5-9-2019

2019 Brain Tissue Replication Using PVA

Justin Wan
Washington University in St. Louis

Phil Bayly
Washington University in St. Louis

Justin Wan
Independent Study Advisor: Philip Bayly
5/9/19

2019 Brain Tissue Replication Using PVA

1. Motivations and Background

1.1 Research Goals in Brain Biomechanics and Traumatic Brain Injuries

The goal of this research was to create and test polyvinyl alcohol (PVA) gel samples to simulate brain tissue. More specifically, PVA gel samples were fabricated to reproduce the anisotropy, isotropy, and elastic modulus of brain tissue. This reproduction of brain tissue will improve on current understanding on the mechanics of brain tissue and brain tissue with altered mechanical properties due to traumatic brain injuries.

1.2 PVA and its Relationship with Anisotropy

As mentioned by the 2011 Hudson paper, *Structure and Strength_ Anisotropic PVA Hydrogels and Spider Mite Silk Fibres,* when PVA freezes, water inside the PVA pushes against the polymer bonds of the PVA[1]. As a result of this, the PVA will become denser in some areas. Following the 2014 paper by Chatelin et al., *Anisotropic polyvinyl alcohol hydrogel phantom for shear wave elastography in fibrous biological soft tissue: a multimodality characterization* [2], when PVA is stretched by 180% of its length while stretched in a Freeze-Thaw cycle, the polymer bonds will become aligned towards the stretched direction, making it anisotropic. With these previous studies in mind, this PVA research was conducted to mimic the mechanical properties of brain tissue and improve our current understanding on brain tissue that experienced traumatic brain injury.

1.3 Personal Objectives and Project Learning Structure

As a personal goal, I wanted to have a better understanding on the solid mechanics behind the PVA gels and learn how to collect data from the PVA when it was tested with the Dynamic Shear Tester (DST). With the data collected, I needed to learn how to analyze the data that was gathered through testing the PVA and understand how the PVA changed with each change Alexa P. (my partner) and I made. To start this process, Alexa trained me in the first few weeks of the semester with how to make the PVA using the previous Bayly Lab PVA procedure. This procedure was adopted from the PVA procedures found in Chatelin's paper, *Anisotropic polyvinyl alcohol hydrogel phantom for shear wave elastography in fibrous biological soft tissue: a multimodality characterization* [2]. The previous Bayly Lab PVA included a Freeze/Thaw cycle that had 4 hour shifts of moving the PVA between the freezer, room temperature, and in the refrigerator throughout the day. After learning how the PVA was made, I learned to induce anisotropy by stretching the PVA to 160% before the third Freeze/Thaw cycle and another 20% before the fourth Freeze/Thaw cycle. However, a new PVA Freeze/Thaw cycle

would soon be developed. This will be explained later on. After understanding how the PVA was made, Alexa trained me in the PVA testing and data analysis. I started by assisting her in the testing and data analyzing, but after a few weeks (February 20th), I was able to test and analyze data on my own.

2. Methodology

2.1 PVA Preparation

The current PVA procedure was adjusted from the previous Bayly Lab PVA procedure and has two steps: creating the PVA and inducing anisotropy to PVA. This PVA recipe uses 92 mL deionized (DI) water and 8 g of 8% wt Polyvinyl Alcohol powder (Mw 89,000-98,000, 99+% hydrolyzed, Sigma Aldrich Product # 341584). Required equipment includes a 1" magnetic stir bar, a Fisherbrand Traceable Kangaroo Thermometer, Fisher Scientific Stirring Hotplate, one 250 mL beaker, 1 VWR 1200 mL beaker, two 65 mm x 95 mm x 25 mm plastic boxes, one circular 100 ml petri dish with a diameter 85 mm and 10 mm height, and one roll of Bemis parafilm. To induce anisotropy in the PVA, the parafilm mentioned above and two different sized custom PVA stretchers are needed.

To start, a water bath 1-2" high was prepared with the VWR beaker and heated on the Fisher Hotplate. 8 g of of the PVA powder was mixed with 92 mL DI in the 250 mL to form a 100 g PVA solution. The magnetic stir bar was placed inside the 250 mL beaker with the PVA solution, which was placed in the water bath. The PVA solution was heated to 90 ℃, and it remained at 90 ℃ for 15 min. To prevent any PVA film from forming on top of the PVA solution, we ensured the magnetic stir bar was continuously spinning.

After 15 minutes, we weighed the PVA solution and refilled the solution with DI water until it was 100 g again. Then, we poured a total of 70 g of solution into the two boxes, 35 g each. The remaining 30 g of solution was placed into the petri dish. Both boxes and the petri dish were then sealed with the parafilm and were subjected to Freeze-Thaw cycles. The Freeze-Thaw cycle consisted of freezing at -27 ℃, thawing at room temperature, and setting inside a refrigerator. The time table for the Freeze-Thaw cycle can be seen in Table 1.

Table 1 **Freeze- Thaw cycle of PVA Throughout a Week**

Day of the Week	Function 1 (9 am)	Function 2 (5 pm)
Wednesday	Creation/Stasis in Fridge	Freeze
Thursday	Thaw	Freeze
Friday	Thaw	Stasis in Fridge
Saturday/Sunday	Stasis in Fridge	Stasis in Fridge
Monday	Stretch/Freeze	Thaw
Tuesday	Stretch/Freeze	Stasis in Fridge

After two Freeze-Thaw cycles, the gelled PVA was removed from the two rectangular boxes and strips stretched to induce anisotropy. One box of PVA was cut into three equal strips of 22 mm x 95 mm while the other box stayed as a single 65 mm x 95 mm strip. Paper towel strips were cut into 45 mm x 20 mm and 45 mm x 63 mm strips. Pencil markings were made 2.5 mm away from the center, as seen in Fig. 1.

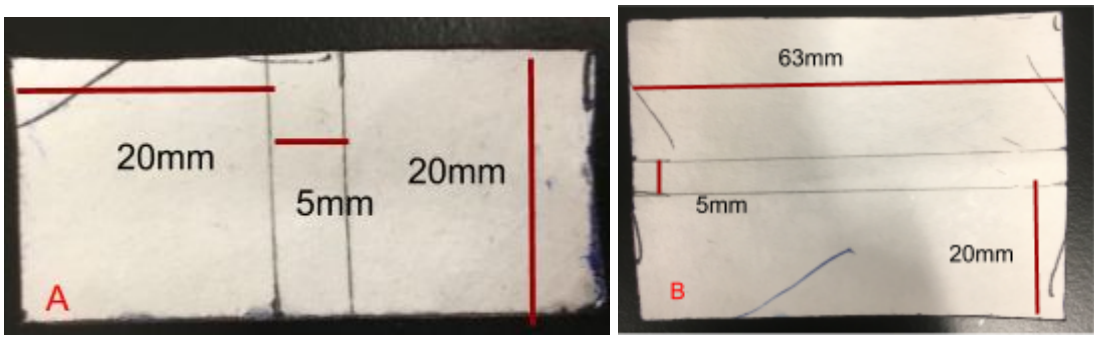


Fig 1. **A/B Paper Towel cut outs.**

These paper towel cut outs were wrapped around the edges of the PVA strips and placed on custom stretchers depending on their sizes, as portrayed in Fig. 2.

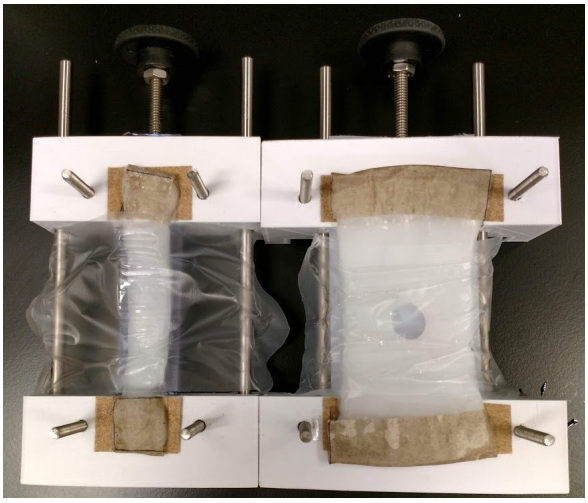


Fig.2 Custom PVA Stretchers developed in Bayly Lab during Summer 2018

The PVA samples were clamped down with screws on the stretcher without tearing the edges. The paper towel was wrapped around the PVA edges to ensure that the PVA would not slip when the PVA was stretched. The PVA samples would be stretched to 180%, going from 5 cm to 9 cm. These values were measured from the edges of both stretchers where the PVA hangs in the air, as seen in Fig. 3.

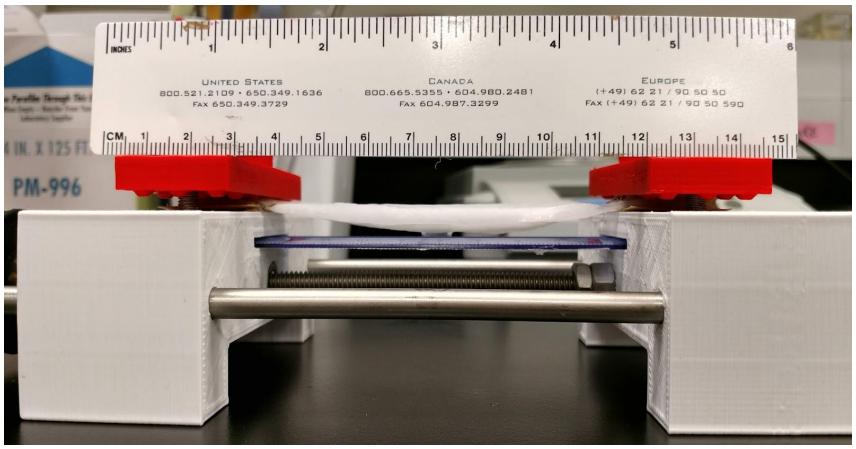


Fig. 3 PVA Stretcher Portrayal

Before and after stretching, the PVA samples were rehydrated with 1-2 mL of DI water and wrapped with parafilm. Then the stretchers were placed in the freezer for two additional Freeze-Thaw cycles. If the samples were not able to stretch to the full 180% before the third Freeze-Thaw cycle, the sample was stretched to 160%, which goes from 5cm to 8cm before the third Freeze-Thaw cycle, and then the remaining 20% before the 4th Freeze-Thaw cycle. At the end of four Freeze-Thaw cycles, there would be three narrow (~16 mm wide) samples, (dubbed narrow body samples), one large sample (dubbed wide body sample), and one control (unstretched) sample inside the petri dish.

2.2 DST Methodology

To prepare samples for the DST, a ⅝" (~15 mm) dia. punch was used to create cylindrical DST samples. At least one sample was punched from each type of gel. When a DST sample was punched from narrow or wide stretched sample, a permanent marker was used to indicate the orientation the PVA was stretched in. When a DST sample was placed in the DST tester (Figure 4), it was tested in two orientations: i) with the sample placed so that the direction of shear displacement is parallel to the direction of stretch and ii) where the sample is rotated by 90 deg so that the direction of shear displacement is perpendicular to the direction of stretch. These are referred to as "parallel" and " perpendicular" orientations in the remainder of this report. For some stretched DST samples, the order of the tests were reversed (i.e. perpendicular then parallel). For control samples, there was no particular orientation, but the sample was rotated 90 deg after the first and re-tested. These are referred to as the "first" and "second" test.

The testing procedures were from Alexa Panrudkevich's adaptation of Jake Ireland's 2017 Dynamic Shear Testing (DST) Procedure document. An Excel spreadsheet designed for PVA data collection was used to track the amount of compression and force that is induced on the PVA samples. A laptop computer with Data Physics SignalCalc Software was used to record the data that the DST induces on the PVA samples. A custom built DST machine induces the aforementioned compression and force on the PVA samples. The DST schematic can be seen in Fig. 4 and the exact specifications for the DST are detailed in the 2011 Okamoto paper, *Viscoelastic properties of soft gels: comparison of magnetic resonance elastography and dynamic shear testing in the shear wave regime*[3] .

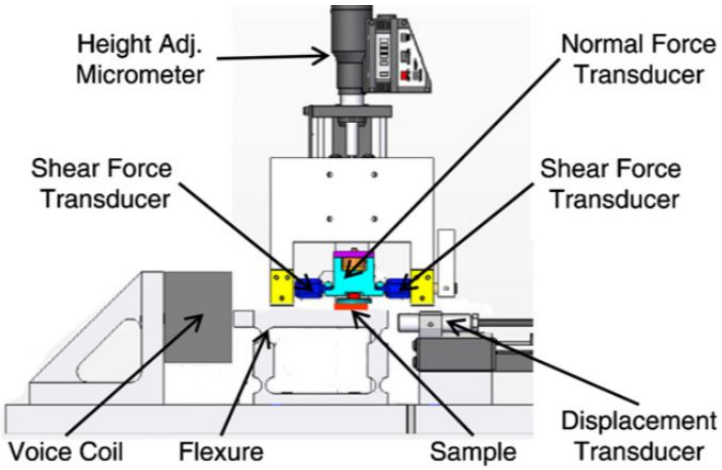


Figure 4 DST Setup from the 2011 Okamoto Paper

To calibrate the DST machine, a steel cylinder with 9.6 g, 18 mm dia., and 5.4 mm thickness was used to measure the force and amount of compression at 4 values: not touching, almost touching, just touching (slight voltage drop), and touching with 1V drop. These values were recorded in the custom Excel sheet, as well as the PVA's creation data, test date, initial mass, diameter, thickness, and time of testing. Then, the SignalCalc software created a .trf file

that stored all the DST data and a pretest without any PVA samples was taken as Trial 1. The SignalCalc software will ask for confirmation three times, as seen in Fig 5.

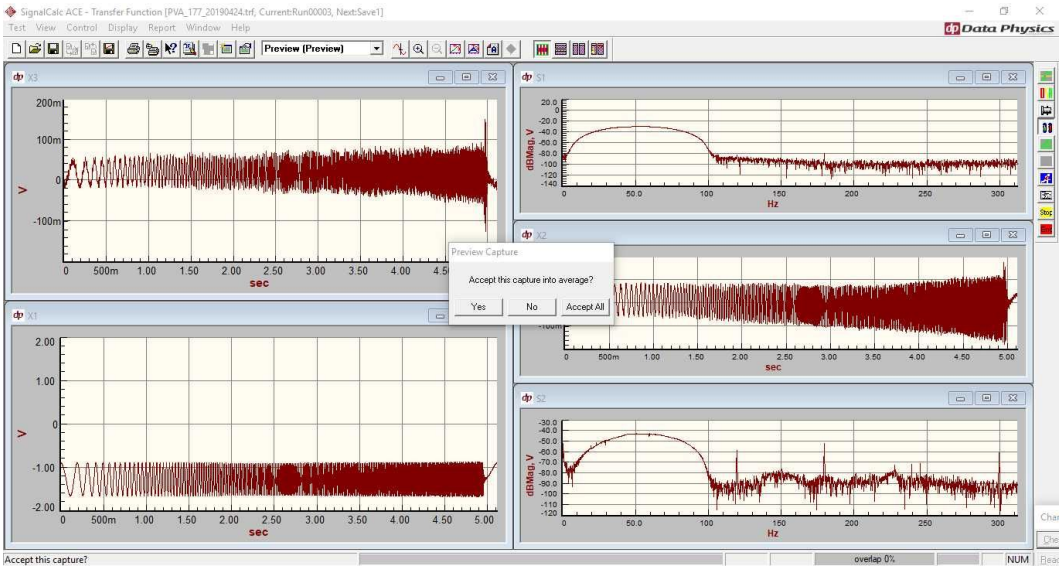


Figure 5 Accepted Trial Shapes for the Pretest

The absolute value of compression and voltage force was recorded using the DST's micrometer and voltmeter. When the PVA sample was inserted into the DST machine in either parallel or perpendicular orientation, a new Check-if Touching .syn file was created and used to test the PVA sample to ensure that the top of the PVA sample was in sufficient contact with the testing apparatus. The PVA was in contact with the DST when it displays sinusoidal wave patterns in the Check-if Touching test, as seen in Fig. 6.

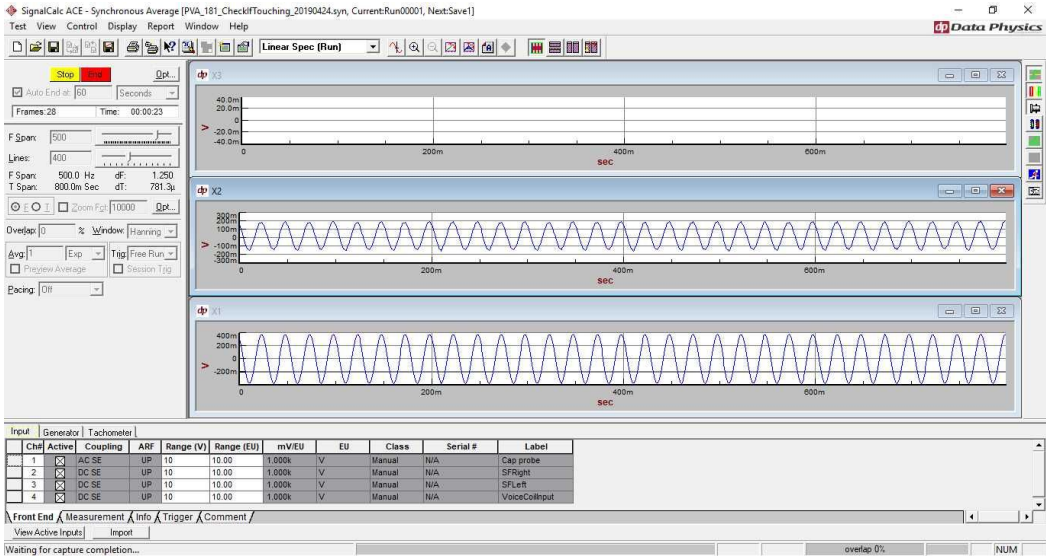


Figure 6 Sine Wave Patterns in Check-if-Touching Test

The absolute compression value and voltage force were recorded in the Excel document. The Excel sheet then produced a prediction of the PVA sample's mass based on the Check-if Touching value, a calculation based on density and volume, and the recorded mass. All three

mass calculations are expected to be within 10% of each other, and if the Check-if Touching weight was not within 10% change, the Check-if Touching test was performed again.

Next, the micrometer was changed from the absolute to the relative setting, which read 0.00 to indicate 0% compression. With all the previous information recorded, the Excel sheet calculated the needed relative micrometer value so the PVA sample would be in -3.0%, 0%, 3.0%, 6.0%, 9.0%, and 12% compression. As such, the micrometer was turned to the -3.0% compression micrometer value, the voltage force values was recorded in the excel sheet, and Trial 2 data was taken. This was repeated until the 12% compression was taken. The PVA was then removed from the DST and its orientation was rotated 90° to either Parallel or Perpendicular depending on whichever orientation it started with. The micrometer was once more set to -3.0%, 0%, 3.0%, 6.0%, 9.0%, and 12% compression with voltage forces recorded, and trials taken until 12% compression was complete. By this step, there were 13 Trials and the final trial was taken as a post-test without the PVA sample in the DST. Like the pre-test, the voltage force and abs micrometer value was recorded in the Excel sheet, along with the final mass of the PVA. When the final 14th Trial was finished, all .trf, Excel, and .syn files were saved.

This testing procedure was repeated for that same stretched PVA sample, but the sample would start with the orientation that was taken after the 7th trial. For example, if the PVA started in parallel orientation and was switched to perpendicular orientation after Trial 7 in the first test, the PVA would start at perpendicular orientation and switch to parallel orientation after Trial 7 in the second test. If the PVA sample was from a control sample, only 1 set of trials was conducted.

2.3 DST Data Analysis

After a PVA sample has undergone testing, a custom Matlab script written by former Bayly lab member Tally P. was used to analyze the .trf file and Excel spreadsheet. The Matlab script would be changed if the sample was taken parallel first, perpendicular first, or if it was unstretched. When the Matlab script finished analyzing the .trf files and Excel spreadsheets, a graph was produced that summarized the results for the sample (Fig. 7). This graph plots a Storage Modulus, which takes calculations on stress and strain that the PVA experiences. This is further discussed in the section 4.1.

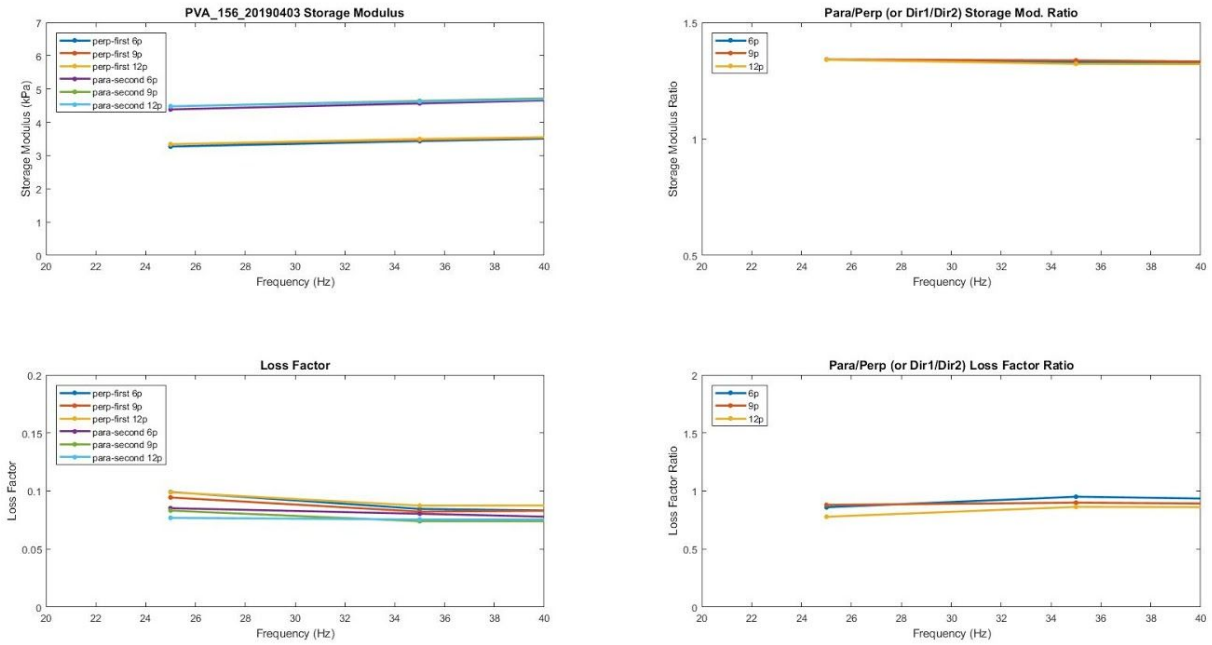


Figure 7 Storage Modulus, Storage Modulus Ratio, Loss Factor, & Loss Factor Ratio of PVA Sample 156 (Anisotropic Para/Perp)

3. Results

3.1 Overview of Samples Created and Tested.

Despite the fact that there was data taken before the new Freeze/Thaw cycle, there were many disparities in the data used, producing only 4 usable tests out of the 9 tests conducted. Furthermore, to keep uniformity, only the samples made with the new PVA cycle will be analyzed. With the new PVA batches made in the Freeze/Thaw cycle from February 6th to April 24th, 60 tests were conducted and information such as storage modulus, storage modulus ratio, etc.were compiled in an Excel summary spreadsheet designed for the lab. 56 tests were found to not have any procedural or testing errors. This breaks down to 11 narrow body PVA gels tested in Perpendicular/Parallel and Parallel/Perpendicular orientation, 9 wide body PVA gels tested in Perpendicular/Parallel and Parallel/Perpendicular orientation, 13 control PVA gels, 6 PVA gels tested in Parallel/Perpendicular orientation, and 1 PVA gel tested in Perpendicular/Parallel orientation. This totals to 40 physical gels made and tested.

3.2 Overall Shear Modulus and Anisotropy.

Figure 8 and Fig. 9 each present a bar graph of the mean values of all 56 accepted storage modulus and storage modulus ratios, where n is the number of samples in Control, Parallel/ Perpendicular, and Perpendicular/Parallel orientation. These graphs use the data found when the PVA was tested in Parallel at 12% compression because 12% compression was the compression value with the most guaranteed contact. Table 2 and Table 3 each present the quantity of, minimum, maximum, mean, and standard deviation of the 56 Control,

Parallel/Perpendicular and Perpendicular/Parallel PVA samples, for the storage modulus and storage modulus ratio. These table values also used the data found when the PVA was tested at 12% compression.

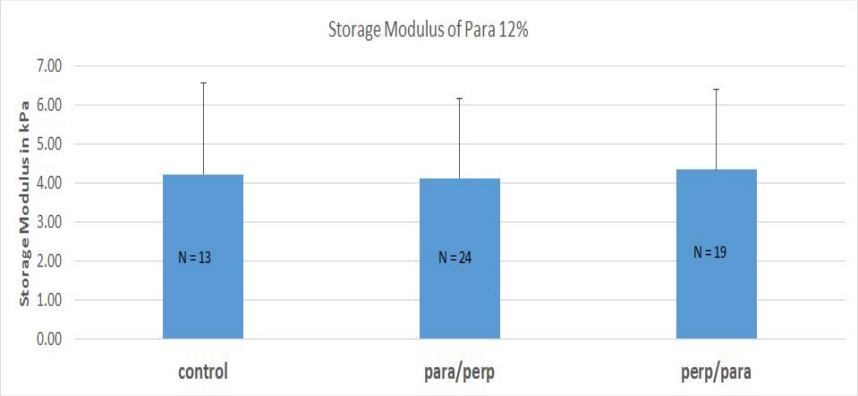


Figure 8 **Mean Storage Modulus**

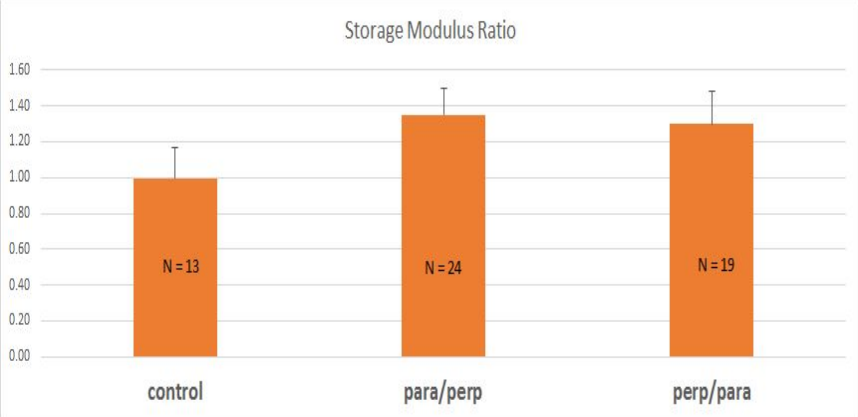


Figure 9 **Mean Storage Modulus Ratio**

Table 2 Minimum, Maximum, Mean, and Standard Deviation of Storage Modulus (kPa)

	Control	**Test order: Parallel / Perpendicular**	**Test order: Perpendicular/Parallel**
Mean Shear Modulus in Parallel Orientation	4.24	4.14	4.36
Standard Deviation	2.36	2.04	2.04
Min	1.89	2.34	2.52
Max	10.12	10.83	10.52

Table 3 Minimum, Maximum, Mean, and Standard Deviation of the Storage Modulus Ratio

	Control	Parallel / Perpendicular	Perpendicular/Parallel
Mean	1.00	1.35	1.30
Standard Deviation	0.17	.15	.18
Min	.65	1.00	.98
Max	1.24	1.72	1.67

4. Discussion and Conclusions

4.1 Shear Modulus and Shear Modulus Ratio in DST

In Fig 7., the graphs display the storage modulus and the storage modulus ratio as the DST passes through 20-40 Hz. In general, the storage modulus measures how stiff the PVA sample is. This data utilizes the force that the DST applies to the PVA and the surface area calculated from the Excel Spreadsheet and calculates the stress that the PVA experiences at some frequency. To add, the % compression and changes in length of the PVA would be used to find the strain at some frequency. Using Hooke's Law, the storage modulus (also known as Young's Modulus), is calculated as a ratio of stress vs strain.

The storage modulus ratio is a ratio of the storage modulus of the PVA when it is in parallel orientation vs. when it is in perpendicular orientation. Having this ratio presents a clear indicator of how anisotropic a sample is, because the closer a sample is to 1, the more isotropic the sample is, and values less than or greater than 1 indicate how anisotropic the sample is.

4.2 Shear Modulus and its Variability

In the top left graphs of the sets produced by Matlab, the storage modulus data for first 6%, 9% 12% and second 6%, 9% 12% compression ratios are expected to demonstrate consistent grouping and should increase sequentially with respect to each other, which Fig.7 demonstrates. As seen by the mean storage moduli for the Control, Parallel/Perpendicular, and Perpendicular/Parallel samples in Table 2, the elastic modulus of most PVA samples are near the 4.14 kPa- 4.36 kPA range.

It was found that there were many variables that change how stiff the PVA samples can get. One such example arose from testing PVA samples. A PVA sample gets stiffer with each successive test due to water loss. In addition, the orientation order that a PVA sample is tested in changes how the storage modulus is analyzed. For example, it is expected that the storage modulus of a PVA sample tested in the Parallel orientation is stiffer than when it is tested in the Perpendicular orientation. These two factors of successive testing and testing in Perpendicular and then Parallel skew how stiff a PVA sample appears. Furthermore, the process of finding the

thickness of the sample by observing shear forces during the Check-if Touching stage of the testing procedure was not always consistent with the thickness of the sample measured with calipers, or the thickness of the sample estimated from the sample mass. If a sample is compressed more than the accepted 10% range, there is also a general increase in the storage modulus. Finally, it was found that the longer a sample stays inside the fridge, the more the sample dries out. As a result, there is an increases in stiffness of the sample.

4.3 Shear Modulus Ration and its Variability

Historically, the storage modulus ratio of stretched PVA samples range from 1.3 to 1.5. However, throughout this semester, the storage modulus has reached a max of 1.72 and 1.67 when the PVA was tested in Parallel/Perpendicular and Perpendicular/Parallel respectively. There are many factors that contributed to this increase.

The first of which was the change in the Freeze/Thaw cycle procedure. Previously, the Freeze/Thaw cycle was a complex timetable of entering the freezer, room temperature and fridge every 4 hours. This timetable did not produce any significant increases to the storage modulus ratio. Thus, the Freeze/Thaw cycle was simplified to the timetable seen in Table 1.

A second change was in the implementation of using a full 180% stretch in the beginning of the third Freeze/Thaw cycle. In the previous version of the BaylyLab recipe, PVA was stretched 160% before the third Freeze/Thaw cycle and another 20% before the fourth Freeze/Thaw cycle. By fully stretching the PVA samples in the third Freeze/Thaw cycle, the PVA samples experienced a general increase in anisotropy.

Finally, Dr. Okamoto made remarks on how hydration could play a part in increasing how anisotropic the PVA samples can be. To test this theory, samples were rehydrated before and after the stretching process during the third Freeze/Thaw cycle by adding an extra 1.0 mL of DI water to the PVA surface.. Then, a new wrapping method to retain water was devised, where parafilm was wrapped underneath and on top of the stretched PVA. A comparison between the new and old wrapping methods can be seen in Appendix A. Initial testing suggests that this idea of hydration retention does increase the storage modulus ratio. However, further testing must be done to confirm this.

4.3 Limitations and Sources of Errors

I. PVA Preparation

Sources of errors do exist and as an indicator, the control samples should not have any degree of anisotropy (storage modulus ratios below or above 1.0) and PVA should not have storage modulus values above 4 kPa. A production error includes how most of the time, the control sample's petri dish does not contain exactly 30 g. Instead, the Control petri dish often has 22-25 g of PVA. This may affect the sample during the testing phase due to being thinner than usual.

II. DST Testing

An inherent testing error is how many PVA samples do not have a flat top surface. As a result of this, a testing error is created where the Check-if Touching process in the testing phase will be flawed. The Check-if Touching test may say the PVA is touching the DST when in reality, only a small portion of the PVA is touching. This leads to the 0% compression of the PVA not being full in contact with the top surface that is touching the DST and not experiencing the full horizontal displacements induced by the DST.

III. Human Error

There have been a few cases where data was accidentally not saved, the Check-if Touching was not updated, or where the sample weight was not recorded. This results in insufficient data needed to make an actual analysis on the PVA. As such, the tests with human errors make up the 4 out of 54 unusable test, whereas the production and testing errors are included in the 50 usable tests. Due to these preparation and testing errors, there are some inconsistencies in the PVA's storage modulus, which is an ongoing issue that is being investigated.

4.4 Conclusion

There are many factors in creating, stretching, and testing the PVA to consistently match the mechanical properties of brain tissue. With regards to the storage modulus of the PVA, the water content inside the PVA proved to be a significant component. The water loss due to consecutive testing or drying from staying in the refrigerator for too long increased the storage modulus. For the storage modulus ratio, there are a few more key elements. When the PVA was stretched 180% before the third Freeze/Thaw cycle, the storage modulus ratio appears to be higher compared to when the PVA was stretched 160% before the third Freeze/Thaw cycle and another 20% before the fourth Freeze/Thaw cycle. From is, it it can be confirmed that there is anisotropy in the PVA that has undergone Freeze/Thaw cycles, and stretching. Nonetheless, the PVA creation, and testing procedures can still be refined to make these PVA be more and more similar to brain tissue.

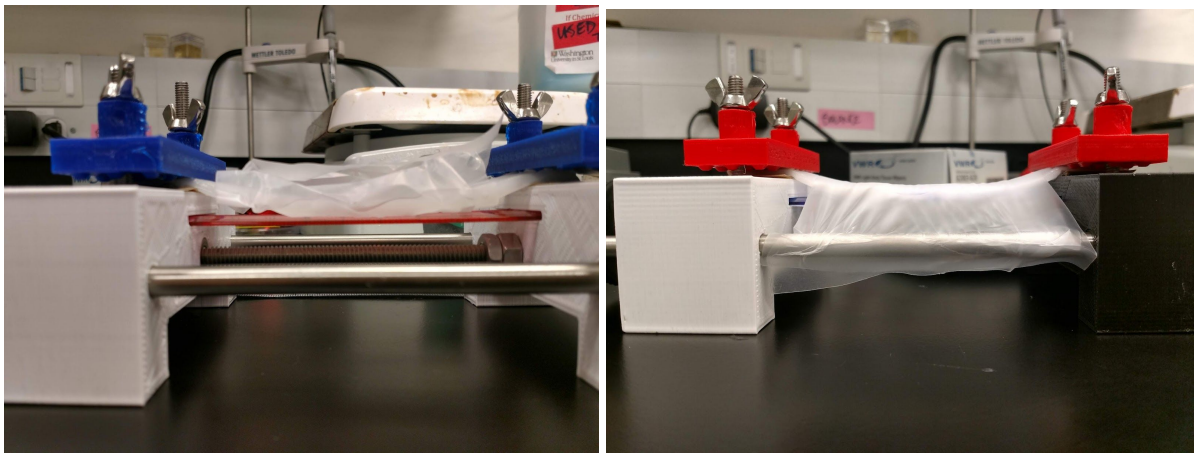
References

[1] Hudson S. 2011 *Structure and Strength: Anisotropic Polyvinyl Alcohol Hydrogels and Spider Mite Silk Fibres* Western Graduate & Postdoctural Studies

[2] Chatelin S. , Bernal M, Deffiux T, Papadacci C, Flaud P, Nahas A , Boccara C, Gennisson J-L, Tanter M, and Pernot M 2014 *Anisotropic polyvinyl alcohol hydrogel phantom for shear wave elastography in fibrous biological soft tissue: a multimodality characterization* IOP Publishing

[3] Okamoto R.J, Clayton E.H and Bayly P.V 2011 *Viscoelastic properties of soft gels: comparison of magnetic resonance elastography and dynamic shear testing in the shear wave regime* IOP Publishing

Appendix A



PVA Comparison between Old Wrapping Method (Left) and New Wrapping Method (Right)