other thrombosis-related conditions such as Pulmonary Embolisms (PE), anticoagulant medications, such as heparin and novel oral anticoagulants (NOACs), and the mobilization of the affected limb are first initially prescribed to break up the clot on its own[25,26]. Inferior vena cava (IVC) filters are inserted into patients within the femoral vein to catch these clot remnants. A balloon catheter is used for treatment in more severe cases of thrombosis.

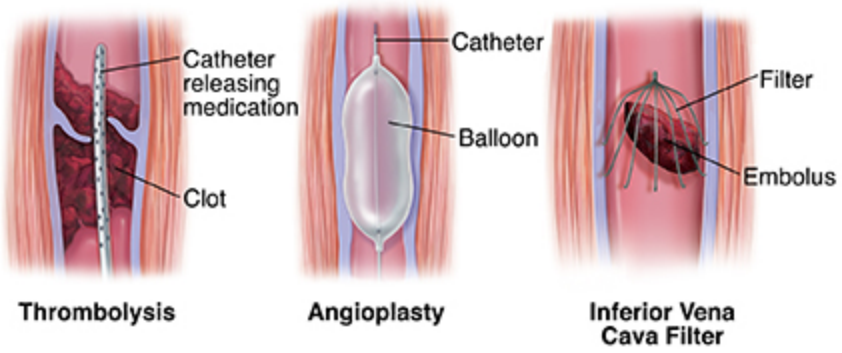

Figure 1. Removal of a blood clot using a balloon catheter[24]

These catheters are inserted into the ipsilateral popliteal vein in the leg and guided using ultrasound or other similar imaging techniques[25]. The catheters initially puncture the thrombus and administer plasminogen in order to initiate the breaking up of the clot. Afterward, a balloon dilator of 12 to 15mm in diameter is expanded in order to break down the thrombus. These fragments are then allowed to travel down to the IVC filter. For some cases after this procedure, a stent is placed to allow for the continuation of blood flow in the damaged area.

1.5 Models for Medical Device Testing

To test these catheters, animal models are primarily used. Baboon models have been utilized due to their similarity in coagulation to humans[27]. Baboons have valves in veins that could be affected by coagulation similar to humans, meaning pathological events through thrombus formation can be accessed. Most commonly, rodent models such as rats and primarily mice are utilized due to the similarities in clot makeup to humans. This becomes useful for modeling each of the three clotting stages for thrombus: acute, subacute, and chronic[28]. Porcine (Pig) blood on its own has also been used to mimic the mechanical properties of human thrombosis. This is due to the similarities of a pig's vascular system to that of the lower limbs of a human, which is where thrombogenesis is most likely to occur[29].

Animal models still have limitations that limit their ability to mimic human thrombosis. Thrombogenesis is required to be induced foreignly, which differs compared to thrombogenesis within humans which is caused by internal stimuli or non-pathological factors such as after surgery[29]. The hemodynamics, vessel size, and coagulation process can also vary significantly between models and compared to humans. While the efficacy of balloon catheters under certain

conditions can be accessed utilizing animal models, how the catheters will respond to a human thrombus can end up differently than expected.

1.6 Proposed Solution

A solution that could address some shortcomings of animal models is the utilization of synthetic hydrogel-based material for thrombi mimicry. Gels made out of silicone, agarose, and gelatin have been utilized to test the efficacy of the surgical procedure involving balloon catheters[30]. These gels are meant to mimic the behavior of human thrombi rather than reproducing the exact mechanical properties. Evaluating this approach, the potential for gels to be used as the primary method to test these catheters begins to appear due to the lack of animal models required.

1.7 Objectives

1. To expand the range of formulation of sustainable hydrogel-based artificial blood clots.
2. To expand the rheological characterization methods from confined compression to dynamic mechanical analysis in shear
3. To compare and interpret the differences between dynamic mechanical compression conducted in compression and shear modes.

CHAPTER 2

Significance

The need for *in-vitro* hydrogel blood clots can be identified when discussing both the development of medical devices and how it affects the efficacy of care for patients

Venous Thromboembolism (VTE), which is the clotting of blood either in the deep veins (DVT) or the lungs (pulmonary embolisms), can affect as many as 900,000 Americans a year with 60,000 to 100,000 of those affected dying from the condition. Even those who survive can be affected by long-term complications such as discoloration, pain, and reduction or even the elimination of blood flow to the affected limb[23]. These factors necessitate the removal of the clot as soon as possible. This is done using primarily balloon catheters surgically.

Blood clots harden over time once they form, initially starting as acute clots, transitioning into subacute clots, before finally solidifying into chronic clots[31]. The mechanical properties of these clots can be measured with compression testing using Young's Modulus and its respective elastic and viscous modulus (E, E' and E" respectively) or shear testing utilizing Shear Modulus and its moduli (G, G', and G" respectively)[32,33]. The ratio of the E'/G' and E"/G" can then be used to calculate the phase angle of the clot, which is in the range of 0 to 90 degrees[34]. An older clot will have a phase angle closer to 0, indicating that it is more solid and vice-versa.

The efficacy of these catheters typically decreases the older a clot is due to more force being required to remove it. To alleviate this, more research and development is being dedicated to increasing this efficacy regarding older, chronic clots. To do this, models are used to test any changes made to these catheters. Currently, the primary method of doing this is with animal blood, specifically from pigs. However, this has proven to be unfeasible for large-scale testing due to the cost, scaling, and availability of the product[35]. Currently, the cost of 50 mL of porcine blood is $148[36]. Additionally, the usage of animal products for testing requires adherence to strict safety and ethical protocols which can further limit usage[37].

These factors make a hydrogel-based alternative attractive. The cost of 125g of agarose and 10g of dextran are currently $254 and $82 respectively, resulting in a lower cost per gram than purchasing porcine blood[38,39]. Additionally, larger sizes of each material are available that

could allow for an ever lower cost per gram. The larger sizes also allow for the making of larger samples and more tests to be performed before needing to be reordered. These synthetic materials are also more widely available and allow for the avoidance of more strict regulation regarding their usage due to their natural origin. All of these factors could accelerate the testing and improvement of these catheters due to the scalability of these hydrogels while simultaneously being cost-effective for companies making these devices.

CHAPTER 3

Materials and Methods

3.1. Introduction

Through an extensive literature review, a clot timeline based on the elastic modulus was defined for the three clotting stages. For the acute stage, which is clots that are less than two days old, a G' of 5 - 20 kPa was found to show the most resemblance[18]. For the subacute stage, which is clots between 2 days to 2 weeks old, a G' of 20 - 200 kPa was used as a reference. Finally, for the chronic stage, which is clots older than 2 weeks, a G' of greater than 500 kPa was utilized for characterization[18]. G" was also desired, though due to a lack of literature, a proper range for each clotting stage could not be concluded. Utilizing a rheometer, which measures the shear stress and strain applied to a material and returns values of both G' and G", a hydrogel-based material could be created that exhibits certain elastic properties while giving viscous properties that would be able to be utilized to define a G" range for each clotting stage[19].

With these parameters in mind, dextran and agarose were decided as the best gels to be used to create an artificial blood clot that could be used for testing. A major reason for this was the fact that a gel made up of these components could be cost-effective, easy to produce, biodegradable, and avoid the use of animal-based products. When crosslinked, the concentration of each can determine the G' and G" of the final gel. For example, a higher concentration of agarose will typically increase G' and decrease G", leading a material that is overall stiffer. Conversely, a high concentration of dextran will have the opposite effect, making a material that is softer[20]. These crosslinks are similar to the links between fibrin within a clot that leads to the coagulation of blood[19].

Table 2. Biomaterials used for each Gel

Label	Dextran from Leuconostoc spp. (Mr 450,000-650,000)	Certified Molecular Biology Agarose
Supplier	Sigma Aldrich	Bio-Rad

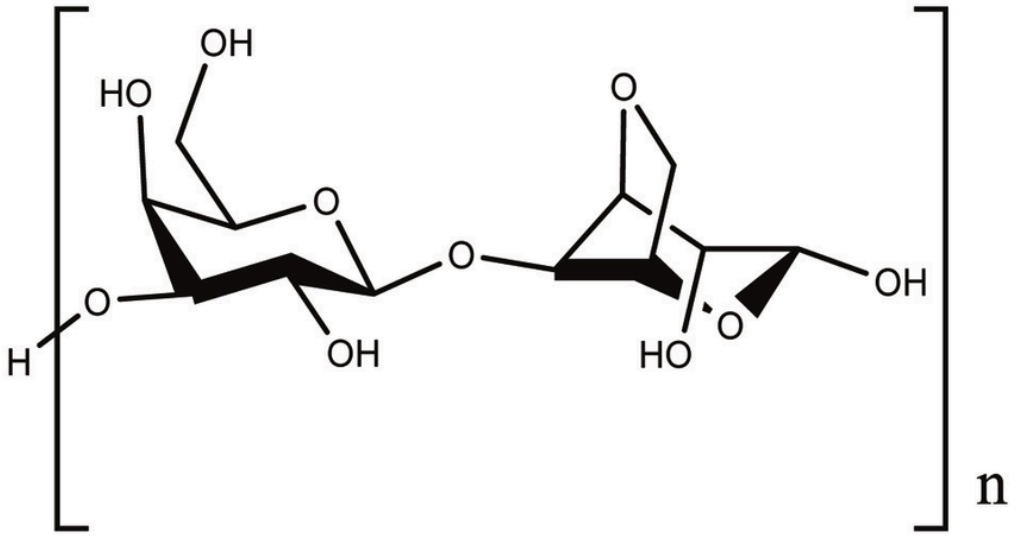

Figure 2. Chemical structure of agarose[21]

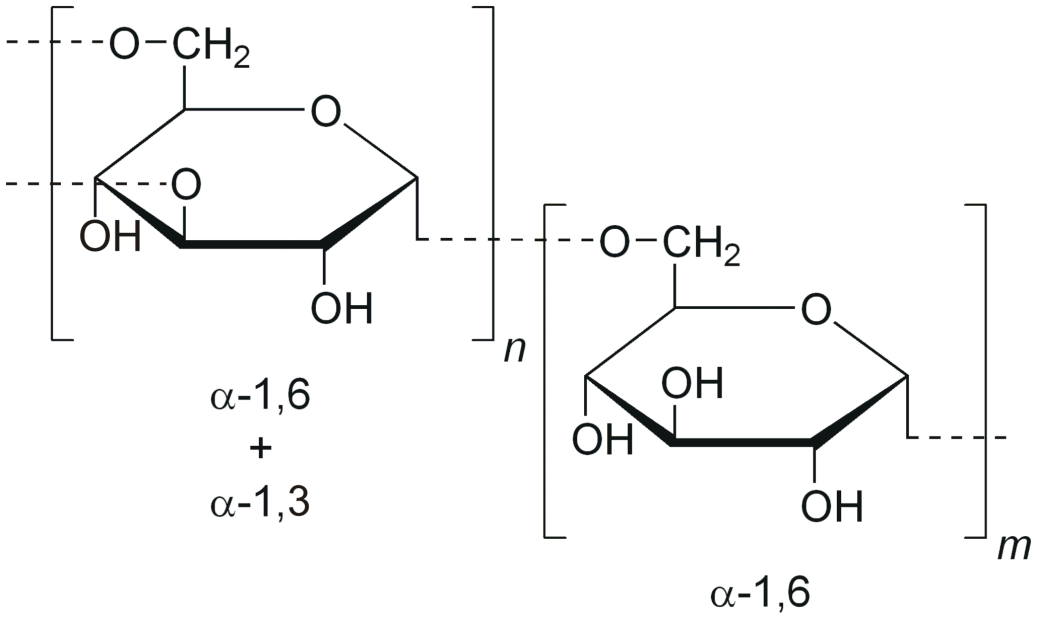

Figure 3. Chemical structure of dextran[22]

3.2. Artificial Clot Creation

a. Saline Procedure

When creating saline to make the gel components in, this protocol was followed:

1. Measure out the desired amount of distilled water that will be mixed into saline

2. Weigh out an amount of salt that is 0.9% of the volume of water
3. Stir the water in a beaker using a magnetic stir plate and bar
4. Pour the salt into the water once it is stirring
5. Wait for the salt to be visually fully dissolved in the water
6. **OPTIONAL:** If using for gel mixing later, keep at 90 ℃ once made

b. Agarose Procedure

To make 85 mL Agarose, this protocol was followed:

1. Weigh out 0.6375g of agarose for A0.75 concentration on scale
2. After the saline has been heated to 90˚C, take the weighed-out agarose and slowly pour it into the flask containing saline
3. The saline should be still heated and kept at a constant temperature with a stirring rod inside the saline as the agarose is being poured in
4. Let the agarose/saline solution continue to stir until there are no “clumps” that have been gelled until the solution is almost translucent
5. Leave on the hot plate and cover the top with a plastic covering

c. Dextran Procedure

To make 70 mL of Dextran, this protocol was followed:

1. Weigh out 7g of dextran on the scale
2. Take the heated saline from the beaker and pour it into another flask where the weighed-out dextran will be poured into
    a. As you are pouring the dextran, do not pour all of it at once since this will cause the dextran to dissolve
3. Similar to the agarose procedure, place a stir rod inside the saline/dextran solution and let it mix until there is no clumping
4. Leave on the hot plate and cover the top with a plastic covering

d. Final Gel Procedure

1. Take two skinny beakers and pour different amounts of agarose and dextran into two separate beakers

2. Next, take a cup, pour the two solutions into the cup, and begin to stir the solutions with one another for the cross-linking to begin
3. Continue to stir until you are going to syringe the solution into the ring on the rheometer plate

3.3. Mechanical Testing

a. Rheometer Procedure

1. Turn on the computer and go to the Rheo application
2. Turn on the vacuum and cooling system
3. Use the 8mm rod and line up with the dashes on the Rheometer
4. Go to the control panel & zero gap it
5. Go to measuring in the top left corner, and do a system check by checking inertia and motion
6. Press gelation/time sweep
7. Right-click the temperature ramp and skip this step
8. Set values for isothermal, temp at 4˚C, and set time for testing for 5 minutes
9. Go to the control panel and set temp at 4˚C and set value
10. To move the measuring device, set the moving profile to Viscoelastic
11. Place the ring onto the rheometer plate after the temperature is at 4˚C
12. Take the gel mixture and syringe and pour it into the ring
13. Move the rod down onto the gel where it barely touches the surface and then close the hood
14. Begin rheology testing
15. After 5 minutes, lift the hood, move the 8mm rod up and off the gel, and then begin cleaning procedures

Making sure the agarose dissolves completely in the solution might be challenging when making a higher-concentration agarose solution. Making smaller batches that use a very small amount of water can also prevent the agarose from solidifying and taking on the desired gel-like qualities. This leads to a difficult-to-work-with solution since it might not be appropriate for rheometer testing. The size of the testing rings and well plates was another problem that was discovered.

The ring and well plate sizes being too tiny can cause errors in measurements. If a ring with a diameter of 12.5 mm is used, for example, there is a chance that the combined solution will seep out during testing, making the data useless.

When in contact with the gel, the rheometer measures the stress (τ) using the force (N) and the area (mm). It almost measured the strain (γ) applied to the material using the horizontal displacement (x) over the height of the gel (y). The equations for that are listed below:

$$\tau = F/A \quad \text{(eq. 1)}$$

$$\gamma = x/y \quad \text{(eq. 2)}$$

The complex shear modulus (G*) is calculated using this strain and strain, and the G' and G" can be derived from this value if the phase angle (δ) is known.

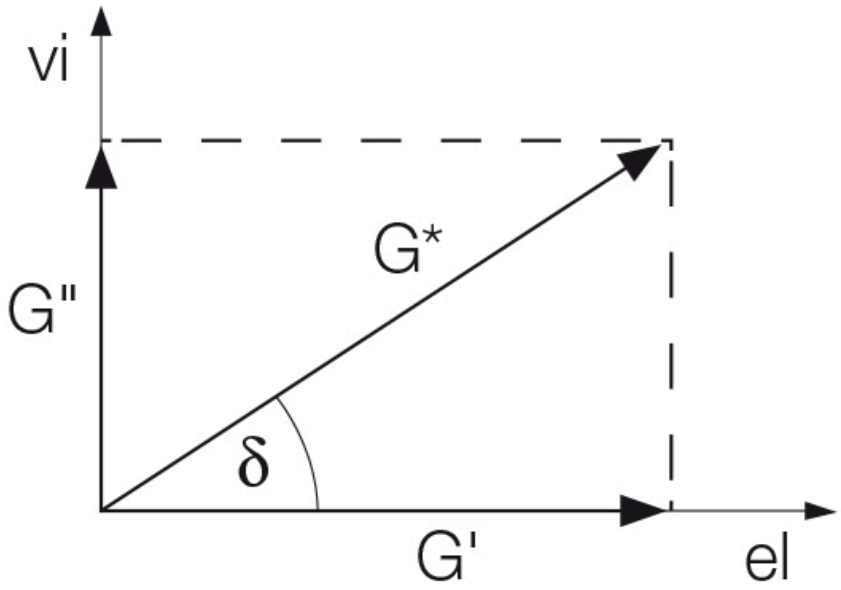

Figure 4. Vector diagram[41] of G*, G', and G"

$$G^* = \tau / \gamma \quad \text{(eq. 3)}$$

$$G^* = \sqrt{G'^2 + G''^2} \quad \text{(eq. 4)}$$

$$\tan(\delta) = G''/G' \quad \text{(eq. 5)}$$

$$\delta = \tan^{-1}(G''/G') * (180/\pi) \quad \text{(eq. 6)}$$

The phase angle can range from 0 degrees to 90 degrees and corresponds to how solid or liquid a material is. A perfectly solid material has a phase angle of 0 degrees while a perfectly liquid material has a phase angle of 90 degrees. Viscoelastic materials fall within this range.

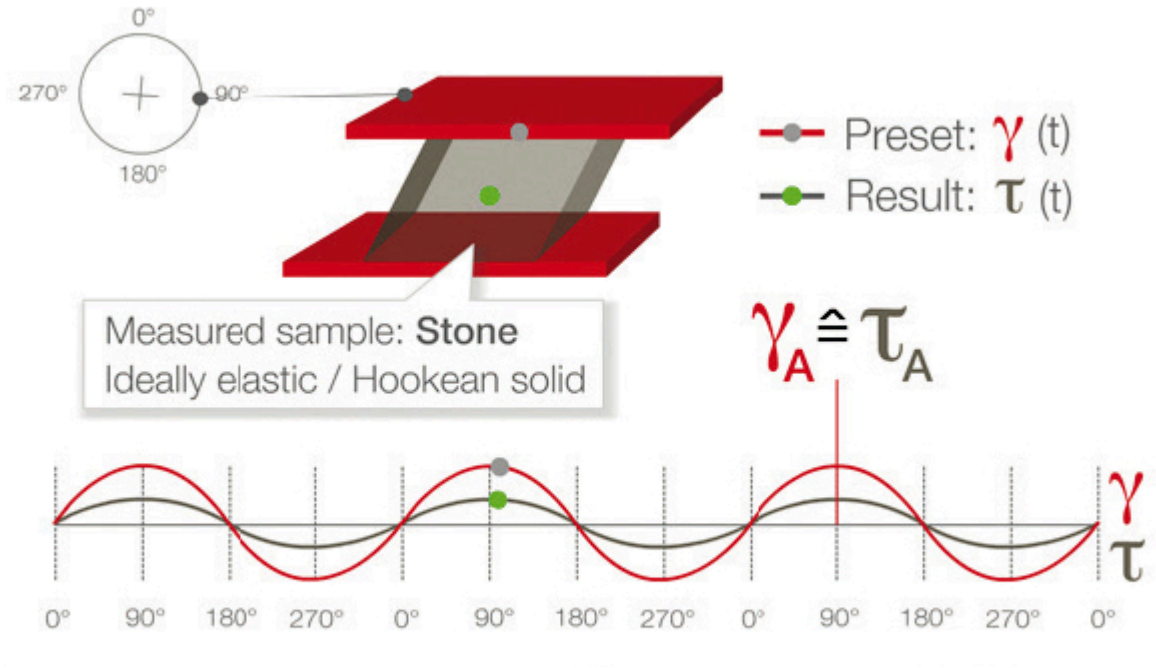

Figure 5. Rheometer analysis of a perfectly solid material[41]

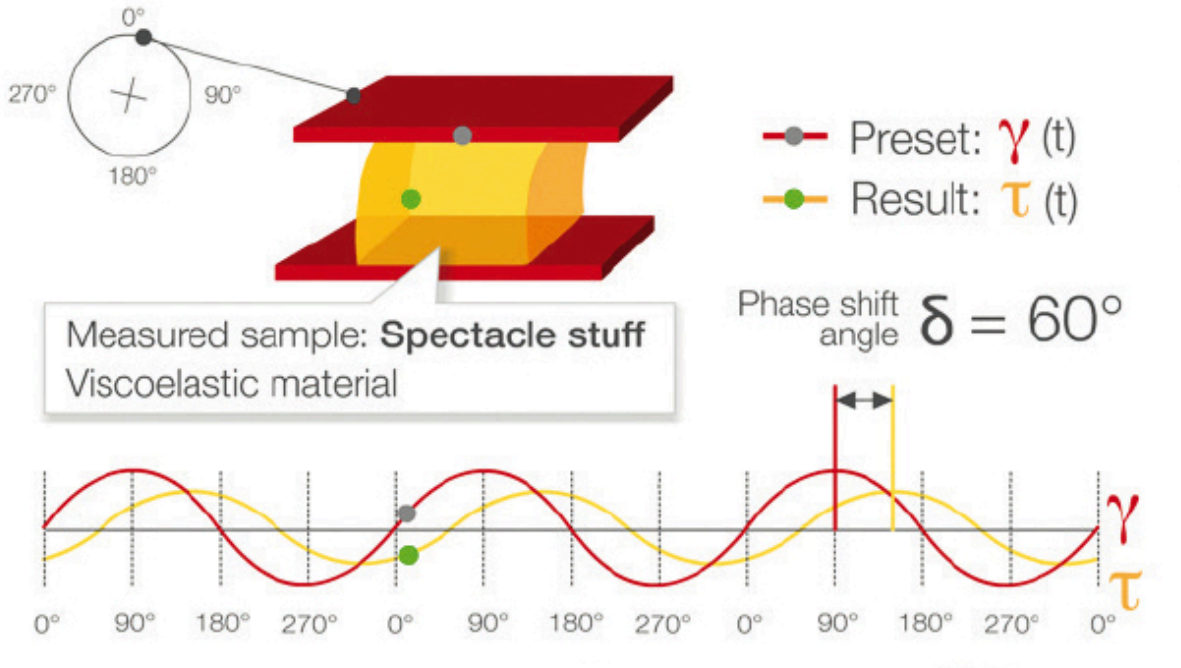

Figure 6. Rheometer analysis of a viscoelastic material[41]

The rheometer measures the stress and strain of a material sinusoidally, with the amplitude of the measurements corresponding to the value of each. As seen in Figure 5, a perfectly solid material

has its wave measurements in phase with each other, meaning that the phase angle is equal to 0. For a viscoelastic material as seen in Figure 6, a phase shift begins to appear between the two measurements, with the value of this shift being the phase angle of the gel. Varying the concentration of agarose and dextran typically will alter the phase angle of gels made.

3.4. Back-up Plan

A set of procedures will be followed in the event that problems occur during the hydrogel's formation, such as the gel's inability to solidify at a particular temperature or concentration. To guarantee that the saline solution is precisely maintained at 90˚C during the mixing procedure for both agarose and dextran, more stringent temperature control measures will be implemented initially. To find the ideal composition that produces the required gel characteristics, the agarose and dextran concentration ratios will also be gradually changed. The gel components will be thoroughly stirred and mixed for longer periods of time to guarantee full dissolution and consistency. Throughout the preparation process, routine sample and viscosity testing will assist in identifying and rectifying any irregularities early, guaranteeing that the gel solidifies correctly.

Alternative mechanical testing techniques will be looked for to guarantee the research's continuation in the event that the rheometer breaks down. If available, access to additional viscoelastic testing equipment, such as dynamic mechanical analyzers (DMA), will be arranged. The hydrogels will undergo manual testing techniques, such as tensile and compression testing, in order to collect preliminary data on their mechanical properties. We'll work quickly to fix and calibrate the rheometer by getting in touch with the manufacturer for the required parts and technical assistance. In order to minimize any delays in key testing, collaboration with neighboring research institutions will also be investigated to obtain access to pooled resources and equipment. Thoroughly recording every step, modification, and observation made throughout the troubleshooting process will aid in identifying trends and underlying causes, enabling effective problem-solving and maintaining the project’s deadline.

CHAPTER 4

Results/Analysis

4.1 Dynamic Mechanical Analysis

Last year's team primarily focused on collecting mechanical properties with the Mach-1. Using this device, dynamic mechanic analysis can be performed to evaluate the E, E', and E" of the gels by applying an oscillating compression force using a load cell at varying frequencies. Once this data was collected, gels were plotted by Young's Modulus from softest to stiffest (smallest to largest), which can be observed in Figure 7.

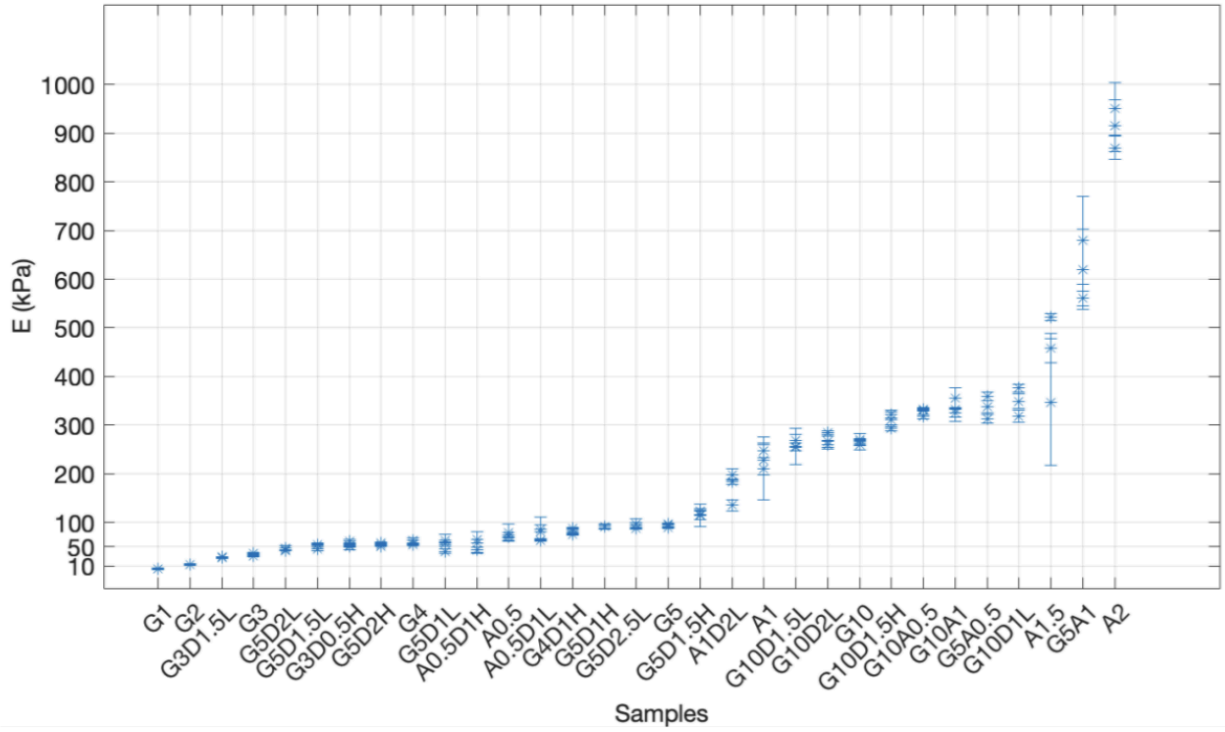

Figure 7. Categorization of gels by Young's Modulus[40].

The frequency applied to the gels can affect the mechanical properties observed by each gel. To test this, we focused on three agarose gels at varying concentrations: 1%, 0.75%, and 0.5%. This was done to limit the number of variables that could alter the frequency results while still overlapping with gels last year's team was able to test in order to compare. Figure 6 below

illustrates the phase angle of each gel at different frequencies.

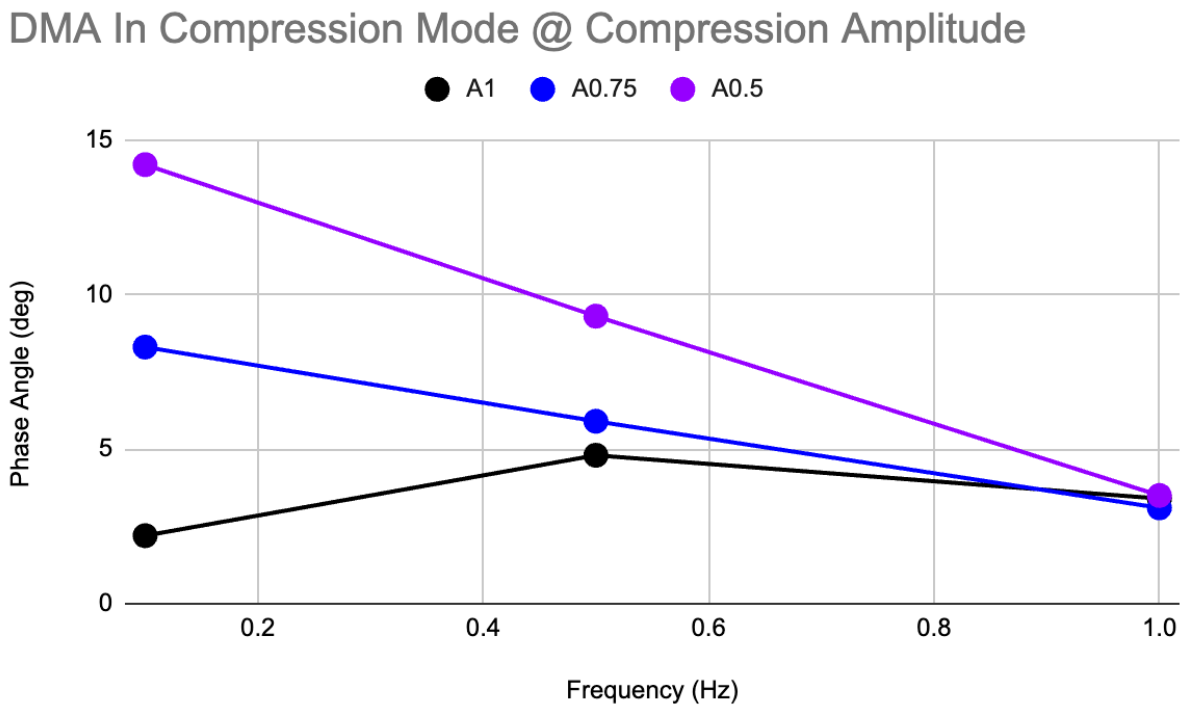

Figure 8. Mach-1 phase angle comparison of agarose gels

At lower frequencies, a noticeable difference in phase angle can be observed between the gels, with the lower concentrations of agarose having a higher phase angle. However, as the frequency of the applied force increases, the definition between the points begins to decrease until it is indistinguishable at 1 Hz.

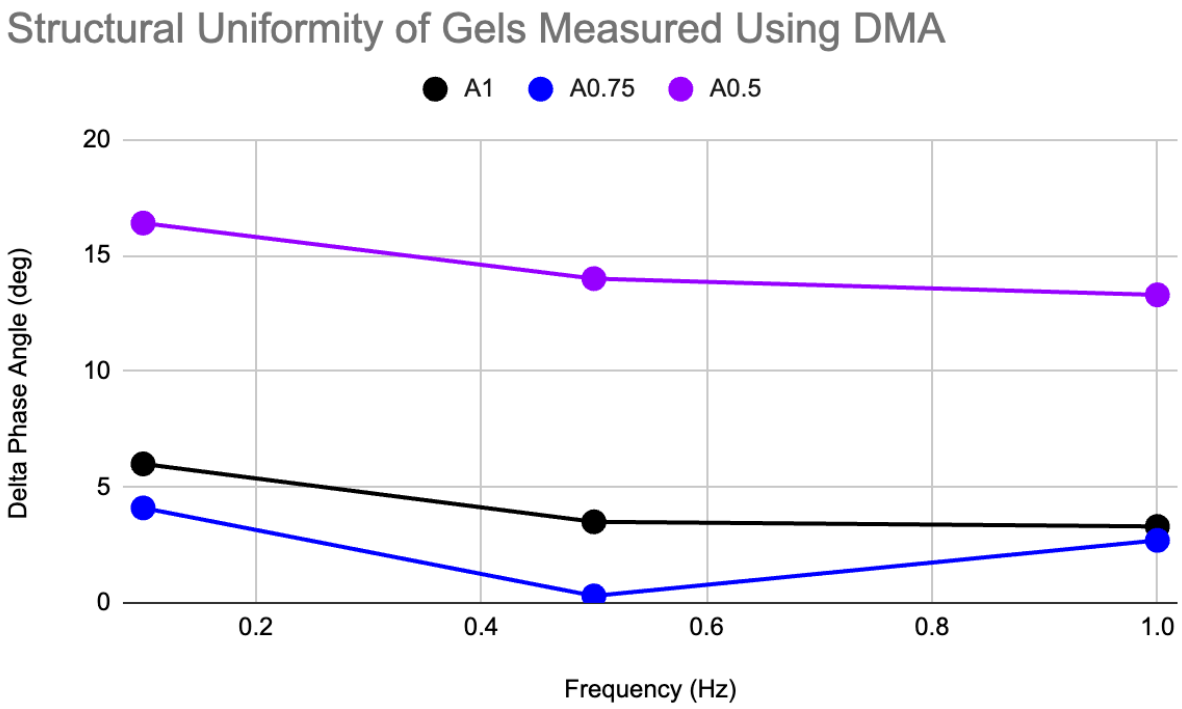

Figure 9. Mach-1 delta phase angle of agarose gels

Since the Mach-1 can only test one side of a material unlike the rheometer, it is useful to test both the top and bottom of the gel to observe if there is a difference in phase angle, which is referred to as the delta phase angle. At lower frequencies, a difference between this delta phase angle between points can be observed, though it is unclear if the concentration of agarose affects this. At higher frequencies, some of this definition is again lost.

4.2 Rheometer Testing Methods

Looking to remove the need for tests on both sides of a gel and to increase the definition of data points at 1 Hz, we shifted to testing with the rheometer. Three types of tests can be performed using the rheometer in order to collect the G' and G" of a gel: a frequency sweep, an amplitude sweep, and a time sweep. A frequency sweep keeps the applied strain on the gels constant while the frequency of the rotational shear force is either ramped up or down at an exponential rate. At lower frequencies, the G' and the G" remain relatively constant, indicating the mechanical properties of the gel at rest, and this can be recorded for comparison. At higher frequencies, the gel softens and begins to behave more like a liquid, causing a crossover where

G” is higher than G’. This corresponds to a phase angle greater than 45 degrees. What frequency this happens and how much the phase angle increases can vary by gel.

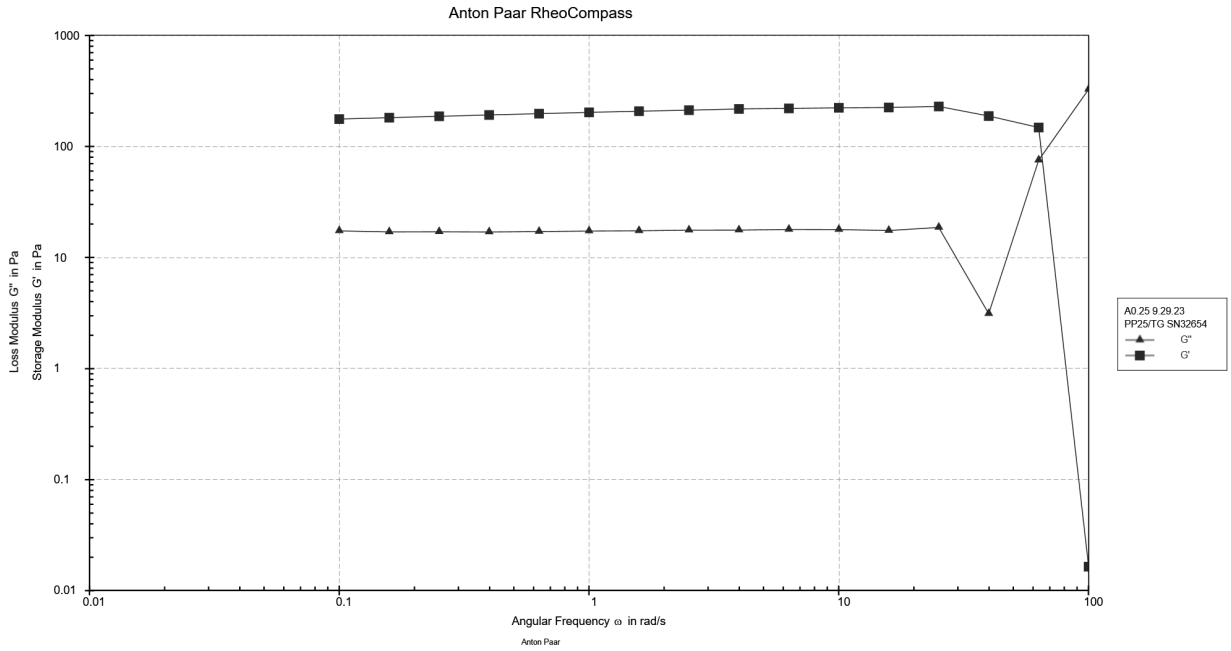

Figure 10. Frequency sweep of the 0.25% agarose gel

Conversely, an amplitude sweep keeps the frequency constant while the applied strain is ramped up or down exponentially. At lower strains, the G’ and G” typically remain relatively constant, again representing the mechanical properties of the gel at rest. As strain increases to a certain point, the gel begins to soften and an increase in G” and a decrease in G’ can be observed until eventually the two crossover. This test can be useful for determining how much force a gel can withstand before breaking down.

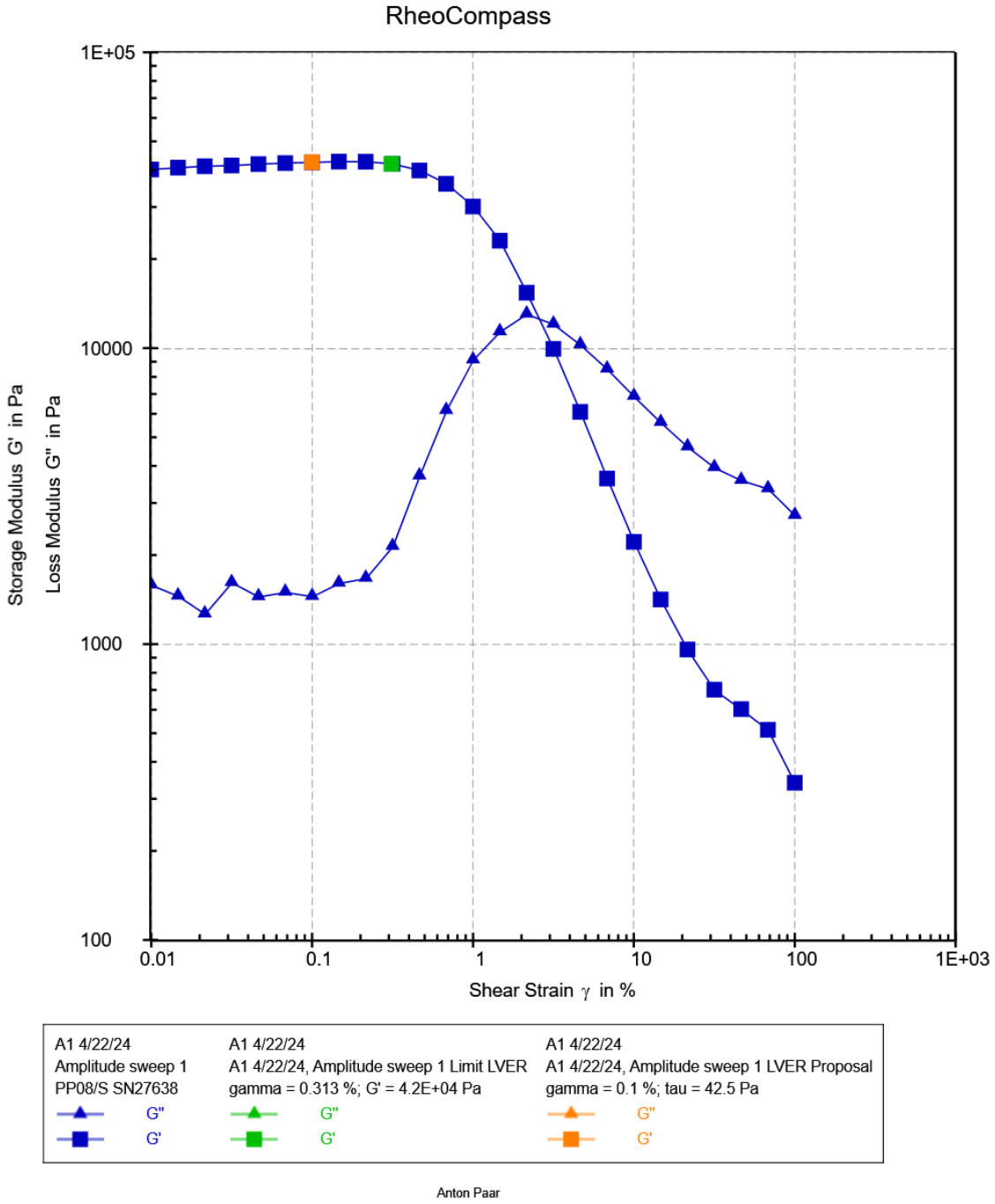

Figure 11. Amplitude sweep of the 1% agarose gel

The final test the rheometer can perform is the time sweep. Both frequency and strain are kept constant while the test is run for a desired amount of time. If frequency and strain are kept sufficiently low enough, a measurement of how the gel behaves at rest can be obtained. This test can also be useful for observing how long a gel takes to solidify, which can be observed if there

is an increasing slope for the G' of a gel. As the gel solidifies, the rate of increase decreases until the G' becomes constant.

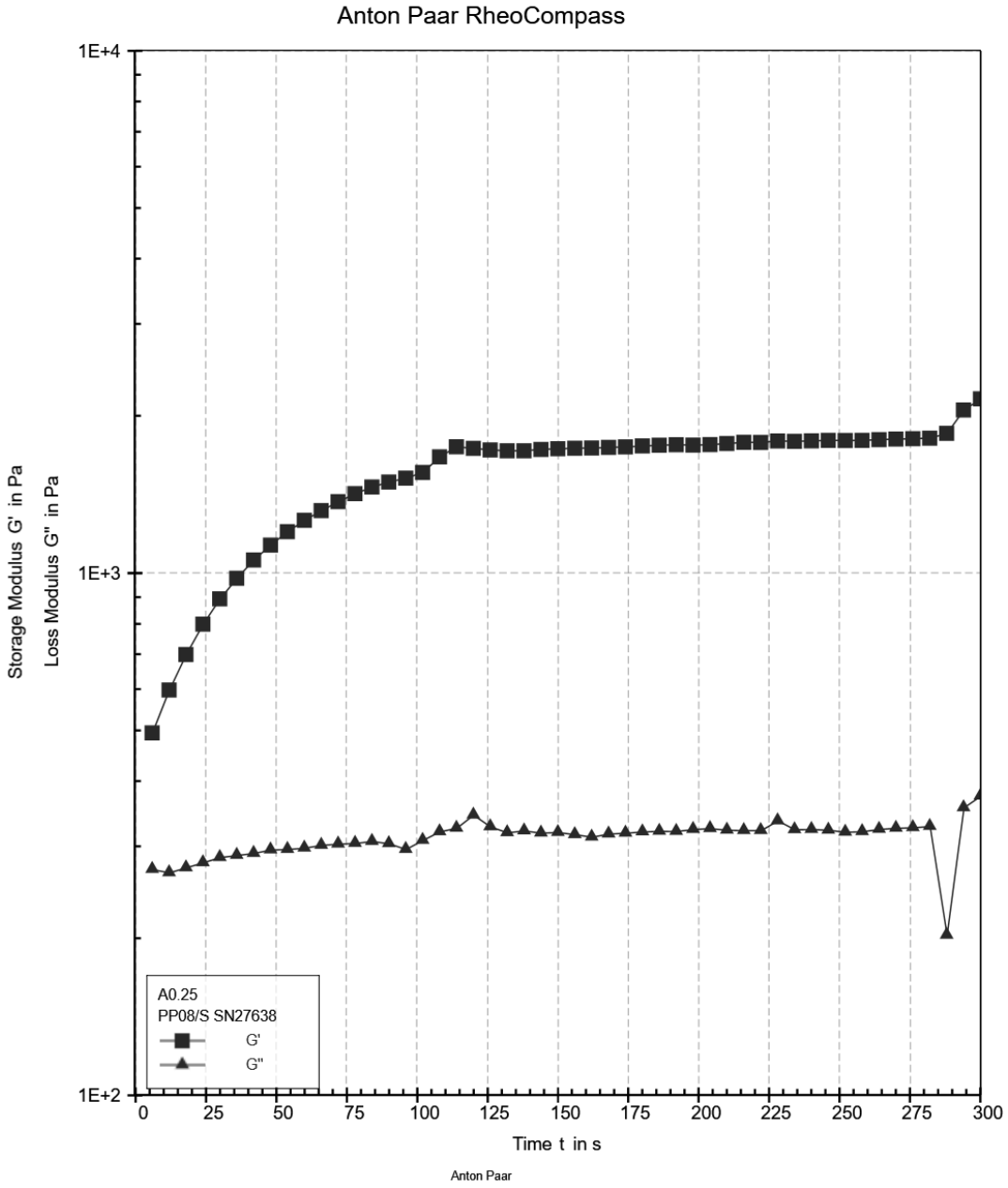

Figure 12. Time sweep of the 0.25% agarose gel

4.3 Rheometer Data

We primarily utilized the time sweep for the collection of our data as this would allow for the observation of the solidification process of any gels made. To compare the mechanical properties while the gel was at rest, a frequency of 1 Hz and strain of 0.5% was utilized. To reduce the risk of outlier data, gels were tested 3 times for their mechanical properties before being averaged in order to calculate the phase angle.

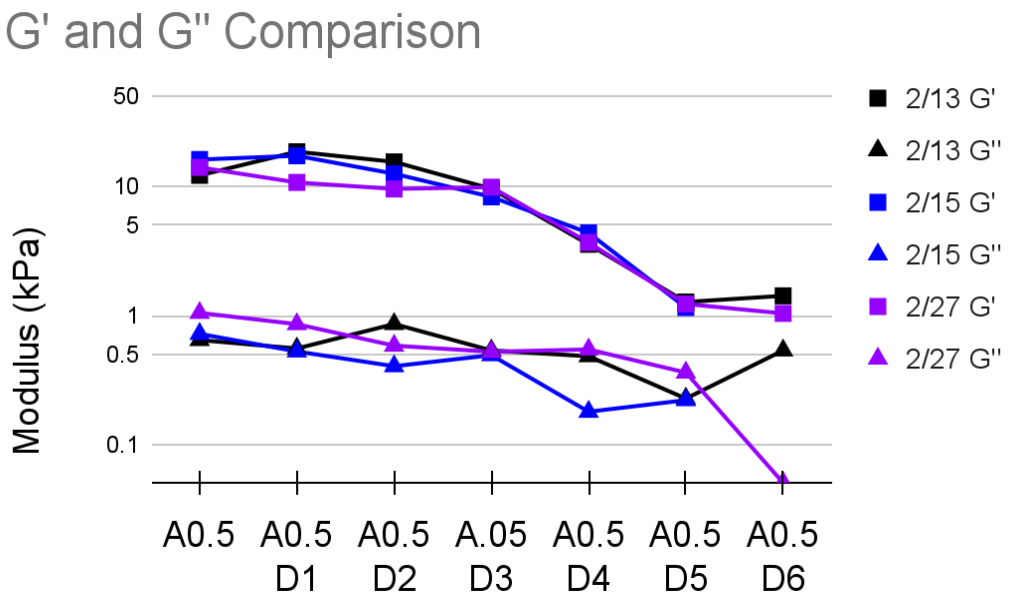

Figure 13. G' and G" comparison of 0.5% agarose gels

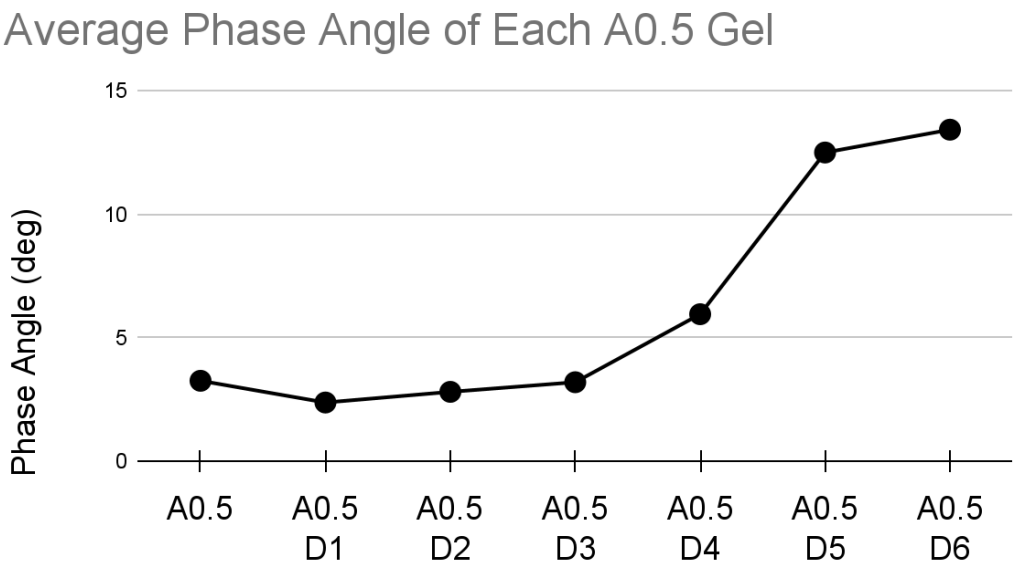

Figure 14. Average phase angle of 0.5% agarose gels

As observed in Figure 14, the effects of adding dextran once reaching the 4% threshold cause an increase in phase angle, indicating that the gel is becoming softer. Utilizing this approach, a comparison between gels of varying agarose and dextran concentrations can be made, as seen below in Figure 15.

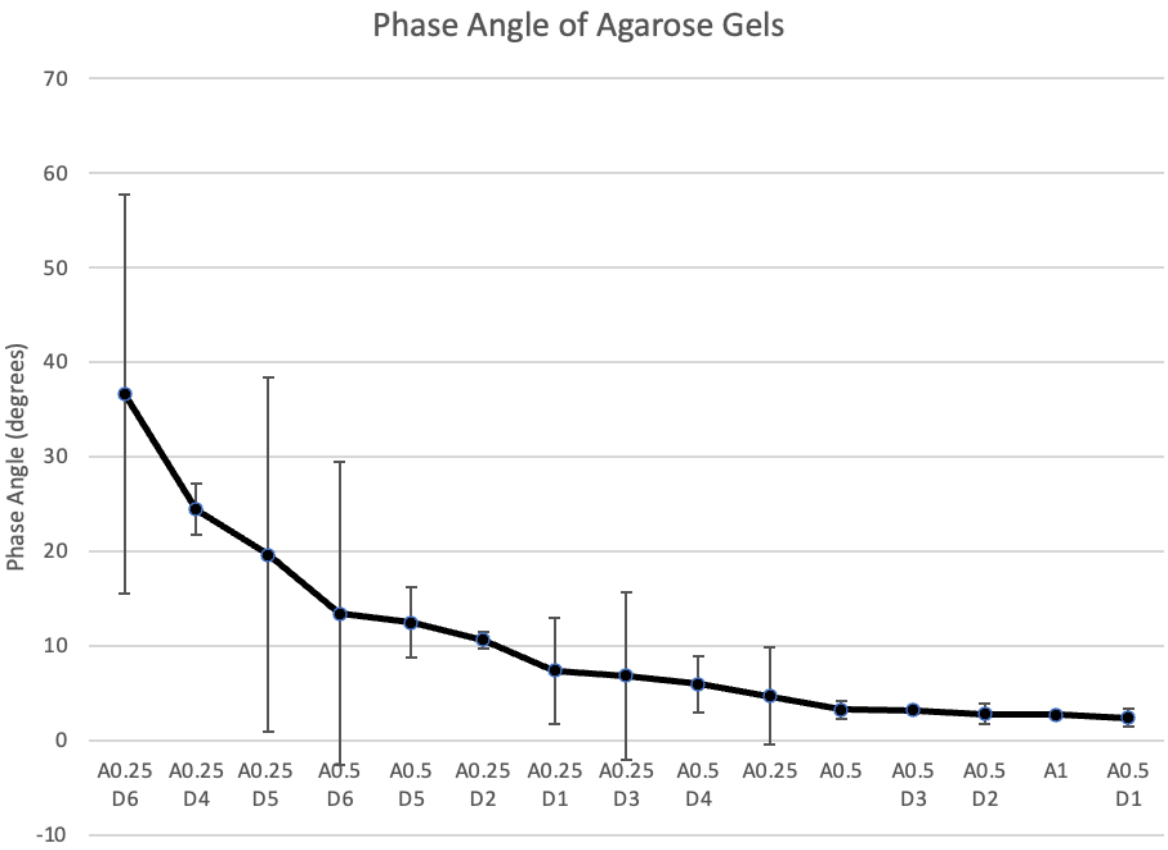

Figure 15. Phase angle of agarose gels

From Figure 15, it can be seen that gels with higher dextran concentrations and lower concentrations of agarose had higher phase angles. The opposite can be observed for gels with lower dextran concentrations and higher agarose concentrations. Note that softer gels typically had more uncertainty for their phase angle, which could cause deviation from the theoretical order of gels based on phase angle.

4.4 Temperature Comparison of Gels

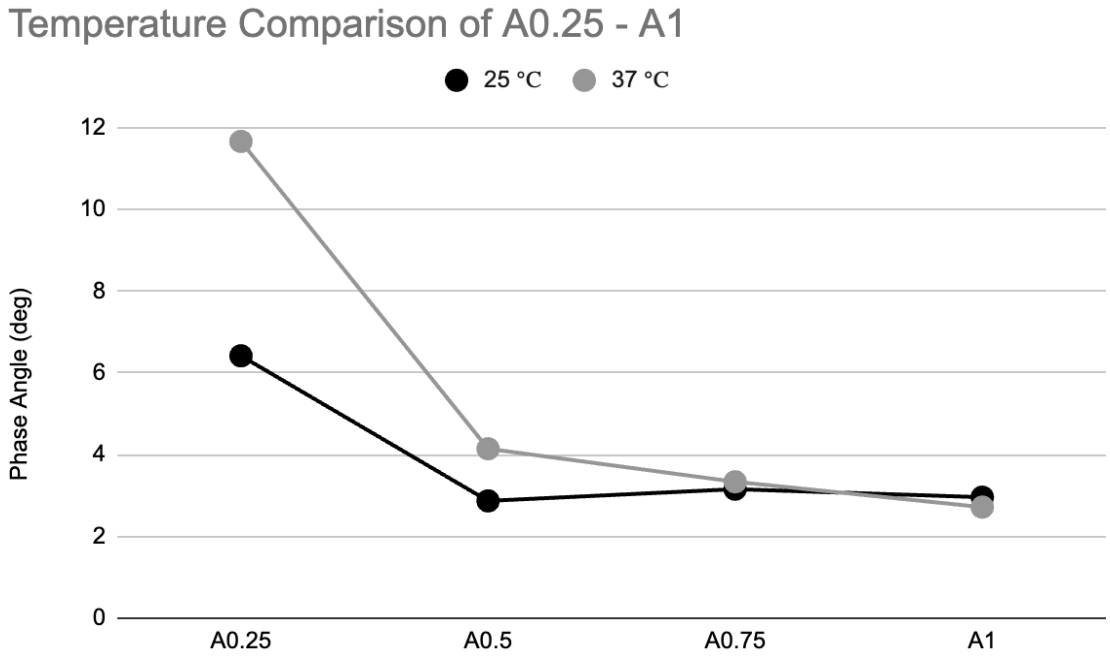

Figure 16. Temperature comparison of A0.25-A1

How the gels behaved in both body and room temperature was also tested. As observed in Figure 16, lower concentrations of agarose had a higher difference in phase angle at body temperature compared to room temperature. For these gels, softening from these temperatures occurred, though the gel does fully melt due to still being below the melting point of agarose (90-95 ℃). This difference becomes negligible at higher concentrations of agarose, depicting that the phase angle remains intact at body temperature and that the gels could be tested in this environment.

CHAPTER 5

Discussion

5.1 Mach-1 Compared to the Rheometer

While the Mach-1 provides valuable information about the mechanical properties of gels, a primary reason we shifted to using the Rheometer for the majority of tests is its ability to get a better definition of gels at higher frequencies and to measure these on two sides simultaneously thanks to its two plate system.

Table 3. Comparison of Mach-1 and Rheometer

Agarose Concentrations (%)	Frequency (Hz)	Rheometry Phase Angle (deg)	Mach-1 Phase Angle (deg)
0.25	1	4.7	N/A
0.5	1	3.26	3.5
0.75	1	3.00	3.1
1	1	2.72	3.4

At a frequency of 1 Hz, which was the primary frequency tested, a clear phase angle difference can be observed with the Rheometer. Conversely, the Mach-1 data is extremely similar and it is difficult to make a conclusion on how the concentration of agarose affects the stiffness of the gel based on this alone. This definition plus the elimination of the need to test the same gel multiple times for uniformity encouraged us to focus on collecting data using primarily the Rheometer. Employing the Mach-1 is still extremely useful for collecting data on the compressive strength of gels, and is helpful if structure and density are desired after the hydrogels within a mixture are crosslinked[42].

5.2 Phase Angle Analysis

Our data showed that increasing the concentration of agarose and decreasing the concentration of dextran caused the phase angle of a gel to decrease. Conversely, doing the

opposite caused the phase angle of a gel to increase. The effects of dextran did not typically affect the phase angle of a gel until it made up at least 4% of the overall composition of the gel. It is still unclear at this point if one component has a stronger effect on the phase angle of the gel compared to another. It should be noted that some gels, such as A0.5 D1, were somewhat outliers as they went against the trends noted earlier by having phase angles lower than gels of higher agarose concentration. This can be possibly attributed to agarose gels having a constant phase angle without the addition of a significant amount of dextran. This was observed by one study that looked at how increasing the concentration of pure agarose affects the shear modulus of the gels[44]. While increasing the concentration increased the overall stiffness and shear modulus as expected, this was at a linear rate. This suggests that the ratio of G' and G" and thus the phase angle were kept relatively constant even as concentration increased. More testing would be needed to confirm that agarose has a constant phase angle without the presence of dextran.

5.3 Preliminary Shelf Life related to temperature stability

As surgeries involving balloon catheters are done in body temperature conditions, it is useful to evaluate if the agarose gels are able to maintain their mechanical properties in this environment. Since body temperature is at the gelling temperature for agarose (34-38 ℃), lower concentration gels may not be able to fully solidify, causing a difference in phase angle. This may also relate to the time it takes for agarose gels to solidify, as it was observed that higher-concentration agarose gels solidified more quickly than lower-concentration gels. Agarose gels are prone to some level of viscosity increase as the temperature increases, though this rate of increase does not accelerate until crossing the gelling temperature of the hydrogel[43]. Only four gels were tested for their durability at body temperature, and it would be useful to test how the addition of dextran affects this resistance.

CHAPTER 6

Engineering Standards

6.1 Ethical Considerations

The goal is to address important ethical issues related to present techniques by creating a hydrogel-based substance using agarose and dextran to mimic the mechanical properties of blood clots for testing balloon metal catheters. Animal blood is currently tested within these kinds of medical device tests, necessitating that businesses obtain and handle this biological material in compliance with FDA rules. In addition to being expensive, this procedure raises ethical questions about the purchase and use of animal blood. By developing a hydrogel with viscoelastic characteristics similar to blood clots, a workable substitute may be presented that reduces the need for animal sources. The 3Rs, Replacement, Reduction, and Refinement, in animal research are considered in controlled, repeatable, and morally sound testing environments that hydrogels can address. This method contributes to the advancement of medical device development in a more economical and compassionate way by reducing the ethical concerns and improving the uniformity and dependability of the testing process.

6.2 Sustainability

Replicating blood clots in medical device testing with a hydrogel-based substance made of agarose and dextran has advantages for sustainability as well. These artificial hydrogels can be generated reliably and sustainably year-round, unlike animal blood, which is a limited resource that is prone to fluctuations in availability and necessitates defined storage conditions. The supply chain is stable and dependable since the components used come from renewable sources. To reduce its influence on the environment and enable safe disposal, the hydrogel is non-toxic and biodegradable.

The reliance on resources that come from animals is reduced and a more sustainable method of testing medical devices is considered by substituting these synthetic materials for animal products. In comparison to the manufacturing method for hydrogels, the synthesis of

animal blood requires a greater use of resources and produces emissions during transportation. The utilization of renewable materials and improved waste management through biodegradability are two ways in which this shift improves the economy. Since their mechanical characteristics can be carefully regulated, synthetic hydrogels are a constant and trustworthy testing medium.

CHAPTER 7

Conclusion

7.1 Overall Findings

We were able to find that the concentrations of agarose and dextran directly affected the phase angle for gels. Gels with at least 4% dextran saw a noticeable increase in phase angle compared to the gels without this component. Conversely, gels with higher concentrations of agarose typically saw a decrease in phase angle, corresponding to these gels becoming more stiff. Every gel tested had a phase angle of under 45 degrees, indicating that the gels still were mostly solid with varying levels of liquid-like behavior.

Gels with increased levels of dextran also typically had higher levels of uncertainty regarding their phase angle. This could potentially affect the reproducibility of these particular gels. However, these gels would most likely be modeled for acute clots, which many catheters on the market already have good efficacy against, so relieving this is not urgent. For gels that could potentially model chronic clots such as 1% agarose, the levels of uncertainty were much more reasonable, suggesting that reproducibility and subsequent testing would be easier.

For more solid gels, there was no noticeable change in phase angle at body temperature compared to room temperature. This suggests that these gels would be feasible to test within these conditions. For softer gels, some solid-like properties are lost but testing could still realistically occur at body temperature if needed. Similar to the phase angle comparison, these gels would model acute clots which already can be easily treated so this is not a major concern for the overall modeling of DVT.

7.2 Future Work

The project will concentrate on various important areas to validate the hydrogel-based material for testing medical devices:

1. **Quantifying Phase Angle**: One main objective is to measure the phase angle of the blood, which is necessary to represent the fluid's viscoelastic properties in our hydrogel models. To make sure the artificial material closely resembles actual blood clots, additional rheological testing will assess the phase angle across a range of shear rates and

frequencies. To improve the accuracy and precision of the phase angle data, this process will include fine-tuning our measuring methods and maximizing the settings on the equipment. Furthermore, in order to forecast the behavior of the hydrogels under various flow circumstances and improve comprehension and management of their viscoelastic characteristics, more research of sophisticated rheological models and simulations will be done.

2. **Classifying Hydrogel Phases**: Hydrogels will be classified according to their mechanical and rheological characteristics, namely phase angle measurements, into acute, sub-acute, and chronic coagulation stages. By precisely simulating each clotting step, each formulation will be matched to the corresponding clotting stage by matching the hydrogel's properties to its viscoelastic characteristics. To make sure each category satisfies the essential standards for viscoelasticity and other relevant mechanical properties, more testing will be conducted. To improve the precision and dependability of the hydrogel properties even further, research on how various environmental factors such as temperature, pH, and ionic strength affect them will be looked into. In order to evaluate how these qualities alter over time and under various storage circumstances, long-term stability testing must be carried out.
3. **Testing with Catheter Models**: Lastly, the categorized hydrogels will be examined to evaluate their performance in real-world medical device testing scenarios utilizing catheter models. Hydrogel clots will be manipulated via balloon metal catheters, and both the hydrogel and the catheter will be closely observed for behavior and response. The creation of standardized testing procedures and evaluation standards for the hydrogels' dependability and efficacy in simulating real-world medical scenarios will be part of this phase.

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