Development of Machines to Engineer Biocompatible Matrices in Ligament, Filtering and 3D Cell Culture Applications

By

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ABSTRACT

This thesis investigates three possible applications of engineered matrices manufactured from Polycaprolactone and Nylon-6 using electrospinning techniques. A method to manufacture bio compatible scaffolds for biomedical applications was studied. The scaffold was manufactured using a Polycaprolactone-PEGDA matrix to increase the viability of growth of cells. Mouse osteoblast cells were cultured on the samples and incubated to check for viability of the cells. The procedure to analyze the samples were not conclusive to show the viability of cells within the samples tested. The use of electrospun Polycaprolactone nanofiber cloth as a functional air filter was tested. As a model for analysis the filters were exposed to a steady stream of air that was polluted by tobacco smoke. The filters were weighed before and after exposure to find the effectivity of filtration. The filters were also imaged using X-ray diffraction to show the effectivity of filtration. The analysis shows that the weight of the filter after filtration was increased by up to 12% by weight. Procedures to manufacture artificial Anterior Cruciate Ligament were investigated. Mechanical properties of the artificial ACL were compared to the properties of ACL harvested from three rabbits. Samples for comparison were made from Polycaprolactone, Nylon-6 and Polycaprolactone-Nylon-6 twisted pair, Polycaprolactone Braided triplet. Prepared samples were broken in a tensile testing machine and the tensile properties were compared to values for the ACL harvested from the host rabbit. The research shows that the samples made were not able to reach the capabilities of the native rabbit ACL.

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PCL - Polycaprolactone

ACL – Anterior Cruciate Ligament

PEGDA – Poly(ethylene glycol) diacrylate

SEM - Scanning Electron Microscope

Chapter 1

Introduction

1.1 Electrospinning

There has been considerable research in the field of electrospinning as a method to produce nanofibers of various forms. The technique of electrospinning is fast, easy and does not require specialized equipment. The setup is portable and can be used in relatively small space. The nanofibers created from these methods are also consistent. This is a repeatable process for a very inexpensive setup [1-7].

Figure (1) shows the electrospinning process [8]. A solution of specific polymer is prepared and atomized into an electric field. The electric field causes the fibers to form a cone as seen in figure (1). The fibers fall toward the negative potential after which they can be collected to be used in desired application.

Electrospinning is a general term for many processes that use the same concepts and properties. The arrangement of the electrodes, dispensing system and collection system defines the process further. In previous attempts success was achieved in spinning using drum, plate and wire setups as seen in figures (1-3). These are very useful in forming fiber cloth that can be used as a building block for other applications. These methods however cannot standardize the topology of the cloth as the collection is random. Though this is acceptable in certain applications the applications here were investigated for specific topology and pore size. This was achieved using dual disc collection methods as shown in figure (4).

The apparatus was set up using stepper motors to rotate two discs on a flat base. The discs were grounded and the positive potential was connected to a suspended nozzle. The polymer solution was loaded into a syringe and dispensed through a tubing system using a syringe pump. After careful observation the height of needle, voltage of the setup, speed of dispensing solution were fixed as the standard. Though the process was seen to form consistent samples, the process is vulnerable still to external conditions. With practice and patience however the user can manufacture samples with consistent precision.

Materials used in electrospinning vary considerably from biocompatible to incompatible material. Nanofibers can be tailored to have different properties that can make them beneficial to specific applications. Natural and synthetic polymers can be used to manufacture tissue engineering scaffolds, filtration membranes and other biomedical uses. By using naturally occurring polymers to make scaffolds the chances of cells to adhere and migrate into the sample is greater [9]. The use of using material to coat onto the existing fibers has also been investigated. The use of collagen, gelatin, elastin and silk fibroin was investigated as an improvement on the fiber alone [10-13].

1.2 Anterior Cruciate Ligament

The Anterior Cruciate Ligament is a common source of injury among athletes and non-athletes alike. The reason for this is exertion to extreme or momentary shock to the knee. This also is seen when the subject is older and has tiered and joints that are not functioning at optimum. This is also seen in children whose body is still growing and adapting to various levels of exertion. Between the years of 2005 and 2015 there were 320 anterior cruciate ligament injuries that were treated in children between the ages of five to fourteen in Vistoria, Australia alone [14].

The current reconstruction techniques of the injured anterior cruciate ligament consists of grafting a donor ligament in the place of the original anterior cruciate ligament. The donor ligament can be taken from the patient's own body or can be taken from an external source. This research explores the viability of manufacturing an anterior cruciate ligament substitute out of biocompatible material to be used as the repair that will in time regenerate into new tissue to replace the original ligament. The substitute will also degenerate over time to end up with a fresh ligament where the injured anterior cruciate ligament used to be. By using an artificial anterior cruciate ligament the patient is not required to go through additional surgery. This is beneficial to the patient since there is a reduced amount of risk and of infection. An artificial ligament also allows for a possibility of engineering a superior quality ligament over time.

1.3 Air filter

Airborne contaminants are a problem every day for people. With the growth in air pollution this issue is becoming a concern for the everyday person. No matter how much one tries to shield themselves from this, the issue still remains. Inhalation of particulate matter of the size of 2.5μm and below is seen to have harmful effects on the health, vision, climate and ecosystems [15-18]. Considerable research has been done on using electrospun nanofiber matrices to filter out microscopic contaminants [19].

When aligned nanofibers are layered onto each other in a crisscross pattern the pores of the resulting mesh can be regulated in size. This allows for a considerable amount of filtering capability tailored to filter out known contaminants. This is the focus of this research. The filters were allowed to obstruct the path of a steady stream of tobacco smoke made to resemble the lung function of an adult male. The filters were seen to provide filtering of 30% by weight of contaminants. These filters were imaged in a scanning electron microscope and x-ray diffraction setup. The filters were seen to filter out carbon and oxygen as the top two elements.

1.4 3D Cell Culture Scaffold

Considerable promise is seen in the use of functional scaffolds to grow cells [20, 21]. The ideal scaffold will be able to allow the desired cells to grow and proliferate into the sample so as to facilitate the implant. Now if the scaffold itself will degenerate with time, and the degeneration is proportional to the growth of cells, in time the implant will completely disappear and the patient will be left with a healthy and useful body part.

The focus of this section is to test a composite matrix of Polyethene glycol diacrylate (PEGDA), Glycated Chitosan and Polycaprolactone to serve as a functional scaffold to facilitate 3D cell culture. The viability of this material was tested over a set of samples using mouse osteoblast cells as a cell culture medium.

On analysis however the tools used to justify the migration of the cells into the sample was not adequate. The cells are present in the sample but after sectioning and imaging the structure of the sample was seen to be compromised. There was not enough evidence to show that the matrix was able to proliferate and adhere the cells.

1.5 Figures

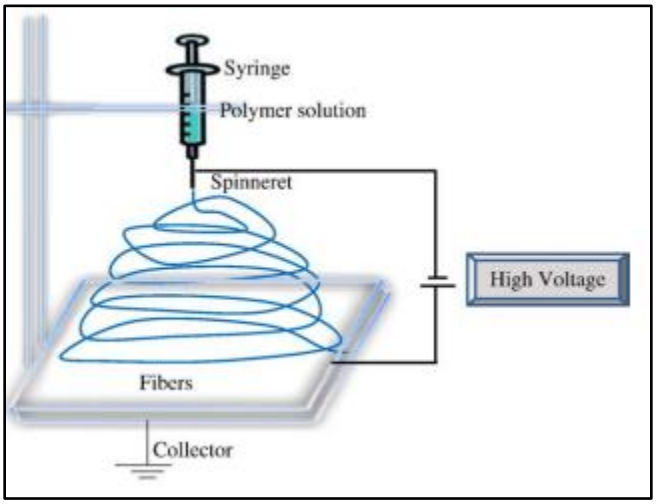

Figure 1: Electrospinning using a plate collector. [8]

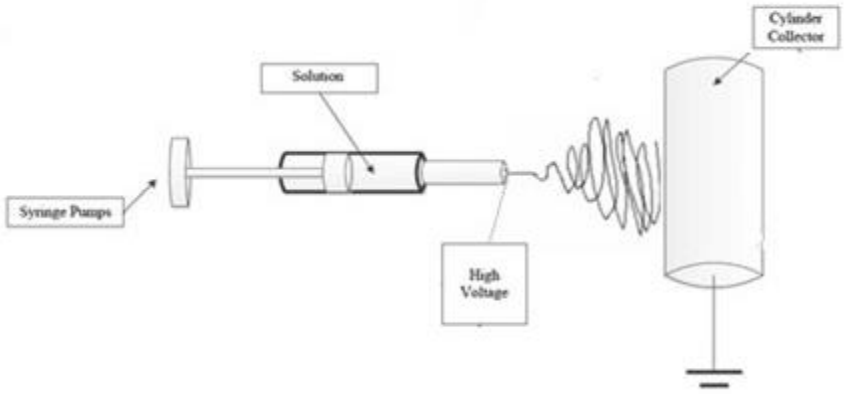

Figure 2: Electrospinning using a drum collector[22]

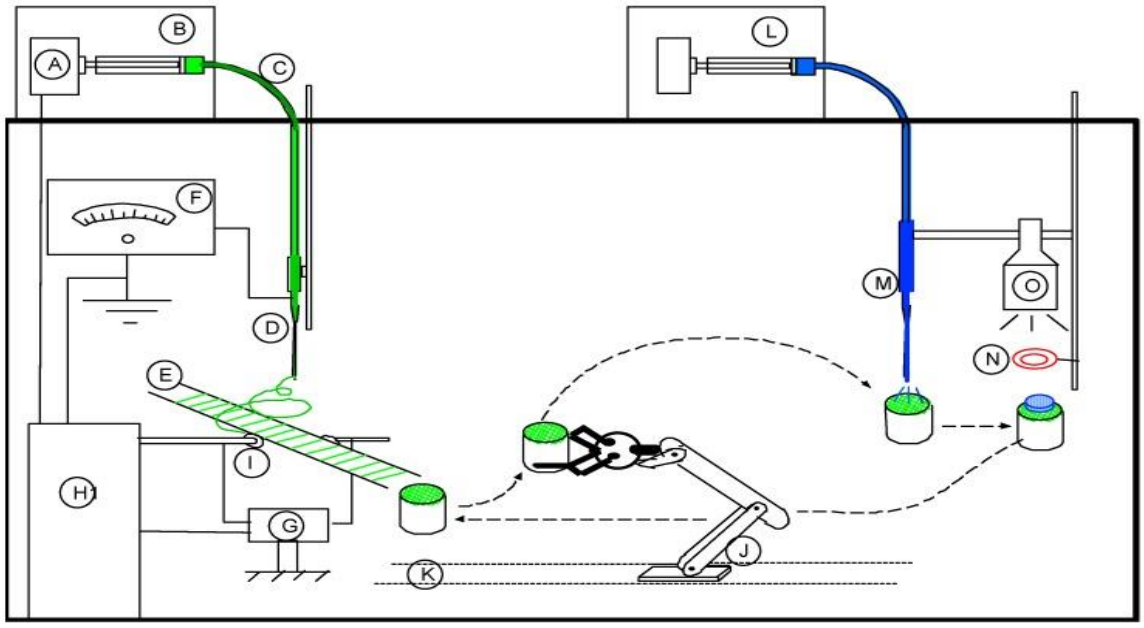

Figure 3: Patented electrospinning process used to create PCL-PEGDA scaffold for tissue engineering application[23]

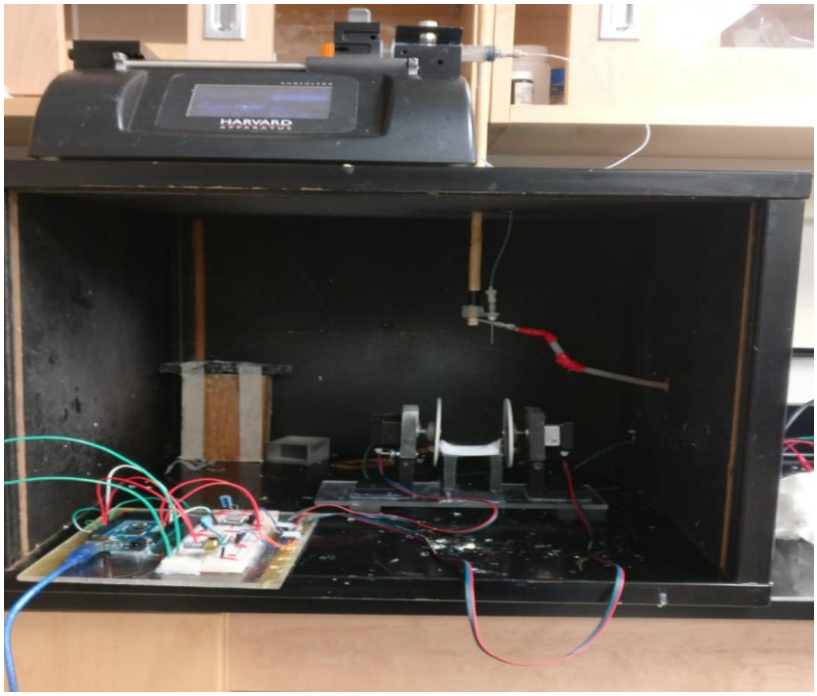

Figure 4: Dual Disc collection system for electrospun nanofibers.

Chapter 2

Tissue Engineered Anterior Cruciate Ligament

2.1 Summary

In this chapter the viability of using a tissue engineered anterior Cruciate Ligament is investigated. The manufactured ACL was made from Polycaprolactone, nylon-6 and Polycaprolactone - Nylon6 twisted pair. These engineered samples were then tested for tensile properties in an Ultimate Tensile Test Machine. The maximum loads and the failure loads were compared to that of Rabbit ACL to show the viability of using engineered materials as replacement to an injured ACL. The stiffness was also calculated statistically and the values compared. It was seen that the maximum load and failure load was consistent with Rabbits of weight between 2lb to 3lb according to Smith [9]. Although the samples tested here were seen to not be a good replacement for the Rabbit ACL broken in this research, the viability of an engineered ACL of another material that can withstand the same loads and characteristics will provide a better quality of life for the patient.

2.2 Background and Specifications

2.2.1 Goals and Objectives

(1) Design a method of fabrication of PCL-Nylon 6 ACL

(2) Research mechanical strength of Rabbit ACL.

(3) Prepared sample ACL for testing

(4) Test the Mechanical Strength of sample ACL to compare with Rabbit ACL

2.2.2 Introduction

The anterior cruciate ligament (ACL) is one of the pair of cruciate ligaments in the knee of a mammal as seen in Figure (2). The name is derived from the arrangement of the ligaments which resemble a crossed formation. The anterior cruciate ligament makes one of four major ligaments in the knee giving 85% of the restraining force to anterior tibial displacement at 30 and 90 of knee flex [24]. The anterior cruciate ligament in a highly organized, dense, cable-like tissue composed of collagens (types I, III and V), proteoglycans, elastins, water and cells [25]. The human anterior cruciate ligament is on average 27 to 32 mm in length with sectional area of 44.4 mm^2 - 57.5 mm^2 [26, 27].

The anterior cruciate ligament starts forming in the gestational period of the fetus in the fourth week between the femur and the tibia in the blastoma [28, 29]. The ligaments come to full form after week 20 of the gestational period where it resembles bundles more parallel when directly compared to the adult ACL [30]. Evidence that the ACL begins to form in the gestational period of the fetus shows that the correct formation of the ACL during this period is crucial to the correct formation of the knee, femoral condyles and the tibial plateau [24].

When analyzing the anatomy microscopically the ACL is seen to present three zones distinctively. The proximal zone is seen to consist of cellular matter of fusiform fibroblasts, collagen type II and glycoproteins namely fibronectin and laminin [31]. The middle zone consist mainly of collagen fibers, elastic and oxytalan fibers. This gives a wide range of motion possibilities with specific fiber clusters taking care of specific degrees of motion

[31]. The distal zone consists of mainly chondroblasts and avoid fibroblasts with less collagen bundles. They are rigid and not as flexible.

The primary function of the ACL is to bring the tibia and fibia back to a bent knee position. This shows that the highest stresses on the ACL is when the knee is in an extended position similar to a person standing or landing from a height with legs straight. Since the position of the legs is straight this causes issues with standing for long periods of time for a person with injured ACL.

2.2.3 Fracture of Anterior Cruciate Ligament

Due to rigorous sports methods and stress the anterior cruciate ligament is the most often torn ligament of the knee with respect to athletes and sports personalities of all levels. Over 200,000 patients are diagnosed with a disrupted anterior cruciate ligament every year in the United States alone [24]. The injury in this region causes diminished quality of life and mobility with considerable pain.

2.2.4 Current methods of reconstruction and their limitations:

In conventional treatments injuries to the anterior cruciate ligament have been treated with biological grafts, allografts or autografts. Autograft matter can be collected form the patient's patellar tendon, hamstring tendon or quadriceps tendon where the hamstring and patella tendons have seen higher viability among surgeons [32]. Allografts are harvested from cadavers and can be harvested from the patella hamstring and Achilles tendons. The harvest from cadavers does not require an additional surgery as in the case of autografts [32].

Autografts and Allografts have disadvantages to its use. Autografts have limited availability due to the fact that the sample is harvested from the patient itself. There is also the need for additional surgery to harvest the matter which requires healing and additional down time. This may also cause donor site morbidity. Allografts have issues with disease and bacterial infection. The samples can also show an unfavorable immunogenic response from the host causing the patient to reject the transplant. Also there are issues with sterilization before transplant as this has been seen to alter the mechanical properties of the graft [33, 34]. This shows promise in arriving at an alternative to the current recovery and rehabilitation options available to the patients.

The use of bracing has also become popular in the treatment of torn ACL. The use of bracing is done by adding external supports to the joint to support the joint in movement. This however is bulky and does not allow for flexibility in the joint as it restricts the movement other degrees of motion.

2.3 Current strategies in tissue engineering of ACL

Methods of anterior cruciate ligament reconstruction using biomaterial scaffolds is crucial. Current methods use biocompatible scaffolds shown in table (1). Biocompatible scaffolds can be tailored to achieve needs of the anterior cruciate ligament. The ideal scaffold will integrate into the body of the patient over time. The scaffold will also degrade over time with ligament cells taking its place. With disadvantages in using these kinds of substitutes to autografts and allografts is that the biomaterials lack the mechanical strength that is needed to withstand the loads that the anterior cruciate ligament is subjected to on a continuous basis. By using a combination of more than one biocompatible material the

properties may be able to be tailored to fit the needs of the patient. This research is a step toward realizing this thought.

2.4 Significance

The use of Autograft requires further surgery on the patient. Though the surgery can be done in a single visit this requires additional physical therapy and healing. With all forms of surgery there is always a risk of infection and by increasing the amount of invasion into the patient's body this risk is increased. In this research we are investigating the use of Polycaprolactone as a viable substitute to an Autograft to be able to decrease the level of risk to a patient who requires an anterior cruciate ligament repair by eliminating the need for additional surgery.

2.5 Material and Methods

2.5.1 Instrumentation and measurements

The samples were manufactured using a dual disc setup using a single nozzle electrospinning technique shown in figure (6). A solution was made of Polycaprolactone using 1:10 ratio by weight in Acetone and sonicated until dissolved. This solution was loaded into a syringe and needle setup made to dispense at 0.5ml/min. Once the setup is ready, the needle is made to be positively charge and the discs are made to be negatively charged with a potential difference of 9 kV. This causes nanofibers of Polycaprolactone to be dispensed and deposit on the dual discs as aligned fibers. The discs are then made to rotate using the dual stepper motors. This causes the deposition of the nanofibers to be continuously formed on the periphery of the dual discs forming a band of nanofiber. Using a ledge to obstruct the band the fibers are transferred onto the ledge and detach from the

dual discs. This clears the dual disc for new fibers as it rotates back to the top. Once sufficient number of fibers were collected on the ledge the fibers are bunched manually and the sample is removed from the collection plate for further testing and comparison.

Figure (7) shows samples prepared by this method. The samples was all prepared using a fixed dispensed volume of polycraprolactone solution. This resulted in samples with similar packing density.

2.5.2 Stepper motor

The stepper motor used for the setup was the 17HD4005-22B from Busheng which is a 24V stepper motor. The motor was selected for due to the low cost and the high durability around voltage and contaminants. The motor was fixed on base manufactured using additive manufacturing. The stepper motors were to be driven using a high voltage source dedicated to the motion of the motors. The motors were engaged using an H-bridge and the control was provided using a potentiometer and the Arduino Uno. The Uno was selected due to its small size and affordable platform.

2.5.3 Arduino Uno

Here's the complete circuit schematics. The stepper motor was used in Full Step Mode thus the 3 MS pins disconnected and just connect the Direction and the Step pins of the drive to the pins number 3 and 4 on the Arduino Board and as well the Ground and the 5 V pins for powering the board. A 100µF capacitor was connected in parallel in order to stabilize the power supply of 24V, 5A power supply to power the motors. Two 17HD40005-22B Stepper Motor from Busheng and its wires A, C, B and D were connected to the pins 1A, 1B, 2A and 2B pins respectively on the driver.

The connections were mirrored to power the second motor so as to keep the two motors in sync. Thus the dual discs were seen to rotate at the same speed and opposite direction. Since the motors oppose each other, the fibers created on the discs would be formed continuously as aligned fibers.

2.5.4 Production of PCL nanofiber matrix

Polycaprolactone is a biodegradable polyester with a melting point of around 60° C. 1.01g of PCL was dissolved in 10.1g of acetone using a sonicator rated at 20khz with 60% intensity for 45 mins. This solution was used as the base to produce the first set of nanofibers. The electrodes were connected to the circular electrodes using brushes as shown in figure (4). The circular electrodes were rated at a constant speed when the PCL solution was dispensed. Loaded into a syringe and dispensed at 100 microliter/min with a circular electrode spinning setup with 11.5KV charge and 10 cm distance between the syringe and electrode. Due to the high potential difference between the needle and the electrodes, the solution leaving the needle was seen to form electrospun PCL nanofibers and these fibers fall forming aligned fibers adhered to the edges of the circular electrodes. The fibers are spun using this setup and the fibers of PCL are seen to be formed consistently on the periphery of the circular electrodes. A perch was provided obstructing the circular path of the adhered fibers to collect the fibers in a continuous fashion. By keeping track of the volume dispensed through the syringe the samples were made to have similar volumes.

The PCL solution was prepared using electrospinning techniques. The fibers were formed on a dual disc setup as shown in the figure (4). The control was provided using a potentiometer to set the speed to the lowest possible each time so as to create more fibers

before collection. The fibers were collected using a fixed perch in the path of the fibers to obstruct the fibers from staying on the dual discs. This setup was used as it is highly automatable and the speed at which the fibers are manufactured is considerably higher. A volume of 1ml solution was dispensed each time to create the fiber bundle and then the bundle was gathered to make the sample for testing.

2.5.5 Production of Nylon-6 nanofiber matrix

Polycaprolactam or Nylon 6 is a crystalline polyamide with a melting point of 215° C. 1g of Nylon 6 was added to 10g of formic acid and mixed using a sonicator rated at 20khz with 60% intensity for 45 mins. The solution was used as a base to produce the second set of fibers. Though the process was set up the same as the PCL fibers the nylon 6 was not seen to create aligned nanofibers. This is because of the high voltage that is needed to create the fibers. The setup being made from metal, could contribute to this issue. The setup was tried to make fibers using glass syringe and plastic syringe. There was a better formation of fibers with the plastic syringes.

The Nylon-6 fibers were not manufactured in the same setup since the high voltage caused the Nylon-6 fibers to be formed in various locations. The nylon-6 samples were made used a plate drying technique. A copper plate was used to coat Nylon-6 solution and then left to dry for 5 mins. Once the time was completed distilled water was drizzled onto the plate. This caused the Nylon-6 solution to coagulate and form a cloth of nylon-6. This cloth was rolled to form the sample for testing.

2.5.6 Production of PCL and Nylon-6 nanofiber matrix

Samples were manufactured using both PCL and Nylon-6 rope. This was done first separately using the previous two methods and then twisted as a twisted pair to be tested

2.5.7 Production of PCL nanofiber matrix with collagen

The PCL samples were also treated with type I collagen. 50ml of collagen was added to each sample and treated under ultraviolet light for 6 hrs after which fibroblast cells extracted from a rabbit host was added on to the samples. The cells were allowed to grow for three days and then ready for testing.

2.5.8 Random trials with PCL nanofiber matrix

Two other random configurations were manufactured and tested to see if samples made of ACL was able to withstand loads. The thickness of the regular sample was increased by 4 times and tested under the same parameters. A braided fiber was also tested.

2.6 Tensile testing of native and fabricated ACL

When any specimen is subjected to tensile stress the length of the specimen is altered. The extent of the change in length varies with the composition of the specimen. Elastic specimens are those that return to the original shape and length after the stress is removed. If the specimen does not return to the original shape and length then the specimen is said to be inelastic. Every elastic specimen has a limit to which the specimen reacts elastically. When specimens are loaded beyond this elastic limit the specimen is no longer elastic.

Tensile testing was performed to find the similarity between the three artificial types of anterior cruciate ligaments and the specimen harvested from the rabbit host. The samples were loaded in the 1010CCH-2.5K-B from Test Resources using specially machined

molds. The ACL was left intact when mounting on the test setup. To keep the bone from moving the bone was drilled and an arrest pin was installed before filling the cavity with bone cement. Once the cement cured, the bone was not allowed to move. The ACL was extended as far as possible without giving tensile load on the ACL. The ACL was then stretched until failure. The ACL samples gave the data found in Figure (8-16)

2.7 Results and Discussion

All data measured in tensile loading of the samples are given in Table 2. The stiffness of the ACL was seen to be 216.2N/mm and the maximum load was seen to be 208.4N. The ACL was seen to have plastic deformation after 170N load and was seen to fail at 2.3mm displacement.

Similarly the PCL samples and Nylon-6 samples prepared were loaded with the same machine to test for tensile strength and failure load. The PCL samples were prepared to have similar width of the rabbit ACL but was broken in batches of three to test for more load. The Nylon-6 sample was also made to have similar diameter of the rabbit ACL but was tested as a single fiber. Figure (8-16) shows the data from these tests.

The parameters measured were also compared to the control values [35, 36] for similarity to fresh rabbit anterior cruciate ligaments since the harvested ligaments were not fresh since the physical properties of the ligaments change quickly once the host dies or the local area is deceased [37]. The data for the ACL sample was seen to resemble rabbits of 5-6 lbs in weight. However the PCL and Nylon-6 samples were seen to resemble the data for rabbits of 2-3 lb in weight. This is in-spite of the diameter being the same for all samples. This may show that the PCL and Nylon-6 on its own is not definitive of the properties of the

rabbit ACL. The diameter needed to provide the same effect of tensile properties was not met with the same diameter of ACL. Though the elastic properties are similar the samples may not be a good replacement for the Rabbit ACL.

2.8 Conclusion and Future Work:

After analysis PCL and nylon-6 on its own does not provide enough mechanical strength to be a viable replacement for a rabbit ACL if the dimensions are to be the same as that of the tissue sample. The Nylon-6 – PCL fiber bundle does not provide the mechanical strength to act as a substitute to the native ACL. This however can be used in the future to tailor specific properties for the ACL that is to replace the tissue sample.

Further research may be needed to check for a different polymer to be woven into a bundle to give better characteristics for a good replacement of rabbit ACL. Another avenue for research is the possibility of using more than one type of fiber to prepare the sample as a woven bundle. This may be prepared using a coaxial needle or by preparing one type of fiber on top of the other type of fiber.

2.9 Figures

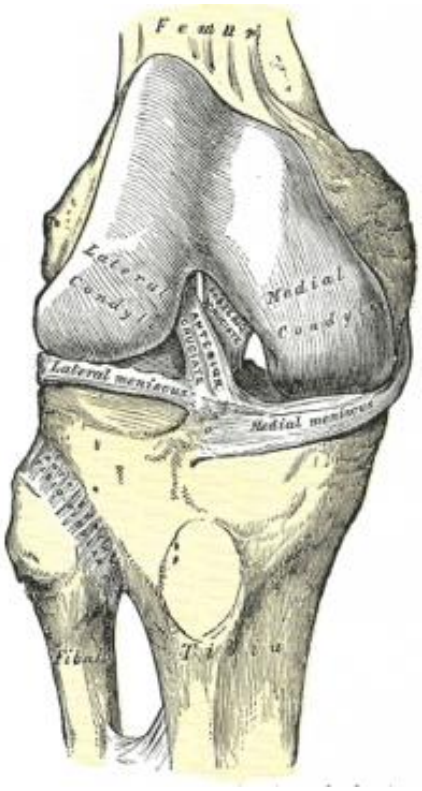

Figure 5: Illustration to show the different ligaments in the human knee[31]

Figure 6: Dual disc setup to create electrospun nanofibers

Figure 7: Sample ACL made of PCL through electrospinning using the dual disc setup.

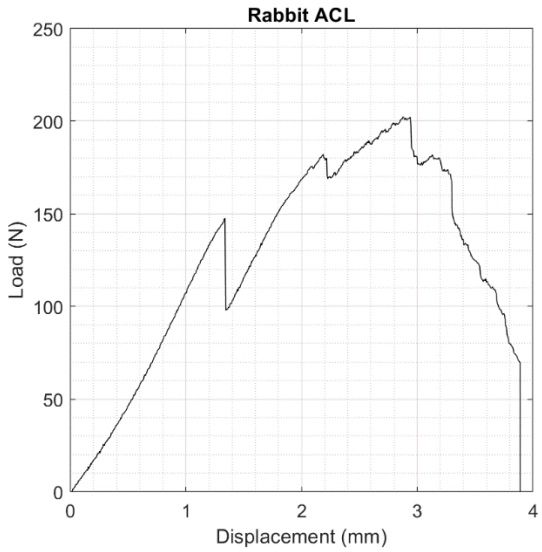

Figure 8: Plot of Load vs Displacement when Testing the Rabbit 1 Sample for Tensile Properties

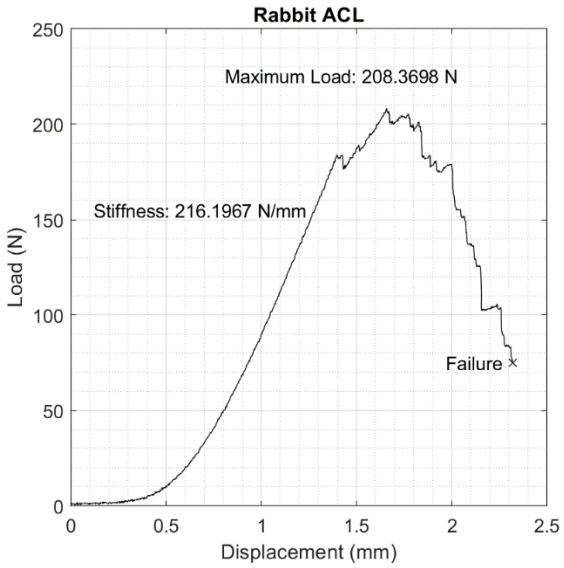

Figure 9 : Plot of Load vs Displacement when Testing the Rabbit 2 Sample for Tensile Properties

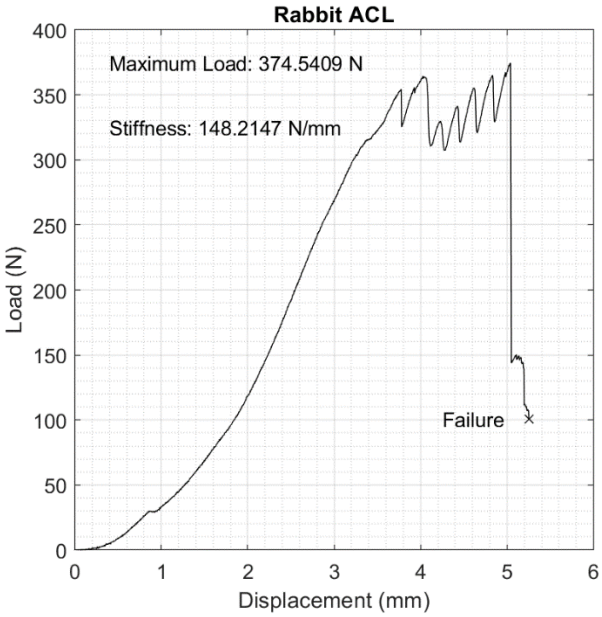

Figure 10: Plot of Load vs Displacement when Testing the Rabbit 3 Sample for Tensile Properties

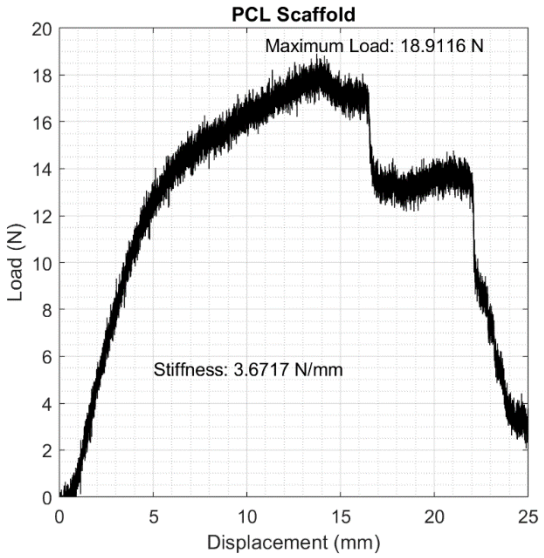

Figure 11: Plot of Load vs Displacement when Testing the PCL 1 Sample for Tensile Properties

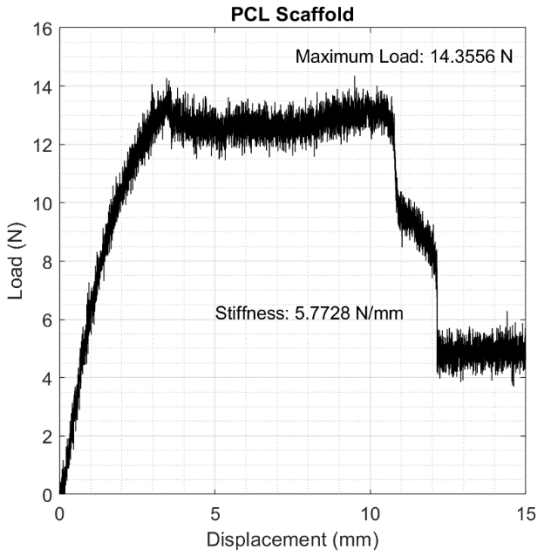

Figure 12: Plot of Load vs Displacement when Testing the PCL 2 Sample for Tensile Properties

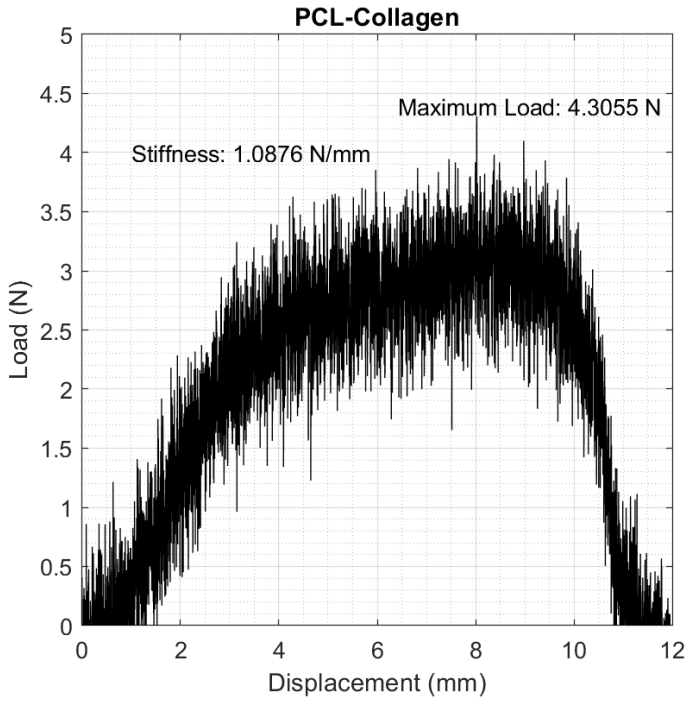

Figure 13: Plot of Load vs Displacement when Testing the PCL_Collagen Sample for Tensile Properties

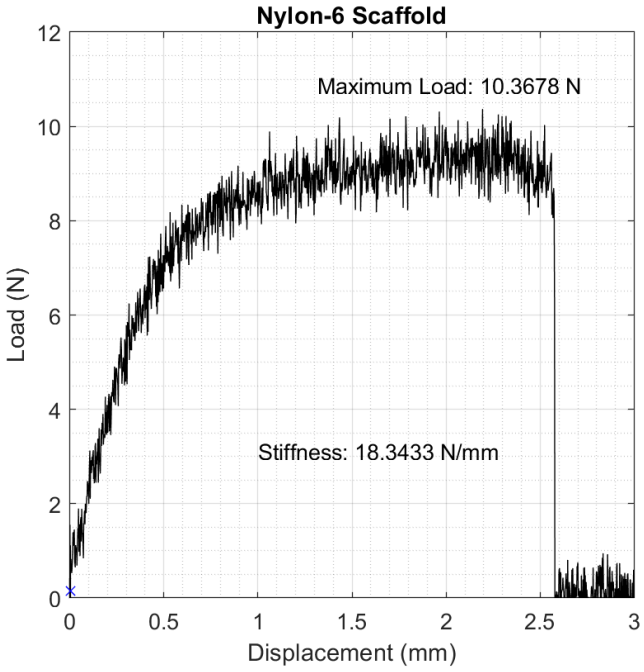

Figure 14: Plot of Load vs Displacement when Testing the Nylon-6 1 Sample for Tensile Properties

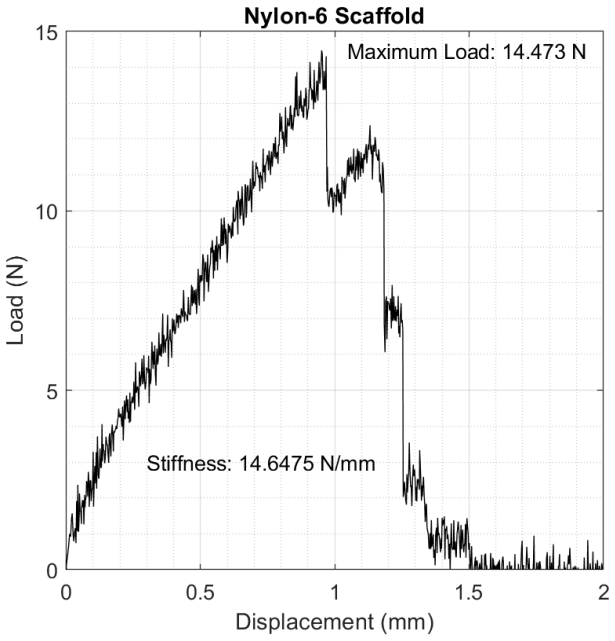

Figure 15: Plot of Load vs Displacement when Testing the Nylon-6 2 Sample for Tensile Properties

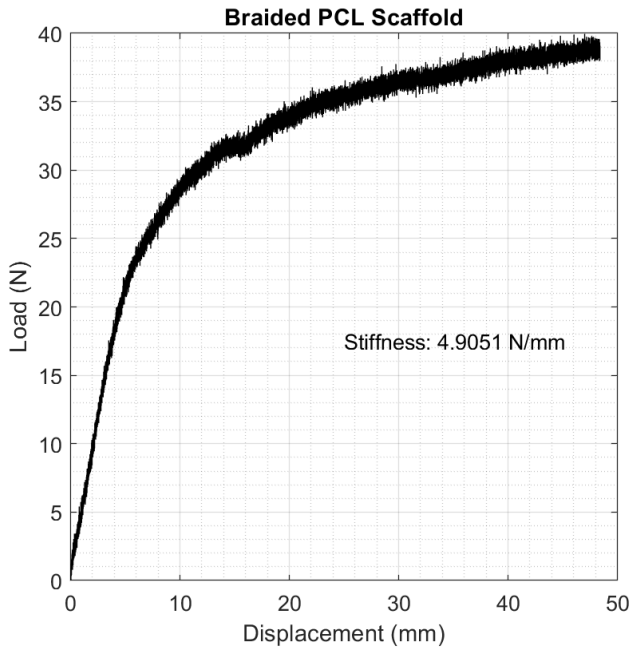

Figure 16: Plot of Load vs Displacement when Testing the PCL_Braided Sample for Tensile Properties

2.10 Tables

Table 1 : Advantages and Disadvantages of Using Specific Biomaterials to Manufacture Fabricated ACL

Biomaterial	Advantages	Disadvantages
Collagen[38-40]	Biocompatible, major component of native ACL. Good tensile strength.	Lacks Mechanical Strength, immunogenic. Limited cell adhesion, sericin coating is immunogenic
Hyaluronic acid[41]	Biocompatible ECM component; can be in sponge or hydrogel form.	Lacks mechanical strength
Chitosan[42-44]	Biocompatible, chemically modifiable. Can be in sponge of hydrogel form. Antimicrobial	Limited cell adhesion and lacks mechanical strength.
Alginate[45-48]	biocompatible, can encapsulate cells, can be in sponge of hydrogel form	Lacks mechanical strength
PDS[49]	Common FDA-approved suture material. Easily manufactured into different forms	Rapid loss of mechanical Strength
PGA[50]	Common FDA-approved suture material. Easily manufactured	Rapid degradation and loss of mechanical strength, biologically inert. Acidic degradation byproduct
PLLA[51]	Slow degradation rate, Easily manufactured	Biologically inert. Acidic degradation byproduct
PCL[7]	Commonly FDA-approved suture material. Easily manufactured	Very slow degradation, biologically inert
PLGA[6]	Degradation rate can be tailored. Easily manufactured.	Biologically inert. Acidic degradation byproduct

Table 2: Parameters measured During the Tensile Test of Each ACL substitue

Sample	**Material**	**Maximum Load** N	**Failure Displacement** mm	**Stiffness** N/mm
1	PCL	14047	1.25	14.65
2	Nylon-6	10.4	2.6	18.34
3$^{\alpha}$	PCL-Nylon-6	NA	NA	NA
4	PCL-Collagen	4.3	10.5	1.09
5$^{\beta}$	PCL-Collagen Dehydrated	NA	NA	NA
7	PCL (thick)	18.9116	22.5	3.67
8	PCL (Braided)	40	NA	4.9
9$^{\gamma}$	Rabbit ACL	NA	NA	NA
10	Rabbit ACL	208.4	2.3	216.20
11	Rabbit ACL	374.5	5.2	148.2

α – Sample 3 was excluded as the braided technique was not standardized.

β – Sample 5 was excluded as the sample disintegrated when dehydrated in heat

γ – Sample 9 was excluded as the femur broke before the ACL gave way

Chapter 3

Aligned Polycaprolactone Fibers as a Functional Air filter for Tobacco Smoke

3.1 Summary

The goal of this chapter is to investigate the viability of a filtering system to be used in conjunction with a tobacco smoking device to be able to filter out possible contaminants in the tobacco smoke. Both in-filter and external setup were tested and the capabilities investigated. The PCL was used to manufacture the air filters. The effect of smoking on the filters were studied by using an automated smoking device that was set up to mirror the lung capabilities of an adult male [52]. The in-filter experiments were seen to have limited benefits as the filters were seen to melt into the cigarette filter. The external filter was seen to have up to 12% increase in weight. Analysis shows that the elements captured on the external filters were predominantly Carbon and Oxygen.

3.2 Background and Specification

3.2.1 Introduction

Airborne contaminants cause issues every day for human beings. With an increase in the amount of air pollution this has become a concerning issue. Inhalation of particulate matter of the size of 2.5μm and below is seen to have harmful effects on the health, vision, climate and ecosystems [15-18]. Considerable research has been done on using electrospun nanofiber matrices to filter out microscopic contaminants [19].

Tobacco as well is still the largest preventable cause of death in the United States according to the Center for Disease Control killing 480,000 Americans each year [52]. Out of these, 41,000 deaths are caused by exposure to second hand smoke [53]. This cause of death can be avoided by using air filters [54]. Aligned electrospun nanofibers pose a unique opportunity in this area of study to serve as a functional air filter to reduce the contaminants in the smoke and provide long-term quality of life to the user. Although this method of filtering may be a step up from the traditional foam filters the most effective way to prevent harm from smoking is still to refrain from inhaling the smoke. This is sometimes very difficult due to habitual smoking and continuing to be in the proximity of habitual smokers.

The ideal air filter will be low cost, high effectivity with low pressure drop across the filter and provide adequate filtering capabilities. The filter will also need to be small in comparison to other filters to be able to be used easily and to be mass-produced. This is where the nanofibers come into play as the propertied of the nanofibers can be highly customizable and the desired properties, once found, can be incorporated. The nanofibers can be formed with relatively smaller diameters ranging from micrometers to nanometers [55]. Nanofibers can be formed from various materials including ceramics, composite materials, polymers, solutions and molten materials. These fibers resemble diameters from micrometers to nanometers and the method is simple using electrospinning [56]. Since the diameter is small the fibers can be woven to form a cloth that has a small pore size to block particles that are larger. The relatively small pore size in beneficial in blocking particulate matter. The cloth will ideally have a large surface are per unit mass with the small pore size. Electrospinning is seen to be a good method to manufacture these fibers [57]. The

small pore size however does not restrict the air flow as the number of pores is far greater. The high porosity helps the air to travel without obstruction resulting in a low pressure drop across the air filter. Thus the applications of this kind of air filter can be vast [58-60].

Due to the concentration of carcinogens and the ease with which the smoke can be controlled and dispensed, the air filters prepared in this research were tested using the smoke from a cigarette. This was used as model to start testing the filtering capabilities of the air filter. With definitive proof that the filter is able to block particles, more tests can be done to see what different changes occur when the layers are increased or the size of the pores are decreased.

3.2.2 Goals and Objectives

(1) Investigate filtration of smaples made from aligned PCL fiber layers filter contaminants from tobacco smoke.

(2) Investigate if position of the filter has an effect on the filtration capabilities of the samples.

3.3 Material and Methods

3.3.1 Smoking apparatus

A smoking apparatus was fabricated to standardize the results. A glass chamber was fashioned to hold a steady vacuum resembling the lung function of an adult male [61] using a vacuum pump. The glass chamber was fitted with a one way check valve to limit the entry of air to one entry and the exit of the air to one exit. This allowed for a steady flow of air from the inlet to the outlet. The inlet was made to fit a cigarette filter and the exit

was connected to the pump. This setup showed to hold a steady vacuum for extended periods of time. An illustration of the setup used is shown in figure (8).

3.3.2 Filter preparation and mounting

The filters were prepared using electrospinning and then layers on top of each other at 90° offsets to form 24 layers. Once this was done a lightly heated punch was used to cut the filters to an 8mm diameter to match the diameter of the filter of a cigarette. This was then weighed and logged for testing. The weights before and after the test is shown in Table (3). Once prepared the filter was then mounted in between the filter taking 2 locations at varying distances from the end of the cigarette. The cigarette filter was cut at these locations using a razor blade. The filters were also mounted in the tubing after the lung machine to compare the filtration capabilities of an external filter. The locations of the filters during testing is shown in Figure (17).

3.3.3 Smoking protocol

The filters were each mounted into a fresh cigarette and at different locations. All cigarettes were Marlboro Lights short cigarettes. The cigarettes were fixed at the inlet of the glass chamber and the vacuum was induced after that. Once the vacuum was induced the cigarette was lit using an open flame. Once lit the flame was removed and the cigarette was allowed to burn without intervention. After the whole cigarette was exhausted the remaining part of the filter was removed and saved in a ziplock bag for further analysis. For the tests that involved an external filter an uncut cigarette was mounted in the same way but the filter was mounted between the tubing after the outlet of the glass chamber.

The filter was kept perpendicular to the flow of smoke in all cases. A total of 20 cigarettes were smoked to arrive at results.

3.4 Results and discussion

The filters that were mounted within the filter were seen to melt as the smoke of the cigarette came in contact with the filter as seen in figure (xx). This may be due to the low melting point of the PCL fibers. The filters mounted in the path of the smoke in tube were seen to survive and filter out contaminants though the specific compounds that the filter was able to block is not determined. Figure (23) shows the filter before the experiment. Figure (18-22) shows the filter after experiment. All images were acquired using the TM3000 Scanning Electron Microscope. The samples before and after were also imaged using X-Ray Diffraction to isolate the compounds found in the surface as seen in Figure (24-30). The starting and ending weights of the filters are shown in table (3).

3.5 Figures

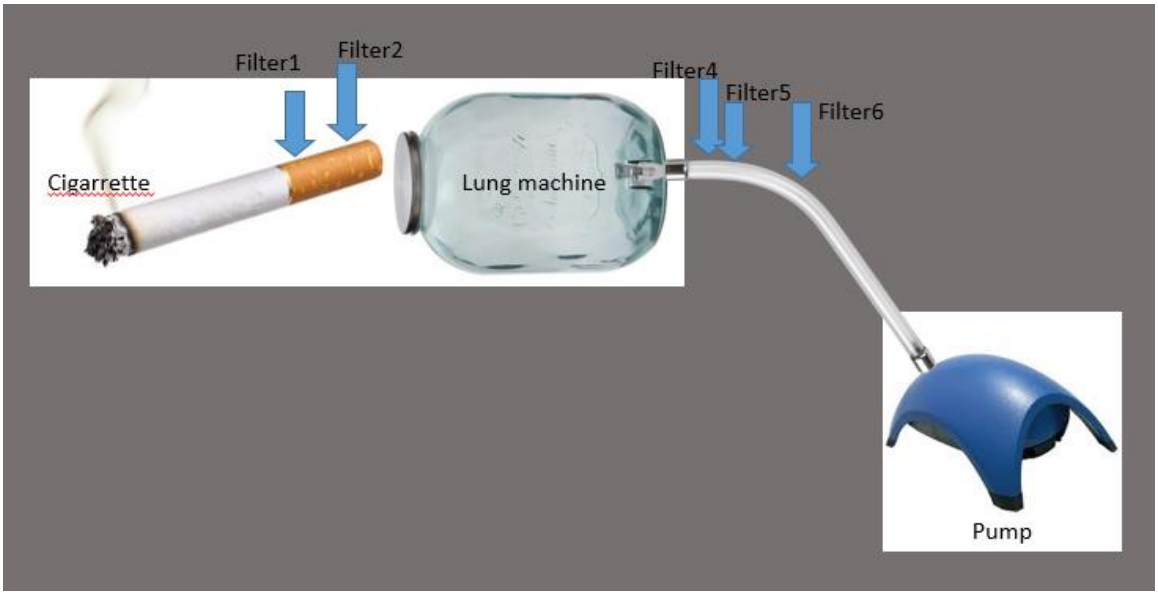

Figure 17

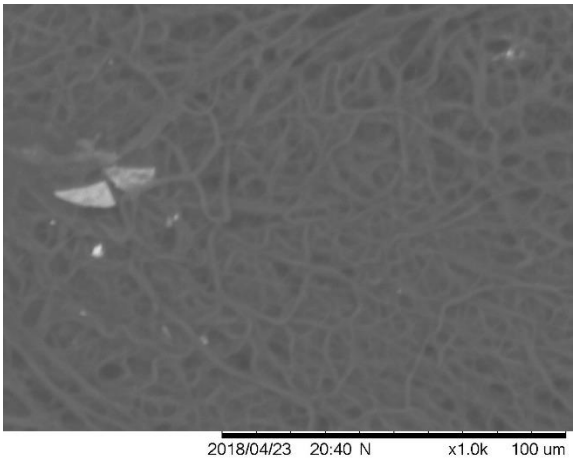

Figure 18 : Surface Morphology After Eperiment – Sample 5

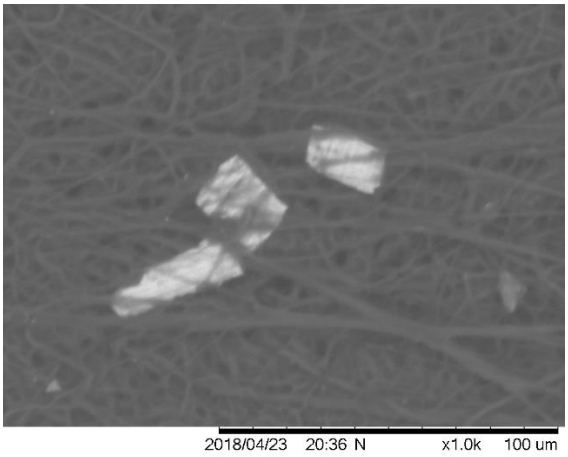

Figure 19 : Particulate Matter Seen in sample After Eperiment – Sample 5

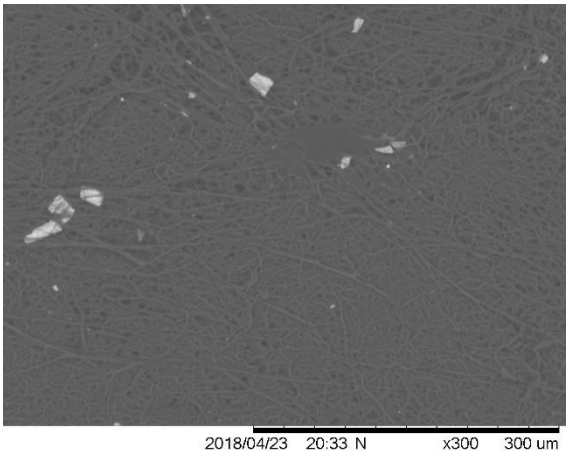

Figure 20 : Surface Morphology After Eperiment – Sample 6

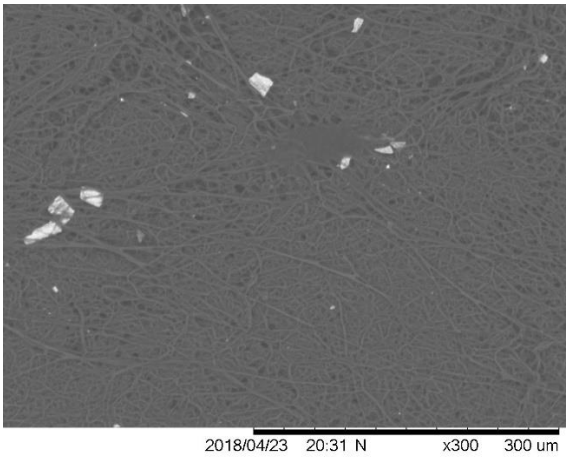

Figure 21 : Surface Morphology After Eperiment – Sample 6

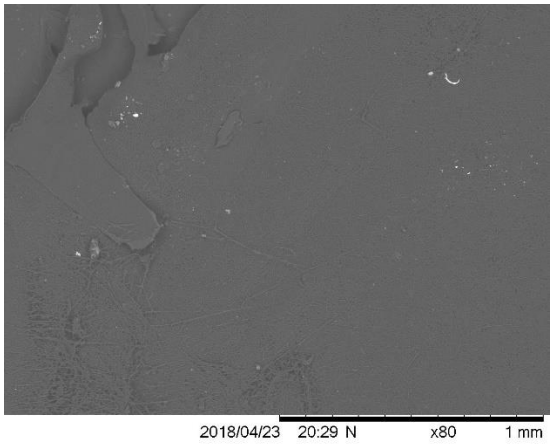

Figure 22 : Surface Morphology After Eperiment – Sample 7

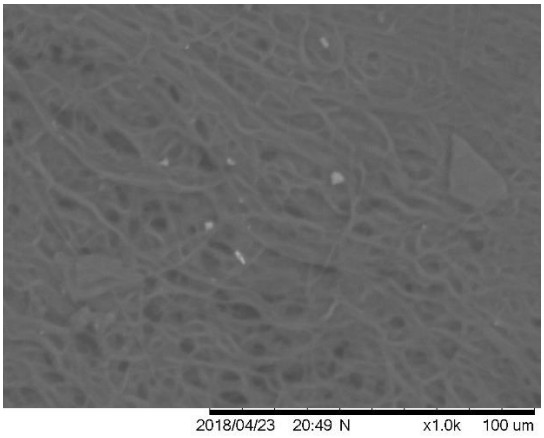

Figure 23 Surface Morphology Before Eperiment – Sample 3

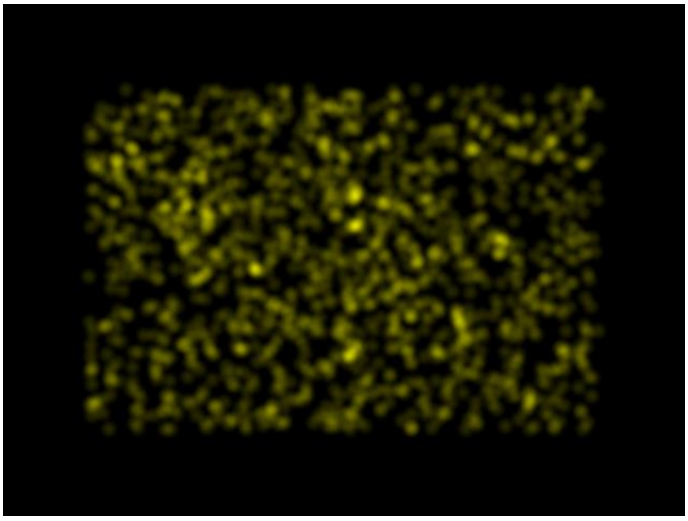

Figure 24 : Oxygen Map from X-Ray Diffraction imaging After Experiment– Sample 5

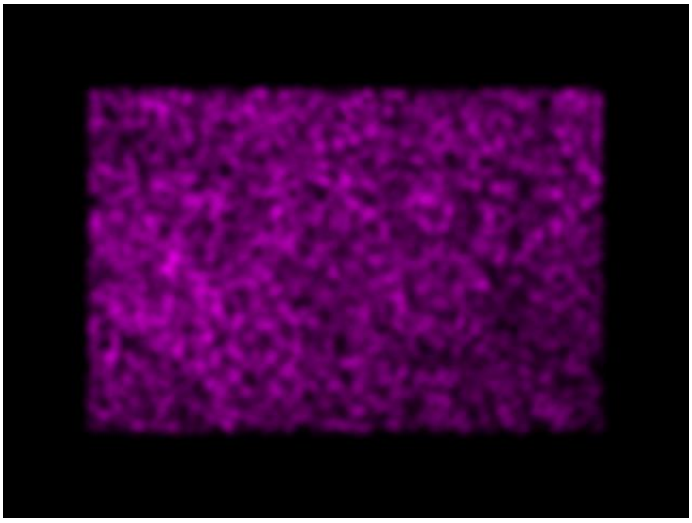

Figure 25 : Carbon Map from X-Ray Diffraction imaging After Experiment– Sample 5

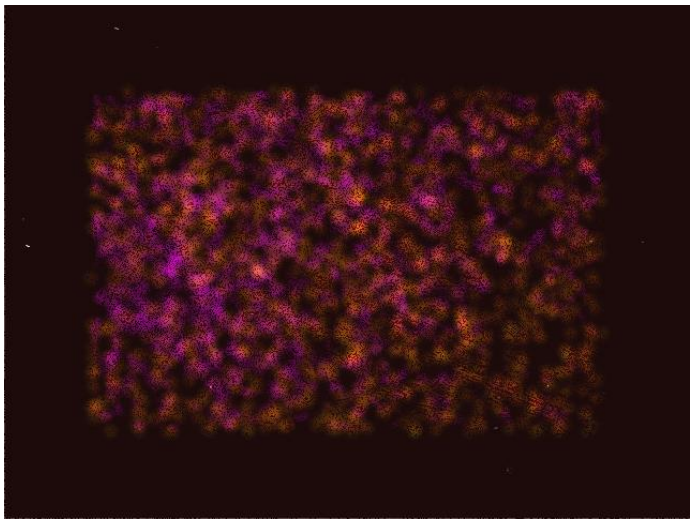

Figure 26 : Combined Map from X-Ray Diffraction imaging After Experiment– Sample 5

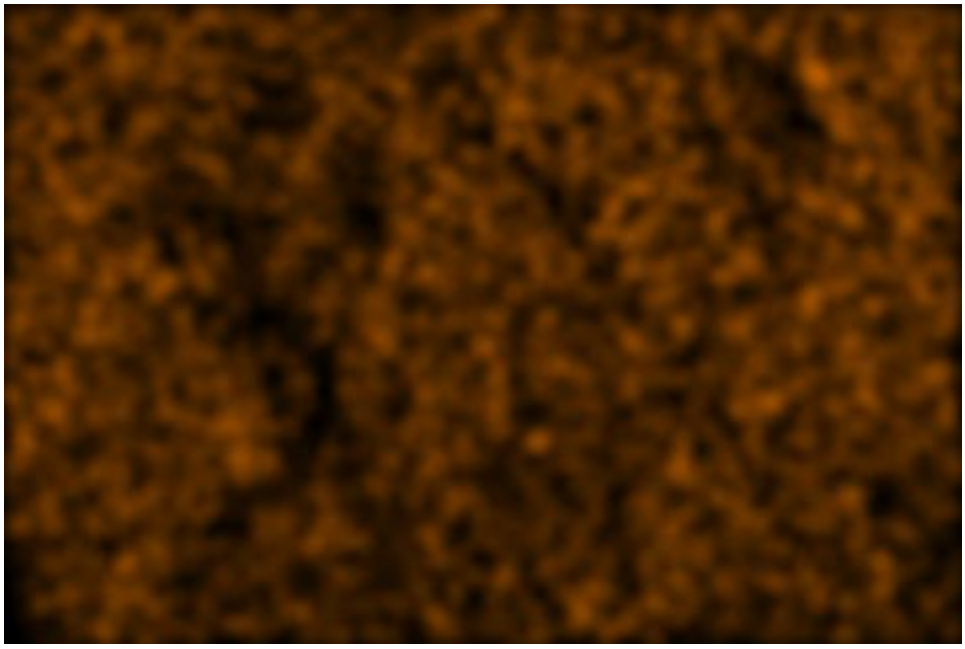

Figure 27 : Carbon Map from X-Ray Diffraction imaging Before Experiment– Sample 5

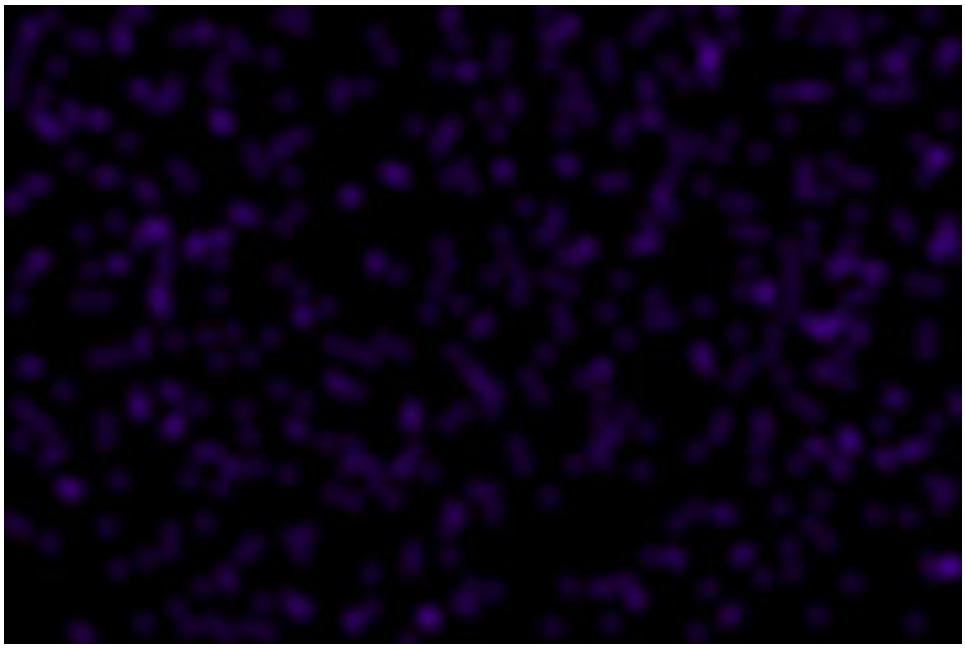

Figure 28 : Aluminum Map from X-Ray Diffraction imaging Before Experiment– Sample 5

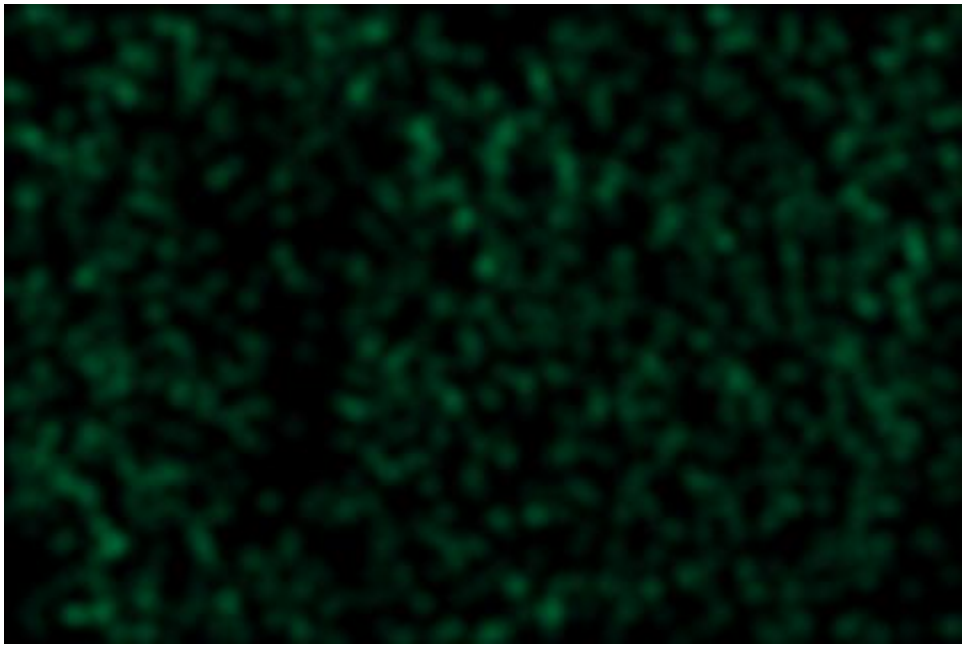

Figure 29 : Oxygen Map from X-Ray Diffraction imaging Before Experiment– Sample 5

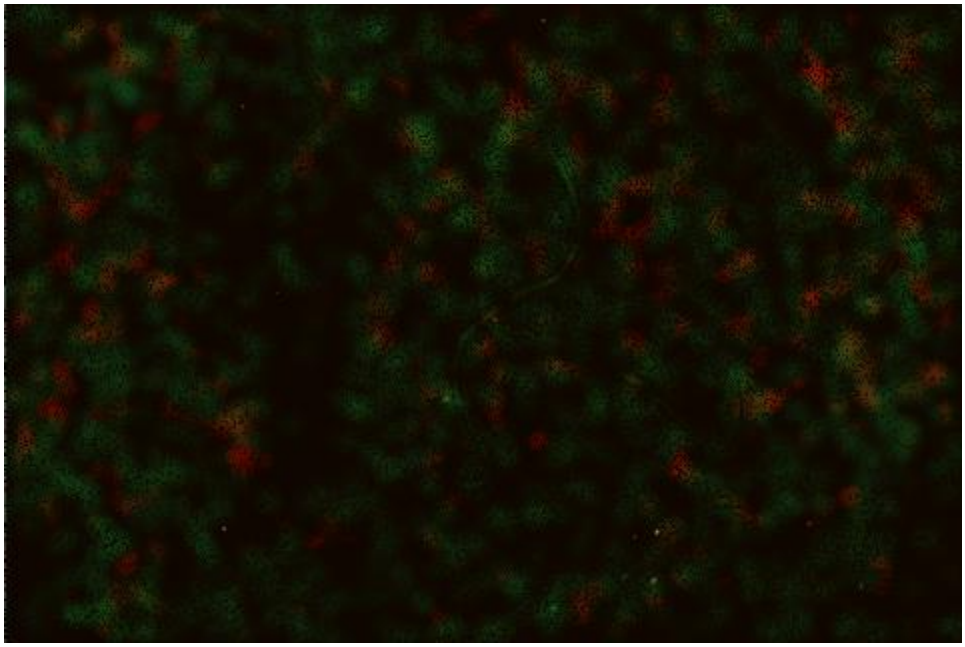

Figure 30 : Combined Map from X-Ray Diffraction imaging Before Experiment– Sample 5

3.6 Tables

Table 3 : Data Showing Weight of the Samples After Steady Exposure to the Smoke from Two Cigarettes

			Mass		
Sample Number	**Material**	**Location**	**Before** $1x10^{-3}$ **g**	**After** $1x10^{-3}$ **g**	**Δm %**
1^{α}	PCL	In-Filter	2.31	Melted	NA
2^{α}	PCL	In-Filter	2.11	Melted	NA
3^{β}	PCL	NA	3.15	Image	NA
4	PCL	External	3.43	3.68	7.28863
5	PCL	External	3.01	3.17	5.315615
6	PCL	External	3.02	3.41	12.91391

α – Sample was seen to be melted after the test.

β – Sample used for control and images.

Chapter 4

Polyethylene Glycol Diacrylate and PCL as a Functional Scaffold for Tissue Engineering Applications

4.1 Summary

The objective of this study was to develop a biomimetic polymer system that can be used as an in vitro model system to evaluate cell migration. The substrate consisted of a photopolymerizable hydrogel based on acrylate-terminated derivatives of polyethylene glycol (PEG) with proteolytically degradable peptides targeted for degradation by specific enzymes involved in cell migration, incorporated into bone, and grafted cell adhesion peptides. An aim was to evaluate the effects of adhesion ligand density on cell migration in this hydrogel system [23].

4.2 Background and Specifications

4.2.1 Introduction

Tissue engineering is a viable field to grow tissues restoring the organ functions in a tailor made fashion for the patient. Tissue engineering uses the growing of cells within scaffolds that are biodegradable and allow for the cells to grow in three dimensions. By using photolithographic processes in micro fabrication the desired size and shape of scaffold can be produced. The research into controlling the porosity of the scaffolds can be considered as important as the porosity is directly related to the cell growth in three dimensions. In this research the photolithographic material used was Polyethylene Glycol Diacrylate

(PEGDA) and the porosity of the material was altered using Polycaprolactone Nanofibers aligned in a composite structure of layers at 90° offset to adjoining layers.

PEGDA scaffolds were seen to be unable to sustain cells when the thickness of the scaffold was greater than 1mm. This proves to be an issue because the 1mm scaffolds were seen to be too thin and not structurally sound to sustain the growth of the cells and give rigidity and shape required to grow the cells. This is due to the lack of porosity in the hydrogel. In this research PEGDA scaffolds were designed to have intricate architecture, pore size and shape, porosity and interconnectivity in order to provide the required structural strength, nutrient transport and micro-environment for cell growth.

4.3 Goals and Objectives

(1) Prepare multilayer samples to test the porosity and the permeability of the PEGDA-PCL Scaffold.

(2) Test for adhesion of Osteoblast cells

4.4 Material and Method

4.4.1 PEGDA Preparation

The researchers in this study fabricated an electrospinning-UV polymerization system. [23]. The system can produce any dimension of cylindrical shape PEGDA-PCL scaffold. Specifically, this study fabricated 10 mm diameter and 1.5 mm thickness PEGDA-PCL scaffold and PEGDA+GC-PCL Scaffold This dimension is selected due to the suitability of cell culturing for biocompatibility tests on each group of samples in 48-well plates. Figure 311 illustrates functional elements of a notional combined ENF production-UV

photopolymerization unit for automatic production of the 3D scaffold. The notional system combines an ENF production unit and UV polymerization unit and a robotic arm for fiber harvesting. Using a system with these functional elements, any number of PCL ENF and PEGDA membranes may be produced in any shape of 3D scaffolds. A substrate may be adapted to produce a 3D scaffold comprising at least two equal linear dimensions, or a circular shape. In this study, we have used circular shape collector. Schematic representation of a PEGDA-PCL scaffold [23]. The process b-c-d can be repeated multiple times to create higher thickness scaffold. PCL pellets (7.69 wt %) were mixed with acetone in an ultrasonic mixer (Sonics & Materials, Inc., Newtown, CT, USA, model # Vibra-cell VCX 130). The sonication process was carried out at approximately 60 C for 30 min. A syringe pump was used to feed PCL solution (2.5 milliliter) into a glass syringe and flow it through a tube to a metallic needle. The metallic disc collectors are spun using speed controlled, stepper motors. The syringe needle is electrically excited by applying a high-voltage ranging from 8.5kV to 9.5kV produced by the power supply. This electrically charged syringe needle for electrospinning synthetic polymer fiber streams is positioned above and substantially centered between the edges of metallic collectors. The distance between the needle and dual disc collectors was approximately 5 cm. The feeding rate of the PCL solution was adjusted to a rate of 0.05 mL/minute. As a result, an electrostatic field is formed between the charged syringe needle and the edges of the rotating metallic disks. This allows for the deposition of aligned fibers on the collector plates and the fibers can be extracted easily. A mold with 10 mm diameter through hole is used to collect fiber.

The samples were prepared using the dual disc setup to create aligned Polycaprolactone Fibers. The PCL fibers were then layered in 90° offsets to form 24 layers of PCL fiber. PEGDA was then formed by treating 65µl of ready PEDGA solution and cured under UV light for 6 mins. The cured PEGDA was then layered onto the sample and another 24 layers were formed on the top. This was done for 3 layers of PEGDA each with PCL 24 layers on either side as shown in figure (32). This was used as the test sample.

Group of samples (PGDA and PGDA_GC) were kept under ultraviolet light overnight then soaked with Fibronectin (0.1 µg/ml) and Collagen (0.01 mol/ml) in 1:1 ratio. Mouse Osteoblast cells ($1x10^5$ cell/ml) were then seeded on all samples using standard cell culture technique. After a 3day incubation period, the cells were fixed. The fixing was done by adding 1 ml of formaldehyde (10%) to each sample for 30 mins, then washing with PBS (1x) twice for 5 min each time and finally adding 100 µl of Alizarin Red (2%) stain for 1 hr. Once fixed each sample was then washed with distilled water thrice.

4.4.2 Sectioning and imaging

After cell culture the samples were imbedded in cryocut fluid, frozen and sectioned at 25 µm and viewed under microscope. All samples were imaged and the adhesion and proliferation was investigated. Sample images are shown in Figure (33-35).

4.5 Result and Discussion

The images were seen to be inconclusive to show that the cells are proliferating or adhering to the samples based on figures (24-26). This is because of the issue we see with the sectioning as when the sections are made close to the Osteoblast cells radius the PEGDA starts to disintegrate and break away. This then does not give a conclusive outcome to this

method. Cells were seen to be present in the sample but there is no way to give conclusive evidence of the location of the cells prior to sectioning.

4.6 Figures

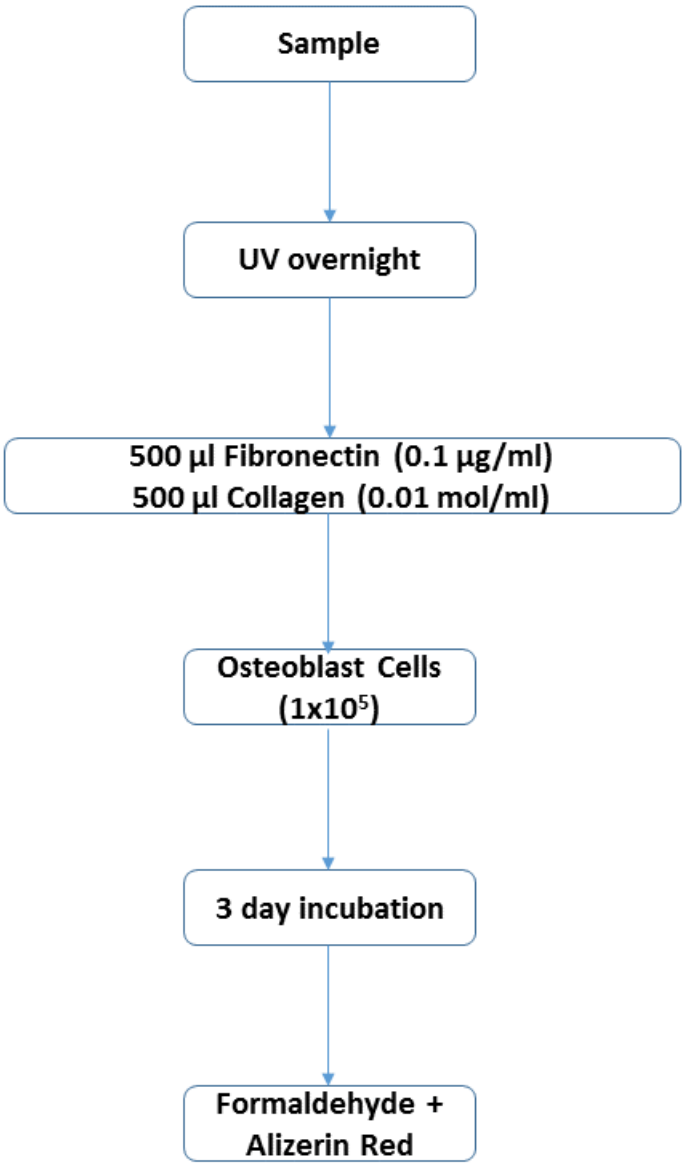

Figure 31 – Flowchart to show steps of cell culture done using Mouse Osteoblast Cells

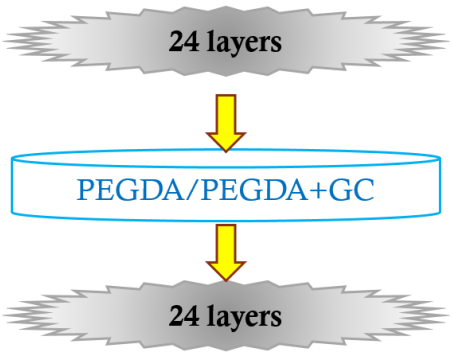

Figure 32 : Illustration to show building block of this study

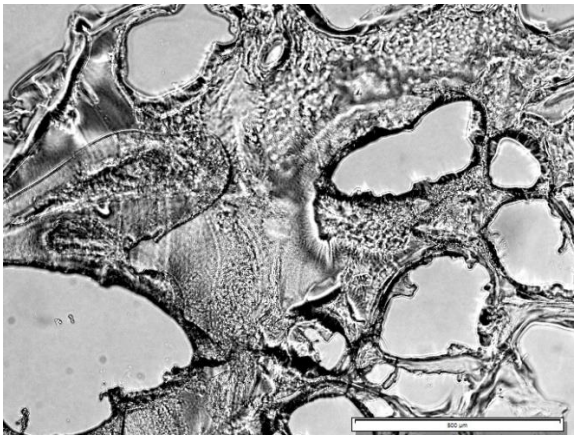

Figure 33: Sectional image of 40PEGDA+65Glycated Chitosan at 40 µm

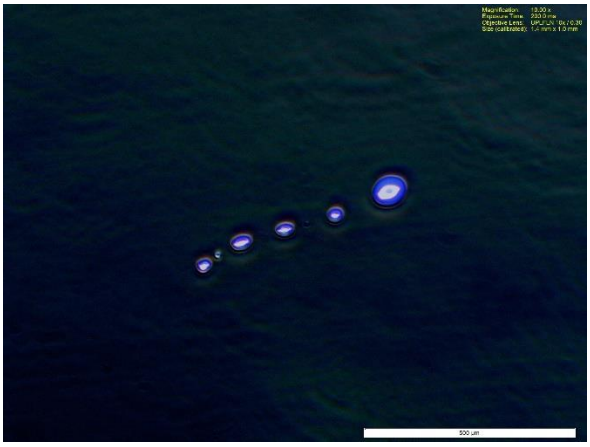

Figure 34: Sections show cell viability after cell culture for three days using DAPI Stain

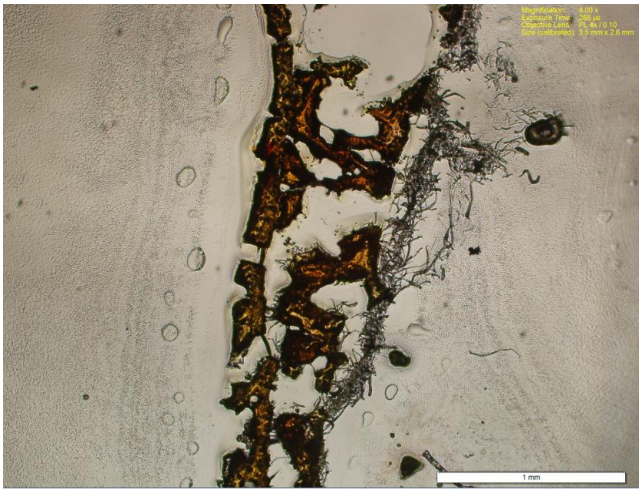

Figure 35: Section image of the complete sample showing disintegration of the sample after sectioning

Chapter 5

Conclusion and Future Work

4.7 Conclusion

The sample ACLs that were prepared with the PCL alone as material for electrospun nanofibers was not strong enough to withstand loads of the knee of a rabbit model. The Nylon-6 was able to withstand more load than the PCL but was not electrospun as the setup was not equipped to do so. There was not much increase in strength by using both PCL and Nylon-6 twisted pair. This may be because the fibers were not intergrated together well.

The melting point of the PCL is not high enough to withstand the tobacco smoke when incorporated into the cigarette filter itself. The external model however was able to block particles of compounds made of Carbon and Oxygen among others.

The use of PCL-PEGDA and PCL+GC-PEGDA as a functional scaffold over which bone cells can be grown is not clear. This is because of the techniques used to image the sample during the evaluation phase.

With this research the use of PCL and Nylon-6 as building blocks to create engineered materials to perform specific tasks is apparent. The machines that were used for manufacture and testing of the samples are all available to the Department of Engineering and Physics to be used further.

4.8 Future Work

With respect to the ACL substitute Nylon-6 shows promise as a base. The nylon-6 samples however could be electrospun to see if the electrospun nanofibers could withstand more load. Once the spinning protocol of the Nylon-6 is formulated, the nylon-6 should also be used as a base over which the PCL should be spun. This may be stronger as the fibers will be more intergrated together. The use of a coaxial spinning technique is also possible to inter-spin two or more types of fibers together. Another avenue is investigation into the use of biomaterial like rope made form plant fiber as a base over which the PCL and Nylon-6 can be spun, the samples may be able to withstand the tensions that an anterior cruciate ligament is subjected to on a constant basis. Another aspect to be investigated is the effect of adding collagen and fibroblast cells onto the samples. This was seen to increase the breaking strength in the sample that was tested. Future samples with collagen must also be freeze dried and tested to see if that would increase the strength of the sample. This is a topic of research that shows promise.

The samples can be testing again to show what particles exactly were blocked and how much of these particles were from the cigarette. The use of this filter model as a generic filter to block pollen, smog and other particles also can be investigated.

By changing the analysis protocol to include separate staining techniques for both the cells and the scaffold, we can better understand the properties of this material that we have created. The sectioning and imaging technique that was investigated here is not conclusive to say definitively that the cells are migrating into the sample. This will be a good place to start investigation.

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