Poly (glycerol sebacate) nanofibers and nanofilms for tissue engineering application

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Poly (Glycerol-Sebacate) Nanofibers and Nanofilms for Tissue Engineering Applications

An Undergraduate Honors College Thesis
in the
Department of Biomedical Engineering
College of Engineering
University of Arkansas
Fayetteville, AR

By

Stephanie Grace Cone
This thesis is approved.

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\[ \text{Signature} \]
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Abstract

In the field of tissue engineering, the development of biodegradable scaffolds that provide both structure and functionality is a major challenge. The use of biological implants, such as decellularized tissues, provides a complete matrix with full tissue functionality, however, problems with immunogenicity often arise when implants from other organisms are used in treatments. As such, there are many benefits to the use of polymeric constructs in tissue engineering. The ability to customize the material, design, and size of an implant provides opportunities for increased structural support and controlled rates of biodegradation.

The selection of polymers for tissue engineering applications requires a strong definition of desired material properties. When designing a biodegradable scaffold, a material may be designed to mimic the properties of the extracellular matrix. This design suggests that an elastomer which is flexible, strong, and entirely biodegradable would be fitting for scaffold applications. Poly (glycerol-sebacate) (PGS) is an inexpensive elastomer which exhibits many of these desirable characteristics.

Within this project, PGS was synthesized by previously published methods. The polymer was combined with PCL in order to make a mechanically robust solution, and nanofibers were fabricated using the PGS/PCL compound solution. Nanofilms were created with both the compound and pure PGS in order to study the difference in surface characteristics between the two.

Key words: Biomedical Engineering, Materials Science Engineering
Section 1: Introduction

Nanomaterials

The advent of nanotechnology has brought many opportunities to the field of biomedical engineering. New imaging, characterization, and assembly techniques have been introduced, along with an entire branch of nanomaterials. Nanomaterials can be defined as materials which have basic components such as grains, particles, or fibers which are less than one hundred nanometers long in one or more dimensions. Nanomaterials have the potential to improve current methods in all levels of medicine, from prevention to treatment.

Nanomaterials provide many new opportunities in the medical field primarily because they exhibit a range of surface properties which is not seen in traditional microscale materials. This is partially due to the increased surface area to volume ratios in nanomaterials and nanostructures. Increased surface area leads to both heightened physiochemical properties and increased surface energetics. With these changes, nanomaterials interact with cells and other nanoscale features with greater strength than their microscale counterparts. The increased available surface area seen with nanomaterials leads to an increase in binding sites to proteins and other organic components, which can result in greater biocompatibility in devices and implants which have a nanomaterial surface.

Nanomaterials can be designed with patterns which encourage the growth and development of native cells in optimal directions. This is due to the similarity in size scale of nanofeatures and biomolecules. The scale of human biological components can be seen below in figure 1. Within natural tissues, an extracellular matrix is created with nanoscale features and
properties \cite{14}. By patterning biomedical constructs with nanoscale features, implants can be designed in a manner which encourages the directional growth of new cells.

**Figure 1**: Size scale of biological components and the appropriate equipment for studying features \cite{8}.

Despite the previously mentioned benefits to using nanomaterials in biomedical applications, there are still some issues with the field. Nanotechnology is a relatively young field, as it was only introduced on a public stage in 1974 \cite{14}. With this young age, there is little information on the long-term impact of nanoscale features in human applications. Some studies have shown nanoparticle interaction to be potentially hazardous to human health, as nanoparticle debris may be taken up by native cells in the body, sometimes leading to issues with
cytotoxicity\textsuperscript{14}. Until these effects can be better understood and controlled, there will be major limits to the applications of nanomaterials in human medicine.

\textit{Nanofibers}

In order to develop tissue engineering scaffolds, biomaterials must be formed into primary structures. One type of base structure which can be created is composed of nanofibers. Nanofibers are strands of material with a diameter which measures in the nanometer scale. Nanofibers have traditionally been created through the process of electrospinning, but the field of centrifugal jet spinning provides a means of creating large amounts of highly aligned nanofibers with much greater efficiency than electrospinning.

Nanofibers are good base structures for tissue scaffolds for a variety of reasons. When nanofibers are compiled into a scaffold, the amount of material packing is relatively low and as such the structure has a high level of porosity\textsuperscript{4}. This is beneficial in tissue engineering because with greater levels of porosity, there is a greater level of host cell colonization. Once the host cells have become integrated into the scaffold structure, the porous nature of the nanofibers also allows for greater interaction with the host extracellular matrix, and nutrients and waste can be exchanged within the scaffold with more efficiency than a tightly packed scaffold would permit\textsuperscript{4}.

The method of electrospinning is widely used as the current standard method of nanofiber production. Electrospinning uses an electric field to direct the flow of a solution through a nozzle, creating nanofibers which are then directed onto a collection vessel\textsuperscript{4}. While this method is well understood, there are potential ways to improve the standards of nanofiber production. The advent of centrifugal jet spinning provides a way to produce nanofiber scaffold more efficiently, with a wider range of materials, and with much greater degrees of alignment\textsuperscript{2}. 
Centrifugal jet spinning is a method of nanofiber production which uses the physical forces of rapid rotation to extrude a stream of polymer nanofiber from a central nozzle\(^2\). An example of a centrifugal jet spinner design and an electrospinnner can be seen in figure 2B below. By using high speeds, in the order of 20,000 to 30,000 RPM, centrifugal jet spinners are capable of producing fibers from ten milliliters of solution in less than a minute, whereas electrospinners produce fibers from around one milliliter of solution in an hour\(^2\). This increase in production speed is a major step in the process of commercializing nanofiber products, as centrifugal jet spun fibers are efficient enough to use in large scale productions.

Figure 2. Apparatus designs for centrifugal jet spinning (A) and electrospinning (B)\(^2,11\).

Another benefit to using centrifugal jet spinning is the alignment of the resulting nanofibers. With electrospinning, nanofibers are simply deposited onto a plate, becoming a tangle of fibers. While there are some versions of electrospinners which deposit the fibers onto a rotating mandrel, resulting in some alignment, the fibers are never tightly packed with consistent alignment. Through the use of centrifugal jet spinning, nanofibers are produced in a single scaffold with a high degree of alignment. A sample of super aligned nanofibers can be seen in the macro-, micro-, and nano-scales can be seen below in figure 3. A major benefit of having
highly aligned nanofiber scaffolds is that the relative strength of a scaffold in the direction of alignment can be very high due to the anisotropic alignment of the fibers.

Figure 3. Super aligned nanofibers as produced by the Disease Biophysics Group at Harvard University. Scale bars for the second and third images are 100 microns and 10 microns, respectively.

When composed of biocompatible materials, nanofiber scaffolds provide new opportunities in many fields within biomedical engineering. While current planned applications include drug delivery and tissue engineering applications, the high levels of cytocompatibility and wide variety of material options associated with centrifugal jet spinning could lead to the involvement of nanofibers in any branch of medicine.

Nanofilms

Nanofilms are an area of interest for many reasons. In biomedical implants, an important aspect of material selection involves the surface characteristics of a device. This is due to the importance of surface interactions between the native tissue and the engineered implant. As such, the addition of a coating to a device can completely change the way it interacts with tissue. In many cases, the addition of coatings which contain either adhesive proteins or growth factors can influence native cells to behave in specific manners. This can lead to native tissue covering a device, which may be beneficial to the acceptance of a therapeutic implant in a patient. If designed properly, these coatings can also encourage specific types of cells to grow in patterns,
which may aid in the development of new tissue with components such as vasculature or natural grain boundaries.

In studying the development of nanomaterials, nanofilms are a valuable topic. Nanofilms can provide a consistent surface with well-characterizable surface properties. Due to the similar size features of nanomaterials and the organic components mentioned earlier in figure 1, nanofilms provide a means of integrating greater biocompatibility to devices, while adding minimal excess volume. The interaction between nanomaterials and natural cells can be seen below in figure 4.

**Figure 4.** Protein adsorption (A), osteoblast attachment (B), and osteoblast differentiation (C) with nanomaterials and conventional materials.

Poly (Glycerol-Sebacate)

In selecting materials for biomedical applications, there are many important characteristics for optimal performance. The chemical environments, temperature gradients, and mechanical stresses found within the human body require implants which can react to many situations in a manner similar to native tissues. Poly (glycerol-sebacate) (PGS) is a robust elastomer with tunable biodegradation rates and controllable mechanical properties. These features qualify PGS as a candidate material for tissue engineering scaffolds.
PGS is an elastomer which is strong, flexible, biocompatible, capable of reversible deformations, and inexpensive\textsuperscript{12}. The properties of this material are similar to those found in collagen and elastin, which are two of the major fibrous components of the human extracellular matrix\textsuperscript{12}. This similarity leads to the opportunity for utilizing PGS in scaffolds which will be absorbed into native tissue over time.

PGS is synthesized through a process of hydrolysis between glycerol and sebacic acid\textsuperscript{12}. Both of these materials are biocompatible and nontoxic, and the Food and Drug Administration has previously approved the use of glycerol as well as polymers which contain sebacic acid in biomedical implants and applications\textsuperscript{12}. As such, PGS is a valid material for use in tissue engineering and medical scaffolds. PGS was designed to contain an ester bond, which provides a hydrolysable bond within the polymer\textsuperscript{12}. This is beneficial in having the polymer degrade easily within tissue environments. The synthesis and final chemical structure of PGS can be seen below in figure 5.

![Synthesis mechanism and structure of PGS](image)

**Figure 5.** Synthesis mechanism and structure of PGS\textsuperscript{12}.

One of the major characteristics of PGS is its elastomeric behavior. This is partially caused by the low amounts of crosslinking found in the polymer. This behavior can be altered by either a change in the curing time or the molar ratios used in the synthesis process, resulting in a rigid polymer which contains a much higher degree of crosslinking\textsuperscript{12}. PGS has been used to
development sheets, foams, tubes, and discs through methods including plate molding and salt fusion molding in previous research projects\textsuperscript{6,12}.

In cell culture studies, PGS has been shown to be a viable substrate for cell types such as fibroblasts, hepatocytes, cardiac muscle, smooth muscle tissue, and Schwann cells\textsuperscript{6}. \textit{In vivo} studies have also been done with PGS, in which implants were determined to dissolve completely within 60 days, with tissue sites returning completely to their natural state\textsuperscript{12}.

The mechanism by which PGS degrades in tissue samples has been determined to be surface erosion\textsuperscript{10}. PGS implants degrade at a rate which has been experimentally found as a function of the degree of crosslinking exhibited by the polymer, which can be controlled by the length and heat intensity of the curing stage in the synthesis reaction\textsuperscript{10}. This information is very valuable, as some biomedical scaffolds may be required to last longer than others, depending on the amount of support required by the local tissue. Different degradation rates may also be desired in PGS depending on the purpose of a specific scaffold. While some implants may be primarily used as a method of drug delivery, where a rapid degradation rate may be needed, other implants may be intended to provide long-term support to tissue while the natural architecture of the muscle heals. With PGS, these varied characteristics can be achieved by altering the synthesis protocol.

\textit{PGS Nanofibers}

When selecting the materials and base structures for tissue engineering scaffolds, there are many factors to consider. The biomaterials must be biocompatible, have similar mechanical properties to natural tissue, and must not create any toxic byproducts. Base structures should
provide optimized mechanical properties, provide a means for controlling the surface area of the scaffold, and be produced efficiently.

The fabrication of PGS nanofibers through centrifugal jet spinning is an efficient process that results in a strong option for tissue engineering scaffolds. The scaffold consists of a tough, biodegradable elastomeric biomaterial with a high ratio of surface area to volume.
Section 2: Methods and Materials

*PGS Synthesis*

PGS was synthesized through a polycondensation reaction of glycerol and sebacic acid. Glycerol and sebacic acid were added to a three neck flask in a one-to-one molar ratio. The polymer was reacted under argon at 120°C for 24 hours. The flask was then moved to a vacuum oven and the prepolymer was cured for 48 hours at 120°C and 40 mtorr. This experimental protocol was developed following methods from previously published work\textsuperscript{12}. The experimental setup for the synthesis reaction can be seen below in figure 6.

*Figure 6.* Experimental setups for PGS synthesis reaction.
Centrifugal Jet Spinning

Nanofiber scaffolds were fabricated by using a centrifugal jet spinner with polymeric solutions. PGS was combined with PCL and dissolved in HFIP. This solution was injected into a centrifugal jet spinner nozzle, and then run at rotational speeds of 20,000, 25,000, and 30,000 RPM. Highly aligned nanofibers would then but cut down the side with a razor blade, and were carefully removed in a single ribbon from the nozzle using tweezers. The ribbons were stored in petri dishes labeled with pertinent information such as date, chemical composition, and rotational speeds. Nanofiber scaffolds were then collected from the nozzle with tweezers.

Through centrifugal jet spinning, super aligned scaffolds of polymer nanofibers were produced. Once spinning was complete, the ribbon was removed from the nozzle without disturbing the relative orientations of the fibers. This process can be seen in figure 7A and 7B below. Following the removal of the scaffold from the nozzle, the fibers were studied at different size scales. This can be seen in figure 8 in the next section.

Figure 7. The process of fiber scaffold creation can be seen in (A) the design of the centrifugal jet spinner, and (B) the removal of a scaffold from a nozzle.
Spincoating

In order to develop nanofilms from polymer solutions, 7% solutions of PCL in HFIP, PGS in chloroform, PGS in HFIP, and a 50/50 combination of PGS and PCL were deposited on glass coverslips. A spincoater was used with two different recipes which are described in Appendix A to create nanofilms of two thicknesses from each solution. The recipes used in the spincoater differed by their maximum rotational speeds, with recipe one reaching 4000 RPM and recipe 15 reaching 6000 RPM.

Mechanical Testing

Mechanical strain testing was performed on the nanofiber scaffolds in order to determine the Young’s modulus of PGS/PCL nanofiber scaffold created at rotational speeds of 20,000, 25,000, and 30,000 RPM. For this test, ribbons of nanofiber scaffolds were mounted within one inch squares of paper using double sided tape. The mounts were placed in an Instron strain tester, and a strain test was performed. Data was recorded on the mechanical force and change in length throughout the test. Calculations were then performed to find the stress vs. strain plots of the nanofiber scaffolds, and then to find the Young’s modulus for scaffolds created at each rotational speed. In order to complete these calculations, the dimensions of each specimen were input into a custom Matlab code.

Scanning Electron Microscopy

Scanning electron microscopy was used to study the features of individual PGS/PCL nanofibers, as well as the general alignment of the scaffolds. Pieces of nanofiber ribbon were mounted on studs and sputtercoated with gold in preparation for the imaging. Images were gathered from scaffolds created at 20,000, 25,000, and 30,000 RPM. Images were taken at three
different length scales in order to compare the alignment as well as the individual fiber properties.

**Water Contact Angle Testing**

Sessile drop water contact angle testing was performed on nanofilms containing all of the nanofilms created in the spincoating step. This test was conducted in order to study the surface properties of the polymer films. Three drops were tested on each nanofilms sample, resulting in 6 data points for every solution. Water contact angles were studied for both the nanofilms created with spincoater recipe 1 and 15, however, the films created with recipe 15 did not provide ample coverage of the coverslips, and the water contact angles were affected by the contact of water with the glass coverslips. Water contact angle testing was also performed on PGS/PCL nanofiber scaffolds for comparison of the polymer properties in nanofibers and nanofilms.
Section 3: Results

The results of this project can be separated into the categories of nanofilms and nanofibers, both of which utilized materials consisting of poly (glycerol-sebacate) (PGS), polycaprolactone (PCL), or a compound of both.

*Nanofiber Results*

Nanofibers can be used as a scaffold for many tissue engineering applications as they exhibit many beneficial physical and mechanical properties. In order to characterize these properties, analytical tests were performed on PGS/PCL nanofiber ribbons which were made at a variety of rotational speeds. The fibers were tested for their water contact angle, Young’s modulus, orientation order parameter, and average fiber diameter.

![Figure 8](image)

**Figure 8.** PGS/PCL compound nanofibers created at 20,000 RPM seen at different length scales. Scale bars are 50, 10, and 5 microns, respectively.

PGS/PCL nanofibers were created at 20,000 RPM, 25,000 RPM, and 30,000 RPM. The resulting ribbons were studied with scanning electron microscopy in order to analyze the effect of rotational speed on the nanofiber characteristics. Images of scaffolds created at these speeds can be seen in figure 9.
Figure 9. PGS/PCL compound nanofibers which were created by centrifugal jet spinning at speeds of (A) 20,000 RPM (B) 25,000 RPM and (C) 30,000 RPM. Scale bars are all 10 microns.

Through analysis of the scanning electron microscopy images such as those in figure 9, an average fiber diameter was calculated for the scaffolds created at 20,000, 25,000, and 30,000 RPM. The average diameters can be seen below in figure 10. With the increasing rotational speeds, decreasing average fiber diameters were observed. When the average values were plotted and a line of best fit was applied, an $R^2$ value of 0.9274 was returned. As seen in the caption in figure 10, a one way ANOVA test showed that the differences in fiber diameters were statistically significant between all of the rotational speeds, with p values less than 0.05 in each case. This correlation confirmed the anticipated pattern of greater speeds leading to lower average fiber diameters.
In order to study the levels of alignment found within nanofiber scaffolds, the orientation order parameter (OOP) was calculated. In a perfectly aligned sample, the OOP would be 1, while in a sample with perfectly random alignment, the OOP would be 0. The anticipated correlation is that with greater rotational speeds, nanofibers should be better aligned, and as such will have higher OOP values. Following analysis of fibers created at three speeds, the OOP values were shown to increase with increasing speeds in PGS/PCL compound nanofibers. The values were plotted in figure 11 below, and when a line of best fit was applied to the graph, it returned an $R^2$ value of 0.899.

**Figure 10.** Average fiber diameters measured for PGS/PCL compound nanofibers which were spun at three different rotational speeds. *$p$ value $< 0.05$.**
Figure 11. Orientation order parameter (OOP) values for PGS/PCL compound nanofiber scaffolds created at various rotational speeds.

The final testing performed on nanofiber scaffolds consisted of determining the Young’s modulus of each ribbon. This was calculated by plotting the stress versus strain plots of each scaffold. The plots from each scaffold can be seen below in figure 12, while the values of the Young’s modulus of each can be seen in table 1. The stress vs strain plots for the 20,000 and 30,000 RPM scaffolds both follow an expected form, with a toe region, linear region, and a plateau at the peak of stress. The linear regions of the stress versus strain plots can be seen below in figure 12. The Young’s modulus values were found for these samples, and can be seen in table 1. The values decreased with the increasing speeds, which correlates with the decreasing fiber diameters. The smaller fibers from the 30,000 RPM tests were weaker than the other fibers, which is reflected by its lower Young’s modulus.
Figure 12. Stress versus strain curves for nanofiber scaffolds created at (A) 20,000 RPM, (B) 25,000 RPM, and (C) 30,000 RPM.

<table>
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Table 1. Young’s modulus values for nanofiber scaffolds.

Nanofilm Results

In order to study the surface properties related to PGS nanofilms, solutions consisting of PGS and PCL were made using both chloroform and HFIP as solvents. Nanofilms were created on glass coverslips using a spincoater. The hydrophobicity aspects of the nanofilms were studied by taking the water contact angles with sessile drop goniometry.

In order to understand the impact of combining PCL and PGS into a polymer compound, nanofilms of both pure substances and a 50:50 compound solution were characterized by their water contact angles. The water contact angle study also provided information on the effect of different solvents on the polymers. As seen in figure 13, the nanofilms made of the same solution
produced similar water contact angles to one another. Overall, the water contact angles of both the PCL and PGS films were lower than the angle reported for PDMS, meaning that both of the polymers were more hydrophilic than PDMS. The compound nanofilms produced water contact angles between those of pure PCL and pure PGS solutions, which suggest that they are exhibiting a combination of surface properties from their constituents.

Figure 13. Average water contact angles from nanofilms of various solutions.
Section 4: Discussion

In this research, nanofibers and nanofilms were fabricated containing a compound of PGS and PCL. These structures were characterized through studying their surface properties, mechanical properties, and topographies. The nanostructures were studied in with specific consideration to their potential as tissue engineering scaffolds.

Nanofibers were fabricated with a compound solution of PGS and PCL through a process of centrifugal jet spinning. These fibers followed many trends commonly found in centrifugal jet spun fibers, such as a decrease in average fiber diameter with increasing rotational speeds. The fiber scaffolds were imaged with scanning electron microscopy, and scaffolds produced at 20,000, 25,000, and 30,000 RPM were shown to contain a network of highly aligned polymeric fibers.

As seen in the results section, as rotational speeds were increased in scaffold fabrication, the resulting nanofibers became smaller in diameter and more highly aligned. Both of these trends were anticipated, as they were also reported in previously published articles with other polymer solutions. Following mechanical testing of PGS/PCL nanofiber scaffolds, it seems that the Young’s modulus decreased with increasing rotational speeds. This trend was expected, as higher speeds produced thinner nanofibers whose properties were not as elastic as the thinker fibers produced at lower speeds.

Nanofilms consisting of pure PGS, pure PCL, and a combination of the two were tested for hydrophobicity with a sessile drop goniometer, and the compound films exhibited water contact angles that were between the angles found with PGS nanofilms and PCL nanofilms. The water contact angles for all of the nanofilms were relatively similar to one another, and there was
a difference seen in the PGS which had been dissolved in chloroform and the PGS in HFIP, so
the data may not have been completely representative of the compound properties of PGS and
PCL. All of the water contact angles measured were lower than the water contact angle of
PDMS, and as such all of the nanofilms were more hydrophilic than PDMS. All of the nanofilms
resulted in water contact angles less than 90 degrees, which means that all of the materials were
hydrophilic. This is beneficial for tissue engineering constructs, as proteins are more likely to
bind to a hydrophilic material than a hydrophobic one. The water contact angles were reported
from a spincoating method which only reached a maximum speed of 4,000 RPM, as the method
which reached 6,000 RPM resulted in streaky films that may not have provided accurate water
contact angle values.

Overall, the fabrication of centrifugal jet spun PGS nanofibers resulted in many of the
desired properties for tissue engineering scaffolds. The scaffolds consisted of a network of
aligned fibers, with diameters that could be controlled by the rotational speeds used in the
experimental protocol. The use of PGS in nanofiber scaffolds resulted in a scaffold with surface
properties which mimic many of the natural properties of collagen and elastin. These features
suggest that PGS nanofibers may be a useful base material for many tissue engineering
applications.
Section 5: Future Work

The development of PGS nanofilms and nanofibers provides the opportunity for many tissue engineering innovations. In the future, further experimentation on the viability of these films and fibers may be done through cell cultures, and eventually mouse models. In the future, test for in vitro cell attachment with PGS nanofiber scaffolds may be completed in order to study the viability of the nanofibers as a tissue engineering scaffold. Comparisons of the growth rates of cells on PGS nanofibers compared to PCL nanofibers will provide information on the biocompatibility of PGS. A study of cells grown on PGS/PCL nanofilms compared to nanofibers will provide information on the influence of a nanofiber scaffold in cell alignment properties. Following the confirmation of PGS nanofibers and nanofilms as tissue engineering scaffolds, a project may be developed to study the ability to use these scaffolds as drug delivery systems. This could be accomplished either through the synthesis of a compound solution of PGS and various drugs, or through creating multi-layered nanostructures.
Appendix A: Experimental Protocol

*Spincoat Recipe 1 Protocol*

The following steps were completed by a spincoater for nanofilms produced by recipe 1.

*Step 1:* Ramp 5, RPM 500, Dwell 5

*Step 2:* Ramp 5, RPM 1000, Dwell 5

*Step 3:* Ramp 10, RPM 1000, Dwell 10

*Step 4:* Ramp 10, RPM 4000, Dwell 60

*Step 5:* Ramp 10, RPM 2000, Dwell 15

*Step 6:* Ramp 10, RPM 1000, Dwell 10

*Step 7:* Ramp 5, RPM 500, Dwell 5

*Spincoat Recipe 15 Protocol*

The following steps were completed by a spincoater for nanofilms produced by recipe 15.

*Step 1:* Ramp 10, RPM 3000, Dwell 5

*Step 2:* Ramp 10, RPM 6000, Dwell 60

*Step 3:* Ramp 10, PRM 3000, Dwell 5
Centrifugal Jet Spinning Protocol

1. Attach nozzle to motor base using set screws
2. Set desired rotational speed through computer interface
3. Supply power to motor to initiate nozzle rotations
4. Fill pipet with 5 mL of polymer solution
5. Inject solution into nozzle while it is spinning
6. Wait for polymer to stop spraying out of nozzle
7. Disconnect power from motor
8. Remove nozzle from motor base
9. Slice nanofiber scaffold down the side of the nozzle
10. Peel scaffold from the nozzle using tweezers
Appendix B: Analysis Protocol

*Scanning Electron Microscopy*

PGS/PCL nanofibers spun at 20,000 RPM
PGS/PCL nanofibers spun at 25,000 RPM
Cone 36
PGS/PCL nanofibers spun at 30,000 RPM
Nanofilm Water Contact Angle Test Images

PCL in HFIP Spincoat Recipe 1

PCL in HFIP Spincoat Recipe 15

PGS in chloroform Spincoat Recipe 1

PGS in chloroform Spincoat Recipe 15
PGS in HFIP Spincoat Recipe 1

PGS in HFIP Spincoat Recipe 15

PGS/PCL in HFIP Spincoat Recipe 1
## Nanofilm Water Contact Angle Values

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</tr>
<tr>
<td>PGS chloroform R15</td>
<td>81.4</td>
<td>81.3</td>
<td>81.35</td>
<td>0.07</td>
</tr>
<tr>
<td>PGS chloroform R15</td>
<td>89.9</td>
<td>89</td>
<td>89.45</td>
<td>0.64</td>
</tr>
<tr>
<td>PGS HFIP R1</td>
<td>53.1</td>
<td>54.5</td>
<td>53.8</td>
<td>0.99</td>
</tr>
<tr>
<td>PGS HFIP R1</td>
<td>50.7</td>
<td>52.3</td>
<td>51.5</td>
<td>1.13</td>
</tr>
<tr>
<td>PGS HFIP R1</td>
<td>51.9</td>
<td>52.4</td>
<td>52.15</td>
<td>0.35</td>
</tr>
<tr>
<td>PGS HFIP R15</td>
<td>63.5</td>
<td>63.3</td>
<td>63.4</td>
<td>0.14</td>
</tr>
<tr>
<td>PGS HFIP R15</td>
<td>70.7</td>
<td>72.7</td>
<td>71.7</td>
<td>1.41</td>
</tr>
<tr>
<td>PGS HFIP R15</td>
<td>66.8</td>
<td>68.2</td>
<td>67.5</td>
<td>0.99</td>
</tr>
<tr>
<td>PGS PCL R1</td>
<td>81.7</td>
<td>83.7</td>
<td>82.7</td>
<td>1.41</td>
</tr>
<tr>
<td>PGS PCL R1</td>
<td>78.5</td>
<td>79.2</td>
<td>78.85</td>
<td>0.49</td>
</tr>
<tr>
<td>PGS PCL R1</td>
<td>80.4</td>
<td>80.2</td>
<td>80.3</td>
<td>0.14</td>
</tr>
<tr>
<td>PGS PCL R15</td>
<td>71.7</td>
<td>71.8</td>
<td>71.75</td>
<td>0.07</td>
</tr>
<tr>
<td>PGS PCL R15</td>
<td>69.6</td>
<td>69.8</td>
<td>69.7</td>
<td>0.14</td>
</tr>
<tr>
<td>PGS PCL R15</td>
<td>65.5</td>
<td>65.8</td>
<td>65.65</td>
<td>0.21</td>
</tr>
</tbody>
</table>
ANOVA Analysis of Average Fiber Diameters

One Way Analysis of Variance

Data source: Data 1 in Notebook1

Normality Test (Shapiro-Wilk)  Passed  (P = 0.813)

Equal Variance Test:  Passed  (P = 0.097)

<table>
<thead>
<tr>
<th>Group Name</th>
<th>N</th>
<th>Missing</th>
<th>Mean</th>
<th>Std Dev</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>20k</td>
<td>24</td>
<td>0</td>
<td>1.136</td>
<td>0.206</td>
<td>0.0421</td>
</tr>
<tr>
<td>25k</td>
<td>24</td>
<td>0</td>
<td>0.812</td>
<td>0.200</td>
<td>0.0408</td>
</tr>
<tr>
<td>30k</td>
<td>24</td>
<td>0</td>
<td>0.700</td>
<td>0.134</td>
<td>0.0273</td>
</tr>
</tbody>
</table>

Source of Variation

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2</td>
<td>2.451</td>
<td>1.225</td>
<td>36.577</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>69</td>
<td>2.312</td>
<td>0.0335</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>4.763</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference  (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

All Pairwise Multiple Comparison Procedures (Holm-Sidak method):
Overall significance level = 0.05

Comparisons for factor:

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Diff of Means</th>
<th>t</th>
<th>P</th>
<th>P&lt;0.050</th>
</tr>
</thead>
<tbody>
<tr>
<td>20k vs. 30k</td>
<td>0.435</td>
<td>8.237</td>
<td>&lt;0.001</td>
<td>Yes</td>
</tr>
<tr>
<td>20k vs. 25k</td>
<td>0.323</td>
<td>6.114</td>
<td>&lt;0.001</td>
<td>Yes</td>
</tr>
<tr>
<td>25k vs. 30k</td>
<td>0.112</td>
<td>2.123</td>
<td>0.037</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Matlab Code for Stress Strain Plots

% Balalab 4/23/2014
% This code is applied to plot the linear region of tensile stress curves
% Make sure you delete all the headers in the *.CSV file
% Rename the file so that there are no spaces or dots
% The file should only start with the data
% calculate stress in column 5
% calculate strain in column 4
% limit spreadsheet to data in the linear region

close all

%import data from spreadsheet
[rawdata,pathname]=uigetfile(’.csv’,’select the data file?’)
raw_data=open(rawdata);

%truncate .csv extension
stringsize=size(rawdata);
filename=rawdata(1:stringsize(2)-4);

% change this file name to match the data set
data=raw_data.Specimen_RawData_125R1S;

%defines data locations in the spreadsheet
stress=(data(:,5));
strain=(data(:,4));

%plots linear region of stress/strain curve
plot(stress,strain);
xlabel('Strain')
ylabel('Stress [MPa]')

%change name for the sample title
title('25K Stress vs Strain')
References


