Abstract

PHB is a novel polymer that has noticed potential to be one of the next important biopolymers used in the medical and bioengineering field. We have started electrospinning this polymer for the first time and demonstrate how its diameter and modulus of elasticity can be controlled by both the concentration of the solution spun and the voltage it is spun at. Both the concentration and voltages have upper and lower limits that limit the size of the fibers produced. We also have demonstrated the affect that the sample's position on the mandrel has on the mechanical properties.

Introduction

Tissue engineering seeks to solve the shortage of tissue transplants by creating a new source for the needed tissues and organs independent of human donors. Current areas of research are focusing on the creation of skin, cartilage, bone, nerves, veins, and retinas. While it is the hope that eventually every area of the body, excluding the brain, will be 'replaceable', it will be a long time before that is realistic. While tissue engineering is in its infancy, creating replacement skin that can serve as an instant clot, bandage, antibacterial, and lattice for re-growth is much more reasonable example of a realistic project.

Tissue engineering includes techniques such as tissue printing that work by laying down cells into a gel; however, in most techniques the cells are seeded onto a matrix and grown to a final developed state. 'Seeding' cells is another way to describe attaching cells to a new material. It is done by washing the material in a bath of cells or by simply placing the cells onto the surface and letting gravity hold them on till the cells attach and move inward. The matrix is often made of a piece of sponge-like material.

One problem encountered thus far with the matrix is the need for small diameter fibers, which are rare in man-made material. If fibers in the natural ECM are on the nm scale, a hundred μm fiber, even if it is made of the same material, will look alien to a cell and likely having negative effects on the properties and vitality of that tissue culture.

Electrospinning comes into play due to its ability to create fibers so thin that the diameter can drop into the needed nm size range. Electrospinning works by creating a potential difference between a syringe containing the material and a grounded mandrel a few inches from it. The material is slowly pushed out of the tip and forms a Taylor cone, which has a very thin jet of polymer flying of the tip and towards the mandrel, when the electrostatic forces overcome the surface tension of the solution. Through the six or so inches of air, the solvent the polymer is in evaporates and the strand thins out to the point that it is a micro- or nano- meter fiber being caught on the spinning mandrel. On one end of the spectrum, if the distance is too large or of if the concentration is too low, the stream will break into droplets. On the other end, if there is an upper limit to how much will go into solution.

Our lab has been looking into using electrospun PHB or polyhydroxybuterate in a scaffold. It is a semi-crystalline thermoplastic that is a copolymer of polyhydroxyvalerate and a natural product of some types of bacteria [1,2]. For this reason there are bacterial enzymes that can quickly break it down [2], which could be useful for selectively removing the PHB after a tissue culture has started producing substantial quantities of it's own ECM. The polymer also degrades quickly in acids, alkalis, and chlorinated solvents [1]. It has similar properties to polystyrene, but is a little less brittle [3]. The HB monomer is a natural component of human blood [1], which is reassuring of its compatibility with tissue cultures.

Since PHB shows promise for the use as a bio-molecule and it is relatively untested in terms of its mechanical properties when nanospun, our lab has decided to do a study to determine its characteristics and how those characteristics can be controlled by changing the solvent concentration. Previously in this lab, TFE has been found to be a very effective solvent for PHB, so for all the experiments, this is the solvent that is used. Preliminary studies have shown 130mg/ml to be the minimum concentration that would spin in solutions created before TFE became accepted as the best solvent, so to find the range of concentrations, we added and subtracted 30 mg/ml to the 130 until the maximum and minimum concentrations were found.

Methods

The set up of the electrospinning process is shown in figure 1. The jet of polymer is created by slowly ejecting the solution out of a charged syringe and having the electrostatic attraction draw the material through the air and onto the mandrel. While it travels through the air, it elongates and the solvent evaporates to created a micrometer or nanometer diameter fiber. The mandrel is set to spin at around 3500 RPM to help collect the fiber as it is created. The platform on which the mandrel sits also has a slower translational motion of about 2 inches at 10.19 seconds per cycle in order to distribute the fibers more evenly.

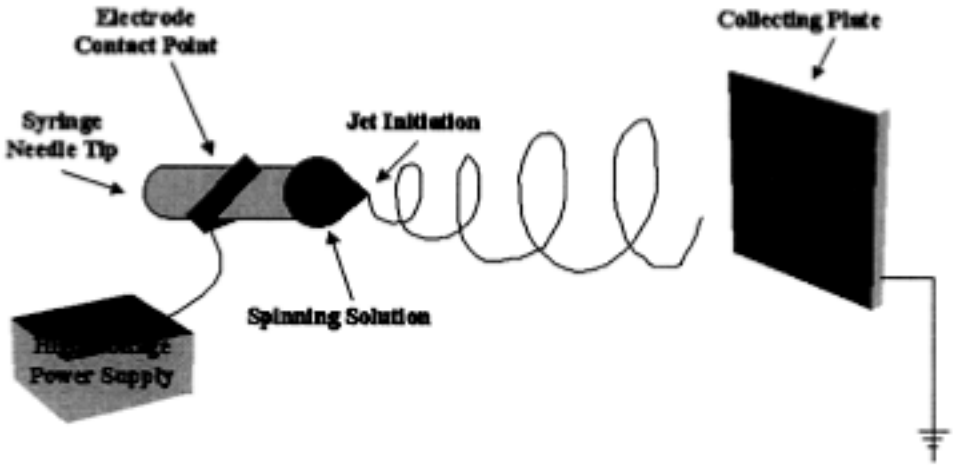


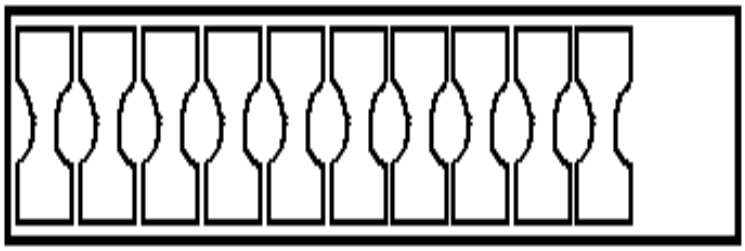
Figure 1. *The classic electrospinner set up was used in the experiment. The only significant difference was the use of a spinning mandrel instead of a stationary collecting plate.*

In this study, PHB, or polyhydroxybuterate, was solvated in 2,2,2-Trifloralethanol, or TFE. Once dissolved, the solution was immediately drawn up into a 5cc syringe, let sit for a few minutes, and then placed into a KD Scientific syringe pump that allowed a measured dispensing at 2.25 ml/hr.

To test the hypothesis that concentration effected fiber diameter with PHB, all the other electrospinning parameters were held constant, including feed rate, distance to the mandrel, mandrel material, syringe, translation speed, PHB source, and voltage. Solution concentrations of 70, 100, 130, 160, 190, 220, and 250 mg/mL of PHB in TFE were created after experimentation using TFE found 50 and 270 mg/mL to be the upper and lower limits of spinable concentrations.

Fiber diameters were measured by taking SEM pictures of each of the fibers at 3000X magnification with a JEOL JSM-820 JE electron microscope and Polaroid film. The pictures were then scanned, digitized, and analyzed with NIH's ImageJ 1.32 in order to calculate the fiber diameter. One hundred measurements per micrograph were randomly selected to find the average and standard deviation, and the measurements were all calibrated using a scale on the micrograph to find the ratio of pixels to micrometers.

We also tested the solution concentration's effects on the mechanical properties of the fiber, using the same mats of fiber that the SEM samples had been taken from. Samples were cut out of the mat using a new scalpel and a "dog-bone" shaped metal template. This particular template was used to ensure that all the samples were the same size and that they would break in the middle, which had the smallest width of .105 in. The gauge length, between the two clamps holding the material, was held constant at .474 in. The alignment of the fibers, due to the mandrel's rotation, should be mostly in the vertical orientation, so set of samples were collected with the stress in both the longitudinal and orthogonal orientation. The longitudinal orientation ran parallel to the principle fiber direction and the orthogonal was perpendicular to that. Due to the shape of the mandrel, the samples were always collected in the pattern shown in figure 2; sample 6 was usually the sample that was from the middle of the mandrel in the longitudinal data set and the orthogonal's data falls into sets of four. The instrument used to measure the fiber characteristics was the uni-directional MTS Bionix200 desktop mechanical tester. The material properties we used to compare fibers were peak stress, peak load, elastic modulus, and strain to failure, all measured and calculated by the MTS TestWorks 4.0 software using the ETB method.



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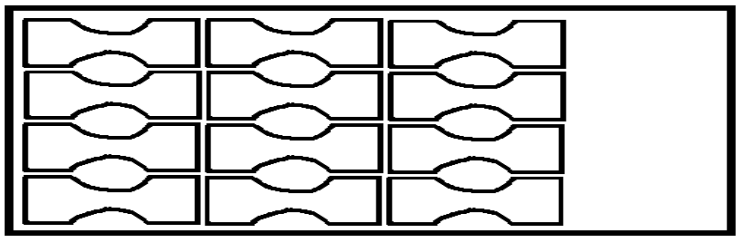


(b)

Figure 2. *The pattern that the longitudinal (a) and orthogonal (b) samples were taken in on the sheet; sample 1 starts on the left hand side of both.*

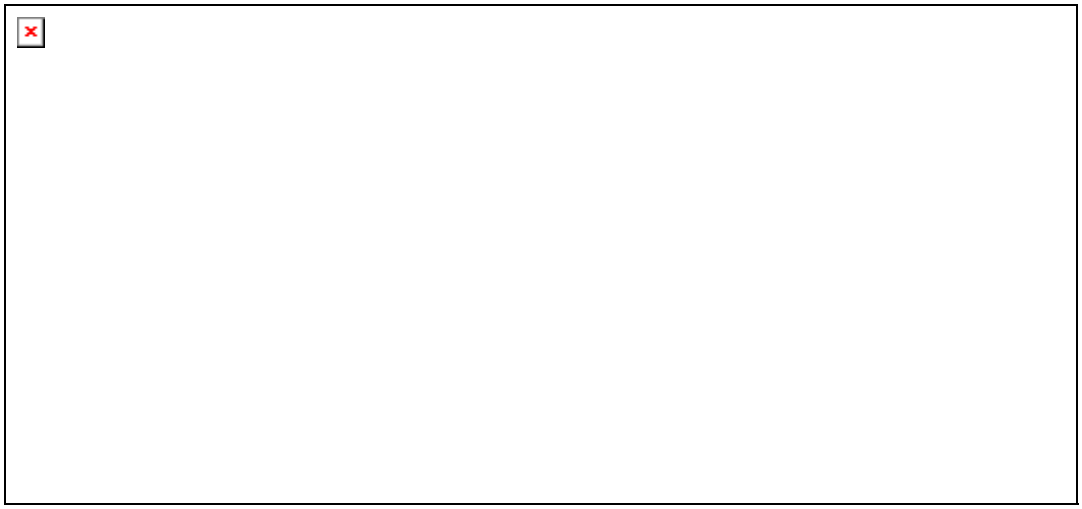
Results and Discussion

The concentration of PHB spun at 22V has a direct relationship to the diameter of the fibers spun. The fifty mg/ml concentration produced the thinnest fibers at an average of .162 μm. 70 mg/ml created fibers at .317 μm; 100 mg/ml, .526 μm, 130 mg/ml, .643μm; 160mg/ml, .566μm; 190mg/ml, .618μm; 250mg/ml, 1.084μm. Though there is variability in diameter and the concentration, a rough estimate of the diameter expected can get gained from the equation diameter(μm) = .0039*conc(mg/ml) + .0332.

Chart 1. The PHB diameter as a linear function of the concentration. The R^2 value

is low for the use of predictions, but it does show a definite correlation.

One of the reasons why the fiber diameter has such a rough correlation to the concentration could be because the diameter of the fibers at each spin had such a large variability. Most mean diameters had a standard deviation of .15 to .34 μm. All the concentrations had extra small fibers that were around or under .1μm. This, naturally, causes the 250 mg/ml concentration to have the largest s.d. at .789μm, and the 50 mg/ml spin to have the lowest s.d. at .089μm.



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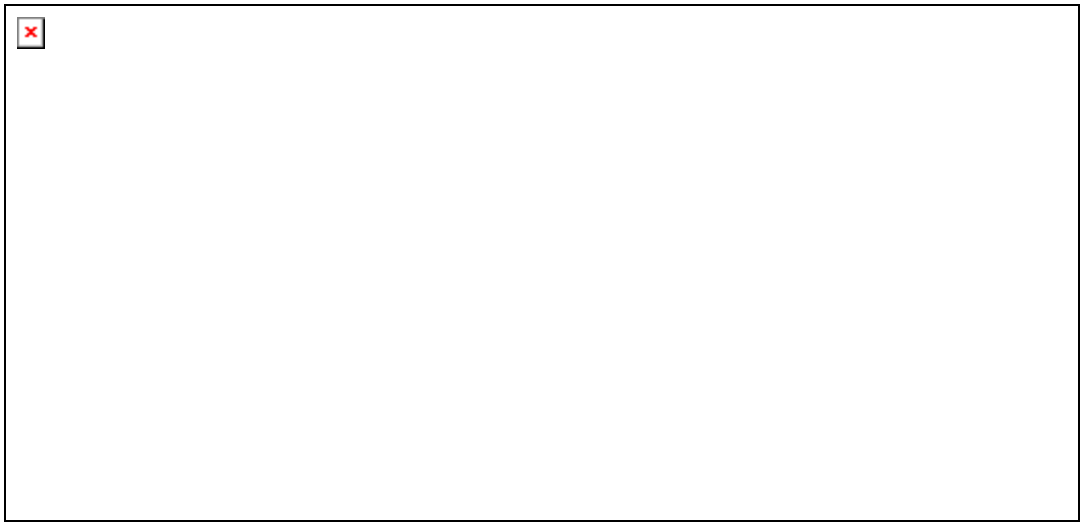


(b)

Chart 2. *The high R^2 value of both the linear tread lines show that the longitudinal*

(a) and orthogonal (b) modulus are directly related to the concentration.

The modulus is very similar for both the orthogonal and longitudinal, though there are some minor difference, like that the orthogonal's modulus decreases more rapidly with concentration.

***Chart 3**. The relative changes of the peak load, peak stress and stain at break with the change of concentration of the longitudinal samples on the left and the orthogonal samples on the right, marked with an 'H'.*

The peak stress could be affected by the concentration, the orthogonal samples would seem to suggest it, but the longitudinal samples only show a rough correlation; more testing would be needed to confirm the pattern. There could also be a pattern in the orthogonal peak load, but it would be only a rough correlation, and given that the longitudinal samples do not show a similar pattern, it is less likely. If anything, the peak load and strain at break are most similar to just the average thickness of the fiber mats, shown in chart 4.

Chart 4. *The average thickness of each of the samples from the fiber mats used for mechanical testing. The pattern is random but useful for corresponding to chart 3's data. The left seven are from the longitudinal testing and the right seven are from the orthogonal.*

Along with trends found over varying concentrations, it was also noticed that within each mat there were patterns in the different mechanical properties, most likely due to the fibers in the center of the mat being more aligned. The 70 mg/mL's data has been graphed to show this, since that concentration had the least noise in the data, probably due to low sheering effects.

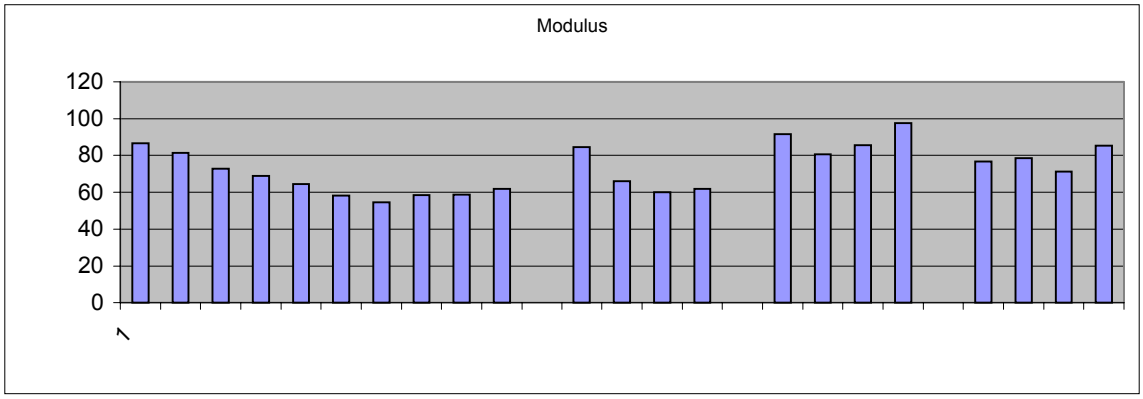


Chart 5. *The modulus of the fiber is affected by the sample's position on the fiber mat. Sample 1 is at the edge of the longitudinal set where sample 6 or 7 is the middle of the mat. Samples with an 'H' are from the orthogonal testing and are in groups of 4, with 1H and 4H always the sample in the edge's corners. See the Materials & Methods figure 2 for a diagram.*

As seen in chart 5, there is a direct and smooth correlation between the modulus and the position on the mandrel and mat. Sample 7, which was straight before the syringe, has the lowest modulus of any of the samples, while sample 1, whose fibers should be the least aligned of all the longitudinal samples, had a modulus similar to the orthogonal samples. This could be because the fibers could be pulled for a longer time until they were aligned before the stress would pull on the fiber lengths themselves, causing them to break. Evidence of this comes from the shape of the sample before it breaks; in the orthogonal samples, the breaking point becomes very thin and thicker compared to most longitudinal samples, where it only becomes narrower for a short period of time before it breaks. In all the different concentrations, the x and (x + 3) samples, which were on the edge of the mats, had a distinctly higher modulus than the two samples in between; the reasons for this are less clear.

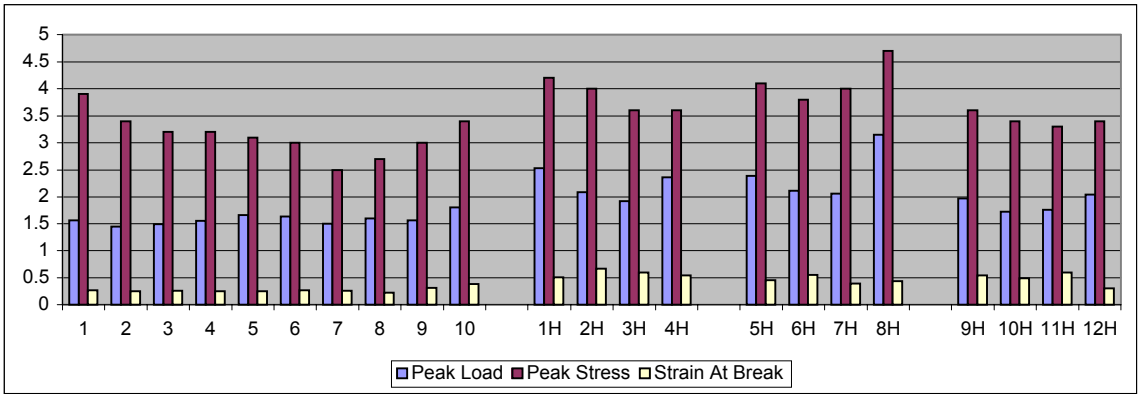
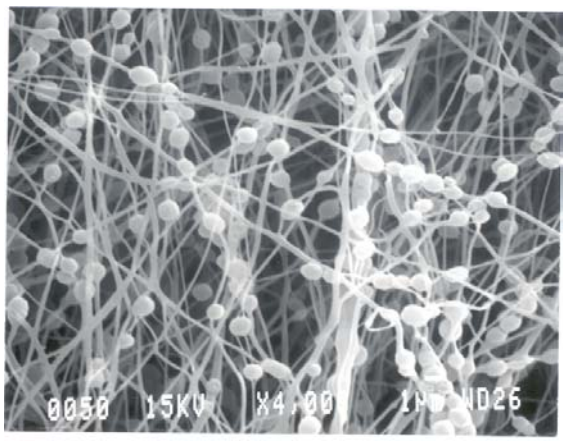


Chart 6. *The relative effects of sample position on the peak load, peak stress, and strain at break in the 70 mg/ml fiber mat.*

The peak stress shows the same pattern along the fiber mat as the modulus does, being higher where the fibers are less aligned, but it has more variability than the modulus. The peak load's pattern is most likely just due to fiber thickness, and there does seem to be a significantly higher strain at break in the orthogonal samples, but within those samples, it shows little pattern.



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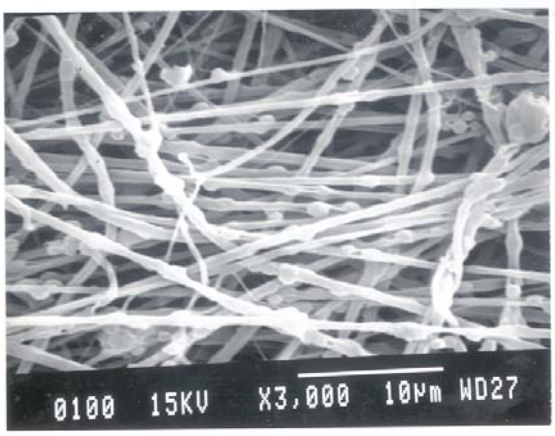

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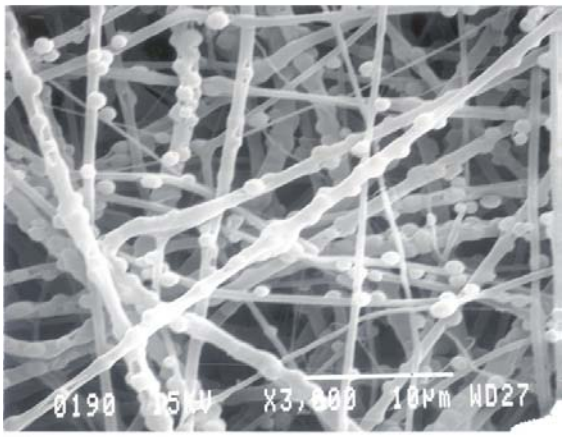
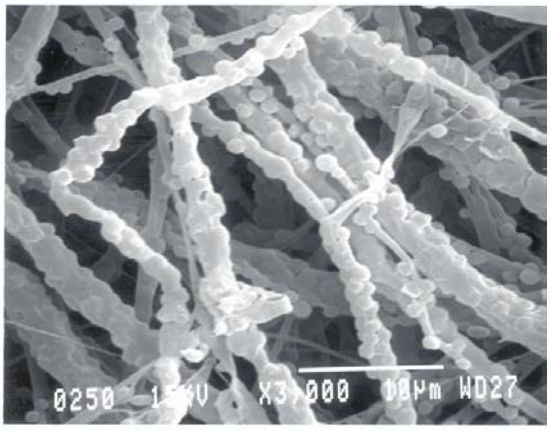


(3C)



(3D)

Figure 3. *Photos of the different concentrations taken at 3000X (4000X for the 50mg/ml) using the SEM. (a) 50mg/ml (b)1000mg/ml (c) 190mg/mL (d) 250mg/mL*

All the concentrations have some of beads in the fibers, and there is not a clear relationship between the number beads and the concentration of the solution. It is unknown whether these develop while the polymer is in the syringe or if they are created in the jet. To try to get rid of the beads, the 160 mg/ml sample, which has a high bead content, was spun at varying voltages. Under inspection using the SEM, we found that the number of beads was not affected, but that the fiber diameter was much larger.

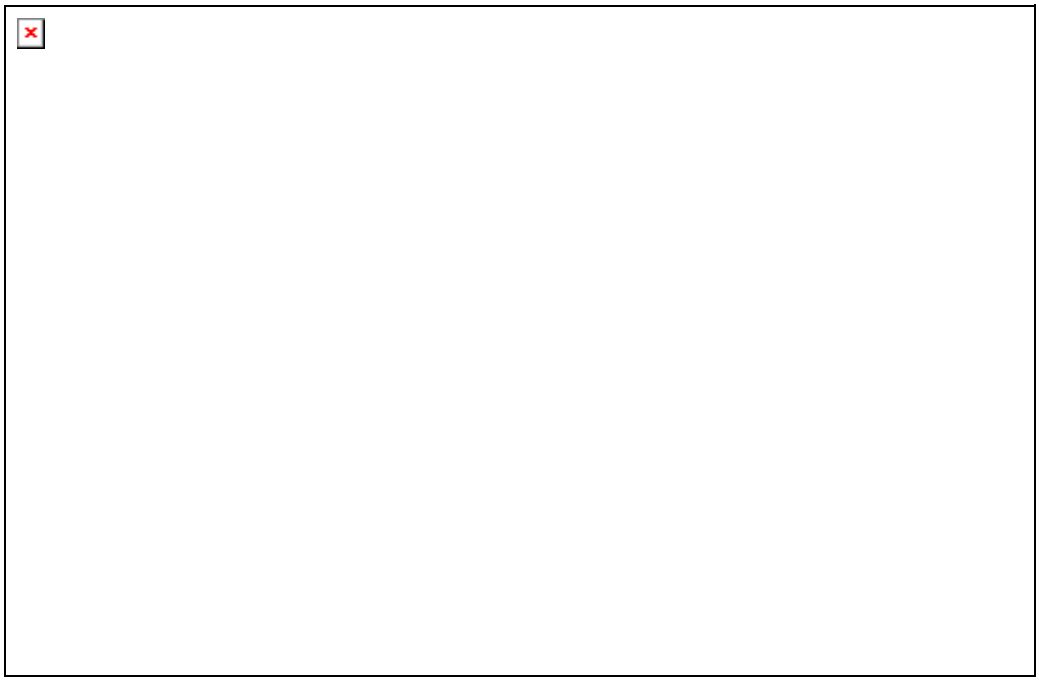


Chart 7. The voltage's affect on the diameter of fibers spun from the 160mg/ml syringe. A roughly linear relationship can be found between the voltages and the diameter of the fibers.

It was found that each of the different concentrations has a slightly different minimum voltage. For 160mg/ml it is around 8kV, while for 70 mg/ml, the lowest possible voltage is around 10kV. The upper limits for the voltage is harder to find since raising the voltage too high creates the risk of sparks, and the diameter did not decrease below the 22kV, so it was not further investigated. Using a lower voltage most notably has the effect of creating fibers much thicker than could be made by changing just the concentration.

Conclusion

PHB can be electrospun from concentrations of 50 to 270mg/ml in TFE. Both the voltage and the solvent concentration can control its diameter, though not very precisely with either. There is variability in the diameter of fibers in any mat of PHB, and there is also beading to different extents in every sample of PHB viewed so far, and there has not been a way found so far to control the beading of PHB. The mechanical properties include whatever effect the beads have on the fibers, and the modulus of elasticity, at least, shows a strong correlation to the concentration of the solvent.

Future studies should be done to test humidity's effect on fiber diameter and properties, since it could affect the rate of solvent evaporation, and there has been some evidence that it does account for some of the variability in the data. Also, preliminary studies were done with NaCl and MgCl2 to see if those would affect the beading, and while neither of them solve the beading problem, either NaCl is creating more beads or MgCl2 is lowering the amount of beads. A sample without salt should be run with those two salts in medium concentrations, along with other salts, to try and characterize their effect on PHB. Third, the samples of PHB collected at the different voltages showed evidence of different mechanical properties, which given their increased diameter, follows logically. A set of those should be created and measure to try to quantify the voltage's effect on the mechanical properties.

References

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