Design of Brain Tumor Phantoms Replicating the Elasticity of Gliomas

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Revised October 31, 2013

Abstract

It is hypothesized that by finding the stiffness of gliomas in the brain, cryogel tumor phantoms can be created which match the elastic properties of gliomas. Magnetic resonance elastography (MRE) is one method that can be used to identify tumors in the brain. Tumor phantoms can be used to help uncover potential weaknesses in these medical imaging systems.[3] Cryogels prepared by the freeze-thaw technique have shown to mimic the mechanical properties of soft tissues and have been extensively used as phantom materials. The effects of varying freeze-thaw cycles and properties of various polymer mixtures in solution were researched in order to produce cryogel phantoms that could model tumor stiffness, compressive Young's modulus(E), and shear modulus(G).

Keywords: brain tumor phantom; glioma elasticity; Young's modulus

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I. Introduction

I.A. Problem Statement

Brain cancer has become one of the top 5 causes of cancer-related deaths in people under the age of 39. Nearly 700,000 people were living with a primary brain tumor diagnosis in 2010, increasing at a rate of about 60,000 people per year. Further, gliomas account for 30% of all brain tumors and 80% of all malignant tumors.[1] A glioma is a type of brain tumor that comes from glial cells. With survival rates near 50% for people between 20-44 years of age decreasing to nearly 5% for those over 65, improvement is needed in order to better diagnose these tumors and treat them in the most appropriate way based on their properties.[2]

Magnetic resonance elastography (MRE) is a new technique used to create images representing the elastic properties of tissue. Recently, it has found potential use in brain tissue imaging. These images can be used to determine the tissue makeup of the brain by differentiating grey matter, white matter, and tumor tissue. Phantoms are important tools for the development and optimization of diagnostic techniques. In order to help uncover potential weaknesses or inaccuracies in the results from MRE imaging, tumor phantoms can be created for use as assessment tools.[3] By using test subjects with known stiffness values, the reliability of in vivo detection methods can be increased.

I.B. Literature Review

The main focus of this project was to design a phantom that replicated the elastic properties of a brain glioma. Gliomas are typically

classified as low or high grade, with high grade gliomas being at least 10 kPa higher in stiffness than low grade gliomas.[4] The main variables in elasticity are the compressive Young's modulus, E, and the shear modulus, G. The relationship between these two variables is shown in Equation 1 below. For this purpose, Poisson's ratio, ν, was taken to be equal to about 0.47 for brain tissue, as it is almost incompressible.[3]

$$E = 2G * (1 + \nu) \quad \text{[Eq. 1]}$$

Based on the research conducted to find typical tumor stiffness, Table 1 below was constructed outlining the ranges found. The average stiffness value was found to range between 30 to 50 kPa, with some low grade tumors having a stiffness of less than 10 kPa. This suggests that some tumors were softer than brain tissue.[26] Low grade tumors are more likely to be benign and high grade are more likely to be malignant. Tumors are classified by grade based on a number of properties including tumor structure and growth pattern.

Table 1: Tumor Stiffness

Modulus (E)	Source
Low Grade: ~35 kPa High Grade: ~50 kPa	Biomechanical modeling of tumor classification and growth[5]
About 30 kPa	Nonlinear elastic registration of brain images with tumor pathology using a biomechanical model[6]
30-40 kPa	Biomechanical modeling of tumor growth: its relevance to glioma research[7]
<15 kPa	Viscoelastic properties of human cerebellum using magnetic resonance elastography [26]

I.B.1. Magnetic Resonance Elastography (MRE)

MRE uses imaging to measure tissue elasticity by gently shaking the tissue, inducing shear waves. A vibration actuator is put in contact with the test subject, which sends transverse acoustic wave vibrations of a known frequency through the tissue.[8] The resulting shear waves are imaged using Magnetic Resonance Imaging (MRI) technology. These images can be used to determine the resultant particle displacement.[9] Elasticity, or shear stiffness, of the tissue can then be calculated using the recorded wavelength of the vibrations.[10]

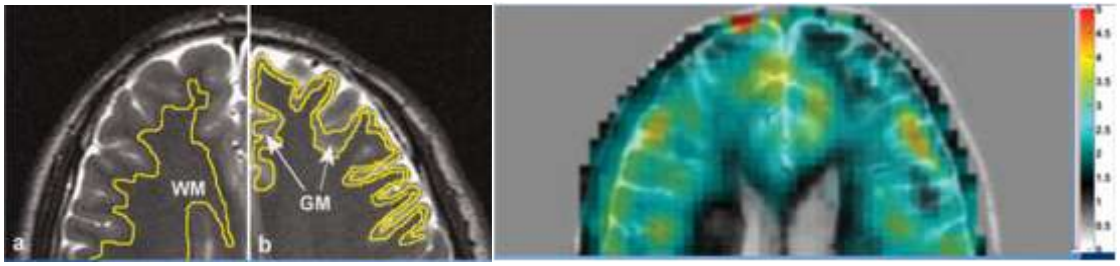


Figure 1: Images showing the boundaries of white and grey matter and the elastic shear modulus (in kPa) for white matter and grey matter from MRE.[11]

I.B.2. Materials

Different polymer combinations were considered for use in this project. All of these mixtures were predominantly composed of polyvinyl alcohol (PVA). PVA is a stable substance; it does not have very high health risks or hazards identified, which makes it easy to work with.[8] It is the most widely used phantom material in medical imaging. One combination that was considered in hopes of replicating high grade tumors elasticity was PVA with alginate (PVA/ALG). Alginate is a naturally occurring polysaccharide that has been widely used in hydrogels for tissue engineering, and is derived from brown seaweed.[12] Another formulation used PVA with polyethylene oxide (PVA/PEO) to replicate low grade tumors stiffness. PEO can be an irritant, but it is also a stable and fairly harmless substance. PEO is a biocompatible polymer which is also widely used in the biomedical field.[13] A third combination used PVA with chitosan (PVA/CHIT) to mimic high grade tumors. Chitosan is very hazardous in the case of eye or skin contact. Goggles and gloves were worn during all handling of this substance.[14] Chitosan has properties which help with rapid blood clotting, so it is used in many homeostatic agents. Chitosan is also a cationic polysaccharide widely used with PVA hydrogels.[15] The molecular structures of these materials are provided in Figure 2.

PVA with polyacrylic acid (PAA) and PVA with aragose were also tested in hopes of reaching high grade elasticity since chitosan proved difficult to work with. These materials were readily available in the lab, and their use in previous testing had shown promise to obtain the desired results. Previous testing done in the labs at Miami University has shown that these materials greatly increase the stiffness of a PVA solution after just one freeze-thaw cycle (FTC). However, previous experimentation did not utilize sonication, where a vibration is applied to the solution to increase consistency and rid it of air bubbles; therefore results were not as precise as desired. Further, we improved upon previous research by lowering the percent composition of agarose and PAA in the solutions in order to target a stiffness of 30-50 kPa after more than one FTC. This was to aid in

easier insertion into a brain model and to allow for a wider range of possible stiffness's that could be obtained among FTCs.

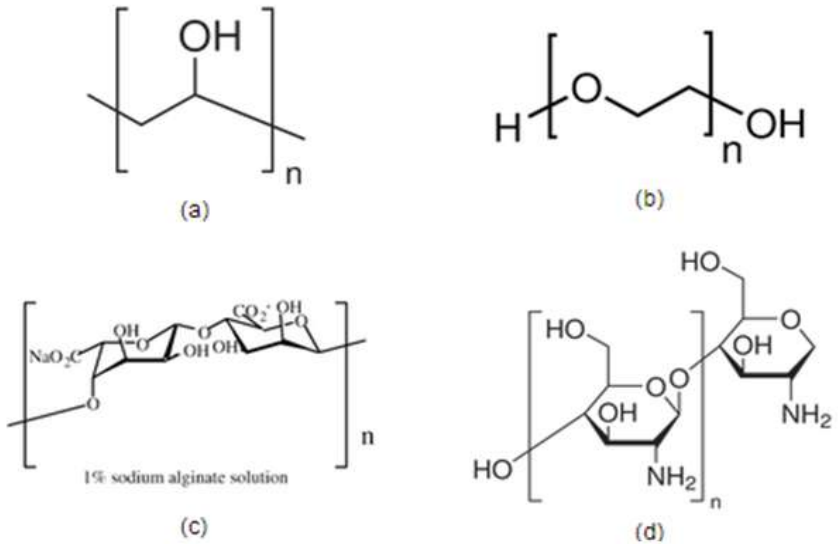


Figure 2: Molecular structures of (a) PVA[14], (b) PEO[16], (c) ALG[17], and (d) CHIT[18].

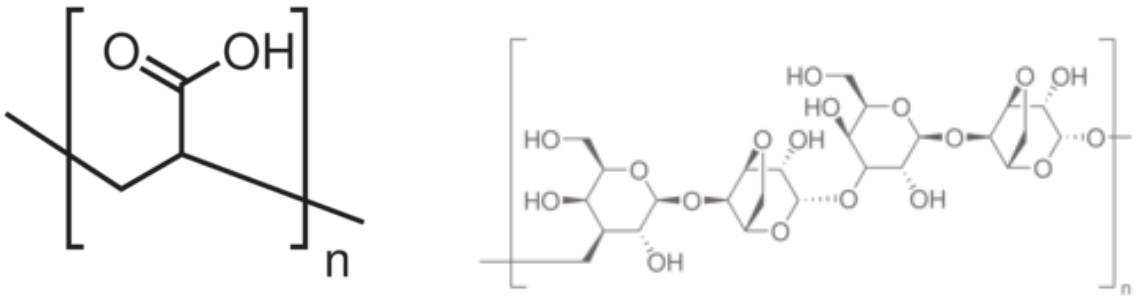


Figure 3: Molecular structure of (a) PAA[19] and (b) agarose[20].

Literature review yielded the following information about possible cryogel stiffness values outlined in Table 2. Although these studies may have been conducted for different applications, and most did not show stiffness ranges within the range desired to replicate glioma stiffness, these results were used to choose the composition of cryogels that could potentially lead to the stiffness ranges targeted in this study.

Table 2: Cryogel Properties

Source	Polymers	Conc. in H_20	FTCs	Modulus (E)	Fig.

A Semi-Degradable Composite Scaffold for Articular Cartilage Defects[22]	PVA/ALG (100 wt% of PVA added of ALG microspheres)	10% PVA 20% PVA	- -	40±10 kPa 140±20 kPa	(4)
Development of Chitosan and Poly (Vinyl Alcohol) Blended Scaffolds for Cell Culture using Supercritical Fluids Technology[24]	PVA/CHIT	100% CHIT 90%CHIT/10%PVA 75%CHIT/25%PVA 50%CHIT/50%PVA	- - - -	4.3 MPa 2.8 MPa 2.6 MPa 1.6 MPa	
Investigation of PVA/ws-chitosan hydrogels prepared by combined irradiation and freeze-thawing[25]	PVA/CHIT	7wt% PVA 3wt%CHIT 1wt%CHIT	1	 ~15-21 kPa ~33-36 kPa	(5)

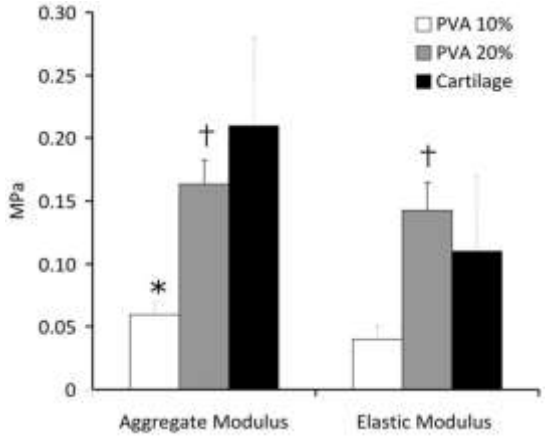


Figure 4: Young's Modulus of various PVA/ALG cryogels[22]

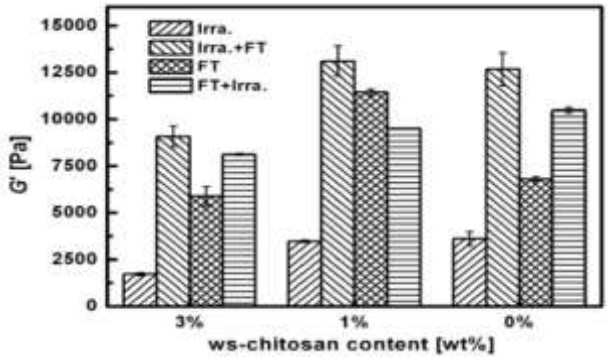


Figure 5: Storage Modulus of PVA/chitosan cryogels[25]

In the case of PVA/PEO blends, studies were found relating their compositions to their crystallinity but not their stiffness. It has been shown that the addition of PEO to PVA would reduce the overall crystallinity of the polymer blend.[21] It is known that as crystallinity is reduced, so is stiffness. This means that higher concentrations of PEO in a PVA/PEO blend would reduce the stiffness of the resultant hydrogel/cryogel.

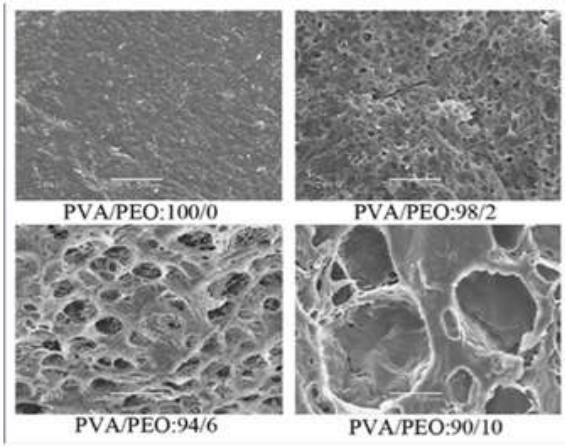


Figure 6: Images showing the effect of increasing PEO concentration in PVA/PEO cryogels.[21]

Based on the properties of these materials, PEO should decrease the stiffness of PVA, and the other materials should increase the stiffness of PVA. Therefore, the goal was to create a low grade tumor phantom using PVA/PEO and a high grade phantom using PVA with either CHIT, ALG, PAA, or agarose.

I.B.3. Freeze Thaw Cycles (FTCs)

To create the cryogels that were used for the study, polymer combinations are mixed together in water to create a solution. By subjecting the solution to freezing temperatures for a certain length of time (freeze-thaw cycles) they become cryogels and gain durable properties. In addition, the ability of a cryogel to resist stress (compression and shear) increases when exposed to multiple freeze-thaw cycles. This allows for some control over the physical properties of the gels. The change in properties is typically more pronounced at up to 12 FTCs. Using more than this number of cycles was shown to induce only minor changes in stiffness. A figure representing a typical freeze thaw cycle and its effects on a gel can be seen in Figures 7 and 8.

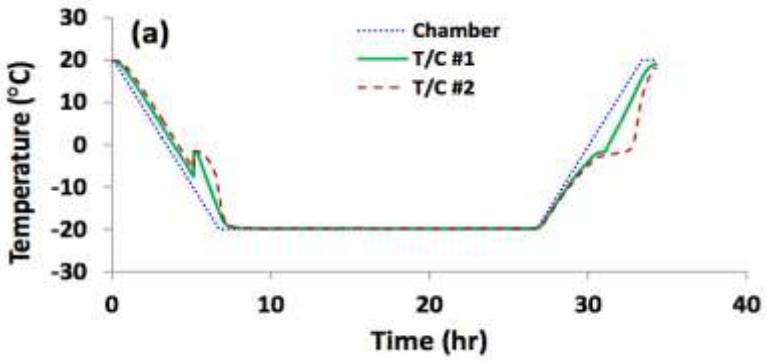


Figure 7: An example freeze-thaw cycle for a large aluminum mold. It shows how the environmental chamber regulates its internal temperature over time.[3]

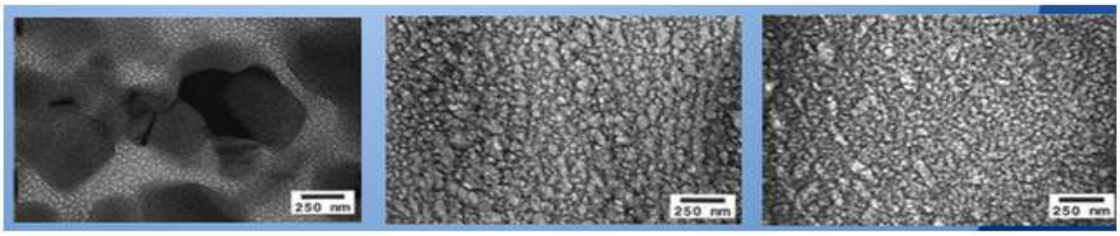


Figure 8: Effects of increased numbers of freeze-thaw cycles (1, 12, and 24 respectively) on PVA gels.[21]

I.B.4. Physical Crosslinking

The crosslinking process due to freeze-thaw cycles was described by Nihal Engin Vrana in his paper on cryogelation of PVA for tissue engineering:

> "Cryogelation is one of the methods of physical hydrogel formation. These gels are formed through processes which force formation of non-covalent bonds such as hydrogen bonds, ionic bonds or by basic entanglement of the polymeric chains and crystallites after freezing and thawing cycles (Figure 9). Most of these gels are reversible gels due to these factors. The gels are very beneficial in the sense that there is no need for addition of any chemical crosslinker or application of UV light, which in some cases cause cytotoxicity and problems due to the remnant chemicals. Cryogels form under moderate freezing conditions in which frozen solvent causes phase separation and acts as a porogen, leading to a gel with high water content. Gelation can occur in each of the three steps of the freeze-thawing process; freezing, storage in frozen state or during thawing. For PVA the most important step is thawing, since this is where most of the gel formation occurs. One of the main aspects of cryogelation is that not all of the solvent freezes under these conditions and there is always a portion of the solvent in the liquid phase. The surface tension between the thawed solvent and the gel phase causes round pores. The conversion between spongy and non-spongy cryogels depends on the freezing regime and the concentration and composition of solute. The physical gelation occurs through formation of a three dimensional non-covalent bond structure, either hydrogen bonds or

hydrophobic interactions depending on the nature of the solutes. The degree of hydrolysis is important for PVA, since a high level of presence of acetyl groups on the chain causes inhibition of bond formation. In order to measure some of the effect of crosslinking on samples, swell testing can be done to measure a swell ratio. The swell ratio is a measure of degree of crosslinking in the gel phase. A relatively low swell ratio indicates greater crosslinking and a more tightly bound structure. High swell ratios indicate that there was a low degree of crosslinking in the gel.[23] This relationship is rather intuitive because the tighter the structure is bound, or the greater the crosslinking, the less space there will be for water to enter the gel."[30]

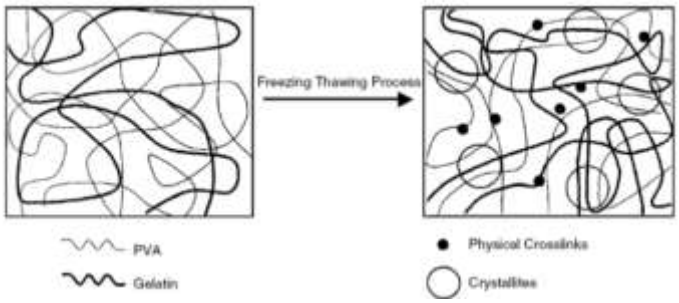


Figure 9: Cryogelation process, Gel formation via entanglement, hydrogen bonding and formation of crystallites.[30]

Of significant importance is the idea that the physical changes in the polymer mixture during freeze-thaw helps promote hydrogen bonding or ionic bonding between polymer strands. These bonds give the gels their particular mechanical properties (ex. stiffness, deformation resistance, and swelling).

I.C. Objectives

The main aim was to create a tumor phantom model that had a range of elastic properties similar to that of a high grade glioma and another which had a range similar to that of a low grade glioma in the brain. Different ratios of polymers were tested in a solution of water. In order to find a mixture that could imitate the elastic properties of both low and high grade tumors, the number and length of freeze-thaw cycles that gels were exposed to was to be determined.

II. Design

II.A. Method

A series of cryogel mixtures prepared over the two semesters were tested. The compositions of the mixtures were based on literature search. Specifically, the polymer mixtures (and ratios) in amounts

that would put the mixture in the elasticity range of gliomas were tested.

In order to prepare the cryogels, the polymers were mixed (by percent weight) together with a constant volume of deionized water. This mixture was then subjected to 85°C for 3 hours to promote proper mixing. Aluminum molds were used to reduce the thermal resistance during the freeze-thaw cycle [Fig. 9]. The prepared solution was then sonicated for 20 min (QSonica S4000), and then poured into the molds (diameter vs. height: 20mm x 4mm). The setup was then subjected to one to ten freeze-thaw cycles (FTC) inside an environmental chamber (CSZ ZPS-16, OH). The prepared gels were maintained in the refrigerator at 4°C inside water and were to be characterized within 24 hours after fabrication. The swelling measurements were performed immediately after the freeze-thaw cycle.[3]

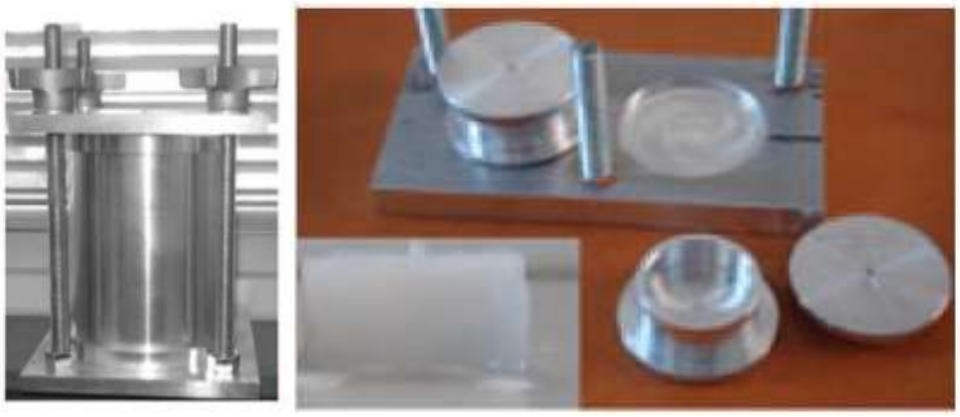


Figure 10: A cylindrical aluminum mold and smaller aluminum molds for tumor phantoms.[3]

An example of a standard profile of the freeze thaw cycles applied to our samples is shown in Figure 11 with a rate of temperature change of 1°C per minute. Samples were held at -20°C for 20 hours for 2 freeze thaw cycles. Cycles were varied to obtain different results for certain samples. The specific cycles used for each sample are described with their respective results.

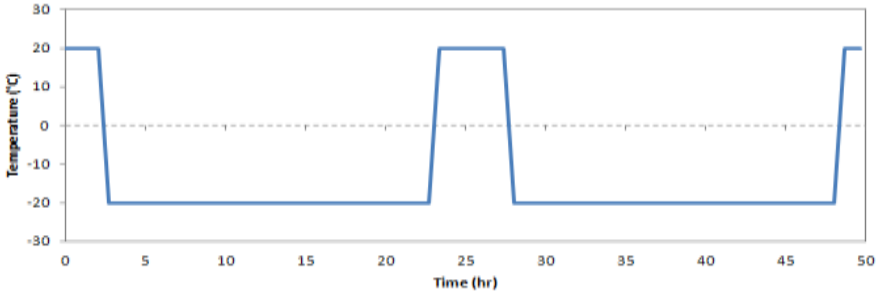


Figure 11: Freeze thaw cycle profile applied to PVA/PAA samples.

The cryogels were put through mechanical testing to determine their elasticity. The samples were tested via Instron compression under controlled displacement (5% strain) within 4 hours of the end of the last FTC. The test consisted of a 50 second ramp-strain phase and a 10 minute stress-relaxation phase. Specifically, the test was conducted to estimate the Young's Modulus (E) of the cryogels. If necessary, these results were then used to modify our polymer composition ratios and numbers of freeze-thaw cycles to better match the tumor properties. A typical example of the resulting stress vs. strain graph is shown in Figure 12.

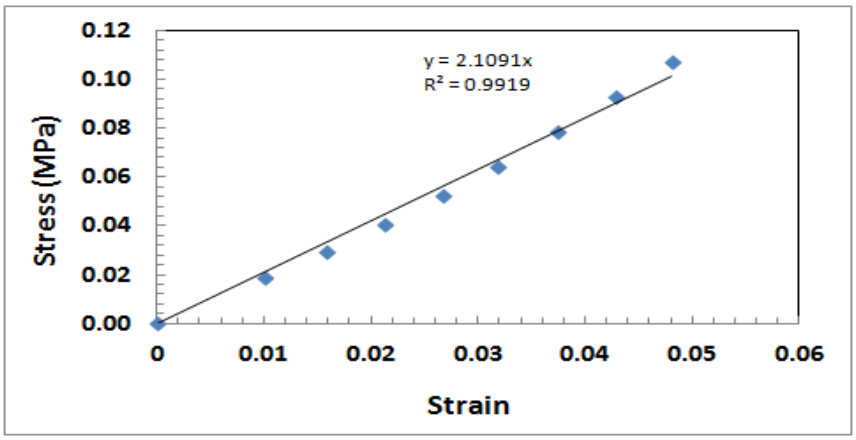


Figure 12: Stress vs. Strain graph where slope is equal to the elastic modulus (2.1091MPa)

For the PVA/PEO gels, a mixture containing 5g PEO (MW 10,000) and 95g PVA (MW 146,000) in 900mL water was used to form a 10% w/w polymer solution (0.5% PEO in dry powder). For the PVA/CHIT gels, 300mL of 10% PVA solution, which was previously prepared, was mixed with 200mL of a 0.5% solution of chitosan (MW 190,000-310,000) in 0.2 M acetic acid. This formed a 6.2% w/w polymer solution (0.2% CHIT in dry powder). For the PVA/PAA gels undergoing two or three 20 hour FTCs, a mixture containing 2.5g PAA (MV 3,000,000) and 50g PVA in 450mL of water was used forming a 10.5% w/w polymer solution (0.5% PAA in dry powder). For the PVA/PAA gels undergoing between five and nine 5 hour FTCs, a mixture containing 0.5g PAA and 49.5g PVA in 450mL of water was used. This resulted in a 10% w/w polymer solution (0.1% PAA in dry powder). For the PVA/agarose gels undergoing ten 5 hour FTCs, a mixture containing 1g PAA and 49g PVA in 450mL of water was used, forming a 10% w/w polymer solution (0.2% agarose in dry powder).

Swell tests were performed by placing gels in water and weighing them each day to measure the water uptake by the gel. The resulting swell ratio was calculated by subtracting the daily weights from the initial

weight, then dividing this value by the initial weight. The swell ratio was then graphed to further assess how the properties of the gel may change over time.

II.B. Constraints

Based on the information found on each material to be used (see Materials), none of them were exceedingly dangerous, making them fairly safe to handle. The process to make the material did not present any great dangers other than to be aware of the temperature of the items while heating. The only constraint in the design was in material selection. The polymers had to be water-soluble in order to be prepared in the molds and there is not a large variety of water-soluble polymers. PVA with PEO, chitosan, and alginate were the best water-soluble polymer options that had not yet been tested at the department, and thus were pursued. Since some difficulties were encountered with CHIT, it was decided that other water-soluble polymers that have already been tested at the department should be used. PAA and agarose are two such materials. Experiments were run with these materials in hopes of achieving the desired results, minimizing some of the inconsistencies from the previous work, and to tailor the formulation to achieve our desired stiffness results.

III. Impact

In evaluating the impact of this design, the global, economic, environmental, and societal facets were considered. Our design had a medium impact globally because such models could be used globally as assessment tools to help improve MRE accuracy. The economic impact was low, although money could be made off of this type of model in the medical technology field if MRE machines require frequent assessment. There was very little environmental impact from our design; however, there could have been high societal impact. By making a tumor phantom that would help to eliminate the weaknesses of the MRE technology, brain tumors could be detected more accurately. The design could help to improve brain cancer treatment so that tumors could be removed in the most appropriate manner.

Table 3: Impact Statement Table

	Impact Statement				
	5 (very high)	4 (high)	3 (medium)	2 (low)	1 (very low)
Global			X		
Economic				X	
Environmental					X
Societal		X			

IV. Results & Discussion

IV.A. PVA/PEO Phantoms

For the first set of FTCs, the samples were held at -20°C for 20 hours, as described in the Methods section. This resulted in an average stiffness of 28.0 kPa after 2 FTC, which was slightly higher than desired. Also, it appeared from the results that the standard deviation was higher than desired, indicating that the homogeneity of the solution needed to be improved. To remedy this, the FTCs used were held at -20°C for only 5 hours in hopes of reducing the stiffness after 2 cycles to fit the low range. The PVA/PEO solution was reheated and sonicated to help promote more thorough mixing. This method was effective and a sample plot and results can be seen below. All of the plots and the results from the first set of samples can be seen in Appendix A of this report.

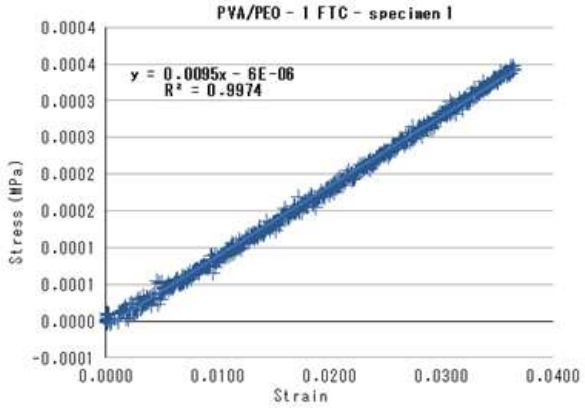


Figure 13: Stress vs. strain plot for PVA/PEO gel held at -20°C for 5 hours.

The slopes from each of these plots were then taken to find the Young's Modulus (in kPa) for each sample. These values can be seen in Table 4.

Table 4: Young's Modulus (kPa) found for PVA/PEO cryogels held at -20°C for 5 hours.

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Average**	**Std. Dev.**
1 FTC	9.5	7.5	6.6	-	7.87	1.48
2 FTC	22.6	14.7	24.3	23.0	21.15	4.36

IV.B. PVA/CHIT Phantoms

For this set of samples, the FTCs performed were held at -20°C for 20 hours, as described in the Methods section. Mixing of this solution was difficult, so a lower than desired CHIT concentration had to be used to improve CHIT dissolving. Prior to mechanical testing, visual inspection of the gels revealed that the chitosan in some gels was distributed inconsistently. Additionally, there were air pockets trapped within some of the gels. The gels under less FTCs were noted to have less consistency in their stiffness's than gels exposed to more FTCs. These inconsistencies in the results can be attributed to the polymer inconsistencies. When preparing the gels, it was noted that the PVA and Chitosan would stratify in solution rapidly. While the solution was mixed as thoroughly as possible prior to introduction to the environmental chamber, it is possible that the polymers separated in the molds before freezing. All of the plots and the results from these samples can be seen in Appendix B of this report.

Table 5: Young's Modulus (kPa) found for PVA/CHIT cryogels held at -20°C for 5 hours

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Average**	**Std. Dev.**
2 FTC	15.8	11.8	29.3	-	19.0	9.2
3 FTC	17.6	17.5	-	12.4	15.8	3.0

Chitosan is a polymer that requires acidic conditions to properly dissolve. Such polymers have reduced mechanical strength because of their affinity for water.[27] Water accumulates around the polymer strands and isolates them from other strands, preventing crosslinking and ideal crystallization while also causing the formation of pores. Normally, PVA alleviates this by assisting chitosan strands in crosslinking.[27] The stratification of the polymers suggests that the PVA was not interacting properly with the chitosan to enable crosslinking with itself. It was also noted that there were many bubbles and air pockets, which could have been caused by water pores retained in the gel. This can explain the low mechanical properties (lower than PVA/PEO) for the chitosan gels.

IV.C. PVA/PAA Phantoms

For the first set of samples, the FTCs performed were held at -20°C for 20 hours, as described in the methods section. For 2 FTC gels, aside from one outlier sample (2), the stiffness's ranged from ~11 to ~14 kPa. For the 3 FTC gels, barring sample 4 as an outlier, the

stiffness's ranged from ~24 to ~ 35 kPa. Standard deviations were above 10 for these tests. This can be attributed to the two outliers. In the second set of testing, more FTCs (5 & 9 total) were used and altered to be held at -20˚C for only 5 hours. The resultant gels were very watery when removed from the environmental chamber, and were found to have very low stiffness values. Additionally, the gels themselves were very fragile, and pieces would break off the gel if handled roughly. The results for these gels were found to be much lower than the initial PVA/PAA gels. This batch of gels had been made with a smaller concentration of PAA than the initial two batches (0.1% PAA as opposed to 0.5% PAA), accounting for the change. All of the plots and the results from these samples can be seen in Appendix C of this report.

Table 6: Young's Modulus (kPa) found for PVA/PAA cryogels held at -20˚C for 20 hours and 5 hours.

	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Average**	**Std. Dev.**
2 FTC (20hr)	12.4	35.7*outlier	14.7	11.3	12.8	1.7
3 FTC (20hr)	33.7	24.6	35.9	10.6*outlier	31.4	6.0
5 FTC (5hr)	4.4	11	-	-	7.7	-
9 FTC (5hr)	-	-	4.2	3.9	4.1	-

PAA is a hydrophilic polymer[28], which means it seeks out and creates hydrogen bonds with water molecules. As a result, like the chitosan gels, it is more difficult for PAA gels to crosslink, so the gels become porous. Hydrophilic polymers increase swelling[28], which is inversely related to crystallinity. Addition of PAA reduces the crystallinity of the PVA/PAA gels. This explains the results obtained for the two and three FTC PVA/PAA samples, which had reduced mechanical properties in comparison with pure PVA and PEO/PVA gels. This also explains the results for the five and nine FTC gels. These gels were less stiff than the initial two trials. The decrease in stiffness can be partially attributed to the (cumulative) less time spent in freezing temperatures (25 and 45 hours compared to 40 and 60 hours). These gels also had similar troubles as the chitosan gels; they had stratified polymer regions. These inconsistencies in stiffness could account for the brittle behavior. When gels were handled and fractured, they would break into granules as opposed to tears in the gel, which could suggest that the polymers separated into

globules within the mixture prior to freezing. In regards to both formulations of PAA/PVA, it has been noted that PAA only ionizes in acidic conditions.[29] The ability of PAA to form ionic crosslinks with itself depends on the pH of the environment.[29] For all of the PAA/PVA gels, the solution was prepared under pH neutral conditions. As a result, the reduced stiffness's of the PAA/PVA gels may be due to the lack of crosslinking between PAA strands that did not completely ionize.

If the pH of the media is closer to the pKa value of PAA, which is reported as 4.25, it results in reduced ionization of PAA and less swelling.[29] In future work with PAA, if gels are produced under acidic conditions, the mechanical properties of the gel will likely improve. It will promote PAA crosslinking and mixture with PVA, which means that there will be more crosslinking, and more homogeneity. This may help to produce a gel that reaches the higher end of the target stiffness values.

IV.D. PVA/Agarose Phantoms

For this set of samples, the FTCs used were held at -20°C for 5 hours. The gels were found to follow viscoelastic stress-strain behaviors. Initially, stress and strain increased linearly with a relatively low slope. However, at a certain point of strain, the stress increased suddenly and continued growing linearly with a greater slope. In order to account for this behavior, the Young's modulus values were calculated using a smaller set of points. The gels' stress was sampled from 2% to 3% strain, and that slope is used as the stiffness value of the gel.
A sample Stress vs. Strain Plot can be seen in Figure 14 and Young's Modulus values in Table 7. All of the plots and the results from these samples can be seen in Appendix D of this report.

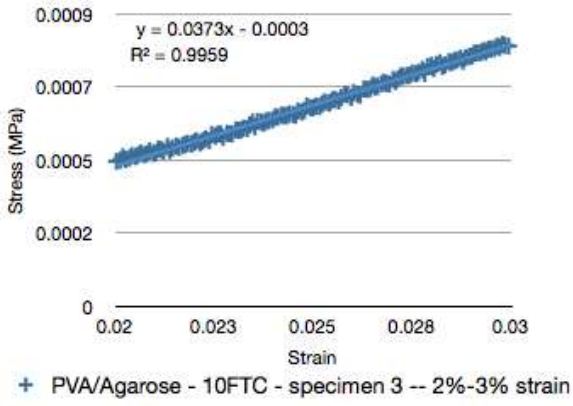


Figure 14: Stress vs. strain plot for PVA/agarose gel undergoing 10-5 hour FTCs.

Table 7: Young's Modulus (kPa) found for PVA/agarose cryogels.

	Sample 1	Sample 2	Sample 3	Sample 4	Average	Std. Dev.
10 FTC	39.7	17.6*	35.0	37.3	37.33	2.35

*outlier

IV.E. Pure PVA Control

Pure PVA samples were prepared so that differences in stiffness achieved from adding different polymers could be seen. A 10% PVA solution was prepared with 10 FTCs, each held at -20°C for 5 hours, so a comparison could be made between PVA and the PVA/agarose samples. Young's Modulus values obtained can be seen below in Table 8.

Table 8: Young's Modulus (kPa) found for pure PVA cryogels.

	Sample 1	Sample 2	Sample 3	Sample 4	Average	Std. Dev.
1 FTC	9.6	15.4	15.9	-	13.6	3.5
10 FTC	45.2	24.4	34.5	32.4	34.1	8.6

IV.F. Swell Testing

Swell testing was conducted on PVA/PEO and PVA/agarose specimens over 7 days of time. Results for these tests are shown below in Table 9 and Figure 15. Data could not be collected on weekends, accounting for the gaps in the data.

Table 9: Swell Testing Results-Swell Percentage

Day	1	2	3	4	5	6	7
PVA/Agarose	66.48%	79.09%	93.19%	97.10%	-	-	95.61%
PVA/PEO	90.44%	116.10%	115.80%	-	-	117.46%	118.96%

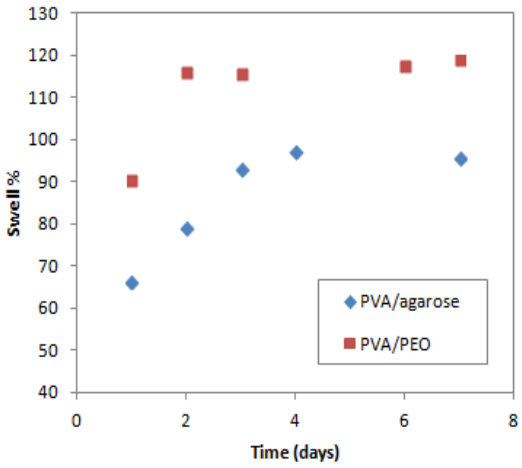


Figure 15: Swell testing results over time

V. Conclusions

Based on the elasticity values found in research for different PVA mixtures, it appears that the hypothesis was correct. A cryogel phantom can be created that will have elastic properties within the range of values found in literature review for both low and high grade glioma.

Using pure PVA as a standard, the qualities of the secondary polymer components can be compared. Pure PVA after 1 FTC averaged 13.6kPA stiffness. The PVA/PEO polymer, after 1 FTC, had an average stiffness of 7.87 kPa. This further suggests that the addition of PEO reduces PVA copolymer mechanical properties[21]. Thus it was concluded that, for low grade gliomas, a 10%PVA/0.5%PEO phantom effectively met the actual stiffness range from the literature of less than 15 kPa when held for one freeze thaw cycle for 5 hours at -20°C. This formulation was easy to prepare and can be used in a brain phantom to assess the ability MRE technology to assess low grade gliomas in the brain.

The Agarose/PVA polymer after 10 FTCs has a higher stiffness than the 10FTC pure PVA gels. By adding 0.2% agarose, the stiffness increased from an average ~34kPa to an average ~37kPa. However, the standard deviation of the PVA gels closes this gap, which means that, statistically, it is not possible to say that the Agarose improved the gel properties (confirmed by student's t-test), although the results were more reproducible than those of pure PVA gels. From these results, it was concluded that a 9.8%PVA/0.2%agarose solution undergoing ten 5 hour FTCs can meet the stiffness range of 30-50kPa for a high grade glioma, as found in some of the literature sources. In the future higher concentrations of Agarose in Agarose/PVA gels can

be created to produce gels that approximate the highest high grade tumor stiffness (~50 kPa). It should be noted that, due to its large, phenyl-based, structure, high concentrations of Agarose will begin to reduce mechanical properties. As such, there can be future experiments to maximize gel stiffness in relation to Agarose concentration. These experiments can help determine the best PVA/Agarose concentration ratio to reach the highest stiffness values in the reported tumor stiffness range. In addition, there needs to be future work with PVA/PAA gels. There is still potential in their use, but future experiments need to formulate them under acidic conditions to see if it would be possible to produce more consistent, stiffer gels.

Swell testing further supported conclusions we drew from the data and our initial predictions for the results. Swell testing results for the PVA/PEO gels indicated a higher swell percentage than the PVA/agarose gels. Lower swell data indicates a higher degree of crosslinking, which was to be expected in the agarose gel that was made to obtain a higher stiffness. In comparison, the PEO gel was intended to be less stiff.

In the future, others working on preparing the brain phantom will have to further research ways of inserting this tumor phantom into the brain phantom for the low grade phantoms requiring only one freeze thaw cycle. We believe that either using some type of membrane to hold the gels in their places while they freeze or injection could be used to accomplish this.

References

[1] "Brain Tumor Facts." American Brain Tumor Association. American Brain Tumor Association, Mar. 2012. Web. 18 Feb. 2013. <http://www.abta.org/news/brain-tumor-fact-sheets/>.

[2] "Brain Tumor Facts & Statistics." San Diego Brain Tumor Foundation. San Diego Brain Tumor Foundation, 2011. Web. 18 Feb. 2013. <http://www.sdbtf.org/facts-bout-bt.html>.

[3] Minton, J. A., Iravani, A., & Yousefi, A. (2012). Improving the homogeneity of tissue-mimicking cryogel phantoms, 39(11), 1–12.

[4] Manuscript, A. (2011). NIH Public Access, 23(5), 497–511. doi:10.1002/ca.21006.MAGNETIC

[5] Drapaca, C. S., & Palocaren, A. J. (2010). BIOMECHANICAL MODELING OF TUMOR CLASSIFICATION AND GROWTH, 115–124.

[6] Kyriacou, S. K., Davatzikos, C., Zinreich, S. J., & Bryan, R. N. (1999). Nonlinear elastic registration of brain images with tumor

pathology using a biomechanical model. IEEE transactions on medical imaging, 18(7), 580–92. doi:10.1109/42.790458

[7] Palocaren, A. J., & Drapaca, C. S. (2012). Biomechanical modeling of tumor growth: its relevance to glioma research, 3(1), 94–108.

[8] Manduca A, Oliphant TE, Dresner MA, Mahowald JL, Kruse SA, Amromin E, Felmlee JP, Greenleaf JF, Ehman RL. Magnetic resonance elastography: non-invasive mapping tissue of elasticity. Med Imag Anal. 5:237-254, 2001. http://digitalcommons.mcmaster.ca/opendissertations/5091

[9] Saeed, Farukh, "Magnetic Resonance Elastography" (2011). Open Access Dissertations and Theses. Paper 5091.

[10] Definition of Magnetic resonance elastography. (2012, June 16). Retrieved October 3, 2012, from Medicine Net website: http://www.medterms.com/script/main/art.asp?articlekey=25364

[11] Green, M. A., Bilston, L. E., & Sinkus, R. (2008). In vivo brain viscoelastic properties measured by magnetic resonance elastography, (May), 755–764. doi:10.1002/nbm

[12] Chhatri, A., Bajpai, J., Bajpai, a. K., Sandhu, S. S., Jain, N., & Biswas, J. (2011). Cryogenic fabrication of savlon loaded macroporous blends of alginate and polyvinyl alcohol (PVA). Swelling, deswelling and antibacterial behaviors. Carbohydrate Polymers, 83(2), 876–882. doi:10.1016/j.carbpol.2010.08.077

[13] Zhe Lian, Lin Ye, Structure and properties of PVA/PEO hydrogel prepared by freeze/thawing method, Journal of Thermoplastic Composite Materials, December 2011, DOI: 10.1177/0892705711430857,

[14] Poly(vinyl alcohol). (2012). Retrieved October 22, 2012, from Sigma-Aldrich website:http://www.sigmaaldrich.com/catalog/product/fluka/81368?lang=en®ion=US

[15] Liu, Y., Vrana, N. E., Cahill, P. a, & McGuinness, G. B. (2009). Physically crosslinked composite hydrogels of PVA with natural macromolecules: structure, mechanical properties, and endothelial cell compatibility. Journal of biomedical materials research. Part B, Applied biomaterials, 90(2), 492–502. doi:10.1002/jbm.b.31310

[16] Poly(ethylene oxide). (2012). Retrieved October 22, 2012, from Sigma-Aldrich website:http://www.sigmaaldrich.com/catalog/product/aldrich/181986?lang=en®ion=US

[17] Jon A. Rowley, Gerard Madlambayan, David J. Mooney, Alginate hydrogels as synthetic extracellular matrix materials, Biomaterials, Volume 20, Issue 1, January 1999, Pages 45-53, ISSN 0142-9612, 10.1016/S0142-9612(98)00107-0.

[18] Chitosan. (2012). Retrieved October 22, 2012, from Sigma-Aldrich website:

http://www.sigmaaldrich.com/catalog/product/aldrich/448869?lang=en®ion=US

[19] Poly(acrylic acid). (2012). Retrieved February 18, 2013, from Sigma-Aldrich website: http://www.sigmaaldrich.com/catalog/product/aldrich/323667?lang=en&region=US

[20] Agarose. (2012). Retrieved March 25, 2013, from Sigma-Aldrich website:

http://www.sigmaaldrich.com/catalog/product/sigma/a9539?lang=en&region=US

[21] Willcox, P. J., Howie, D. W., Schmidt-Rohr, K., Hoagland, D. A., Gido, S. P., Pudjijanto, S., Kleiner, L. W. and Venkatraman, S. (1999), Microstructure of poly(vinyl alcohol) hydrogels produced by freeze/thaw cycling. J. Polym. Sci. B Polym. Phys., 37: 3438–3454. doi: 10.1002/(SICI)1099-0488(19991215)37:24<3438::AID-POLB6>3.0.CO;2-9

[22] Scholten, P. M., Ng, K. W., John, K., Serino, L. P., Warren, R. F., Torzilli, P. A., & Maher, S. A. (2012). A Semi-Degradable Composite Scaffold for Articular Cartilage Defects. NIH Public Access, (212).

[23] Plastics, C. E. (n.d.). Standard Test Methods for Determination of Gel Content and Swell Ratio of, 09.

[24] Marina, L., & Silva, C. (2008). Development of Chitosan and Poly (Vinyl Alcohol) Blended Scaffolds for Cell Culture using Supercritical Fluids Technology.

[25] Yang, X., Liu, Q., Chen, X., Yu, F., & Zhu, Z. (2008). Investigation of PVA/ws-chitosan hydrogels prepared by combined γ-irradiation and freeze-thawing. Carbohydrate Polymers, 73(3), 401–408. doi:10.1016/j.carbpol.2007.12.008

[26] Zhang, J., Green, M. a, Sinkus, R., & Bilston, L. E. (2011). Viscoelastic properties of human cerebellum using magnetic resonance elastography. Journal of biomechanics, 44(10), 1909–13. doi:10.1016/j.jbiomech.2011.04.034

[27] Wang, Tao, Mahir Turhan, and Sundaram Gunasekaran. "Selected properties of pH-sensitive, biodegradable chitosan–poly(vinyl alcohol) hydrogel." Polymer International. 53.7 (2004): 911-918. Web. 15 Apr. 2013.

[28] Berger, J., M. Reist, et al. "Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications." European Journal of Pharmaceutics and Biopharmaceutics. 57.1 (2004): 19-34. Web. 15 Apr. 2013.

[29] McGann, Michael, Clement Higginbotham, et al. "The synthesis of novel pH-sensitive poly(vinyl alcohol) composite hydrogels using a freeze/thaw process for biomedical applications." International Journal of Pharmaceutics. 372.1-2 (2009): 154-161. Web. 16 Apr. 2013.

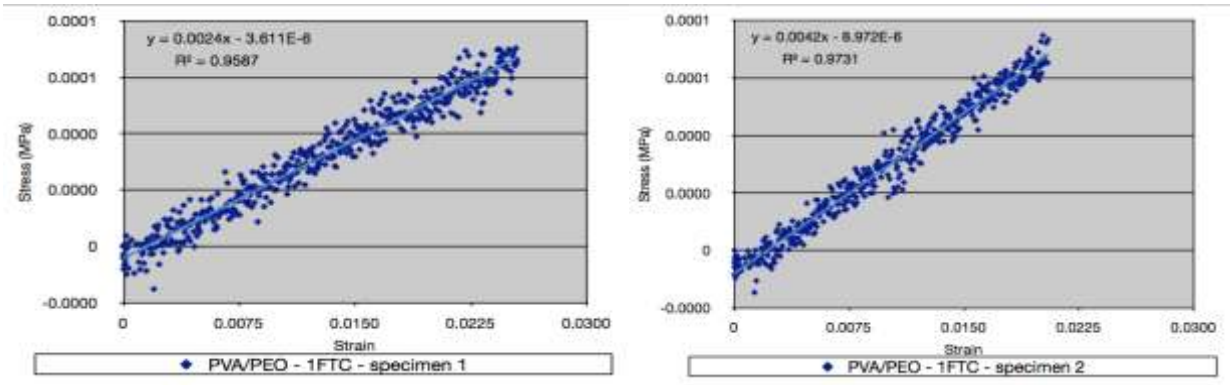
[30] Use of Poly Vinyl Alcohol (PVA) Cryogelation for Tissue Engineering: Composites , Scaffold Formation and Cell Encapsulation Nihal Engin Vrana (M . Sc . BTech) Use of PVA Cryogelation for Tissue Engineering : Composites , Scaffold Formation and Cell. (2009).

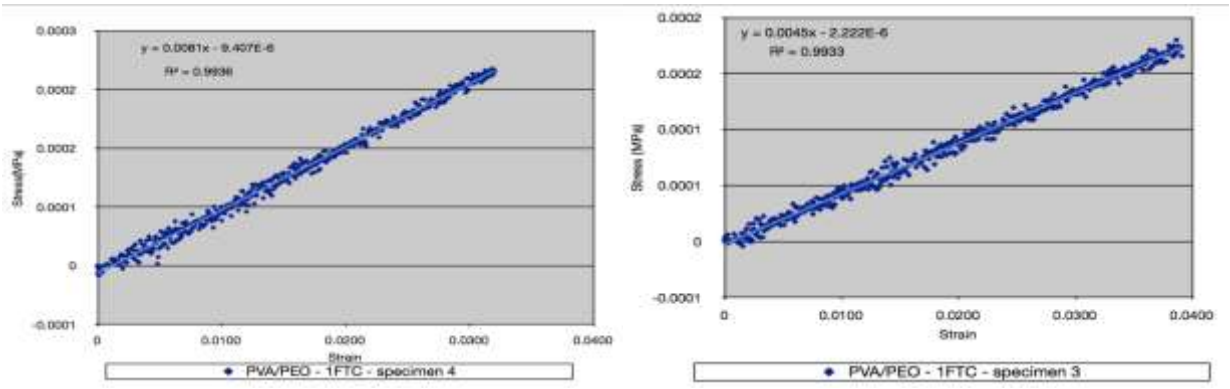
Appendix A: Stress vs. Strain Plots for PVA/PEO

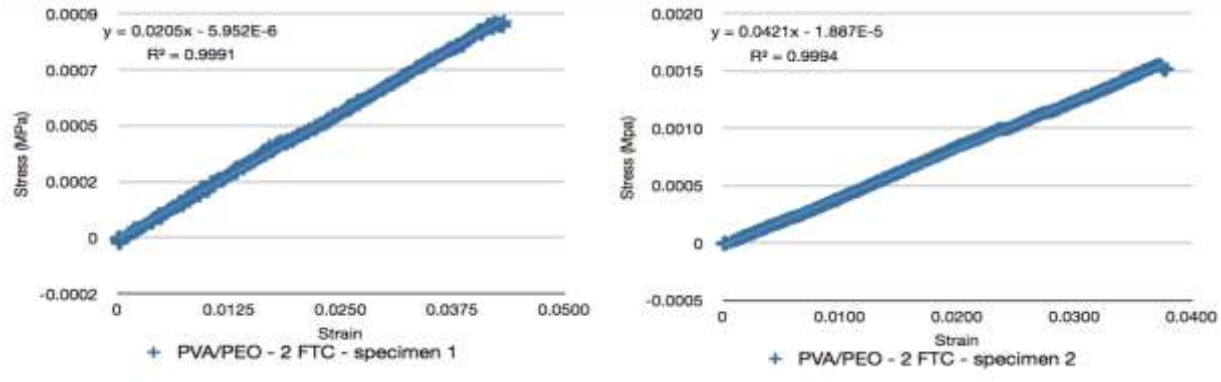
Table A.1: Young's Modulus (kPa) found for PVA/PEO cryogels held at -20°C for 20 hours.

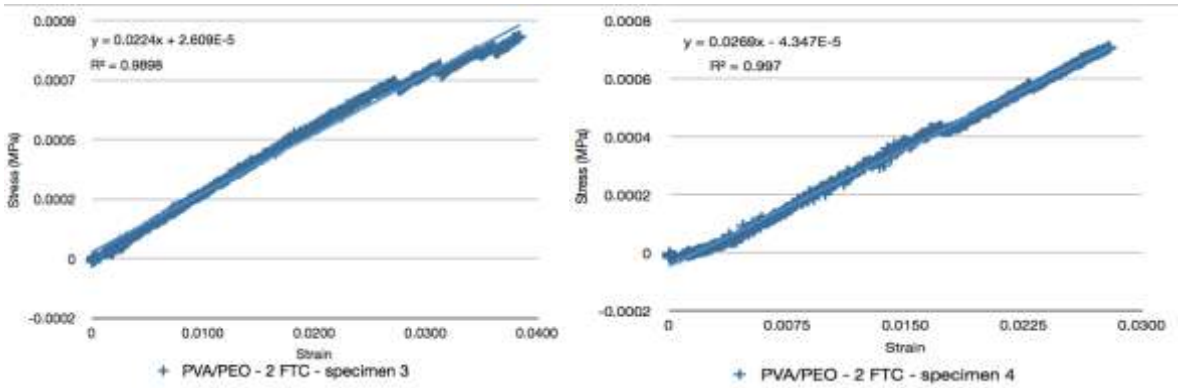
	Sample 1	Sample 2	Sample 3	Sample 4	Average	Std. Dev.
1 FTC	2.4	4.2	4.5	8.1	4.8	2.4
2 FTC	20.5	42.1	22.4	26.9	28.0	9.79

A.1: Plots for PVA/PEO held at -20°C for 20 hours

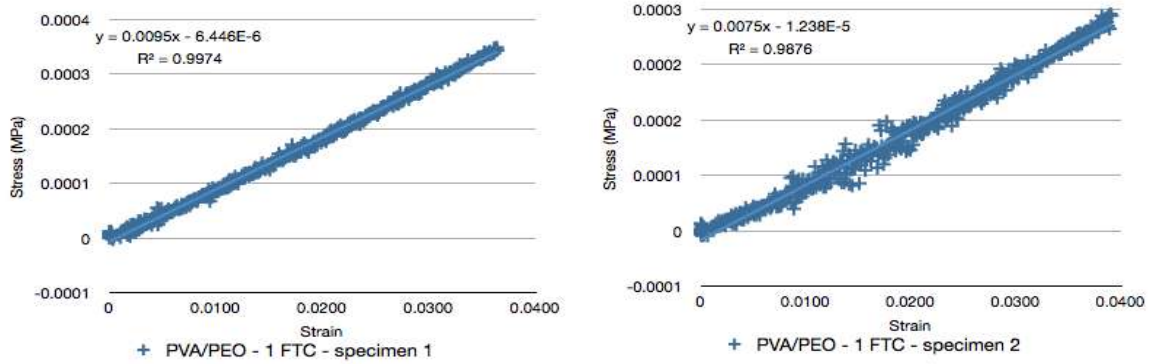


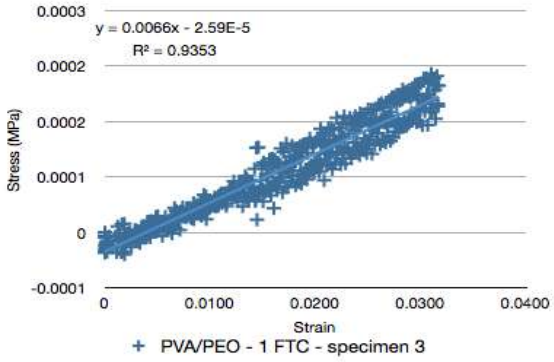


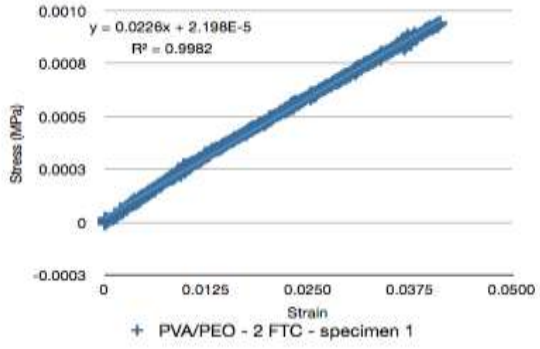


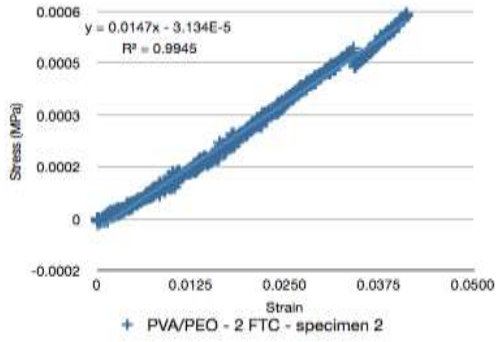


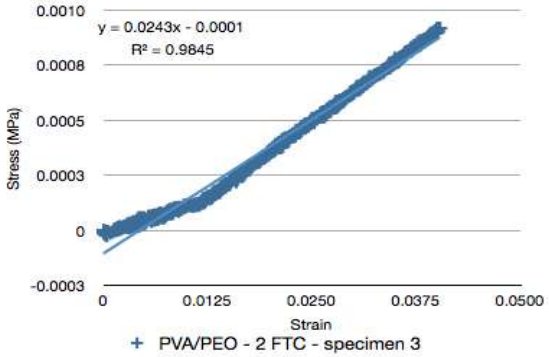
A.2: Plots for PVA/PEO held at -20°C for 5 hours

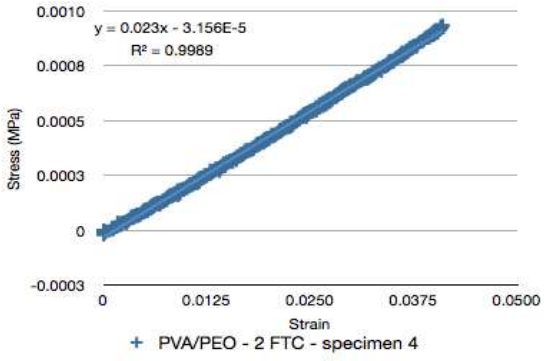




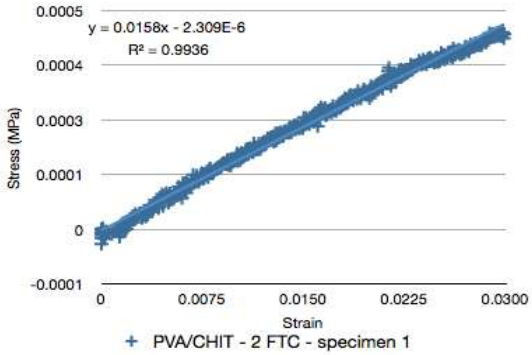


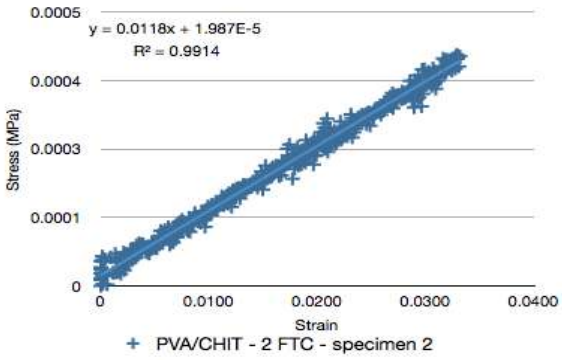


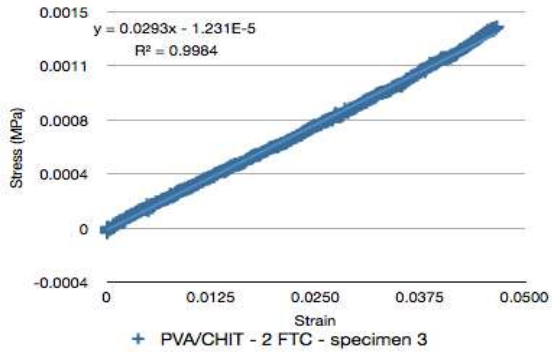


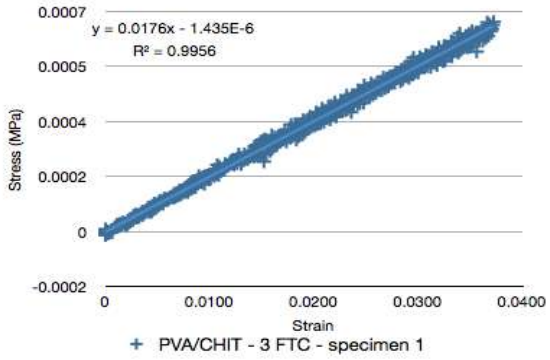


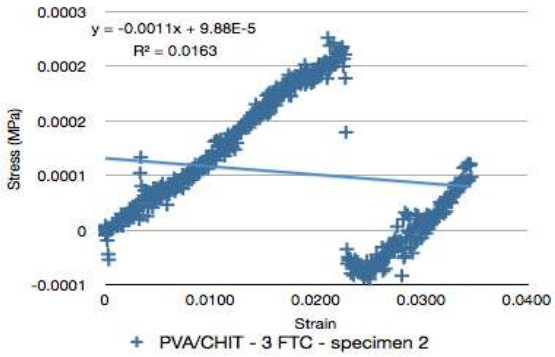
Appendix B: Stress vs. Strain Plots for PVA/CHIT


y = 0.0158x - 2.309E-6
R² = 0.9936
Stress (MPa)
Strain
PVA/CHIT - 2 FTC - specimen 1


y = 0.0118x + 1.987E-5
R² = 0.9914
Stress (MPa)
Strain
PVA/CHIT - 2 FTC - specimen 2


y = 0.0293x - 1.231E-5
R² = 0.9984
Stress (MPa)
Strain
PVA/CHIT - 2 FTC - specimen 3


y = 0.0176x - 1.435E-6
R² = 0.9956
Stress (MPa)
Strain
PVA/CHIT - 3 FTC - specimen 1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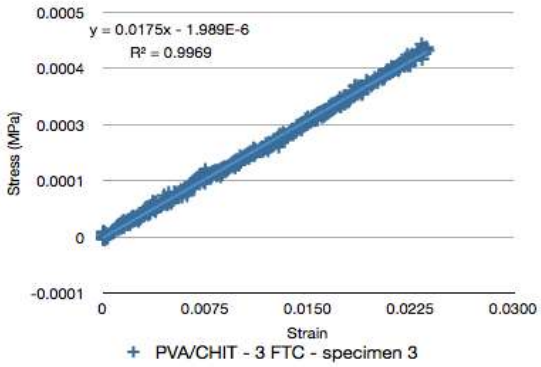
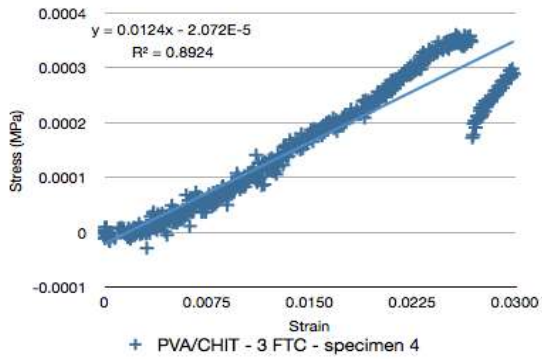
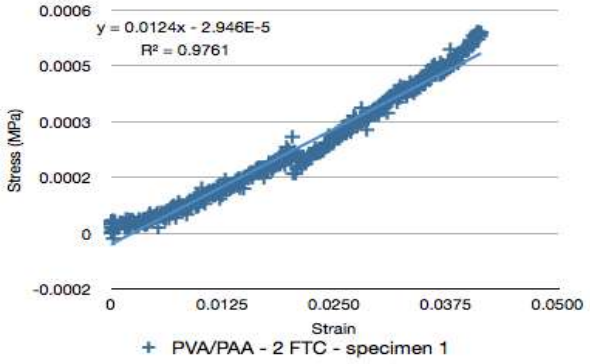
R² = 0.0163
Stress (MPa)
Strain
PVA/CHIT - 3 FTC - specimen 2

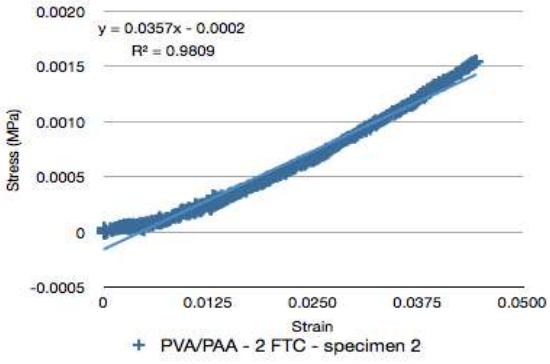

y = 0.0175x - 1.989E-6
R² = 0.9969
Stress (MPa)
Strain
PVA/CHIT - 3 FTC - specimen 3

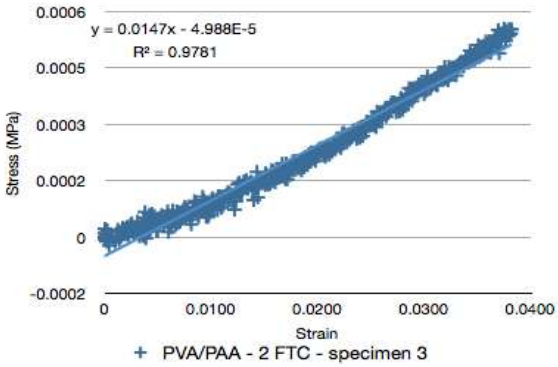

y = 0.0124x - 2.072E-5
R² = 0.8924
Stress (MPa)
Strain
PVA/CHIT - 3 FTC - specimen 4

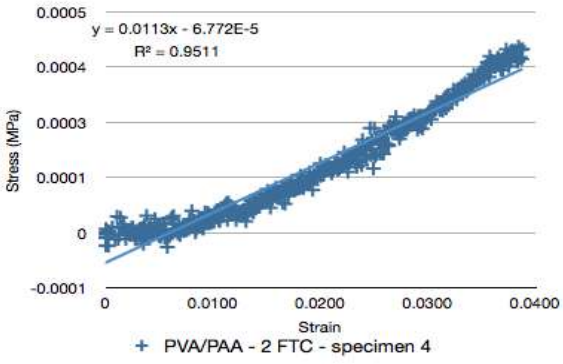
Appendix C: Stress vs. Strain Plots for PVA/PAA

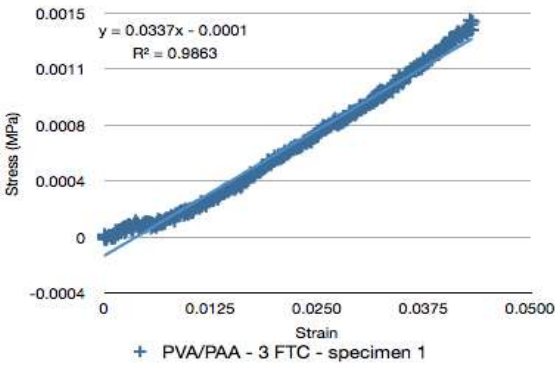
C.1: Plots for PVA/PAA held at -20°C for 20 hours

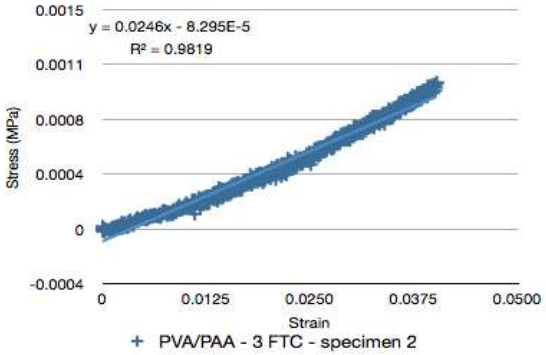


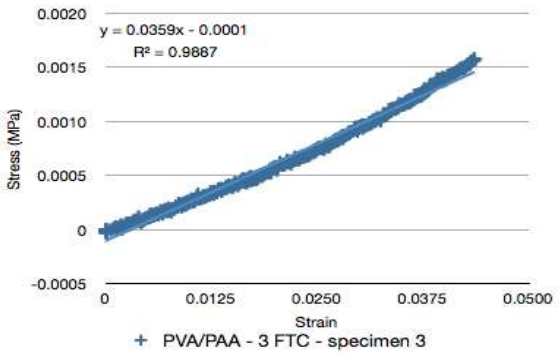


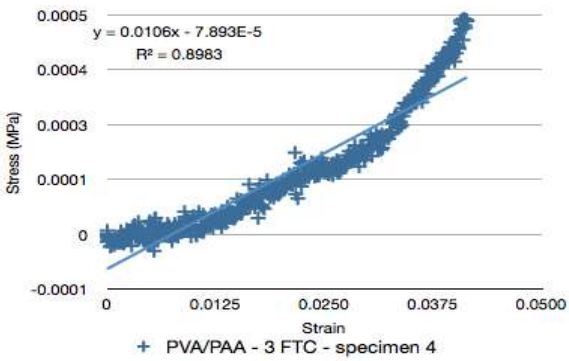




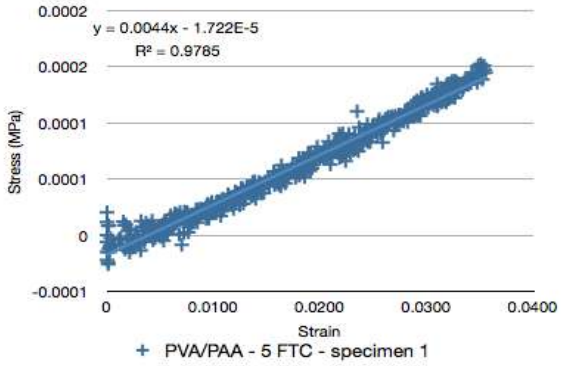


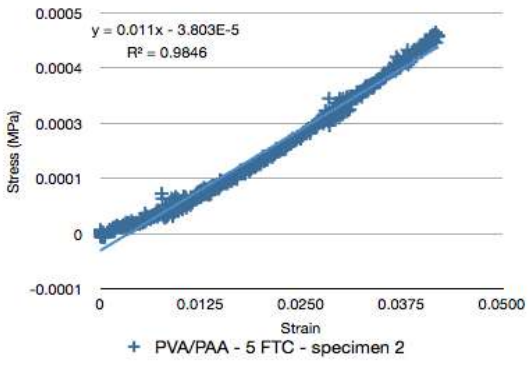


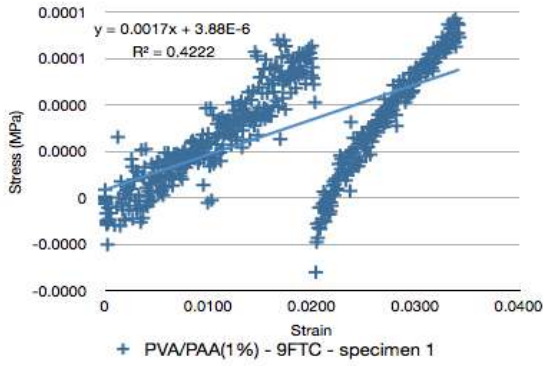


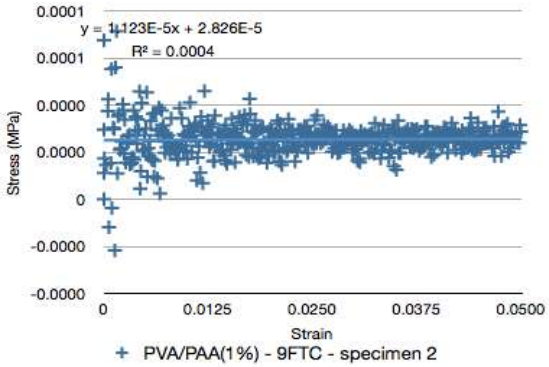


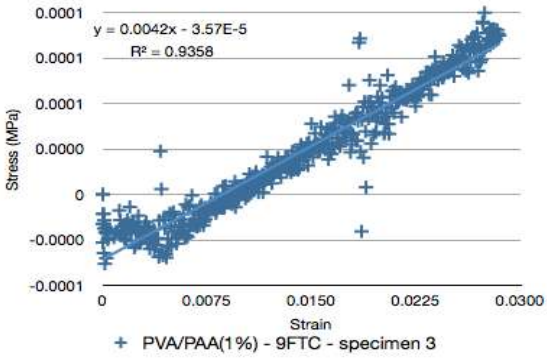
C.2: Plots for PVA/PAA held at -20°C for 5 hours

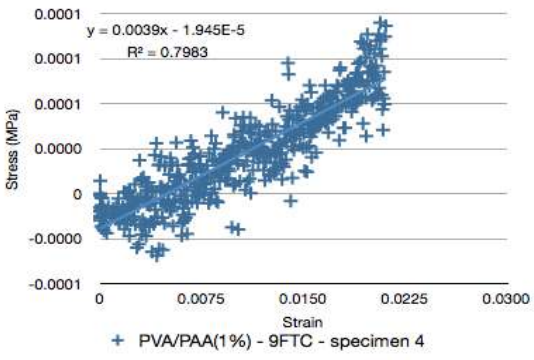




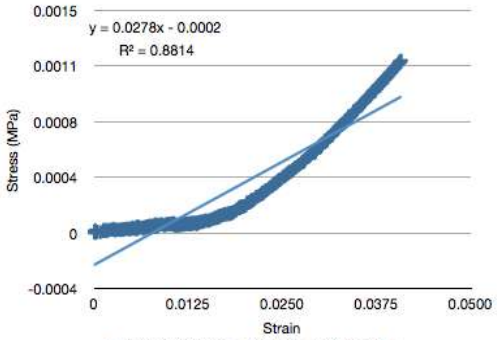




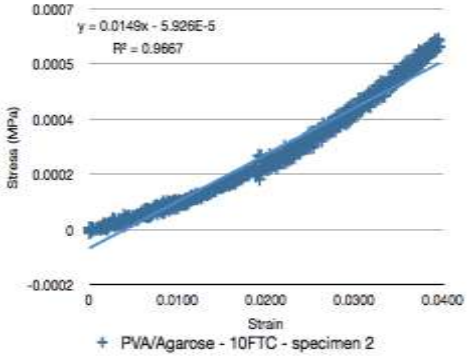




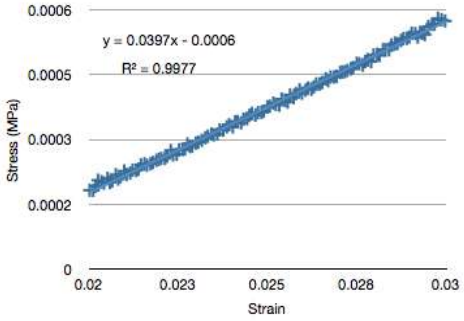
Appendix D: Stress vs. Strain Plots for PVA/agarose



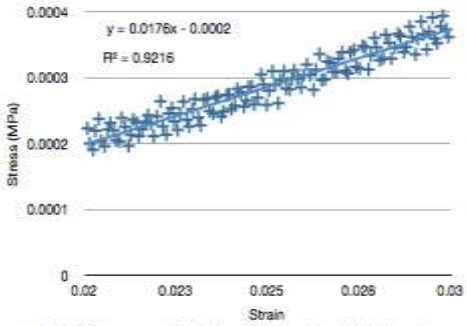
PVA/Agarose - 10FTC - specimen 1



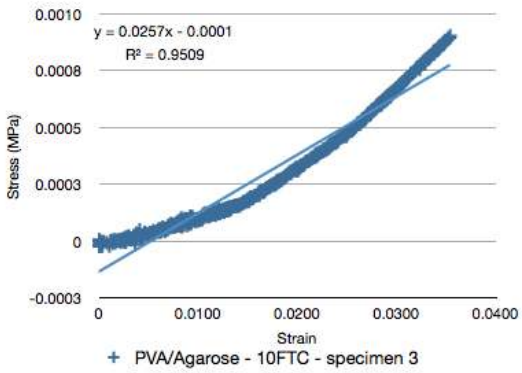
PVA/Agarose - 10FTC - specimen 2



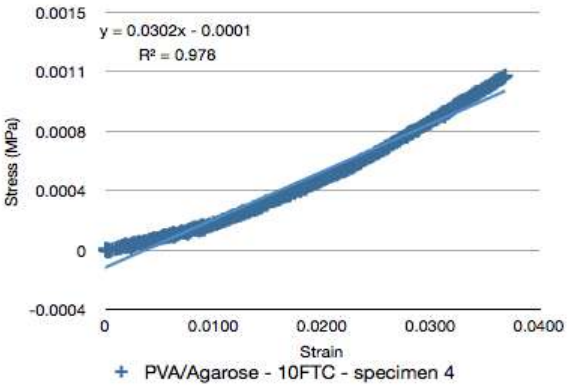
PVA/Agarose - 10FTC - specimen 1 -- 2%-3% strain



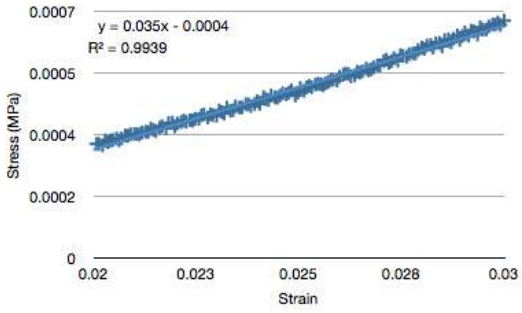
PVA/Agarose - 10FTC - specimen 2 -- 2%-3% strain



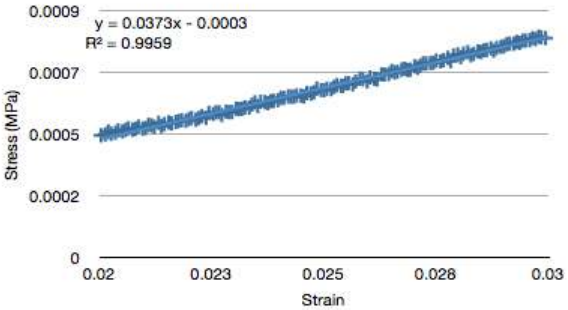
PVA/Agarose - 10FTC - specimen 3



PVA/Agarose - 10FTC - specimen 4



PVA/Agarose - 10FTC - specimen 3 -- 2%-3% strain



PVA/Agarose - 10FTC - specimen 3 -- 2%-3% strain