Mechanical analysis of Three Coaxial Electrospun Synthetic Biopolymers

Roy Anderson[1], B.S., Mentor: Wei Tan[2], Ph.D.
[1]University of Maryland, Baltimore County, United States, randers2@umbc.edu
[2]University of Colorado, Boulder, United States, wei.tan-1@colorado.edu

Abstract:

Cardiovascular disease is one of the leading causes of death in North America. Management techniques such as vascular grafting are essential to preventing death from cardiovascular disease. Synthetic vascular grafts are ideal, but currently the leading material is approximately 43% successful. There is a need for creating mechanically stable synthetic biopolymers for vascular grafting. Vascular grafting is a very important and effective treatment technique for treating heart disease. Mixtures of polymer solutions with a fixed ratio of 1:5 between a hydrophobic, synthetic, biodegradable polymer (PU, PCL or PLA) and hydrophilic, natural-derived gelatin. The hybrid composite nanofibers demonstrate highly-interactive layered structure between the hydrophobic core and the gelatin sheath.. Rheological analysis was conducted to characterize the mechanical properties of this material. The linear viscoelastic region (LVR) was found for all three biopolymers, meaning further information can be found from this material which could lead to more material testing. Following the success of finding the LVR of all three polymers, frequency sweeps were conducted on two out of the three polymers to test the mechanical stability. Frequency sweeps illustrate that while the polymer has the potential to withstand the frequency of a heartbeat and other complex frequencies of the body. Preliminary data illustrates that PU:Gel displayed a higher complex modulus each biopolymer could withstand the average pressure of blood flow, and the frequency of a heart beat. Future tests regarding the effect of crosslink chain lengths on the mechanical property of biopolymers will be conducted.

Keywords: Biopolymers, Mechanical Properties, Vascular Graft, Rheology, Electrospinning

Introduction:

According to the center for disease control, 610,000 people die of heart disease annually, which is 1 out of every 4 deaths in the United States. Cardiovascular disease (CVD) is the leading cause of deaths in North America. [1] Management techniques as well as lifestyle changes are major factors in helping to prevent death from CVD. Vascular grafting is a procedure that redirects and dispenses blood flow in a particular area of the body, creating a new passageway for blood flow. Vascular grafts are essential in treating patients with heart disease and correcting tissue defects, and are also essential in treating major blockage or stenosis of blood vessels. [2] Vascular grafting is an important feature of vascular and transplant surgery and has become an increasingly common feature of tissue engineering. Such procedures may be successfully applied to a bypass graft such as coronary artery. There is a constant need for alternatives using material obtained from the patient's body, particularly the lower leg autologous vein or artery tissues to use as vascular grafts. Autologous grafts utilize various healthy areas of the vein of the patient, typically the lower leg, to create a vascular graft. Currently, synthetic materials, such as polytetrafluoroethylene (PTFE) have not matched the capacity for beneficial change of native blood vessels, particularly in the cases of medium or small diameters settings defined as less than 6 millimeters. [3] Previous studies have shown that standard PTFE grafts are only successful as femoro popliteal bypass grafts at 45% after 5 years of use, while autologous vein grafts display between a 60-80% success rate. [4]

Synthetic materials have been employed in vascular graft design for the flexibility of tailoring their mechanical properties.However, studies have shown that "Synthetic vascular grafts account for approximately half of all end stage renal disease in the United States." Synthetic grafts are associated with higher rates of failure and complications. "Synthetic vascular grafts are the most common type of permanent dialysis graft used in the United States", but the drawbacks are great morbidity and expense. [5] Previous studies indicate that although 42% to 60% of synthetic vascular grafts remain patent at 3 years, many require intervention to maintain patency to correct stenosis, or blockage and strictures that prevent blood flow, and thromboses, or the formation of blood clots." [5] For the success of a vascular graft, high compliance is critical, because the compliance mismatch between vascular graft and neighboring arteries at the site is a major cause of graft failure due to at the junction of mismatch compliance disturbed blood flow leads to adverse cell responses which leads to increased stiffness of the graft which leads to failure. Synthetic grafts are essential in cases with extreme trauma, such as the use of prosthetic grafts in complex military vascular trauma. Surgeons are currently searching for the best method for bypass grafting for military members with extensive wounds involving multiple extremities caused by improvised explosive devices. [6]

In this case surgeons do not have the ability to use other parts of the patient's body, and synthetic grafts are needed for successful bypass surgeries. There is a need for improved mechanical properties in synthetic vascular grafts to increase the success in vascular grafting, which is vital in the health of a patient in need new passageways for blood flow. Currently surgeons are apprehensive towards the use of vascular grafting because of their tendency of early failure. Successful vascular grafts must closely mimic the mechanical properties of arteries and valves. The mechanical properties of arteries affects the blood flow and changes in blood flow are associated with harmful body effects such as hypertension and atherosclerosis.[7]

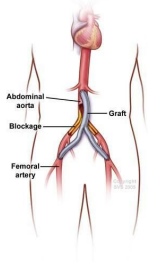

Fig 1: Displays an external view of atypical vascular graft

Biocompatible, biodegradable hydrophobic polymers like polycaprolactone (PCL), polyurethane (PU) and polylactic acid (PLA) have been used in tissue engineering applications including vascular regeneration. In spite of the high mechanical stability of these polymers, they lack the innate reactive sites for cell adhesion. The hydrophobic nature of PCL, PU and PLA tends to attract platelet and plasma protein adhesion, leading to the aggregation and intimal enlargement of the artificial blood vessels [8]. Synthetic polymers such as PU, PLA, PCL have easily reproducible mechanical properties, but are hydrophobic, meaning the polymers does not interact with water and other polar fluids which promotes a low level of cell attachments. The body consists of 60% of water, therefore having a hydrophobic material interacting with the body create a potential situation where the material rejects the water and cells needed for cellular growth and proper healing and function. Natural occurring polymers such as Gelatin (GE) does not cause a foreign body response, and is interacts very well water and other polar substances. This phenomenon is called hydrophilic. Creating a combination with a synthetic polymer inner core and a Gelatin outer core creates a natural synthetic polymer that incorporates the advantages of having a reliable inner foundation, and an outer surface that the body will not reject. GE essentially creates a viscous balance to the elastic synthetic polymer. A way to combine this synthetic polymer to the natural polymer is by the method of electrospinning. Previous studies show PLA electrospun with GE increases the water stability and cell responses when interacting with the synthetic bio-polymer which is ideal for a vascular graft. [14] Studies show that a GE blend with PU creates a highly porous structure that allows for proper cell growth, the study also illustrated a pore size comparable to blood vessels. [15] While a blend PCL and GE greatly increases the stiffness and Elastic modulus of the material when compared to only PCL material. [16]

Polymers are chains of repeated molecules. Polymers are important materials in vascular grafting because they have the potential to exhibit viscoelastic properties. Viscosity is defined as "A measure of the internal resistance of friction when a material moves against itself" [8] . Elastic refers to the ability to retain shape after deformation. [9] Polymers alone in the body are generally soft and brittle and not ideal for vascular grafting. therefore a method called cross-linking is used to enhance the mechanical properties of the polymers. Crosslinking is the process of bonding two or more molecules using covalent bonding. In the body, proteins naturally present in the body can contain cross-links generated by enzyme-catalyzed or spontaneous reactions, such cross links are important in generating mechanically stable structures. While in polymers, cross-linking increases the elasticity as the molecular weight increases more connections are formed in the material, essentially creating a longer chain of materials, that results in increasing elasticity at high deformation rates. [9] Cross-linking has been shown to improve the strength and thermal stability of the Polylactic acid. [10] Finding a cross-linked polymer that can enhance blood flow and have a significantly higher mechanical properties and compliance to PTFE would be highly beneficial for patients and surgeons. Figure 2 below illustrates the nanostructure of these biopolymer after crosslinking. Each material displays a different polymer to gelatin ratio. As PCL:GE, illustrates the highest amount of gelatin to polymer content, as indicated by the blue arrows, Polyurethane has largest amount of polymer content when compared to the gelatin. While polylactic-acid (PLA): Gelatin illustrates a balanced amount of polymer to gelatin content.

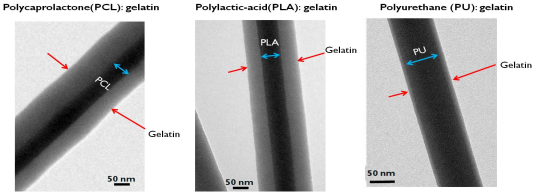

Fig 2: TEM images of the nanostructures

Purpose

The purpose of this study is to use rheometry to analyze three electrospun, co-dissolving solvent synthetic natural blend polymers consisting of Polyurethane, Polycaprolactone, and Polylactic acid separately mixed with gelatin and cross-linked with genipin to analyze their respective mechanical properties and determine which of the three is the most advantageous for vascular grafting.

Background

Electrospinning

Electrospinning is an electrostatic fiber formation utilizing electrical forces to produce fibers from polymer solutions. [17] The process uses a high amount of voltage and current to convert a liquid solution into a solid polymer. A direct current voltage at a range of several tens of kilovolts is needed to create the process. Electrospinning is conducted at room temperature, and consists of three essential components: A high voltage power supply, a metal or pipette tip i.e. a spinneret, and a grounded collecting plate. The high voltage sources injects charge of a constant polarity into a polymer solution.[17] The polymers are dissolved in solvent and accelerated towards a collector of opposite polarity. Polymer solutions held by surface tension at the end of a capillary tube is subjected to an electric field that attains a critical value at which electrical force overcome surface tension forces. This process leads a charged jet of polymer solution that is ejected from the tip of the needle.. [17] The unstable, rapid whipping of the jet occurs in the space between the tip and the collector which leads to evaporation of the solvent, leaving the polymer behind. Figure 3 provides an illustration of the electrospinning process. Coaxial electrospinning provides a unique approach to construct polymer mixture, wherein, the polymers are highly interactive at the nanoscale to bring about novel properties. Coaxially electrospun fibers in comparison with coated or blended fibers have shown enhanced biocompatible and mechanical properties for tissue engineering and regenerative applications. Few attempts have been made using coaxial electrospinning to develop small diameter artery grafts. In coaxial electrospinning, two compartments containing either different polymer solutions (shell) and a non-polymeric Newtonian liquid (core), initiate a core–shell jet. As a result, the core–shell jet solidifies and fibers are deposited on a counter-grounded electrode . In most fabrication conditions, the shell fluid is able to be processed with electrospinning, while the core fluid is not electrospinnable . [17]
the elastic region using the following equation:

$$E = G*(1+v) \quad (8).$$

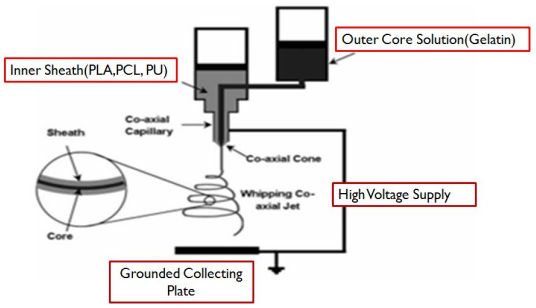

Fig 3: Displays an overview of the electrospinning process[20]

Introduction to Rheometry

Rheology is the science of flow and deformation of materials under control testing conditions. [18] [9] Rheometry is important for characterizing the dynamic material properties of complex material structures such as polymers after they are processed. Two rheological experiments that are important in determining polymer performance are strain sweeps, and frequency sweeps. Frequency sweeps tells the user how time affects the sample while strain sweeps are important in determining physical parameters such as the Shear modulus, (G), which is illustrated in the equation below, equations [1]-[8] are all provided by reference 8:

$$G = \frac{Shear\ Stress}{Shear\ Stain} = \frac{\tau}{\gamma} \ (1),^{[8]}$$

Where shear stress (τ) and strain (γ) are defined in the equation below.

$$\tau = Shear\ Stress = \frac{Force}{Area} \quad (2).$$

$$\gamma = \frac{change\ in\ length}{original\ length} = \frac{l - l_o}{l_o} \quad (3).$$

Using equations [1]-[3], the storage modulus (G'), which is the measure of the elasticity of the material and the loss modulus G" which illustrates the amount of energy lost from the placing a shear stress on the polymer, and viscosity which is the ratio of shear stress to shear strain. G' and G" are calculated using the following equations:

$$G' = Storage\ Modulus = \frac{\tau}{\gamma} cos\delta \ , (4).$$

$$G'' = Loss\ Modulus = \frac{\tau}{\gamma} sin\delta \ (5).$$

$$tan\ \delta = \frac{G'}{G''} \ (6).$$

Using G' and G'' the complex storage modulus (G*) can be calculated using the following equation

$$G^* = G' + iG'' \ (7),$$

Finding the dynamic modulus is extremely important in studying the viscoelastic properties of polymers. Strain sweeps are important in characterizing viscoelastic behavior [19].
Finding the storage and loss modulus can be easily converted to the elastic storage and loss modulus (E' & E") finding the elastic modulus of the synthetic polymers, they can be compared to the mechanical properties of arteries. The complex shear modulus found in equation [7]can be used to converted to

where v is poisson's ratio. [18] From those comparisons we can determine which synthetic biopolymer will theoretically perform better as a vascular graft. Strain sweeps establish the extent of how far the material can be stretched without becoming mechanically unstable. After the fluid's linear viscoelastic region (LVR) has been defined by a strain sweep, its structure can be further characterized by a frequency sweep at a strain of critical strain. The importance of the LVR is that within this region the material response is constant, at this region the complex polymer material can be characterized. The linear viscoelastic region is shown in figure 4. Frequency sweeps provides more information among particles or droplets. In a frequency sweep, measurements are conducted over a range of oscillation frequencies at a constant oscillation amplitude and temperature. [9] The information generated from rheometry studies will lead to further understanding about which material will operate better as a vascular graft, which will lead to more reliable and cost effective vascular grafts.

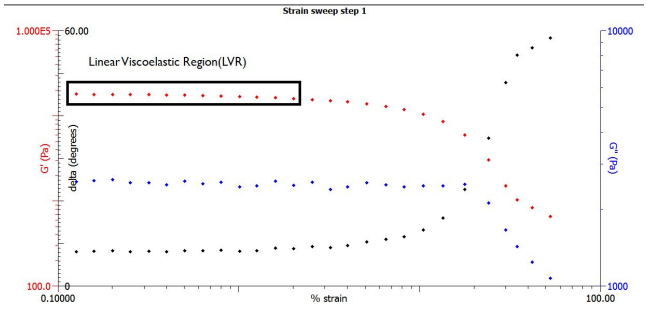

Fig 4: PLA:Gel Strain Sweep with highlighted linear viscoelastic region

Methods

Glass slide biofunctionalization & salinization

In order for the biopolymer to properly attach to the glass slides, treatment of the glass-slides must be conducted. Slides are immersed in 20% sulfuric acid (Sigma Aldrich St.Louis, MO) for approximately 30 minutes in a glass container. The slides are then washed in deionized water three times, changing the water at five minute intervals. Slides are immersed in acetone for 30 minutes, then immersed in Aminopropyltriethoxysilane overnight. Glass slides are then washed in acetone three times changing to fresh acetone every five minutes, then washed in deionized water three times changing the water every five minutes. Lastly the slides are immersed in glutaraldehyde for 1 hour followed by two washes in deionized water.

Coaxial electrospinning fabrication

The apparatus used for obtaining coaxial fibers is shown in the supplemental information. Core hydrophobic polymer solution was passed through the inner needle of 22 G (0.71 mm in internal diameter) and the sheath gelatin solution was passed through the outer needle of 16 G (1.65 mm in internal diameter). A dual syringe holder was used to place the polymer solution loaded syringes. This was designed to extrude the solutions simultaneously. To create the cross linked polymer, a 1% weight per volume sheath solution must be created. The sheath solution consists of PLA, PCL, or PU. Pellets of solid sheath solutions were measured to be approximately 0.1 grams, which is then dissolved in 10 mL of HFPA are stored in disposable vials. To create a 5% wt/volume core solution, 0.5 g of bovine gelatin were measured and dissolved in 10 mL of HFPA. Solutions are placed on a shaker to dissolve for approximately 12 hours. Coaxial spun materials were fabricated by an electrospinning apparatus. The sheath and core solutions are stored separately in 5mL syringes which is connected to a blunt ended needle that serves as the spinneret charged by a high voltage power supply. The 5mL syringes are connected by an 8 cm piece of Teflon tubing.

To foster the collection and detachment of the polymer from the electrospinning apparatus, an 8 x 8 cm square sample of cardboard was cut using a box cutter and dull scissors, which is then tightly wrapped in aluminum foil. The collector stage is lifted by a Styrofoam stage with a hole in the middle of the stage. The bottom of the collector is connected to a negative alligator clip which grounds the collector. 22 mm sample slides were placed on the thin sheet of aluminum foil before the electrospinning process is initiated. Air bubbles are were purged by increasing the flow rate of the pumps until visible bubbles are eliminated.Also the positive voltage alligator clip is then connected to the top of the coaxial cone needle to complete the electrospinning circuit. The necessary voltage to conduct electrospinning is 12 kV while the syringe pumps are set to a rate of 1 ml/hour. The PCL/PU/PLA combined with GE are collected on the 8 x 8 cm piece of cardboard wrapped in aluminum. The nine slides are carefully cut preserving the polymer formed during electrospinning. An image illustrating the look of a polymer after electrospinning is illustrated in figures 5 and 6.

Fig 5: 5-Hour Electrospun PCL:GE.

Crosslinking of electrospun fibers

After electrospinning, each sample is labeled and placed into a Petri dish where it is cross-linked with a solution of 2 mL of 0.25 g genipin in 100% ethanol for 24 hours. Following that the material is then hydrated with 250-500 μl of water. The material thickness on the glass slides are then measured using a digital caliper. In order for accurate rheometry analysis to be conducted, the thickness of the material must be above 0.5 mm.

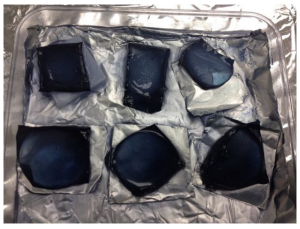

Fig 6: PCL:Gel samples after crosslinking

Rheometry Analysis

To find the linear viscoelastic region rheological analysis was conducted on the ARG2 (TA Instruments, New Castle, DE). Two essential tests that were conducted are strain sweepsand frequency sweeps. The strain sweep establishes the extent of the material's linear viscoelastic region (LVR). Determining the LVR is essential in gaining new information about complex materials. Strain sweep measurements were conducted at 10 points each decade of strain. For example, there were be 10 readings between the 0.1% - 1% strain. Strain sweep measurements spanned from 0.1 % strain to approximately 50 % strain. Frequency sweeps were conducted at a stress strain range of constant oscillation frequency, and illustrate the time dependencies on viscoelastic properties. Oscillatory frequency sweep tests were conducted with logarithmic step increases between 0.1 to 50 Hz to identify the linear region for all materials. Samples are adhered with tape to ensure that the sample does not slip. Each strain sweep experiment was initiated at 2N and recorded to confirm that each material is placed under the same conditions. While each frequency sweep was operated at a pressure of 100 Pa. Raw G' and G" data was collected for each frequency sweep and strain sweep. The LVR was then observed by finding the region where there was little to no change in G' and G". For each material, the G' and G" in the LVR was then averaged for each sample. The complex modulus was then calculated using equation [6].

Fig 7: AR-G2 rheometer

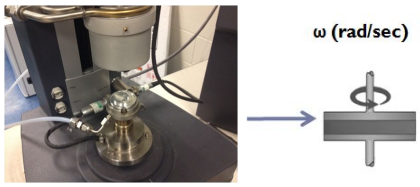

Fig 8 : Parallel plate testing mechanism used for AR-G2

Results

Preliminary strain and frequency sweeps

Preliminary sweep data has established a linear viscoelastic region for all three biopolymers, The average LVR G', G", and G* for each material is shown in Table 1 below. The storage modulus (G') for the PU:Gel was determined to be highest at 39.7 kPa compared to 28.7 for PLA:Gel and 18.9 kPa for PCL:Gel. The storage modulus is an indicator of mechanical stability. PU:Gel had the greater loss modulus at 5.7 kPa, when compared to a 4.5 kPa G" for PLA:Gel and 2.8 kPa for PCL:Gel. Due to the differences in storage and loss modulus, PU:Gel had the greatest complex modulus (G*) at 40.2 kPa, compared to 29.0 kPa for PLA:Gel, and 18 kPa for PCL:Gel. The complex moduli of all three biopolymers were calculated to be above the average pressure of blood flow which is approximately 16 kPa. due to this important finding frequency sweeps have been conducted for PCL:Gel and PLA:Gel.

Table 1: Comparing G', G" , and G" for the three synthetic biopolymers.

	PCL:GEL (kilopascals) (kPa)	PU:GEL (kilopascals) (kPa)	PLA:GEL(kilopascals) (kPa)
Number of Samples	5	3	3
Average linear Viscoelastic Region (LVR) Storage modulus (G')	18 ± 9 kPa	39.7± 20 kPa	28.7 ± 16 kPa
Average LVR Loss Modulus (G")	2.8 ± 2 kPa	5.7 ± 4 kPa	4.5 ± 3 kPa
Average Complex Modulus (G*)	18.2 ± 9 kPa	40.2 ± 20 kPa	29.0 ± 15 kPa

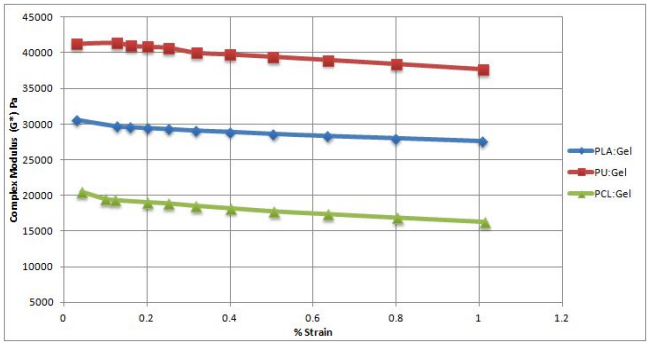

Fig 9: Illustrates Complex modulus vs. Strain for all three biopolymer LVR

Preliminary frequency sweeps have illustrated that the material increases in complex modulus as the angular velocity increases, illustrating a gelled system, as in all cases of frequency sweeps the storage modulus were higher than the loss modulus. Figure 10 displays the complex modulus as a function of frequency for the average of three PCL:Gel and PLA:Gel frequency sweeps. Figure 10 illustrates the increase in complex modulus as the frequency increases. The frequency range that this experiment was conducted under provided information about the long-term material behavior of these three polymers. PLA: Gel displays a much higher complex modulus as the frequency increased. Each frequency sweep illustrated stable mechanical properties within the frequency range of a heart beat, which is estimated to be approximately 1.0-1.6 Hz.

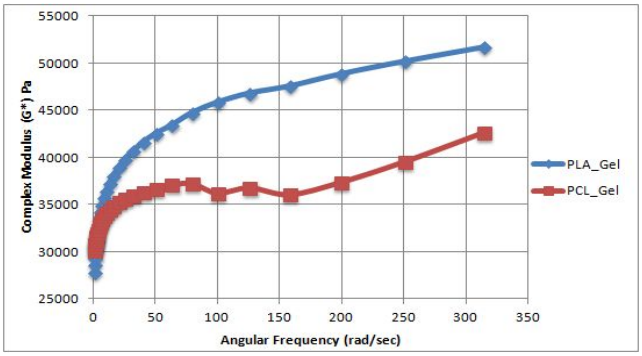

Figure 10: Complex modulus as a function Angular Frequency

Discussion

This preliminary strain sweep data illustrated expected results, the TEM images display that PU:Gel blend had the largest amount of polymer, thus assuming that the material would be more mechanically stable than PLA:Gel and PCL:Gel. Each strain sweep illustrated similar steps in degradation, illustrating a linear viscoelastic region until approximately 1% strain, then displaying strain softening, meaning that as the material increases in strain the material becomes softer and behaves closely to a liquid. PU:Gel illustrated more rigid viscoelastic properties than the other two synthetic polymer blends. In the case of an artery, it is ideal to have more rigid structures, in terms of complex modulus, PU:Gel would be the ideal synthetic biopolymer.

Frequency sweep data also illustrates that the LVR was successfully found in the cases of PCL:Gel, and PLA:Gel for the response throughout the test was generally linear. Illustrating that further mechanical tests can be conducted within the LVR to determine more information regarding the biopolymers. Figure 7 illustrates the difference in complex modulus as a function of strain within the linear viscoelastic regions. For a majority of the frequency sweeps for the biopolymer PCL:Gel, there were constant small spikes of G' and G" between the angular velocity of 100 - 200 rad/sec, which approximately 15.9 - 31.8 Hz. This frequency is caused by a resonance frequency of the AR-G2. The resonance frequency is the natural frequency of the physical parameters caused by the rotating objects. The resonance frequency is the natural frequency of the physical parameters caused by the rotating objects. This resonance frequency was only found during experiments with material thickness between 0.5 and 1 mm illustrates the effect of material thickness on rheometry testing.

The raw strain sweep data illustrates similar mechanical responses between the three polymers, the polylactic acid:gelatin (PLA:Gel) blend displayed a slightly consistently longer LVR when compared to the other two.While PU:gel has the greater complex modulus which means a more rigid and mechanically stable structure, PLA:Gel illustrates very constant behavior at longer rates of strain than PU:gel, and PCL:gel. Displaying a constant LVR over a long period of strain is an ideal mechanical property to display. The figure below illustrates the constant G' which is shown by the red dots, and the loss modulus being illustrated in the blue dots. Figure 11 illustrates potential high resistance to strain by the long LVR, this mechanical characteristic is ideal for a vascular graft.

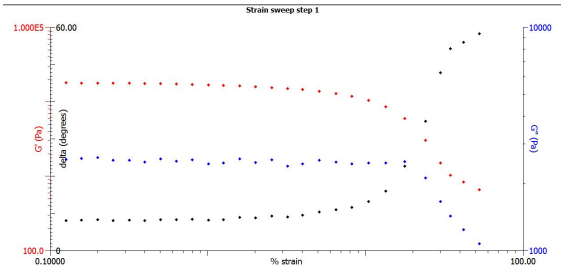

Figure 11: Preliminary strain sweep of PLA:GE

Future work

Further rheometry on all three polymers will be conducted to verify the data, and generate frequency sweep data for polyurethane:gelatin. Following that rheological analysis on PCL:Gelatin polymer blends with different cross-linking agents. Poly propylene glycol diglycidyl and Poly ethylene glycol diglycidyl are known to have longer cross linking lengths than genipin. Longer chain crosslinkers are known to improve mechanical properties of polymers. Learning about the effects of cross linking lengths on the microstructure and mechanical properties of these biopolymers. Following that study the three biopolymers will be created into cylindrical shapes in the form of vascular grafts, where their mechanical properties will be assessed using uni-axial tensile testing.

Conclusion

There is a need for creating mechanically stable synthetic biopolymers for vascular grafting. Vascular grafting is a very important and effective treatment technique for treating heart disease. Improving synthetic vascular graft performance will lead to more patients having the ability to have this treatment. Mixtures of polymer solutions with a fixed ratio of 1:5 between a hydrophobic, synthetic, biodegradable polymer (PU, PCL or PLA) and hydrophilic, natural-derived gelatin. The hybrid compositenanofibers demonstrate highly-interactive layered structure between the hydrophobic core and the gelatin sheath. Varied interactions between the core materials and the sheath lead to the difference in the sheath thickness, core-sheath structure. Rheological analysis was conducted to characterize the mechanical properties of this material. The linear viscoelastic region (LVR) was found for all three biopolymers, meaning further information can be found from this material which could lead to more material testing. Following the success of finding the LVR of all three polymers, polymers to test the mechanical stability. Frequency sweeps illustrate that while the polymer has the potential to withstand the frequency of a heartbeat and other complex frequencies of the body. Preliminary data illustrates that PU:Gel displayed a higher complex modulus each biopolymer could withstand the average pressure of blood flow, and the frequency of a heart beat. Future tests regarding the effect of crosslink chain lengths on the mechanical property of biopolymers will be conducted.

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