Controlled Deposition of Polymer Matrix Using Electromelt Spinning

by

Alicia Thomas John

Master of Science in Engineering Physics - Electrical Engineering

University of Central Oklahoma

Edmond, Oklahoma

2019

Master Thesis

Submitted to the Faculty of

the Graduate collage of the University of Central Oklahoma

in partial fulfillment of the requirements for Degree of

Master of Science

May, 2019

Controlled Deposition Of Polymer Matrix Using Electromelt Spinning

Thesis Approved:

Dr. Morshed Khandaker, Chair

Dr. Weldon Wilson

Dr. Alaeddin Abuabed

ACKNOWLEDGMENTS

First and foremost, I would like to thank God Almighty for giving me strength, knowledge, ability to undertake this research and complete it satisfactorily. Without his blessings, this achievement would not have been possible

I would like to express my sincere gratitude to my advisor, Dr. Morshed Khandaker, for his continuous support throughout my master's research, for his patience, motivation and immense knowledge. His office door was always open whenever I had trouble or questions. He patiently observed my work and steered me in the right direction when I needed it. He even came on weekends just for my study and that deserves a true respect from the bottom of my heart. I could not have imagined having a better advisor and mentor for my master study.

I am gratefully indebted to Dr. Weldon Wilson , Dr. Robi Hossan , Dr. Alaeddin Abu-Abed and Dr. Abduella Ait Moussa for their valuable comments and support during my research.

I also like to acknowledge the Department of Engineering and Physics, the College of Mathematics and Science, and Graduate collage at University of Central Oklahoma for all the encouragement and enthusiasm for my research.

I would like to thank my research mates, especially the biology students, who did the cell culture for my research. They were always ready to help me and always encouraged me. I sincerely appreciate your cooperation and guidance.

Finally, I must express my profound gratitude from my deep heart to my beloved parents, Thomas P John and Annamma John, for their love, unfailing support and continuous encouragement throughout my research and writing. This accomplishment would not have been possible without them.

ABSTRACT

Electrospinning is a widely used technique to produce fibers from polymer solutions using high electric field. With its simplicity and low-cost, all kinds of polymers can be processed using this technique. But there is a limitation to produce precise 3D structures and inability to control the pore size and non-uniformity. There is an alternative approach called melt electrospinning, which is a processing technique to produce fibers and fibrous structures from polymer melts in the range of micrometers to millimeters. Electromelt technique is solvent-free and can build precise and uniform scaffolds with pre-determined pore size. The efficiency of both processes depends on the instrument parameters and solvent properties: fiber diameter, uniformity of the fibers, solution viscosity, flow rate, tip to collector distance, ambient parameters.

The goal of this study is to compare the morphological and biological characteristics of the 3D scaffolds produced from electrospun and electromelt technique. There are three objectives for this study. The **first objective** was to produce 3D scaffold using the electrospinning process and analyze the scaffolds morphologically. The polycaprolactone (PCL) scaffolds were produced using the electrospinning system. The **second objective** was to develop a melt electrospinning system using Newport actuators for controlling the scaffolds in the three dimensions within the range of 100 to 500 microns and its morphological analysis. Obtaining the desired precise movement using the actuators and selecting a suitable flow rate determines the feasibility of the system. The system used a programmable -syringe pump system, to control the ejection of fluid to maintain constant flow rate, preventing the formation of any non-uniformities. The area roughness, height, pore size and fiber diameter of the structures were analyzed under SEM and Profiler. The **third objective** was to compare the morphological characteristics of the results from both electrospinning and melt electrospinning.

This study successfully developed an electromelt system that can produce rectangular and cylindrical PCL scaffolds with consistent porosity. From the morphological analysis using SEM and profiler, the scaffolds produced from electromelt process has observed to have very precise porosity than the electrospun process thus concluded that melt electrospinning is more efficient technique to produce 3D scaffolds. From the *in vitro* cell analysis of the scaffolds produced, the cells were able to attach to the scaffolds very easily and observed a noticeable growth of cell, proliferation and infiltration through the pores as the end-product is applied for bio-medical applications. Thus electromelt can provide a consistent solution to the problems of electrospun.

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LIST OF ABBREVIATIONS

PCL : polycaprolactone

3D : Three dimension

UV : Ultra violet

HV : High voltage

kV: Kilovolt

ESP : Enhanced System Performance

EEPROM: Electrically Erasable Programmable Read- Only Memory

DC : Direct Current

LCD : Liquid Crystal Display

SMC : Single Motion Controller

R: resistance

I : current

V: voltage

GPIO : General Purpose Input Output

USB : Universal Serial Bus

RS : Recommended Standard

CHAPTER 1

INTRODUCTION

1.1 SUMMARY

Electrospinning is a widely used technique to produce fibers from polymer solutions using high electric field. With its simplicity and low-cost, all kinds of polymers can be processed using this technique. But there is a limitation to produce precise 3D structures and inability to control the pore size and non-uniformity. There is an alternative approach called melt electrospinning, which is a processing technique to produce fibers and fibrous structures from polymer melts in the range of micrometers to millimeters. Electromelt technique is solvent-free and can build precise and uniform scaffolds with pre-determined pore size. The efficiency of both processes depends on the instrument parameters and solvent properties: fiber diameter, uniformity of the fibers, solution viscosity, flow rate, tip to collector distance, ambient parameters[1, 2].

A crucial parameter is the uniformity of the fibers, preventing the appearances of beads or pores within the fiber structure and the solution viscosity which solely depends on the selection of the polymer solution. Flow rate of the polymer solution in the syringe should be very low as this would in turn gives sufficient time to cure high flow rates are not recommended as it can result in beaded and non-uniform structures. Selecting the appropriate collectors, determining the optimum tip distance for efficient collection of the fiber along with maintaining constant temperature and humidity are other processing parameters which results in overall scaffold production.

This research is to develop a modified melt electrospinning system using Newport actuators for controlling the scaffolds in the three dimensions, thus making the fiber diameter consistent and controllable. And selecting a suitable chemical solution, which is biocompatible, biodegradable

and that possess good mechanical properties, can cure faster also determines the feasibility of the system. Different solutions such as Gelatin, Polycaprolactone (PCL) with acetone were tried with various concentrations. Syringe pump system is used to control the ejection of PCL solution, this helps to maintain constant flow rate, preventing the formation of any non-uniformities. The solution is then collected in the base which moves according to the actuator stages, while the base is heated using UV light, accelerating the normal curing time of the solution. The whole system is enclosed for optimal performance of the UV light.

1.2 ELECTROSPINNING

Electrospinning technique is widely used for producing fibers and fibrous structures by the influence of high electric field. All kinds of solutions can be used in this process like natural polymers and synthetic polymers. Electric field causes the elongation of the solution to fibers, which are then collected in the substrates called collectors. This method is very popular and initially used in textile industry and then advanced in the biomedical field. [3, 4]

Unlike the traditional spinning method, electrospinning method yields very thin fibers with large surface area through a different scheme by using tens of kVs to generate the electric field required to elongate the polymer solution into the fiber. This electric field tends to weaken the surface adhesion and tension in the solution by charging them and then repelling the similar charged particles, along with the evaporation of the solvent, this will distort the structure of the solution. There are two different types of electrospinning system, depending on the position of the needle or the syringe containing the solution: Horizontal (Figure 1.1b)or Vertical system (Figure 1.1a). A typical electrospinning system would contain the following components:

a) A metallic needle

b) High-power voltage supply

c) Collector

d) Syringe pump

The high voltage power is supplied between the needle tip and the collector, the positive electrode being connected to the needle tip and the ground connected to the collector. More than 5 kV is applied usually, but this can vary depending upon the characteristics of the polymer solution. This electric voltage causes evaporation of the solvent and tends to alter the stability of the solution by charging the solution and then repulsing action takes place. This would force the solution to enter a bending stage by stretching the solution jet. The grounded collector can collect the fiber deposited in different forms. The collector can be a rotating drum or a linear stage depending upon the applications of the fiber.[5]

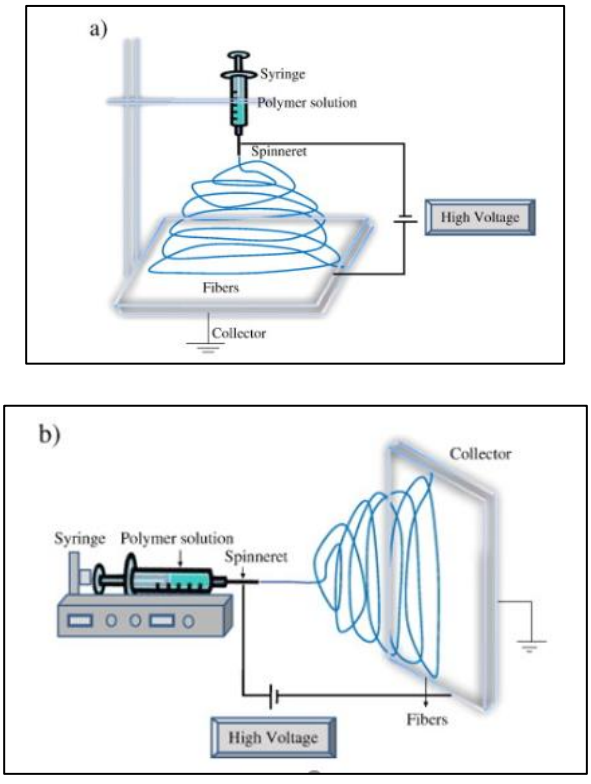


Figure 1.1 : ELECTROSPINNING[2, 5]

Theoretically, there are four different regions within the electrospinning method:

a. The base region

b. The jet region

c. The splay region and

d. The collector region

The base region is the region of the needle end where the charged solution is contained.

The jet region is the region where the solution tends to follow a straight line and the splay region denoted the region where the jets splits into many nano fibers. The collector region is where the fibers deposits. [5, 6]A schematic illustration of these regions are shown in Figure 1.2:

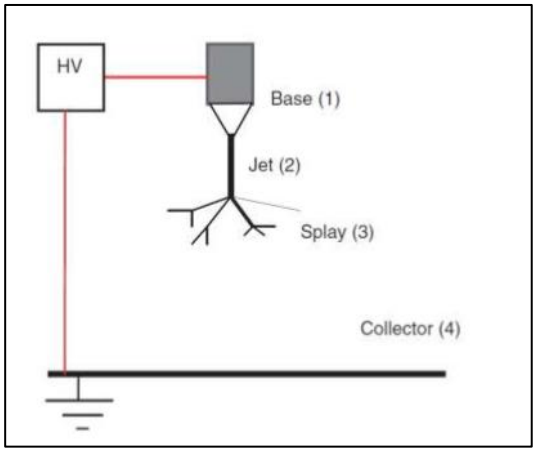


Figure 1.2: JET ANALYSIS OF ELECTROSPINNING[6]

Even though this promising technique undergoes a simple operation, there are several parameters that must be accounted for the efficiency and fiber morphology. They are classified as solution parameters, process parameters and ambient parameters.

Solution parameters: Viscosity, conductivity, surface tension, polymer molecular weight, dipole moment, and dielectric constant are the most vital parameters that are considered as the solution parameters. Fluctuating one parameter can by and large influence other arrangement properties, so it is hard to segregate the impacts of the arrangement properties freely.[2, 4, 5]

There is a minimum concentration level that must be maintained in order for the electrospinning to occur. If the concentration is very low, then there is a very high percentage of electro spraying to occur instead of electrospinning, which results in forming beads and spindles in the structure.

Ideal solution viscosity is required as low viscous solution will not produce fibers and very high viscous solution faces trouble in forming jets. Various studies were conducted by producing fibers from different viscous solutions and were analyzed. The solution with more than 1000 centipoise viscous were tend to produce good nanofibers.[2, 4, 5]Figures 1.3 and 1.4 describes the effect of viscosity and flow rate to the produced fibers.

Process parameters: These parameters include flow rate, distance between the tip and the collector, applied voltage and the collectors. The flow rate of the polymer solution is one of the critical parameters that affects the process of the fiber production. Slow flow rate is recommended as this would enable the dissipation of the solvent to be slower and reducing the possibilities of beads, bubble or spindle formations, that occurs in cases of high low rate. An optimal flow rate should be maintained for the efficient performance of the process.

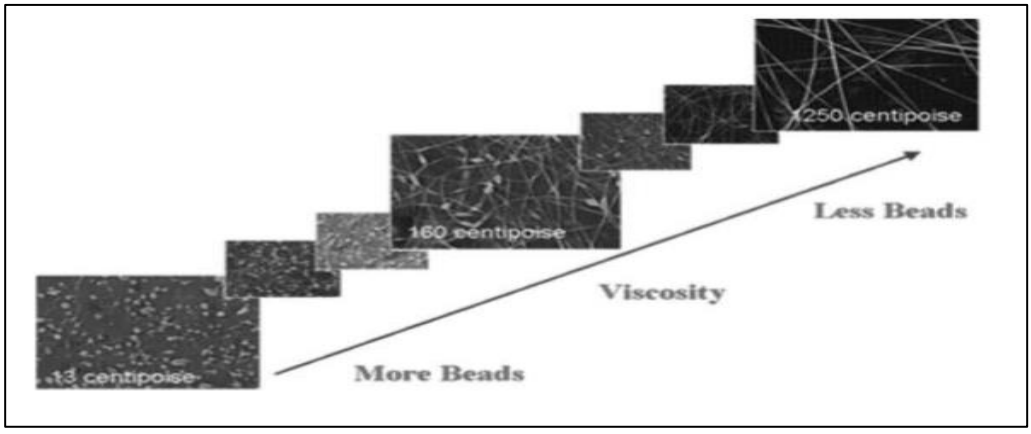


Figure 1.3: VISCOSITY ANALYSIS[2]

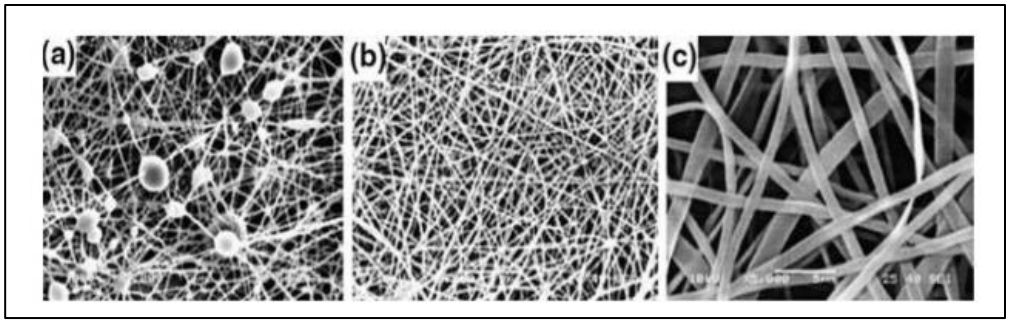


Figure 1.4: FLOWRATE ANALYSIS[2]

Numerous studies were conducted on the effect of voltage variations on the fiber structure. The applied voltage should not be very less as it has to charge the solvent and weaken the surface adhesion, converting into repulsing action. It was observed that when the voltage was increased, the repulsing force increased and hence the fiber diameter was decreased. Also, very high voltage is not recommended as it can also trigger the formation of beads and bubbles inside the fiber which affects its morphological characteristics.

As a contactless system, the distance between the tip and the collector should also be maintained properly. The collector should be a reasonable distance with the tip as this could facilitate the dryness of the fibers before reaching the collector. The optimum distance was identified by various trials for the production and it is also dependent on the solution used. The collector is a conducting substance or material used to collect the nano fibers, mostly aluminum foil is used. Different types of collectors are also used for different applications such as rotating rod, wire mesh, conductive paper or cloth, parallel or gridded bar.[2, 4, 5]

Ambient parameters: Ambient parameters such as humidity and temperature are observed to have a large impact on the fiber diameter and morphology. Generally low humidity can dry the

solution easily and can boost the speed of evaporation, whereas high humidity leads to thick fiber production by neutralizing the repulsing action and can also increase the formation of pores in the fibers. Low temperature can reduce the speed of drying the solvent and also can affect the fiber diameter whereas high temperature can decrease the fiber diameter. Hence, low humidity and high temperature is recommended.[2, 4, 5]

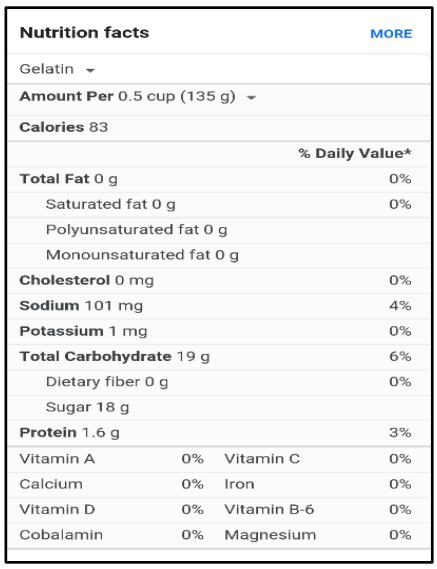
1.3 MELT ELECTROSPINNING

Melt Electrospinning technique is another technique used for producing fibrous structures in the range of micrometer to millimeter, by using molten polymer solution. This method is solvent free and the solution is melted by applying an electric field in addition to the high electric field. The basics of this process is very similar to the electrospinning process: Electric field causes the elongation of the solution to fibers, which are then collected in the substrates called collectors. This method solves the issue of evaporation of the solvent and is known to produce scaffolds with high precision. Isolating the two electric power sources is the only thing that needs to be considered during the technique. This thesis is testing the capability of the process and the analysis of the resulting scaffolds. [7-9]

1.4 MATERIALS

A. GELATIN

Gelatin is a translucent, colorless, flavorless protein , derived from various animal body parts by boiling skin, ligaments or bones with water. Gelatin is usually used in various cosmetics, as a thickener for puddings, candies, cakes, icecreams etc. As gelatin when dissolved with boiling water, the mixture turns out to be a very high viscous solution, biodegradable and bio-compatible and hence adaptable for the project. The nutritional facts of Gelatin is described in Figure 1.5:



Nutrition facts MORE

Gelatin

Amount Per 0.5 cup (135 g)

Calories 83

	% Daily Value*
Total Fat 0 g	0%
Saturated fat 0 g	0%
Polyunsaturated fat 0 g	
Monounsaturated fat 0 g	
Cholesterol 0 mg	0%
Sodium 101 mg	4%
Potassium 1 mg	0%
Total Carbohydrate 19 g	6%
Dietary fiber 0 g	0%
Sugar 18 g	
Protein 1.6 g	3%

Vitamin A	0%	Vitamin C	0%
Calcium	0%	Iron	0%
Vitamin D	0%	Vitamin B-6	0%
Cobalamin	0%	Magnesium	0%

Figure 1.5: GELATIN[10]

B. POLYCAPROLACTONE (PCL)

Polycaprolactone is one of the earliest, semi crystalline biodegradable polyester which finds major application in tissue engineering. PCL exhibits very good mechanical properties with excellent resorbability , long time degradation and low melting point which fits with our application, and hence serves as a good choice. To enhance the curing time and improve crack resistance, mostly PCL are used with other chemicals, which are blended together to homogenous solution for the production of scaffolds in tissue engineering. The formula and nutritional facts of PCL is depicted in Figure 1.6.

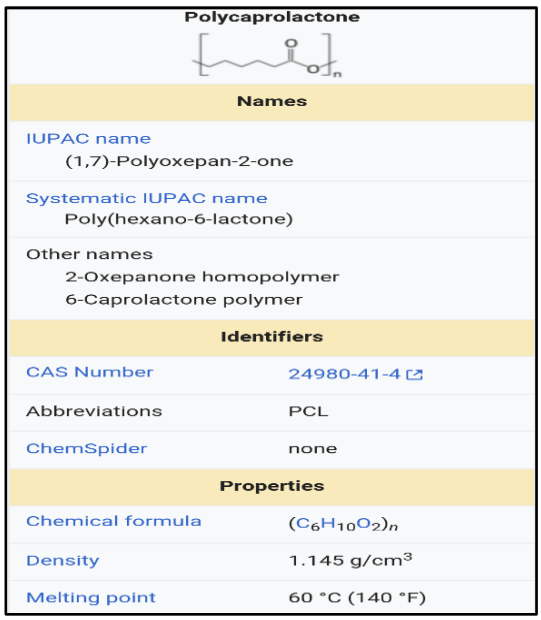


Figure 1.6: PCL[11]

1.5 DEPOSITION ALIGNMENT

There are three different types of alignment of PCL deposition : Unidirectional and Bi-directional deposition and random.

Unidirectional deposition of PCL fibers can only direct in one directional and there have been several studies conducted on it. In Khandaker et al. [12, 13], have conducted study on the unidirectional PCL fibers and their effect on the fracture of cement/implant surfaces. They produced unidirectional fibers using a custom-made electrospinning unit and deposited the fibers in a drum collector. They also successfully achieved bi directional fibers using grounded plate collector and carried out shear test and tensile test. They concluded that the unidirectional fibers have more fracture toughness than bi directional. In Han et al., unidirectional PCL fibers were produced using the melt electrospinning technique and then used for their applications for dually self -composite PCL production.[14]

In Cooper et al., PCL blended with chitosan was used for the study of restoring damaged nerve cells using the fiber and fibrous scaffolds for the cell adhesion and proliferation. They also compared the result with randomly oriented fibers and cast films of the same composition. They found that the PCL-chitosan unidirectional fibers were exhibiting bipolar morphology and supported the cell growth and guided the cells along the fiber orientation.[15]

In Yun et al., PCL fibers were produced using electrospinning process in bidirectional orientation and were treated with silver nanoparticles and silica to produce fluorescence based biosensor. They used aluminium foil as the collector and placed at a distance of 20 cm from the needle tip and obtained the fiber upon which was their study conducted. They concluded that PCL fibers with the incorporation of silica and silver can act as a fluorescence biosensor with increase sensitivity.[16]

1.6 SCIENTIFIC SIGNIFICANCE

Aligned fibers are always desired for the cell scaffolds eventhough random orientation is appropriate, aligned fibers can control the cell guidance in the desired direction more better than the random fibers.[17] In different cell studies conducted, the gene expression of the fibroblasts cells were examined and observed that the cells were oriented perfected in aligned fibre and exhibited very little orientation in the random fibers.[18, 19] This cell adhesion depends on the fiber diameter and the cell type. A minimum diameter of 0.97 microns is needed for the cell orientation.[20]. Regarding the cell proliferation, fibers were produced using electrospinning and then sterilized before treatment of cells. After the study, they found aligned fibers exhibiting slightly higher cell proliferation then the random ones.[21]

1.7 PROBLEM STATEMENT

Generally, scaffolds are used in tissue engineering applications like air filtration, gas absorption, hence forth the scaffolds should be bio-compatible with uniform porosity. This study aims to produce very defined and precise fibrous scaffolds using melt electrospinning system as aligned fibers and scaffolds tend to promote cell growth, proliferation and filtration greatly than non-aligned structures. Controlled diameter and pore size are the main focus of this system which also promotes environmental protection as a solvent -free technique. Enhanced cell growth and proliferation can have huge impact on the biomedical industry as they can be used for tissue regeneration, drug delivery etc.

1.8 MOTIVATION AND GOALS

The motivation of this thesis is to build a system that can develop polymer matrix produced by controlled deposition using electromelt process, with only few microns spacing. There are several parameters that need to be found out for building the system from scratch like flow rate, solution viscosity, actuator movement , space between the tip and the base. The goals are to analyse the parameters of the produced polymer matrix morphologically using Profiler and Scanning electron microscope.

1.9 OBJECTIVES

This study has 3 main objectives, which are:

1) To produce cross fiber production using the electrospinning technique
2) To build a melt electrospinning system that produces 3D rectangular and circular scaffolds of 100 - 500 microns
3) Comparison of the morphological and biological analysis of the produced scaffold using Scanning Electron Microscope and Profilm Profiler [diameter, layer spacing and area roughness]

1.10 ORGANIZATION

Chapter 1:

1. Discusses the important concepts underlying and the need of this thesis
2. Discusses the goals and objectives of the thesis
3. Discusses the instruments used in the thesis

Chapter 2:

1. Discusses about the concept underlying the electrospinning technique
2. Discusses about the electrospinning system and the materials and methods used
3. Discusses about the results obtained and the conclusion obtained.

Chapter 3:

1. This chapter discusses about the concept underlying the melt electrospinning technique.
2. Discusses about the components used to build the system for non-photo sensitive materials
3. Provides detailed description of the programming involved with the actuators and motor syringe pump system.
4. Provides the design simulation
5. Describes the experiments conducted to find the required viscosity and flow rate of the solution
6. Discusses about the results obtained and the conclusion obtained.

Chapter 4:

1. Morphological and biological analysis of the 2D rectangular structure produced using electrospinning and melt electrospinning.
2. Morphological analysis of the 2D circular structure produced using electromelt system with PCL solution
3. Morphological analysis of the 3D rectangular and cylindrical structure produced using electromelt system with PCL solution.

CHAPTER 2

CROSS FIBER PRODUCTION USING ELECTROSPINNING SYSTEM

ABSTRACT

The goal of this study is to develop PCL scaffolds using electrospinning technique in two different architectures: rectangular and circular structures. With the syringe pump system and the high voltage supply, temperature and the flow rate were controlled. PCL pellets were used with acetone as the material. Using glass slides, rectangular structures were obtained in unidirectional with multiple layers and using plastic mold, circular structures were obtained in bidirectional with multiple layers. These structures were analyzed under SEM for the topography and *in vitro* cell analysis were conducted. From the SEM images, the structures turned out to be non-precise in diameter and pore size and not aligned. But, the *in vitro* studies were successful, as the cells grew and adhesion and proliferation were also successful. These structures are suitable material for biomedical application but their non- alignment will pose a serious issue.

2.1 INTRODUCTION

Electrospinning is the method of producing fiber utilizing electric voltage between the solution droplet at the end of the needle and the collector, thus converting the droplet into charged strings of fiber in the range of few hundred nanometers. This process does not require any other complex mechanism or complicated system architecture, thus simplifying the operation and can also combine different solution types natural as well as synthetic. Although this technique was only known in the textile industry before decades, the recent years have seen the massive exploitation of this method in other requisite fields. The main advantage of this technique is the production of

very thin nano fibers through a contact less strategy , also the high surface area to volume ratio, high porosity, small fiber to fiber distance, low start up cost, ease of material combination are its well-known advantages. Due to the versatile advantages of this method, the resultant Nano fibers include medical drug delivery, filtration, wound dressing, tissue application, biological scaffolds, environmental protection etc.[5, 22]

Despite these characteristics, this technique is been affected by many limitations such as inefficient morphological parameters, non-aligned scaffolds, controlling the porosity, non-uniformity within the layers, shrinkage and distortion. The nano fibers produced by this conventional method completely lacs orientation and hence been a hindrance for many practical applications of the scaffolds. There have been various attempts to eliminate these undesirable limitations and produce an efficient system which can produce uniform scaffolds.

2.2 MATERIALS AND METHOD

2.2.1 PREPARATION OF THE POLYMER SOLUTION

Although different polymers are available, Polycaprolactone (PCL) is chosen as the polymer for this experiment, which is mixed with acetone to obtain the solution. PCL pellets (pellet size~3 mm, average Mn 80,000) from Sigma Aldrich is used and using the sensitive weighing scale in the laboratory, measured 0.1 g of PCL. Acetone (laboratory reagent $\geq$ 99.5 %)is also weighed and 10g of Acetone is taken. Instead of mL, I took gram scale of acetone. PCL beads is then added into the acetone solution and then mixed using the sonicator, which uses sound waves of 20 KHz and it is set to 60% of its amplitude. This sonicator mixes the solution and the time which it takes depends on the polymer used. I have currently set to 45 minutes for the viscosity we need. For

high viscosity, we can change the concentrations of the PCL beads and acetone and also increase the time for sonication.

2.2.2 FLOW RATE/ SYRINGE PUMP SYSTEM

The commercial Harvard Apparatus syringe pump system is used for the flow rate, in Figure 2.1. The polymer solution when done is poured into the syringe and is connected to the tube using the adapters. One of the adapters is then attached to the needle. The syringe is placed into the socket and secured using the bolts. The flow rate of 25µL/min is already set by different trials. The needle is in turn fixed by attaching into the wooden piece.

2.2.3 HIGH VOLTAGE SUPPLY

The high voltage required for the electrospinning process is supplied as shown below. After several trials, the voltage is fixed to be 9kV. For safety precautions, High voltage glouses are used while operating on this voltage supply. The green wires connected from the voltage supply is the ground wires and are connected to the collectors. The HV component is displayed in Figure 2.2.

Figure2.1: SYRINGE PUMP SYSTEM

Figure 2.2: HV SUPPLY

2.2.4 DISTANCE FROM THE TIP TO THE COLLECTOR

The distance between the tip and the collector plays a vital role in the fiber collection. If the collector is placed very far, the fibers may not be able to deposit perfectly to the collector. If placed near, the fiber formation can get affected and thus will affect the formation of fibers. With various trials the distance was found be good in the range of 20-30 cm.

2.2.5 COLLECTORS

Two different collectors are attached to this system: a rotary drum and the parallel electrodes.

Parallel electrodes are using for collecting fibers in a parallel manner and can be used for wound applications, animal study and bacterial study. The parallel electrode is connected into a wooden block and can be moved to obtain the fiber structure by keeping the needle stationary. Rotary drum is connected to a DC motor supply which requires a 12 V power supply to function. Also, A key pad is connected which can increase/decrease the speed of the rotation depending on the applications needed with the fibers. In both the collectors, the fibers can be made in a layered

structure, by forming one above the other. For the system, parallel electrode collctor is used as shown in Figure 2.3.

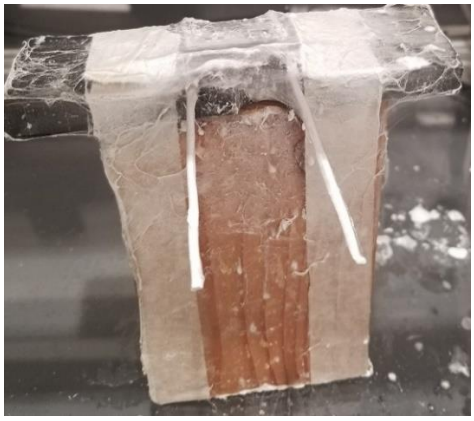


Figure 2.3: PARALLEL ELECTRODE COLLECTOR

2.3 CUSTOM- BUILD ELECTROSPUN SYSTEM

A custom made electrospun unit was build in this study to produce uni & bi-directional fibers which is shown in Figure 2.4.

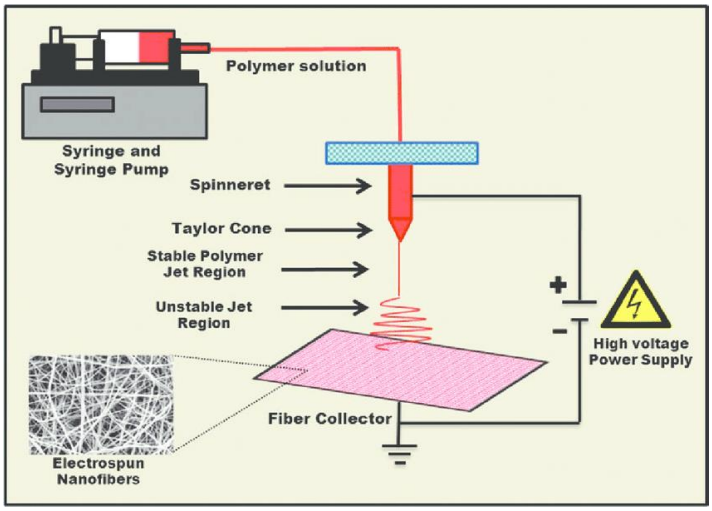


Figure2.4: MELT ELECTROSPINNING SYSTEM[23]

After supplying high voltage, fibers gets deposited across the needles unidirectional which can be obtained using glass slides or plastic, acrylic molds held by the tweezers which are already autoclaved or sterilized. Uni-directional fiber deposition can be directly obtained with the glass slide whereas the bi-directional fiber deposition was not direct as the position of the electrons pose a threat for the glass slide to go through and collect in in the perpendicular direction. Hence ring like structures smaller in diameter than the gap of the electrodes were used, so it can be inserted below the fibers and then pulled up to get it deposited and then the mold is rotated 90 degrees manually to obtain it in bi-direction.

2.4 OPERATING PROTOCOL

The two parameters that have to figured out for this system is the appropriate flow rate and the HV value. Initially decided to obtain a value for the flow rate for the efficient performance of the system. As the solution was not that viscous, with little pressure it can eject faster. So, decided to start with lowest speed in the range of nl/min. Started with 5 nl/min and checked to observe that the solution was not that ejected properly in time, it was very slow. Hence decided to increase it to 50 nl/min just to observe if it makes any changes and that was also not that good. Started in the range of ml/min. Checked the speed of 10ml/min and that was of high speed and lots of solution was ejected. Tried to decrease the speed by 5 ml/min. Again the speed was high enough to eject more solution. Tried 1 ml/min and observed that the speed is still higher for the solution viscosity as we only need one drop at a time for the electric field to act on it. Decided to go down to 0.01 ml/min as the attempt to figure out the flow rate and that was good but small drop. Increased at the rate of 0.01 ml/min to see the best flow rate possible. At 0.05 ml/min it was good to see medium sized drop coming out of the needle and hence after many trials decided to go with 0.05 ml/min as the appropriate flow rate for this system.

Regarding the voltage, several research papers suggested the value of around 3kV- 20 kV as necessary for the electrospinning technique. Started with 3 kV and the flow rate of 0.05ml/min. Observed that the drop was affected but not in such a way to obtain good fibers. Electric field was acting upon it but very slightly and didn't obtain a good result with drop not completely transforming into the fiber. Increased to 5 kV to observe the change in the droplet, which was better than the 3 kV but still not appropriate as the drop had more electric field but not that that much as to elongate the fiber. Decided to jump to 10 kV and observe the droplet. This trial was good, drop were completely changed into fibers but at some point, the fibers were not forming as the jet was getting slightly broken by the influence of the electric field. Hence, decided to decrease the voltage to 9kV and observed that the voltage was good enough for the fiber as the jet was not affected. And the fiber production was good was 9 kV and flow rate of 0.05 ml/min.

2.5 RESULTS

A. DESIGNED SYSTEM

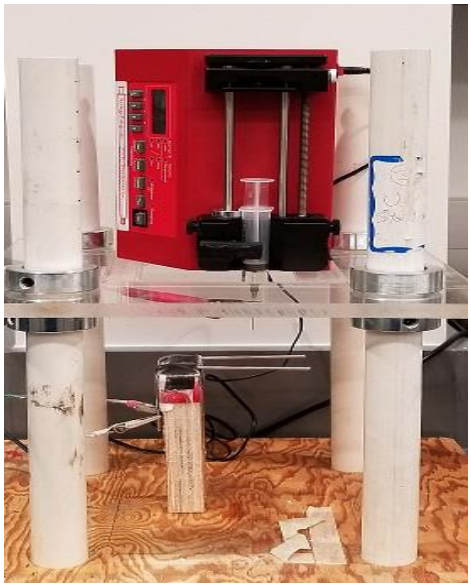


Figure 2.5: ELECTROSPUN SYSTEM

B. UNIDIRECTIONAL PCL LAYERS

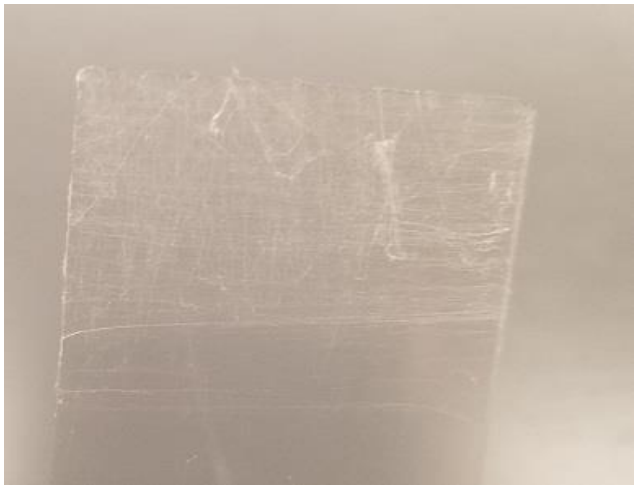


Figure 2.6: UNIDIRECTIONAL RECTANGULAR SINGLE LAYER

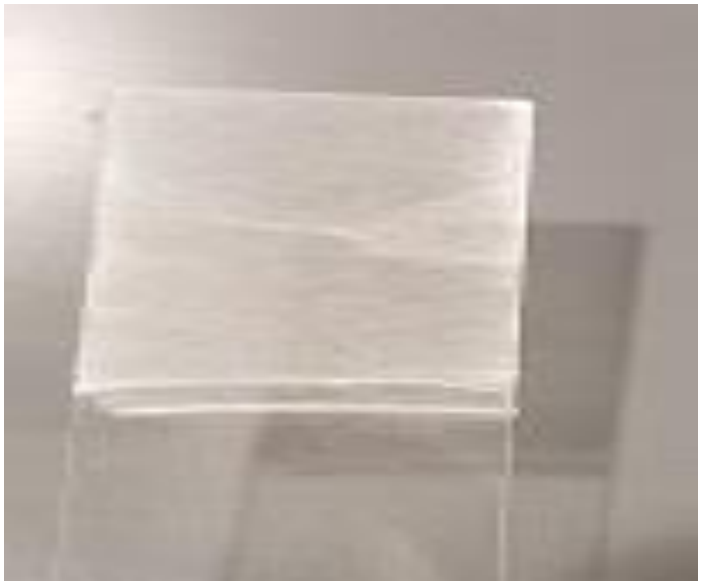


Figure 2.7: UNIDIRECTIONAL RECTANGULAR MULTIPLE LAYER SAMPLE-1

Figure 2.8: UNIDIRECTIONAL RECTANGULAR MULTIPLE LAYER SAMPLE-2

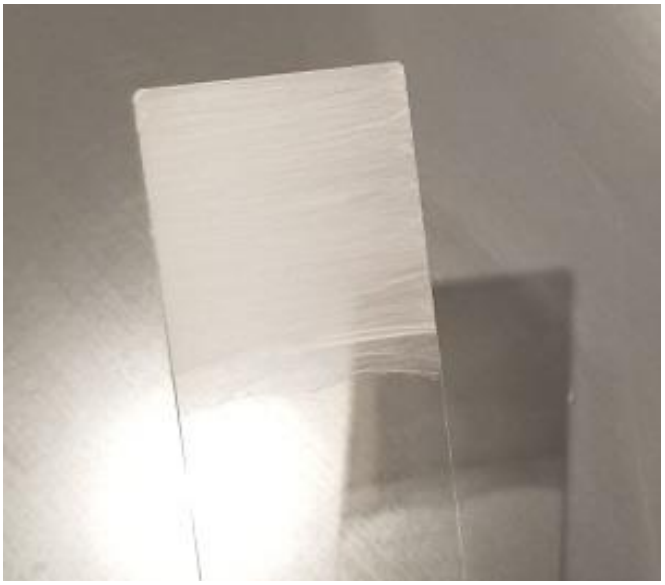


Figure 2.9: UNIDIRECTIONAL RECTANGULAR MULTIPLE LAYER SAMPLE-3

PCL fibers were produced using electrospinning process and using parallel electrodes as the collector. Used glass slides to collect the fibers in the same direction manually. As shown in Figure2.6 the glass slide only has one layer of fiber in it and even with naked eye it can observed that the fiber is very thin and not aligned. Another glass slide was used to obtain 6 layers , one on

top of the other but that was getting even more thicker and not in an aligned manner, forming like a bandage or a small piece of cloth. . The Figures 2.7-2.9 have 6 layers of fibers on them.

C. BI-DIRECTIONAL FIBER

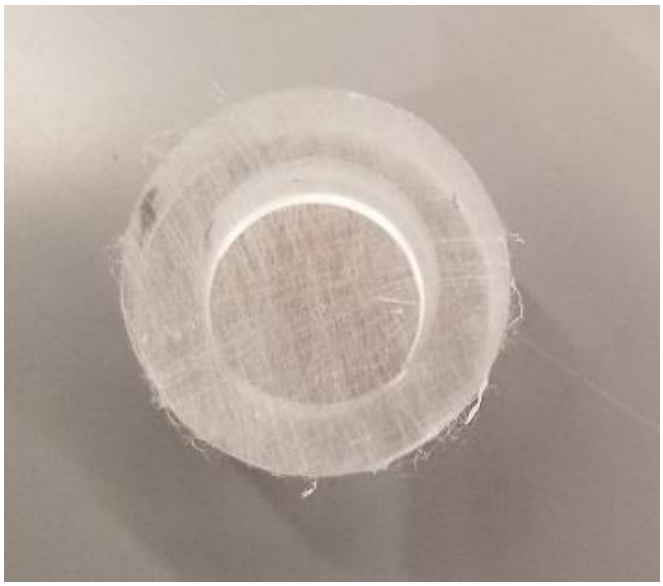


Figure2.10: BIDIRECTIONAL CIRCULAR SAMPLE -12 LAYERS

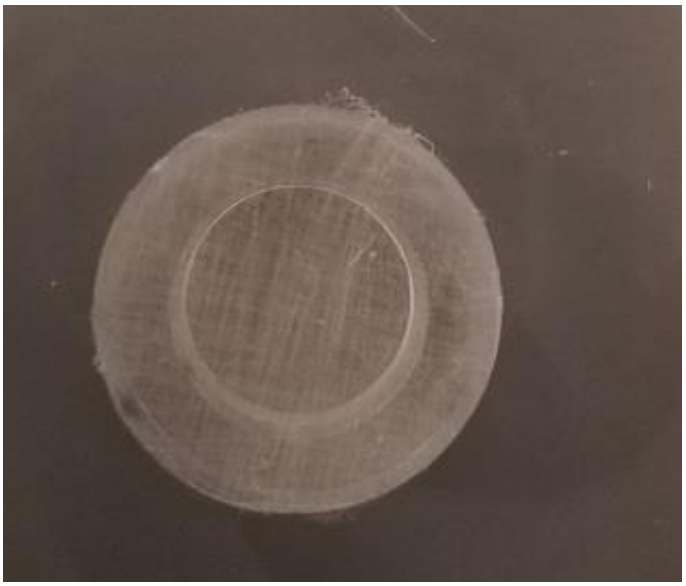


Figure2.11: BIDIRECTIONAL CIRCULAR SAMPLE -18 LAYERS

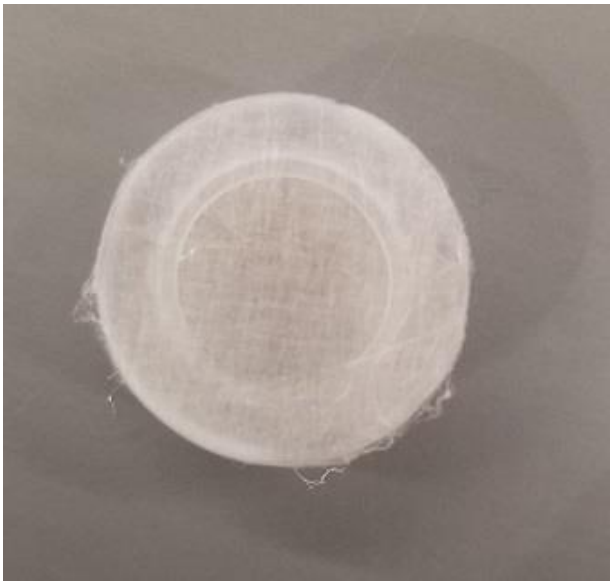


Figure2.12: BIDIRECTIONAL CIRCULAR SAMPLE -24 LAYERS

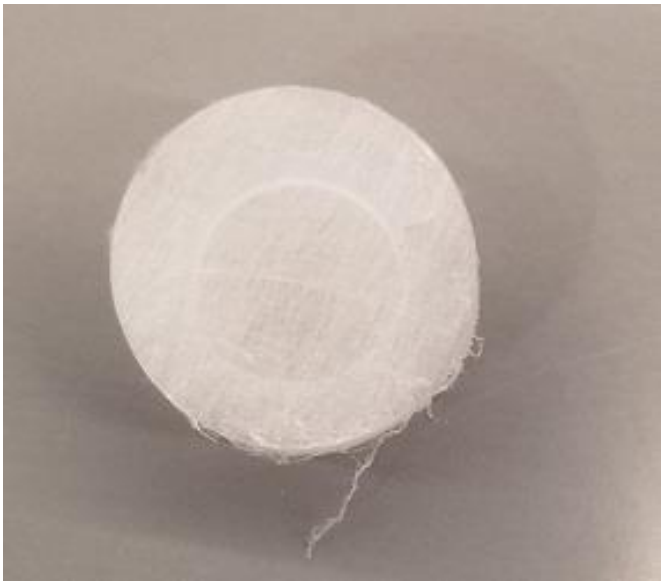


Figure2.13: BIDIRECTIONAL CIRCULAR SAMPLE - 30 LAYERS

The results shown in Figures 2.10-2.13 are produced using the same process as before, but bidirectionally, Manually collected fibers on this plastic mold, and used tweezers to move the mold in right angles to obtain the crisscross structure. Made 4 samples with different layers in it: 12 ,18

, 24 and 30 layers respectively. As the layers increases, the fiber deposition turns to be opaque resembling a cloth .

Both the fibers structures developed were analyzed and the morphological characteristics were interpreted in Chapter 4.

2.6 CONCLUSION

This chapter discusses about the electrospinning process, the principle behind it and the parameters involved with it. The goal of this chapter was to produce 3 unidirectional PCL samples of 6 layers and four bidirectional samples with layers 12, 18, 24 and 30,using the electrospinning system.

The electrospun system was build using a commercial Harvard Apparatus syringe pump mounted and the HV supply with parallel electrodes as the collector. After several trials, the parameters were set up with the flow rate as 0.05 ml/min and the high voltage as the 9 kV. The fiber was produced across the electrodes. Using glass slides and plastic molds the unidirectional and bidirectional fiber structures were obtained respectively.

From the microscopic observation, the bidirectional and unidirectional fibers are not consistent in the fiber diameter and the pore gap. Also, precision of the structure is at stake for the samples produced. But, on the other hand, from the cell analysis, cells growth in the PCL layers and cell adhesion and proliferation were better in increased layers, hence the samples produced are suitable for biomedical applications.

CHAPTER 3

CROSS-FIBER PRODUCTION USING MELT ELECTROSPINNING

ABSTRACT

The goal of this study is to develop a melt electrospinning system using Newport Actuators with high precision and to produce 3D scaffolds with rectangular and circular structures. The first objective is to build the melt electrospinning system with appropriate gap between the layers, flow rate and temperature. The fiber diameter and pore gap are controlled by the actuators, through different trials, flow rate and temperature was determined. PCL pellets were used with acetone as the material and through the developed system, PCL scaffolds were produced, one dimensional and two dimensional structures with rectangular and circular configurations. These scaffolds were then analyzed using SEM and Profiler for the topography and roughness. *In vitro* cell analysis were conducted, cells found to grow abundantly and cell adhesion and proliferation and infileration were found to be highly increased in the scaffolds than the electrospinning process. Hence, the scaffolds can be used as a potential material for tissue engineering applications.

3.1 INTRODUCTION

In electrospinning, fibers with consistent density and thickness can be achieved due to the repulsive charges around the collected fibers. However, the thickness of such substrates is less than 1 mm. A great part of the research and industrialization have been conducted in the solution electrospinning in view of the thick fiber diameter, high viscosity and complex system for high temperature and high voltage requirements for the melt electrospinning. Melt electrospinning is another form of electrospinning by using polymer melts as the solutions to produce fibrous structures. They are very similar to 3d printing and eliminates the usage of volatile solutions, unlike the conventional electrospinning process. While electrospinning depends on evaporation, melt

electrospinning relies on cooling to solidify the formed substrate. Besides, molten polymers are viscous and non-conducting, thus eliminating the chances of electrical instability and the cons of solution accumulation during the process. This method thusly produces more precise and predictable fiber scaffolds with the arrangement of moving collectors with defined shape and thickness, resembling a 3d printing process.[7, 9, 24-26] Comparison of solution electrospinning and melt electrospinning is provided below:

Table 1: ELECTROSPINNING vs MELT ELECTROSPINNING[27]

Technique	Solution electrospinning	Melt electrospinning
Solidification mechanism	Mass transfer (solvent evaporation)	Heat transfer (cooling)
Solvent-free	No	Yes
Efficiency	Lower	Higher
Environmental friendly	No	Yes
Modeling	Easier	Harder
Diameter of fibers	Smaller	Larger
Viscosity limitation	No	Yes

3.1.1 PRINCIPLE OF ELECTROMELT PROCESS

Melt electrospinning is a promising technique to produce fibrous scaffolds with specific design, shapes and defined thickness and more efficient contrary to the solution electrospinning. This method is considered more safer and eco-friendly as it avoids the expelling of gases from the solution and biomedically safe with enhanced drug loading capability. The modus operandi of this process is similar to the solution electrospinning and is described in detail. The polymer solution is made more viscous than its counterpart and is then melted to form the polymer melt which is then ejected through the spinneret which is charged by the high-power electric source and between the collector. The electric power charges the solution and weaken the surface adhesion of the solution, thus distorting the solution structure. This creates the repulsive force and elongates the

ejected solution. Instead of solution evaporation, the ejected jet cools down and solidifies either in the air, before deposition or after depositing on the collector.[8, 22, 28, 29]

3.1.2 COMPONENTS AND CONTROLLING PARAMETERS OF ELECTROMELT SYSTEM

Alike the solution electrospinning, there are both vertical and horizontal configurations for melt electrospinning. [9, 26, 27]A typical melt electrospinning contains the following components as shown in Figure 3.1:

1. A metallic needle / spinneret
2. High power voltage source
3. Polymer melting sub-system/electric heater
4. Collector

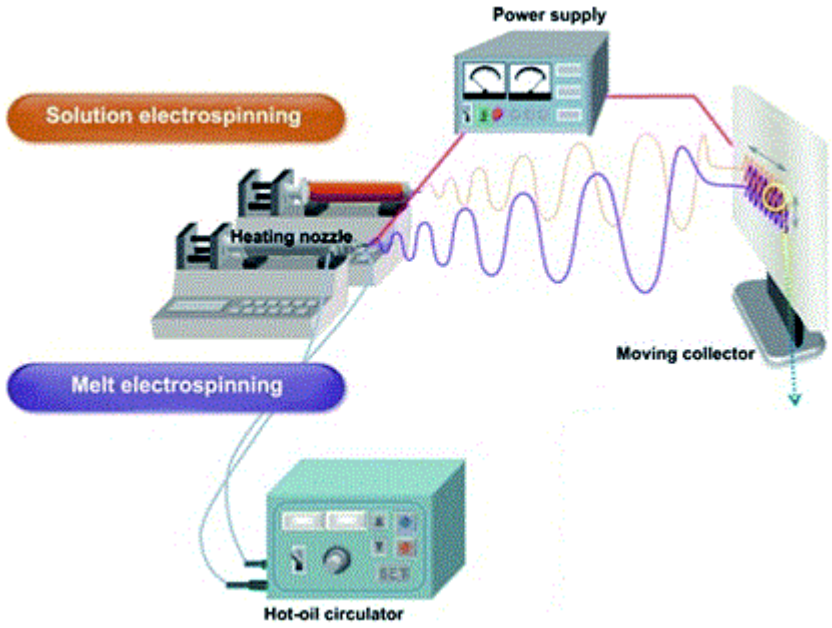


Figure 3.1: MELT ELECTROSPINNING[30]

A. Spinneret: The spinneret or the metallic needle flat tipped for efficient production of jets. Many experiments were conducted to fabricate a sheet of a polymer without using a spinneret and turns out having so many taylor cones with the region, which disrupts the homogeneity. Also, several cross sections for the spinneret were tried and observed to be possible options like

triangular and cruciform other than the typical cylindrical one. The upgrading of this electrospinning process is to increase the outputs and more surface area to volume ratio with less time, which can be achieved using multiple spinnerets simultaneously. The polymer solution should be continuously supplied to the spinneret without any variations without any fluctuations in the flow rate required.[8, 29]

B. Heating sub-system: Electrical systems, laser, hot air and circulating fluids are used for melting the polymer solutions. Each system has their own drawbacks and safety concerns and hence electrical systems are mostly preferred. This heating system should be completely isolated from the high voltage source, even though both connections are directed towards the spinneret. That is another factor to be taken care of in this system as it requires highly sophisticated configuration.

Hot air can be blowed to the tip pf the spinneret, put will not provide a uniform and precise control of the temperature. Circulating fluids solves this problem, as they provide uniform control. Water or oil is heated and the polymer solution with the syringe is dipped into it to achieve the hot temperature required. Water can be used for polymers with less melting point and oil for high melting points. Nevertheless, both these strategies require high safety precautions: water is conductive so should be isolated from the syringe and oil can contaminate the scaffolds. Laser can be also used with or without the spinneret to achieve a uniform heating with less heating time but this will result in more complicated system.[8]

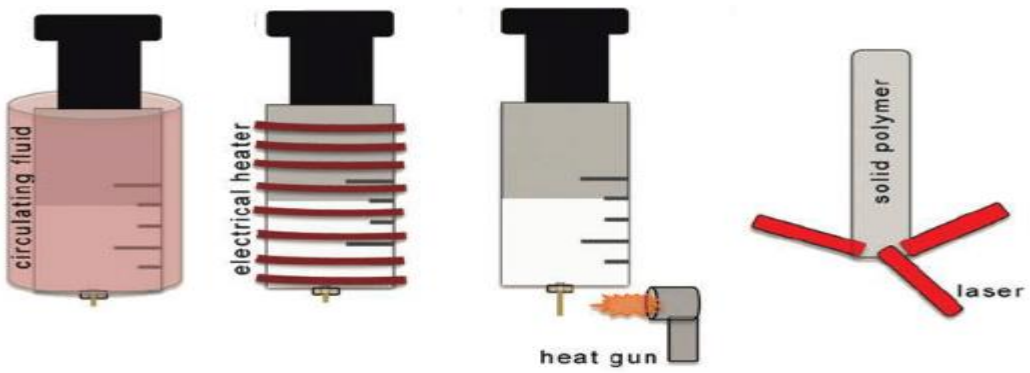


Figure 3.2: TYPES OF COLLECTORS[8]

C. Collectors: Different substrates can be used as collectors being required to be conductive as they need to provide electric field with the spinneret. Usually metals are considered as copper, aluminium , brass etc, Some studies tried to deposit the scaffolds or fibers into solutions but then the high voltage polarities are reversed and uniformity was not achieved in this study.[8]Figure 3.2 describes the different collectors generally used.

D. Process Parameters: There are several parameters that should be considered for the efficient production and uniform fiber morphology and those include Material parameters, viscosity, applied voltage, jet characteristics, jet speed, fiber diameter and ambient parameters.[8, 27]

E. Material Parameters: Molecular weight and Molecular chain are very important factors. Very high mwt results in thicker fiber with high viscosity but affects the crystallization of the melt. Usually, very low flow rates are required but high viscous melts may result in blocking the nozzle with low flow rates.[8, 27]

F. Solution viscosity: This is another significant parameter to be considered for the optimal performance of the process. Viscosity shouldn't be too less as it can enhance the formation of beads and may not even produce fibers and shouldn't be very high, There should be a threshold value for which the electric force should be able to act on the solution to weaken the surface tension and adhesion and to be able to elongate the jets.[8, 27]

G. Applied voltage: As related to the viscosity of the fluid, there should be a threshold voltage level determined so as the electric filed can convert the droplets to jets and facilitate the process. The applied voltage should be greater than this threshold voltage and usually twice as the voltage used for solution electrospinning is required for melt electrospinning. Ie in place of 5-20 kV, the voltage applied should be around 20-100 kV is recommended in achieving the voltage. The voltage shouldn't be that high enough to breakdown the jet formation from the syringe, which will not produce fibers, in turn provides hindrance to it. In melt electrospinning, another major contradiction appears as a heating system is also incorporated. The voltage connected to the spinneret should not affect the heating system of the polymer[27][8].

H. Jet characterisitics: In solution electrospinning, the jets have both stable and instable region and even whipping of the jets also occurs. This whipping was once thought to make the fiber more precise along with the electric field. But in melt electrospinning, the jets only have stable region and there is no whipping in this process. Low viscous melt can cause this whipping which is not desired in this process. The electric charge on the solution is not much higher to cause the jet refinement which in turn prevents any electric instabilities and melt will be always in the stable mode.[8, 27]

I. Jet speed: The jet speed is much lower compared to the solution electrospinning.[27]

J. Fiber fineness: The diameter of the fibers from the solution counterpart results in the range of 100-1000 um and the pore size to be around 500um. Air can penetrate through these as well as liquids, cells or other particles. In melt electrospinning, usually above 1 um is achieved by temperature increased and add more salts to the polymer solution and decreasing the fiber diameter but unfortunately more complex methodology have to be followed.[8, 27]

K. Ambient parameters: In the solution electrospinning, ambient parameters like temperature and humidity played a vital role in the evaporation of the solution which in turn affected the fiber diameter and pore size. However for melt electrospinning, due to the absence of a solution, ambient parameters doesn't have a strong impact on the solution but when there is excess humidity this can cause the air to breakdown, as air is the conducting media between the electric voltage and collector this will indirectly cause non uniformity or can even cease the process. So high humidity should be avoided.

Many studies were conducted to observe and analyze the effect of high temperature in the melt electrospinning process and found that when the temperature of the tip is increased, this can in turn control the fiber diameter. But very high temperature can solidify the jet very quickly even before getting deposited in the collector, hence moderate temperature and low humidity are the preferred ambient parameters.[8, 27]

3.2 MATERIALS, INSTRUMENTS AND METHOD

3.2.1 PREPARATION OF THE POLYMER SOLUTION

Polycaprolactone (PCL) is chosen as the polymer for this experiment, which is mixed with acetone to obtain the solution. PCL pellets (pellet size~3 mm, average Mn 80,000) from Sigma Aldrich is used and using the sensitive weighing scale in the laboratory, measured 0.1 g of PCL. Acetone (laboratory reagent $\geq$ 99.5 %)is also weighed and 10g of Acetone is taken. Instead of mL, I took gram scale of acetone. PCL beads is then added into the acetone solution and then mixed using the sonicator, which uses sound waves of 20 KHz and it is set to 60% of its amplitude. This sonicator mixes the solution and the time which it takes depends on the polymer viscosity needed.

3.2.2 ELECTROMELT SYSTEM DESIGN

A schematic illustration of the melt electrospinning system for the non-photo sensitive system is shown in Figure 3.3.

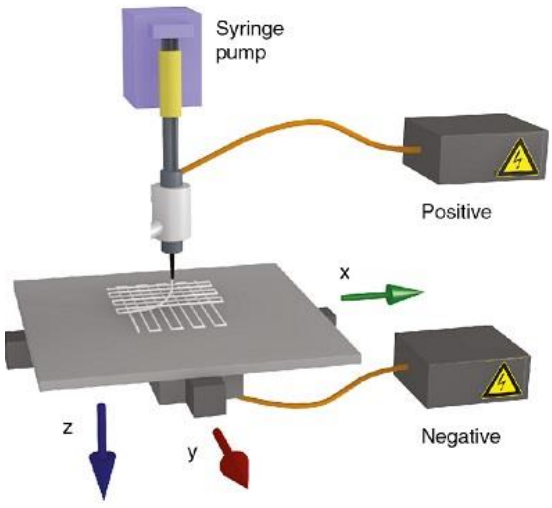


Figure 3.3: SCHEMATIC REPRESENTATION OF ELECTROMELT PROCESS[31]

The actuator stages acts as the base for this system. Three actuators represents the three different directions x, y and z as shown. The z axis will be mounted in the vertical direction and will be responsible for the height of the scaffold. The syringe pump system will also be mounted on the top, either together with the z axis or as a separate unit. Typically, there are two voltage connections involved, only one is shown in the picture. One is high voltage system, which makes the collector as the grounded one and the needle tip as positive for the electrostatic repulsion to occur. The second connection is the voltage required to melt the polymer solution, which depends on the melting point of the polymer used. So, high voltage cannot be applied to the polymer solution. Appropriate voltage should be selected for the heating system and should be isolated from the high voltage connection. The suitable collector should be selected and the distance between the tip and the collector is another parameter that must be evaluated for the efficient performance of the system.

3.2.3 INSTRUMENTS

A. FLOW RATE/ SYRINGE PUMP SYSTEM

The syringe pump system is used for the flow rate. The polymer solution when done is poured into the syringe and the syringe is in placed in the proper slot in the syringe pump system. There are teo different methods of application: Horizontal and Vertical. When horizontal, the syringe pump system will be paced horizontal with the syringe and then tube connected from the syringe goes to the vertical direction, which is connected to the needle, also in vertical direction. Vertical application eliminates the tube and the system will be placed vertically as well as the syringe and the needle. The syringe pump used for maintain the flow rate is shown in Figure 3.4.

Figure 3.4: SYRINGE PUMP SYSTEM

B. HIGH VOLTAGE SUPPLY

The high voltage required for the electrospinning process is supplied as shown below. After several trials and for several applications, the appropriate voltage should be obtained. For safety precautions, High voltage glouses are used while operating on this voltage supply. The green wires connected from the voltage supply is the ground wires and are connected to the collectors.

C. VOLTAGE FOR MELTING PROCESS

The melting point of the PCL is found to be 60 C. So, temperature should be provided in the system enough for the PCL solution to melt before collecting on the collector. Appropriate voltage should be found and provide the positive of the voltage to the region above the needle tip for melting and the ground connection to the collector.

D. COLLECTORS

Glass slide is used as the collector for the scaffold which is connected with a copper tape to make the glass conducting. The green wire from the HV system is connected to the glass slide.

E. NEWPORT ACTUATOR SYSTEM

There were two types of motion controllers available for me to work: SMC 100cc motion controller, which can only control one axis at a time, and the ESP 301 motion controller, which can control three axes at the same time.

SMC 100 CC/PP MOTION CONTROLLER:

The SMC 100 CC/PP is a motion controller which can control only one actuator at a time, and can efficiently work only if connected to an external power source. The abbreviations in the name of the controller represents the motor specifications used : CC stands for the DC servo motors where as PP stands for the DC stepper motor configurations. There is also a SMC-RC keypad along with the motion controller, which can control the actuators manually. Figure 3.5 depicts SMC 100CC/PP motion controller.

Figure 3.5: SMC 100CC/PP

NEWPORT MOTION CONTROLLER:

The ESP301 is used as a stand-alone controller to drive an ESP motion device. It can control and drive up to 3 axes of motion in any stepper and DC motor configuration. The ESP301 is available with a front panel with LCD display and manual control buttons. A menu allows the user

to change velocities, accelerations and more, without a computer interface. Figure 3.6 depicts the Newport Motion Controller.

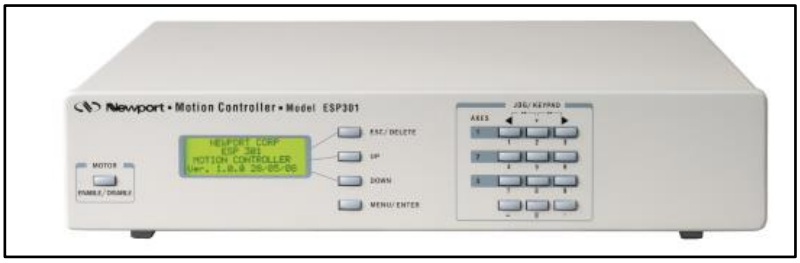


Figure 3.6: Newport Motion Controller

In case of SMC 100CC, the motion controller seems to very complex and required an external voltage supply to function. Also, it appeared to be very much outdated and finding the home position of the axes seemed to be difficult. There were different lights shown as indicator and each of them meant very different faults. Trouble shooting will be hard for a non-engineer so dropped this controller and focused on the ESP 301.The structural details of the machines and programming characteristics of ESP 301 is discussed elaborately in this section.

NEWPORT LINEAR STAGES:

Linear motion stages provides automated positioning and are available in either single axis or as an XY stage. The stages are provided linear motion using linear motor inside the actuator, which is considered to be the most precise actuation technology to control down to nanometers level. A pair of linear stage from Newport Company were used for mounting the collector, for collecting the fabricated scaffold in bi-directional. Another linear stage was used to control the z direction or the height of the scaffold. Figure 3.7 represents a Newport linear stage.

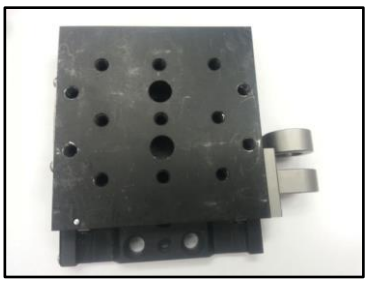


Figure 3.7: Newport linear stage

NEWPORT ACTUATORS:

An actuator is a mechanical component that provides controlled or limited positioning or movements, operated either by manually, electrically or by fluids. They require a control signal and a source of energy. There are mainly linear actuators and rotational actuators: Linear actuators are used for positioning in a straight line applications like push-pull, Rotational Actuators coverts energy to rotational motion or positioning. I'm using three ESP actuators, ESP refers to Newport stages with an EEPROM (ESP chip), that contains all stage information like motor type, travel limits, maximum speeds, etc. Actuators are used in this system to move the linear stages in accordance with the program code used for producing the scaffold in few nanometer range.

Figure 3.8: Newport Actuator

Figure 3.8 shows the Newport Actuator. Initially learned the PDF files on the Newport motion controller and actuators, learning the basics of the actuators and the motion controller. I understood about the Motion controller, the front key panels and also the rear panel in which all the ports are

located; communication ports : IEEE, USB and RS 232 and GPIO axis ports to communicate with the computer and driver ports: the ports which controls the actuators. It had only two driver ports but we needed three ports so we bought the third one eventually for the z axis. There was already a CD ROM along with the purchased motion controller which installs the ESP 301 software to communicate with it.

Also, learned about the actuators, its safety considerations, terminology, symbols and definitions, about the HOME and JOG position which is very frequently used. HOME is the origin position that the axis can be programmed into, can choose three different homes for the three axis. Before the programming, the axis have to be at their respective home positions. It is also recommended to do a home search before, to see if it has already assigned home. JOG is movement of the axis not under the program control, by using the buttons of the motion controller and also using the Jog tab in the software. Figures 3.9 and 3.10 shows the software tabs and the command terminal of the software respectively. More details of the software is shown in Appendix A.

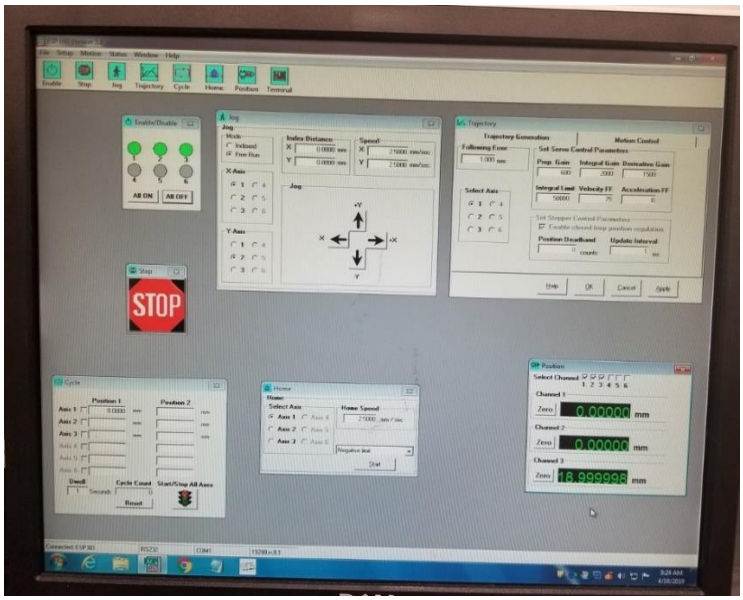


Figure3.9: ESP301 SOFTWARE TABS

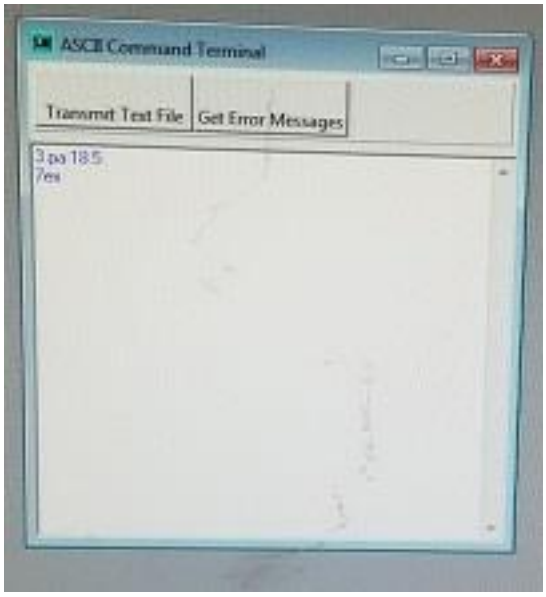


Figure 3.10: COMMAND TERMINAL

3.2.4 OPERATING PROTOCOLS

A. WITH NEWPORT ESP301:

Understood about the motion controller and the actuators from the user manual and learned there are so many commands to move the actuators. Came across the commands for the most simple positioning such as **0R – search for home, PA- move to an absolute position, PR- move to a relative position , EX- execute a program ,DH- define home, LP- list a program, TE- tell error, TB- tell buffer, JL- set low jog velocity , JH- set high jog velocity, AC- set acceleration, AG-set deceleration, ID- stage identifier, VA – set velocity, QP- quit program and WS – wait for stop.** After learning the purposes of these commands and the modes these should be used, a sample program was tried just to move 1 mm and then incrementing the measurement by 1 mm. Unfortunately, the actuators didn't move. So, tried to understand all sorts of error from the user manual, corresponding to the codes the controller displayed in the error message tab. It displayed 103 which indicates the following error threshold of the first axis was exceeded. Following error threshold means the difference between the real value which is entered through the program and

the theoretical value which the controller computes from its own cycle. If for any axes, this difference exceeded the pre-set maximum following error, that will make this error code. From the description of the error, understood the FE command value might be set up earlier and have to change it for the application. So, used **1 FE?** To know the value currently, it is set and it displayed **0.5.** I changed it to 1.0 using the command **1 FE 1.0.**

After resolving this error, the next error code displayed was 104 which indicates the controller detected a value greater than the positive hardware limit for the first axis. It is the limit to which the actuator can move in the positive direction and hence used the command **1 ZH?** To understand the pre-set value and it displayed **25H** and changed it to **23H** using **1 ZH 23H.** After sorting the two errors that popped up, tried the program again but still it showed the x03 error so got confused whether it didn't change or should the controller be resetted completely for the new values to be accepted. Checked the current value of the FE command and it showed 1.0. So, it changed the value without resetting but now have to figure out the best value that could solve this threshold error, and with the calculations , decided to upload the value 5.0 using the commands **1FE 5.0.**

Now decided to understand the pre-set values of all the important parameters that could give me a clear idea of the parameters that the actuators are set in. Decided to find the absolute position of the frist axis using 1 PA? and it showed 0.0000 as it matched with the hardware position and now need to check the velocity of the first axis which it is already set in , using 1 VA? command and it displayed 2.5000 units/s. Was so curious and decided to find the maximum velocity the first actuator can attain by using the command 1 VU? . It displayed 5.0000 units/ s as the maximum velocity the first axis can attain. Now it is the turn to understand the home search speeds for the two axes. There are two types of speeds in which the axis can search for its home- low speed and

the high speed. OH for high speed and OL for low speed. By using the commands 1 OH? and 2 OH?, the home high speed for both the axes were identified as 2.5000 for both of them. And 1 OL ? and 2 OL? Determined the low speed as 0.6250000 for both the axes.

As continued to execute the program, it still encountered the same error 103, for the following error threshold and thought about another approach, there is another command that can be used for changing the threshold error ZF. By using the command, 1 ZF? , the preset value was identified as 3H and then changed to 5H by 1 ZF 5H. these setting are saved but to make sure these are saved for the future purposes, used SM command which saves the current settings to the non-volatile memory. After some thinking, changed the velocity of the first axis to 10 units/s and the maximum velocity of the first axis as 20 units/s by using 1 VA 10.00 and 1 VU 20.00. Also changed the FE command value to 50.0000 by using 1FE 50.000 as needed to avoid that error entirely.

Again, tried to execute the program, but now the error codes displayed were very different. The code was 105 and 205, which indicates the negative hardware limit of both the axes were exceeded. This is the value to which the axes can move in the negative direction depending on their current position. I used command 1 ZF? And 2 ZF? To understand the current values and that were 2H for both. So, changed to 3H by using 1 ZF 3H and 2 ZF 3H respectively. And now both the axes worked. Another problem encountered was sometimes after the execution, it didn't respond as too much programs were saved to be uploaded in the task manager and hence used XX command to erase the program. 3XX means erase the program #3 from the memory and also had to use XM command which completely erases the program memory of the controller and it shows the value 59904.

So Table 2 shows the data of the values that is saved as the current setting for the applications.

Table 2: COMMANDS, AXIS and VALUES PRE-SET FOR THE SCAFFOLDS

AXIS	COMMANDS	VALUES
1	PA	29.99999
2	PA	9.99999
1	VA	2.50000
2	VA	2.50000
1	VU	5.00000
2	VU	5.00000
1	OH	2.50000
2	OH	2.50000
1	OL	0.62500
2	OL	0.62500
1	FE	1.00000
2	FE	1.00000
1	ZF	3H
2	ZF	3H
1	ZH	25H
2	ZH	25H
1	DH	0.00000
2	DH	0.00000

Conducted a small trial program and after the result figured out to change the home position back to 0 for both axes using DH. We can program it to reach home after the execution or manually turn the knobs, which is not possible every time. Tried to run another program and it had some errors back with the already discussed parameters. After figuring out the parameters and their values , which actually was more familiar with the device and its operation and hence, moved onto the next important phase of the thesis- programming the actuators for the scaffold shapes.

a. RECTANGULAR STRUTURE PROGRAMMING

Rectangular structure was very easy to make with 20 x 7 mm dimension. Created programs for different pore size, 1000, 500 and 250 microns. They seemed to be perfect was easy to check the software as it displayed the values and that was very easy to understand the errors if it had, unlike the circle. And then figured out the second layer to be in the opposite orientation ie 7 x 20 mm to give a perfect mesh cross-section as required. The results are depicted in the results section and the program codes are described in Appendix B.

b. CIRCULAR STRUTURE PROGRAMMING

For the circle code,

$$(x - h)^2 + (y - k)^2 = r^2$$

The above equation was used to develop the circle coordinates centered at (h, k) with radius r, which would serve as the values we need for the program code. First mission was to find the appropriate center and radius for the circle. Used Microsoft excel to plug in the equation and used it as a tool to find the circle coordinate values. As a circle is needed with 10 mm diameter, I tried the center as (5,5) and radius 5 mm.

So, the equation becomes

$$(x - 5)^2 + (y - 5)^2 = 25 \qquad (3.1)$$

Decided to develop the code for 1000 microns and then decrease it to 100 microns. As 1000 microns as 1mm increments and decided to input x values starting from 0, with 1 increment, to 10 mm and obtain the value of y coordinates by converting the above equation to a quadratic equation and then using quadratic formula to derive the y coordinates.

The above equation when converted into a quadratic equation with the known values of x, becomes:

$$(y-5)^2 = 25 - (x-5)^2 \qquad (3.2)$$

$$y^2 - 10y + 25 = 25 - (x-5)^2 \qquad (3.3)$$

$$y^2 - 10y + (x-5)^2 = 0 \qquad (3.4)$$

The Quadratic Formula: Given a quadratic equation in the following form:

$$ax^2 + bx + c = 0$$

...where a, b, and c are the numerical coefficients of the terms of the quadratic, the value of the variable x is given by the following equation:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Figure3.11: QUADRATIC FORMULA[32]

By using this quadratic equation, the y values can be found by:

$$y = \frac{10 \pm \sqrt{(10^2 - 4(x-5)^2)}}{2} \qquad (3.5)$$

But when substituted the values, the circle coordinates turns out to be negative and then not defined. For trying different center values, the radius value was fixed as 5 mm. Tried the center as (7, 5).

$$(x-7)^2 + (y-5)^2 = 25 \qquad (3.6)$$

$$(y-5)^2 = 25 - (x-7)^2 \qquad (3.7)$$

$$y^2 - 10y + 25 = 25 - (x-7)^2 \qquad (3.8)$$

$$y^2 - 10y + (x-7)^2 = 0 \qquad (3.9)$$

Now the quadratic formula for this equation will be:

$$y = \frac{10 \pm \sqrt{(10^2 - 4(x-7)^2)}}{2} \qquad (3.10)$$

Again, the coordinates turns out to be not defined. For the third attempt I the center as (5,7).

$$(x-5)^2 + (y-7)^2 = 25 \qquad (3.11)$$

$$(y-7)^2 = 25 - (x-5)^2 \qquad (3.12)$$

$$y^2 - 14y + 49 = 25 - (x-5)^2 \qquad (3.13)$$

$$y^2 - 14y + (x-5)^2 + 49 - 25 = 0 \qquad (3.14)$$

$$y^2 - 14y + (x-5)^2 + 24 = 0 \qquad (3.15)$$

Now the quadratic formula for this equation will be:

$$y = \frac{14 \pm \sqrt{(14^2 - 4(24 + (x-5)^2))}}{2} \qquad (3.16)$$

After obtaining the y values, it was coded appropriately for the first layer and for the second layer, the x and y axis are flipped. So the equation becomes:

$$(y-5)^2 + (x-7)^2 = 25 \qquad (3.17)$$

$$(x-7)^2 = 25 - (y-5)^2 \qquad (3.18)$$

$$x^2 - 14x + 49 = 25 - (y-5)^2 \qquad (3.19)$$

$$x^2 - 14x + (y-5)^2 + 49 - 25 = 0 \qquad (3.20)$$

$$x^2 - 14x + (y-5)^2 + 24 = 0 \qquad (3.21)$$

Now the quadratic formula for this equation will be:

$$x = \frac{14 \pm \sqrt{(14^2 - 4(24 + (y-5)^2))}}{2} \qquad (3.22)$$

And this equation gave the positive defined values and hence the circle was fixed as the center at (5, 7) and radius 5 mm.

Started working on the circle, the idea was to develop more layers by building one on top of the other. So, developed a code for the circle with 1mm space apart and executed the program with the software to observe if any errors arise. Successfully cleared the errors and also developed the code for the second layer. The circle was difficult to just observe and the see if it is really producing the shape as needed. So, got the idea of using a pencil in place of a syringe and observe for the design and shape. Attached a pencil to another setup such that the tip of the pencil touches the base. The actuator's linear stages serves as the base with small piece of paper attached to it , to get the imprint from the pencil. Used pencil first to check if the system works perfectly or any change has to be made as by naked eyes it is difficult to get the image of the precise movements. Hence, this serves as the circle code for 1000 microns. The image of the structure obtained with pencil is showed in Figure 3.13

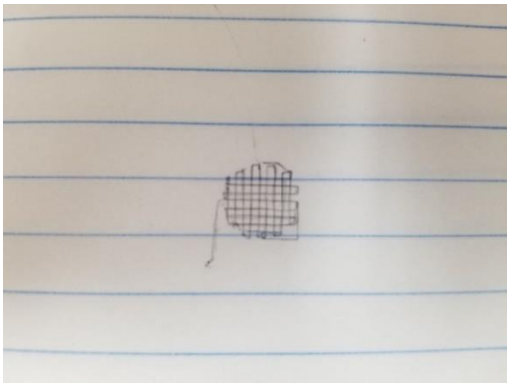


Figure 3.12: CIRCLE CODE TESTING WITH PENCIL

In this image, the two layers are cleared visible. Even though the second layer is printed in the same height because of the pencil application, for the scaffolds that has to be produced, the second layer should be on top of the first layer, hence requiring certain height difference between the layers. And hence the circle structure code was developed with the height difference of 1000 microns and this can be tested only with the actual setup and solution.

Reducing the micron dimensions to 500 microns, which means only increment of 0.5 mm between the values. Tried with the circle equations used for the 1000 microns with x values from 0 to 10 mm and it turned out to be undefined. Hence tried to keep the center as fixed and changed the radius to 7 mm. The equations for center (5,7) and radius 7 mm are:

$$(x-5)^2 + (y-7)^2 = 49 \qquad (3.23)$$

$$(y-7)^2 = 49 - (x-5)^2 \qquad (3.24)$$

$$y^2 - 14y + 49 = 49 - (x-5)^2 \qquad (3.25)$$

$$y^2 - 14y + (x-5)^2 = 0 \qquad (3.26)$$

$$y^2 - 14y + (x-5)^2 = 0 \qquad (3.27)$$

Now the quadratic formula for this equation will be:

$$y = \frac{14 \pm \sqrt{(14^2 - 4(x-5)^2)}}{2} \qquad (3.28)$$

This worked and it gave the circle coordinates for y. and then flipped the coordinates to obtain the equation for x as

$$(x-7)^2 + (y-5)^2 = 49 \qquad (3.29)$$

$$(x-7)^2 = 49 - (y-5)^2 \qquad (3.30)$$

$$x^2 - 14x + 49 = 49 - (y - 5)^2 \quad (3.31)$$

$$x^2 - 14x + (y - 5)^2 = 0 \quad (3.32)$$

$$y = \frac{14 \pm \sqrt{(14^2 - 4(y - 5)^2)}}{2} \quad (3.33)$$

As 1000 and 500 microns were developed successfully, the dimensions left was 100 microns, which only has 0.1 gap . As challenging , tried to make circle code for 10 microns which only has 0.01 gap between the adjacent layers. And the circle was same as that of 1000 microns, centered at (5,7) with radius 5mm.

All the codes of the circle with 1000, 500, 100 and 10 microns are provided in the Appendix A section.

B. CONTROLLING PARAMETERS

For electrospinning system, 1g of PCL was taken and mixed with 10g of acetone. But for the melt electrospinning system, more viscous solution is needed for the desired topography. Different trials were conducted by varying the amount of PCL and acetone. From logical thinking, the amount of PCL was increased by decreasing the amount of acetone. First trial was conducted on 0.1 g in 5 g and sonicated for 30 minutes. After sonication, the solution was poured into a syringe and then tried to make adjacent lines in a glass slide and checked if the lines remain distinct or spreads out. But the lines completely spreads. So tries 0.25 g in 4 g and sonicated for 45 minutes and tried again on the glass slide but was unsuccessful. Increased the concentration of PCL to 0.3 g and tried in 4 g. After sonication, the amount of solution produced is very less and hence decided to try 6 g from later on. As that trial also didn't succeed, tried a higher concentration 0.6 g of PCL

in 6g of acetone. This was very better than the previous trials but still spreads little bit. So tried 0.78 g in 6 g of acetone as the most suitable one.

The needle was already chosen for the application to be of 0.01 mm diameter needle with the foresight of 10 microns. As the vertical application is needed, decided to mount the syringe pump system vertically with the syringe directly connected to the needle. Tried several flow rates before choosing one. First trial was 250uL/min using the nanofiber needle but that spreads too much. Now, decreased the flow rate from 250 to 100uL/min. So for the 0.01 mm diameter needle, this was perfect as the needle hole was very small and it needed some pressure to push the solution through the needle. Now incorporated the voltage across it, and saw 9kv is too much and that makes the solution to behave very weirdly. After some trials , decided to choose 2 kV as the high voltage value. Now only one more parameter left. Melting voltage, at first decided to start at 5 V but that was causing the same problem of evaporation. Hence decided to decrease the voltage to 4 V.

Started producing layers on the glass slide, whose one side is connected to copper for the ground connection. Successfully produced first layers for 1000 microns pore gap and the fiber diameter was very very small. Then decided to decrease the pore gap to less microns, to 500 microns and 100 microns were also successful. But the problem was with the second layer , when the height was increased by 100 microns, the liquid from the needle was just clotting around the needle instead of ejecting as a fluid and forming the scaffold, which posed a serious problem. Was not able to increase the voltage as that will cause a strong problem for the evaporation of the solution.

And also incorporating two voltage sources was very hard. Had to try glass syringe and water bath once as it was not safe to be around the machine. After many suggestions and help from the professor, decided to buy a hotend part of a 3D printer for our application. This hotend consists of

a thermistor , which when applied to certain range of voltage, converts the electric power to thermal energy. And this was what needed for the system.

The hotend came with several tips of different dimensions like 0.5, 0.4, 0.3 and 0.2 mm. Decided to try 0.4 mm with the same visocity. But as the hole was kind of bigger, with the flow rate of 100 uL/min, that completely spreads the solution apart forming like a sheet instead of the scaffolding structure. Hence, needed to change the flow rate. Decided to see what impact can 50uL/min can do. That still causes the spreading but not that much. Hence reduced it to 30 uL/min and it worked perfectly well. Tried the 1000 microns and was a successful. For the 500 microns, as the tip hole was around 0.4 mm/ 400 microns, decided to switch the tip to 0.2 mm, the smallest one available. 500 microns was a success but still it was very thin and was not able to peel off the glass slide for cell study and analysis.

If needed to make it thicker, then more liquid should be ejected or height should be increased. but that means that could spread and may not produce the scaffold. So, planned to increase the height of the tip from the collector. As the experiments proceeded and thicker scaffold is desired, decided to increase the viscosity again by increasing the concentration of PCL to the same amount of acetone. Tried 1 g of PCL in 6 g of acetone and that was perfect for the scaffolds which became thicker in a good way with the flow rate of 30uL/min.

Now started to produce the scaffolds more thicker and used 0.4 mm hole tip for this application . The z axis would move up in the vertical direction for the second layer and hence tried the second layer but the solution didn't come out. So, the thermostat after continuous heating, attained high temperature that made the solution to get vapourised stage. So for the second layer the voltage was reduced to 3 V. The best part with the thicker scaffolds was that, for the program of 1000 microns

gap, the end result had only 500 microns gap as the solution spread some distance in the course of thickness.

But the problem with the system is, it cannot move more than 2000 microns. If the height of the tip is more than 10 microns, the layer forming cannot attain the shape of the base forming or in some cases, it was observed the solution spreads completely.

3.5 RESULTS

The designed melt electrospinning system is shown below with all the connections. Figure 3.14 shows the hotend part with the tube and the needle tip writing the first layer of the scaffold. This distance between the tip and the slide is 2mm.

Figure 3.13: ELECTROMELT SYSTEM

Below are the results obtained from the system both unidirectional and bidirectional scaffolds.

Figure 3.14: UNIDIRECTIONAL SINGLE RECTANGULAR LAYER

The two pictures shows the first layer of the unidirectional rectangular layer , programmed for the 1000 microns gap size. From the Figure 3.15, the fibre diameter is very small and found to be around 100 microns from the SEM images. The second image shows one of the line detached from the glass slide but then loses its shape of the rectangle.

Figure 3.15: UNIDIRECTIONAL SINGLE RECTANGULAR LAYER

Figure 3.16: RECTANGULAR MESH STRUCTURE ATTEMPT-1

This figure shows the attempt of rectangular mesh ie the first layer and then the second layer on top of it but was not that successful because the second layer was scratching the first layer and thus distorting the first layer and hence decided to increase the height to more than 10 microns. Then decided to program for an outline to preserve the shape of the sample. And also tried the thicker scaffold with increased viscosity, for these samples, the fiber diameter was increased to around 500 microns and the gap size was decreased to 500 microns even though the program for 1000 microns was used.

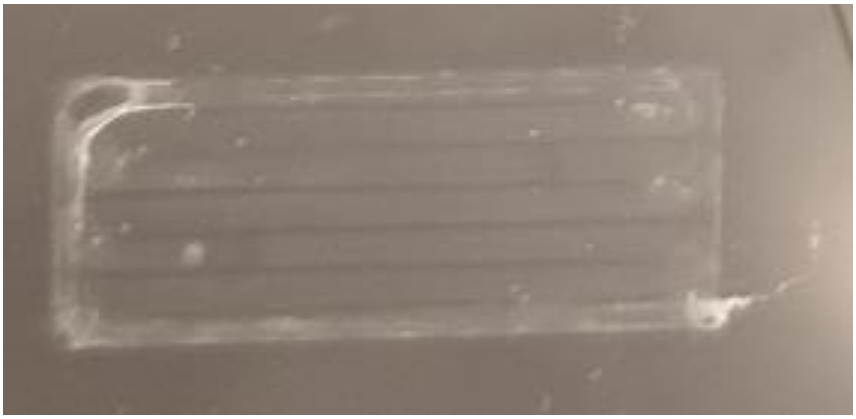


Figure 3.17: UNIDIRECTIONAL RECTANGULAR SINGLE LAYER WITH OUTLINE

As seen, these scaffolds are very thicker and after dried they can be peeled off and used for cell study and analysis. The Figure 3.18 serves as the first layer of the rectangle and the fig represents the second layer of the rectangle successfully produced and the fig represents the rectangular mesh scaffold formed .

Figure 3.18: RECTANGULAR FIRST LAYER WITH INCREASED THICKNESS

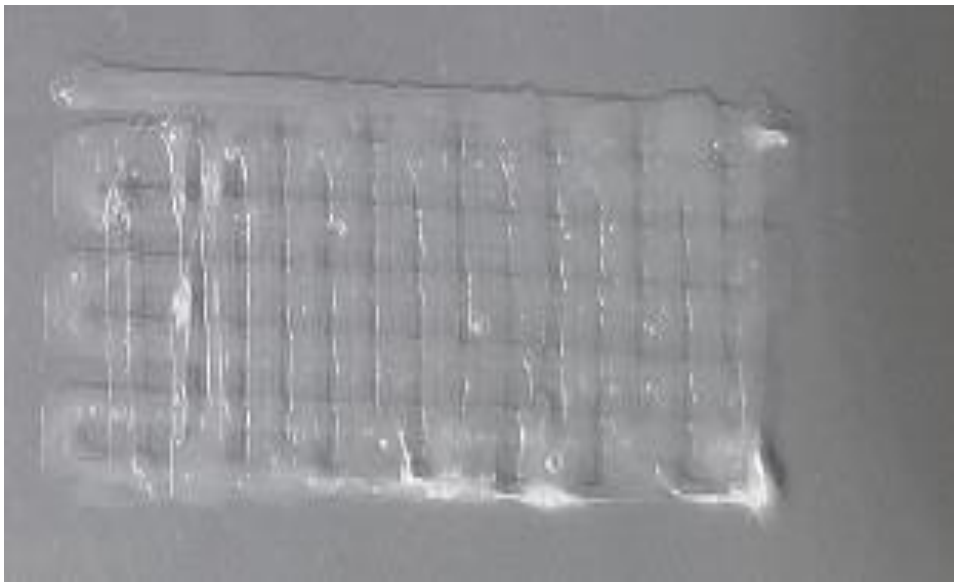


Figure 3.19: RECTANGULAR MESH DOUBLE LAYER WITH THICKER FIBER

The first layer of the circle is formed is showed in Figure 3.21 with less thickness and after obtaining thickness along with the second layer formed a successful circular scaffold as shown in Figure 3.22

Figure 3.20: CIRCULAR FIRST LAYER

Figure 3.21: CIRCULAR MESH WITH DOUBLE LAYER

3.7 CONCLUSION

The objective of this chapter was to develop a electromelt system using the Newport actuators for the precision of the scaffolds and to produce rectangular and circular scaffolds. The morphological and biological characteristics of the scaffolds will then be analyzed. The actuators were programmed for different dimensions such as 1000, 500, 100 and 10 microns for all the three axes: x, y and z directions. With different trials, created the suitable viscous solution of PCL and acetone: 0.78 g of PCL in 6 g of with 45 minutes of sonication if small fiber diameter is needed and 1 g of PCL in 6 g of acetone I with 45 minutes of sonication if large fiber diameter and thick scaffold is required. Evaluated the voltage values for both the systems: 2kV for HV system and 3-4 V for heating system. Also conducted several trials for the appropriate flow rate of the solution and found 30uL/min is the best for the system.

Unidirectional first layers of rectangular structure was developed with small and large fiber diameter. Large diameter is preferred, fiber diameter: 830 um and pore gap 510 um and then rectangular structure with 2 layers were developed of fiber diameter 812 um and pore gap 520 um(thick fiber) In case of thin fiber rectangular mesh, the fiber diameter was 279 um and the pore gap as 533 um. . Circular structures were also created. With the same fiber diameter and pore gap as the thicker rectangular structures. *In vitro* cell analysis showed that the cells really likes the scaffold, and grew in them. Cell adhesion and proliferation were done and cell viability test were also calculated. Hence the scaffolds are suitable for tissue engineering applications.

CHAPTER 4

COMPARISON OF THE MORPHOLOGICAL AND BIOLOGICAL CHARACTERISTICS OF ES AND EM SCAFFOLDS

ABSTRACT

After obtaining the results from the electrospun and electromelt systems, their morphological and biological characteristics are evaluated using SEM (for measuring the fiber diameter and pore size), Profiler (for measuring the area roughness and the height) and the fluorescence microscope (for evaluating the cell growth, cell adhesion and the cell proliferation).

Careful analysis has shown that the results from the electrospun system are non-aligned and not uniform in the fiber diameter and pore size whereas the results from the electromelt system are aligned with precision and very uniform in diameter and pore size. Both the results are found positive in the cell study with abundant cell growth, adhesion and proliferation. Thus, both are suitable for nerve, bone, muscle, skin and tendon tissue, generally tissue engineering applications but electromelt scaffolds proved to be better with uniform diameter and pore size.

4.1 INTRODUCTION

Biomedical applications such as stem cell transplantation and cell regeneration, requires three dimensional porous scaffolds. Many characteristics of these scaffolds should be analyzed carefully for the successful application and morphology is such an important characteristics. For fibrous scaffolds volume, area, size and internal structure have to be observed as they have an influence over the cell growth . There are various imaging methods available for measuring the artifacts of the scaffolds. With these advanced imaging methods, different local areas of the same sample can be visualized. Scanning electron microscopy, Transmission electron microscopy, Atomic force microscopy and Optical microscopy are the prominent imaging methods used .In optical

microscopy, the sample preparation and the equipment cost are cheap, but the limiting resolution is only around 200nm, which makes it excluded for the nanofiber measurements. The principle behind electron microscopy relies on the interaction of the sample with the electron beam, after which secondary electrons, back-scattered electrons and fluorescence are obtained. SEM utilizes the secondary electrons/reflected electrons to scan the sample whereas TEM uses the transmitting electrons to scan the sample. Both techniques are similar in regards with the components used but differs depending on the application. For TEM, the height of the samples should not be higher than 150 nm and for high-resolution imaging, it reduced down to 30 nm. Moreover, they are very expensive and requires trained user as it involves complex procedure. For SEM, there is no limitation for the size of the samples and the only requisite is SEM only function in vacuum. Recently, LVSEM was developed which is a two-chamber system which has high vacuum separated with the low vacuum. The main advantage is the high depth of sharpness obtained from the SEM images, making SEM a useful tool for evaluating the morphology of the nano fibrous scaffolds. Comparing SEM with AFM, AFM can work in any ambient conditions and can provide 3 dimensional images of the structure. But the sample should be uniform in their roughness for using with AFM. Samples with different roughness ratio when measure using AFm cannot give a clear image of evaluation.[33-35]

Profilometry analysis were done to quantify the roughness of the sample. There are contact and non-contact profilometry process. Non-contact profilometry is used in this study as less maintenance is required and will not be affected by the sample roughness and also for small steps, they are faster.[36]

4.2 COMPARATIVE MORPHOLOGICAL ANALYSIS OF ELECTROSPUN AND ELECTROMELT RESULTS USING SEM and PROFILER(only for electromelt systems)

A. UNIDIRECTIONAL RECTANGULAR STRUCTURE

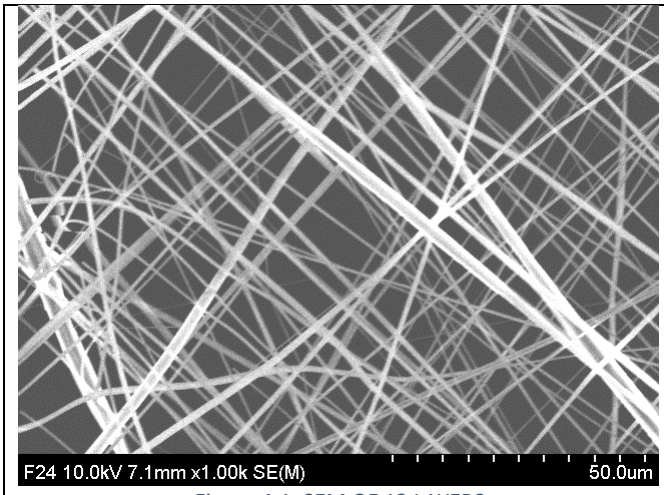


Figure 4.1: SEM OF 12 LAYERS

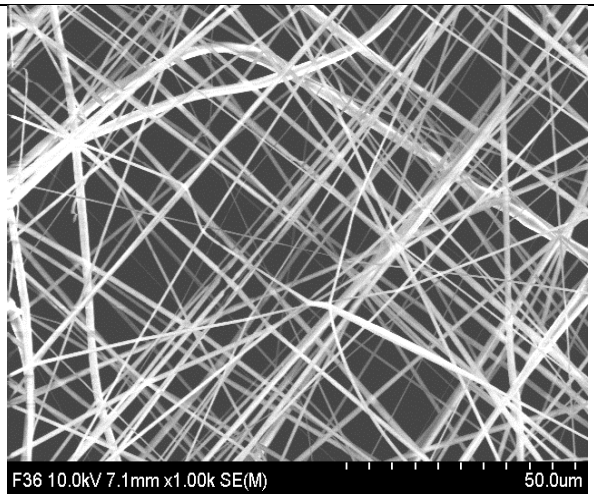


Figure 4.2: SEM OF 18 LAYERS

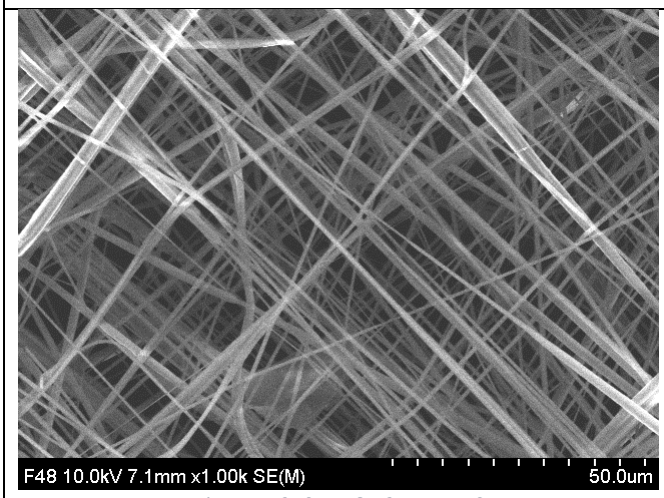


Figure 4.3: SEM OF 24 LAYERS

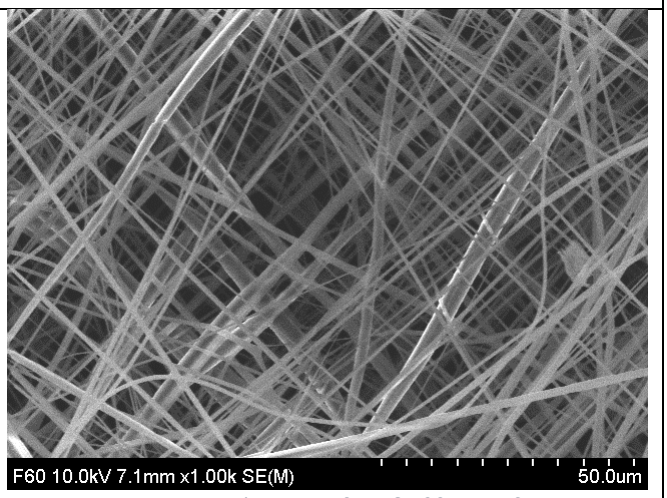


Figure 4.4: SEM OF 30 LAYERS

The SEM images of the samples from the electrospun system are shown above. Figure 4.1 discusses about the 12 layers, as seen the fiber is randomly oriented and fiber diameter is not consistent throughout the sample. It varies from different ranges. The pore size is also not

consistent . Same is happening in case of 18 , 24 and 30 layers. They are not consistent and as the layer increases the pores are looking as if they are sealed because of the dis orientation.

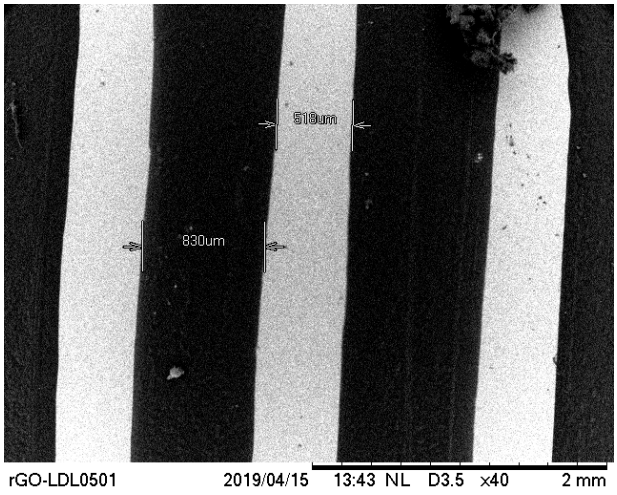


Figure 4.5: SEM OF THICK SINGLE LAYER RECTANGLE

This SEM image of the electromelt sample is shown above with the vertical single layer (thick diameter). This picture clearly outlines the defined and aligned structure with consistent fiber diameter 830 um and pore gap size 510 um.

B. RECTANGULAR DOUBLE LAYER MESH STRUCTURE(ELECTROMELT)

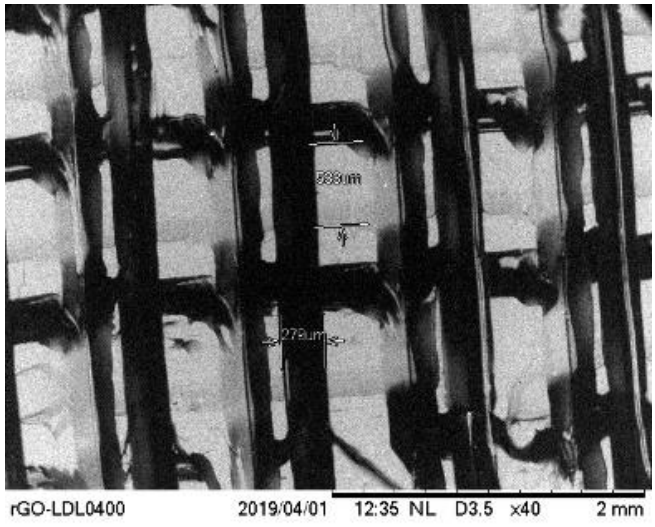


Figure 4.6: SEM of rectangular mesh with thin fiber diameter

This Figure 4.6 illustrates the SEM image for the rectangular mesh structure produced using the electromelt system(thin diameter) and this was programmed for 500 microns. The picture shows that the fiber diameter is consistent with 279 um and the pore gap uniformly at 533 um.

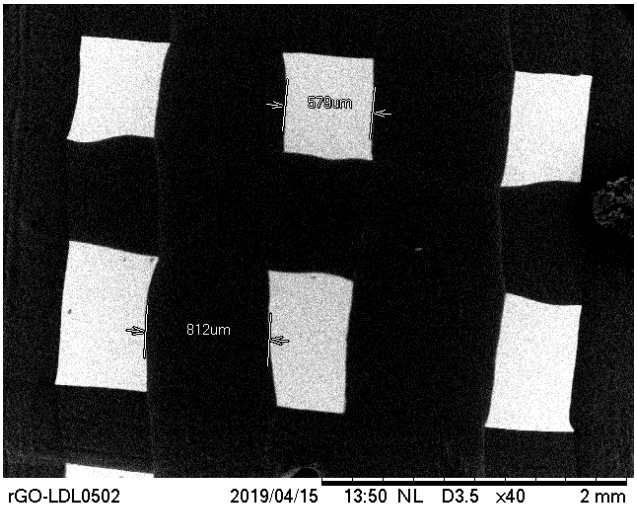


Figure 4.7: SEM of circular mesh

The Figure 4.7 illustrates the SEM image of the rectangular structure formed with thick diameter. Again consistent in diameter and pore size . Fiber diameter : 812 um and pore gap 520 um. Used the program of 1000 microns to obtain the 500 um gap for thick fiber.

4.3 MORPHOLOGICAL ANALYSIS USING PROFILER (ELECTROMELT)

The Figures 4.8 and 4.9 demonstrates the profiler image for the thin fiber rectangular mesh and the data below represents the area roughness data and it can be concluded that height of 6 microns is only achieved in this case.

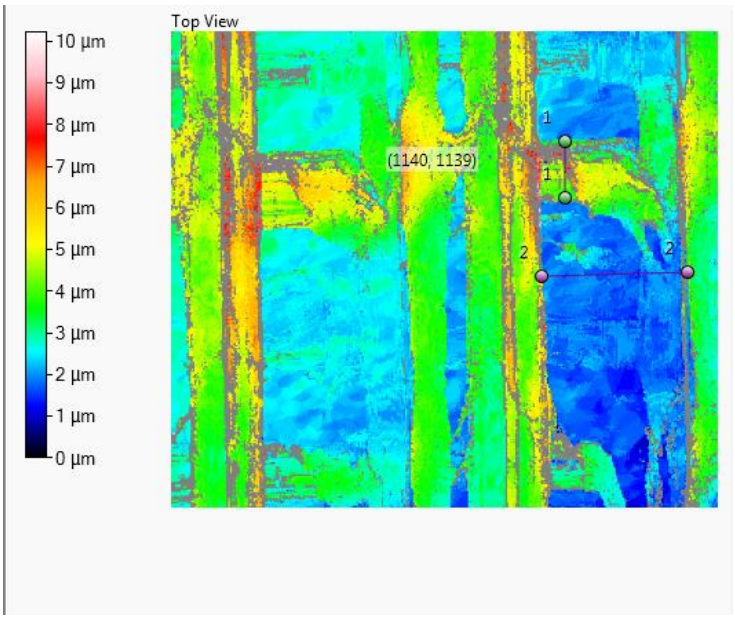


Figure 4.8: PROFILER DATA OF THIN RECTANGULAR MESH

Area Roughness

General			
Average	3.634	µm	Mean height
Minimum	1.097	µm	Minimum height
Maximum	4.995	µm	Maximum height
Range	3.898	µm	Maximum - Minimum
ASME B46.1 3D			
Sp	1.36	µm	Peak height
Sv	2.538	µm	Valley depth
St	3.898	µm	Maximum peak to valley height
Sa	0.2525	µm	Arithmetic mean height
Sq	0.32	µm	Root mean square height
Ssk	-0.3233		Skewness
Sku	3.923		Kurtosis
EUR 15178N Amplitude			
Sp	1.36	µm	Maximum peak height
Sv	2.538	µm	Maximum pit height
St	3.898	µm	Maximum height
Sa	0.2525	µm	Arithmetic mean height
Sq	0.32	µm	Root mean square height
Ssk	-0.3233		Skewness
Sku	3.923		Kurtosis
ISO 25178 Height			
Sp	1.36	µm	Maximum peak height
Sv	2.538	µm	Maximum pit height
Sz	3.898	µm	Maximum height
Sa	0.2525	µm	Arithmetic mean height
Sq	0.32	µm	Root mean square height
Ssk	-0.3233		Skewness
Sku	3.923		Kurtosis

Figure 4.9: PROFILER IMAGE OF THIN RECTANGULAR MESH

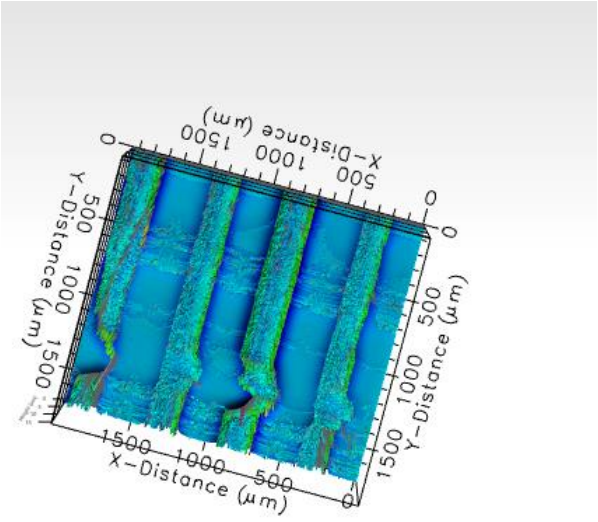


Figure 4.10: PROFILER IMAGE OF THE FIRST LAYER OF THICK RECTANGLE

Area Roughness

General				
Average	3.566	µm		Mean height
Minimum	0	µm		Minimum height
Maximum	16.28	µm		Maximum height
Range	16.28	µm		Maximum - Minimum
ASME B46.1 3D				
Sp	12.71	µm		Peak height
Sv	3.566	µm		Valley depth
St	16.28	µm		Maximum peak to valley height
Sa	0.4296	µm		Arithmetic mean height
Sq	0.6704	µm		Root mean square height
Ssk	1.123			Skewness
Sku	12.59			Kurtosis
EUR 15178N Amplitude				
Sp	12.71	µm		Maximum peak height
Sv	3.566	µm		Maximum pit height
St	16.28	µm		Maximum height
Sa	0.4296	µm		Arithmetic mean height
Sq	0.6704	µm		Root mean square height
Ssk	1.123			Skewness
Sku	12.59			Kurtosis
ISO 25178 Height				
Sp	12.71	µm		Maximum peak height
Sv	3.566	µm		Maximum pit height
Sz	16.28	µm		Maximum height
Sa	0.4296	µm		Arithmetic mean height
Sq	0.6704	µm		Root mean square height
Ssk	1.123			Skewness
Sku	12.59			Kurtosis
ISO 25178 Spatial				
Sal	23.26	µm	s=0.2	Autocorrelation length

Figure 4.11 : PROFILER DATA OF THE FIRST LAYER OF THE RECTANGLE

Figures 4.10 and 4.11 represents the profiler data of the first layer of the thick rectangle and shows the height of the sample as around 20 microns.

4.4 WETTABILITY AND DEGRADABILITY TEST ON THE RESULTS FROM ELECTROSPUN AND ELECTROMELT

Wettability test was conducted to evaluate the measurement of absorbance capability of the scaffolds. Degradability test was conducted to evaluate the degradability of the scaffolds produced from both the systems. As both systems used the same polymer, the test will clearly reflect on the degradability of the samples. For this test, one sample from each system were taken. Samples from the electrospun system were taken and punched using a puncher to separate out a small portion for the test.Then measured their weight and then inserted into the PBS solution for seven days. After the first day, the value was measured and recorded and then waited for a week. The results are recorded as a table below:

Table 3: WETTABILITY and DEGRADABILITY TEST OF PCL(EM system)

Test	Initial weight	1 week	Percentage
Wettability	0.0017	0.039	129.412%
Degradability	0.039	0.0268	31.28%

4.5 CELL ANALYSIS ON THE RESULTS FROM ELECTROSPUN

The results from the cell culture proved that the scaffolds are compatible with the cells as the cells grew abundantly and multiplied. Both rectangular and circular structures were analyzed and the images are depicted in Figures 4.13-4.16. We used Alexa 488 kit, with DAPI and Hoechst dyes, cultured the cells and scaffolds for about 3 days and then proceeded with adding Edu and fixing the, at the end of staining. From the figures, it depicts the cells are growing in a random fashion.

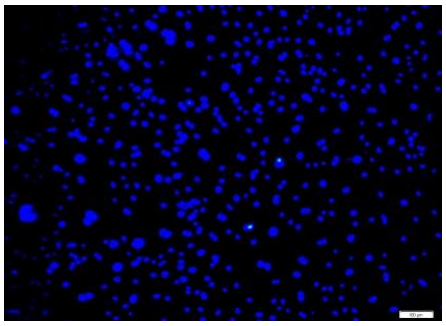


Figure 4.12: CELL ADHESION

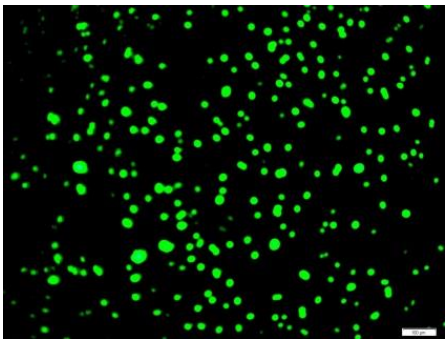


Figure 4.13: CELL PROLIFERATION

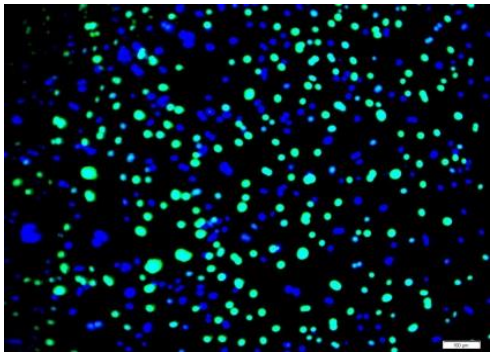


Figure 4.14: COMBINED IMAGE

4.6 CELL ANALYSIS ON THE RESULTS FROM ELECTROMELT

The results from the cell culture proved that the scaffolds are compatible with the cells as the cells grew abundantly and multiplied . Both rectangular and circular structures were analyzed and the images are depicted in Figures 4.13-4.16. We used Alexa 488 kit, with DAPI and Hoechst dyes, cultured the cells and scaffolds for about 3 days and then proceeded with adding Edu and fixing

the, at the end of staining. The figures were taken from a group of 5 samples. We chose 5 because this number makes the results statistically significant.

A. RECTANGULAR MESH:

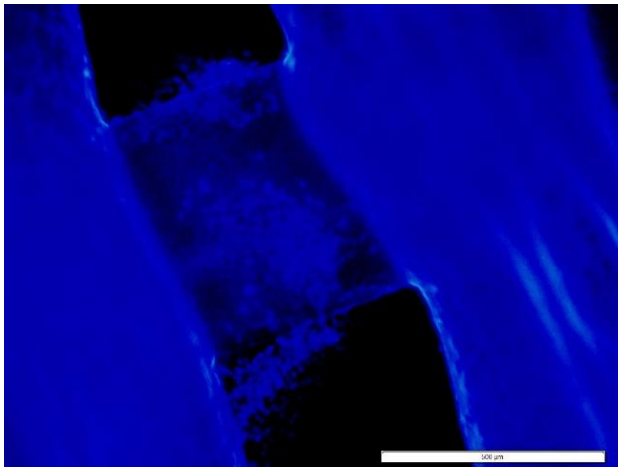


Figure 4.15: ADHESION IMAGE OF THE RECTANGULAR MESH

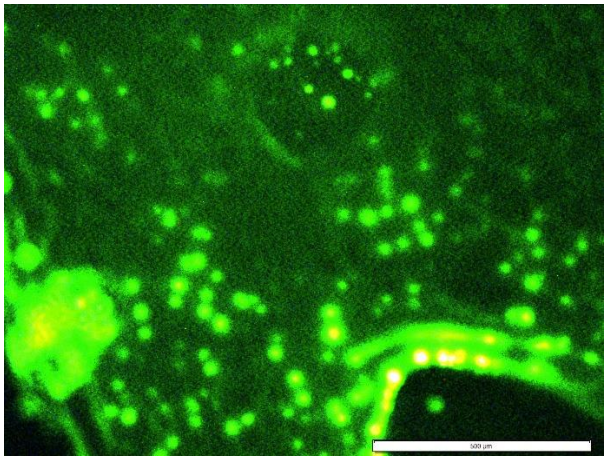


Figure 4.16: PROLIFERATION IMAGE OF THE RECTANGUALAR MESH

As seen from the pictures, the cells count for adhesion and proliferation is high which implies that the scaffold is bio-compatible and it provides a good environment for the cells to attach , grow and

multiply in. Challenges faced were trying focus the fluorescent microscope as the scaffold has multiple layers and the cells like to migrate in between layers.

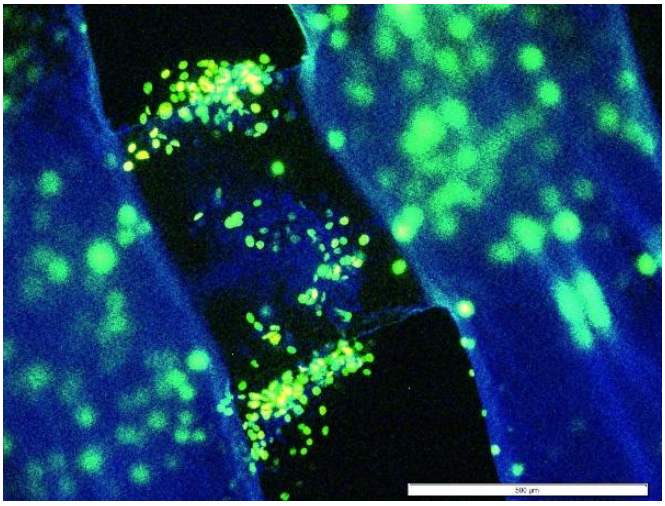


Figure 4.17: ADHESION+PROLIFERATION

B. CIRCULAR MESH

The Figures 4.16- 4.18 reveals the adhesion , proliferation and the combined picture of both process, similar to the rectangular mesh.

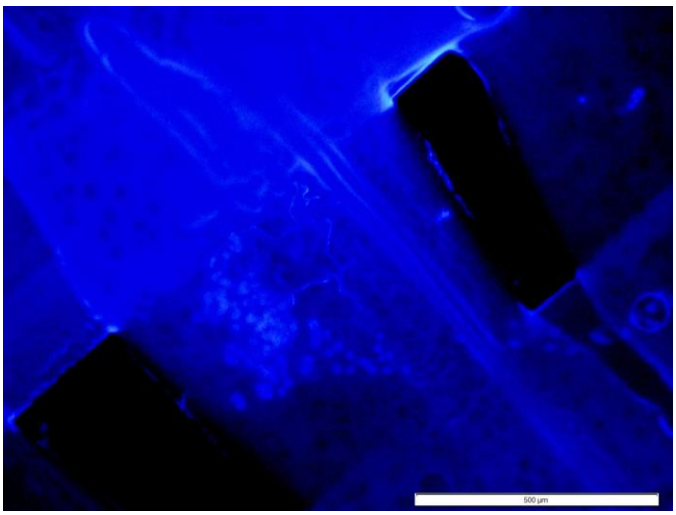


Figure 4.18: ADHESION IMAGE OF CIRCULAR MESH

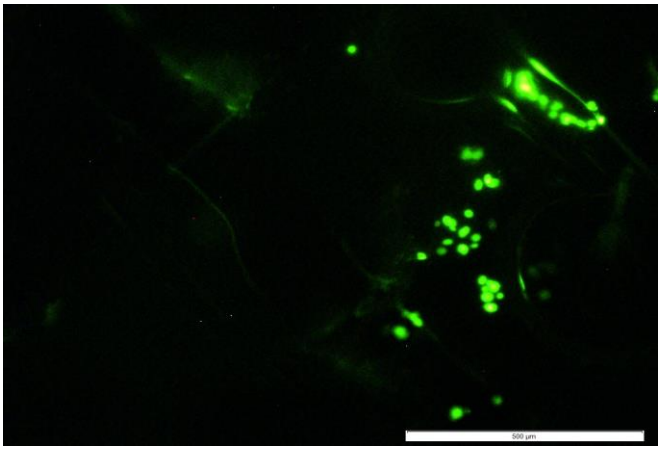


Figure 4.19: PROLIFERATION PIC OF CIRCULAR MESH

4.7 CELL VIABILITY TEST ON THE RESULTS FROM ELECTROSPUN AND ELECTROMELT

Cell viability test is the test which shows the cells are active or not. As the cell adhesion and proliferation measures their ability to grow and multiply, cell viability measures if the cells when put in an in vivo media , survives or not. The in vivo media is very similar to human body, If the test turns out to be positive, then its likely to survive in human body.

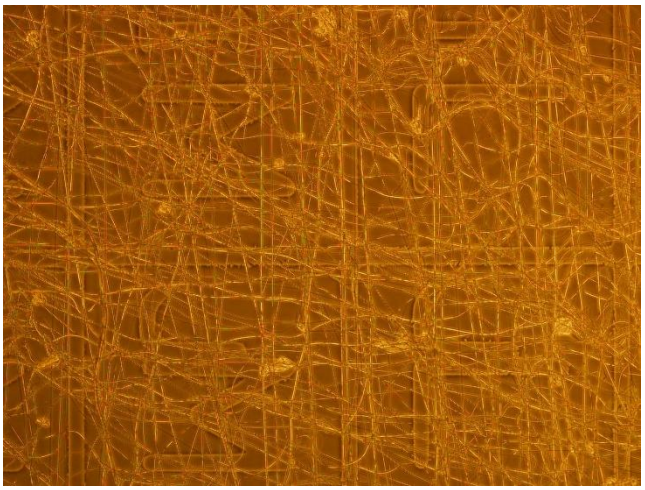


Figure 4.20: CELL VIABILITY TEST FOR 12 LAYERS

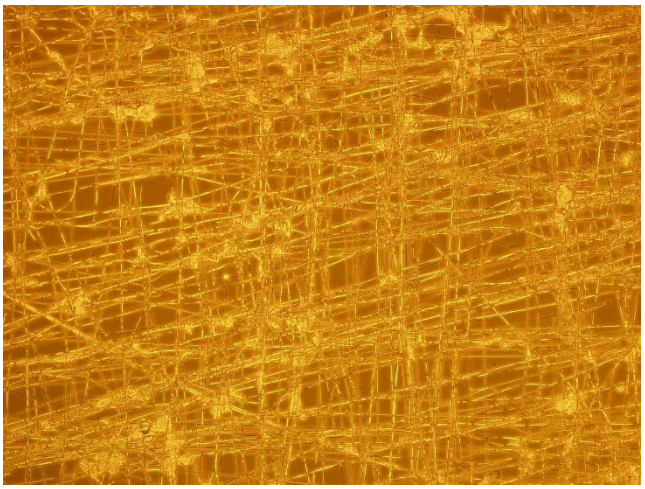


Figure 4.21: CELL VIABILITY OF 18 LAYERS

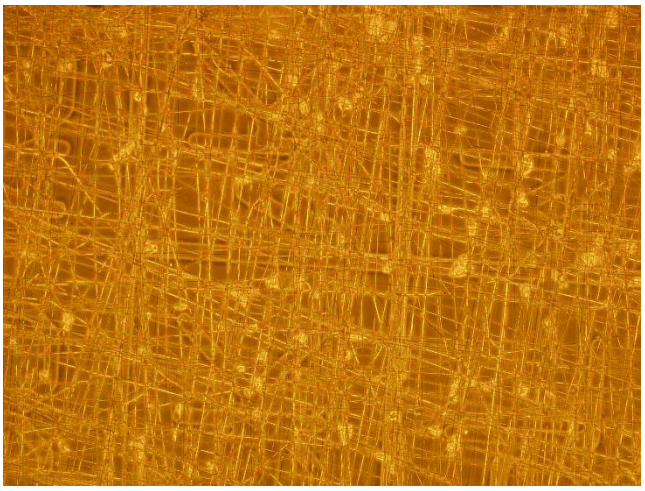


Figure 4.22: CELL VIABILITY OF 24 LAYERS

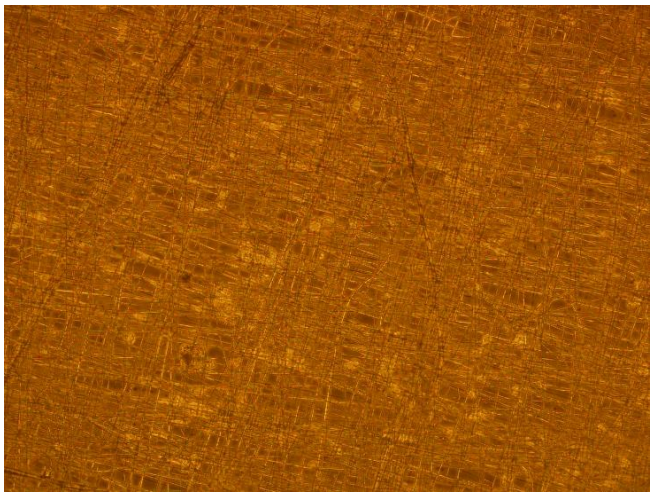


Figure 4.23: CELL VIABILITY OF 30 LAYERS

The cell viability results from the electromelt system were conducted in a different way . The scaffolds were placed in a media similar to human body with the cells. This test was conducted for 4 days. If the cells are found to be alive after 4 days, there is a higher possibility of cells surviving in the human body. The Table 4 below shows the number of cells active. As the number is greater than 0, this reveals that the cells can survive in the scaffold with the human body ambience.

Table 4: CELL VIABILITY RESULTS OF EM SYSTEM

Sample:	**1**	**2**	**3**
Cell count	2.907	1.653	1.634
	2.802	2.141	1.646

4.8 CONCLUSION

Morphological analysis of the scaffolds provides more details about the internal structure. The samples obtained from the electrospun and electromelt were observed and analyzed by SEM images, which provides information about the topography of the sample and the cell analysis were also conducted. Cell adhesion and proliferation were observed using a fluorescent microscope and analyzed the cell growth and adhesion tend to increase for more layers of PCL. Degradability test were conducted by placing the sample in the PBS solution and test a day after and after 1 week. Cell viability tests were also conducted to analyze the cell attachment after 48 hours.

From the cell analysis of ES structures, the cells grew in a random fashion whereas in the EM scaffolds the cell growth was guided in a direction, which is preferred more in case of cell

regeneration. Also, cell viability test was positive for both the applications as the cells were active in the media after 4 days.

CHAPTER 5

PROBLEMS AND FUTURE WORKS

Problems faced in the Chapter 2 regarding electrospun system are the manual collection of the fibers in the glass slide and the plastic mold due to the non- aligned formation of the fibers. The fibers produced are not able to pronounce in which direction they are oriented. Also, during the sample collection, it was very difficult to maintain consistent thickness of the fibers in the system.

Future works associated with the chapter 2,electrospun system will be creating a electric sub-system along with the system that have the potential to control the alignment of the fibers automatically and that will make it more easier to obtain the fibers easily in an aligned fashion and thusly it will be easily to maintain a desired thickness.

Problems faced in the chapter 3,electromelt system are inability to attain scaffolds with more height. The maximum height attained was 50 microns. Also, incorporation of UV light for the non-photo sensitive materials is also a problem that has to be solved as UV light should be placed above the sample no more than 8 cm. With the present system, shadow of the hotend may affect the curing of the solution under UV light.

Future works regarding the electromelt system will be producing scaffolds with at least 1.5 mm height as it is the standard height. For that, more than 70 layers should be made in a structure alternatively with first and second layer. Also, the development of the system so it can be used for both photo-sensitive and non-photo sensitive materials will be huge step forward.

5.1 SOLIDWORKS MODEL OF THE PROPOSED SYSTEM

SolidWorks is a program that allows for 3D rendering and simulated testing of individual parts and assemblies of multiple parts. A 3D model of an object can be constructed using variety of tools, and can use the inbuilt applications such as Simulation Xpress tool and FloXpress tool to apply loads and to examine the way fluids flow through it respectively. The created models can be used for milling tools and 3D printers to actually create the part from a variety of different sorts of materials.

Before starting to build the system, I was asked to model it in SolidWorks to get an outline, add or remove parts, include more suggestions regarding the materials, dimensions and structures involved. Creating the model was really helpful in discussing my ideas to others and finding suitable materials for the parts required. First, I created all the models base, top cover, UV lamp, Actuators, Stages, Motor, Syringes, Tube, and Needle separately in the "Sketch" and then combined all together in the "Assembly".

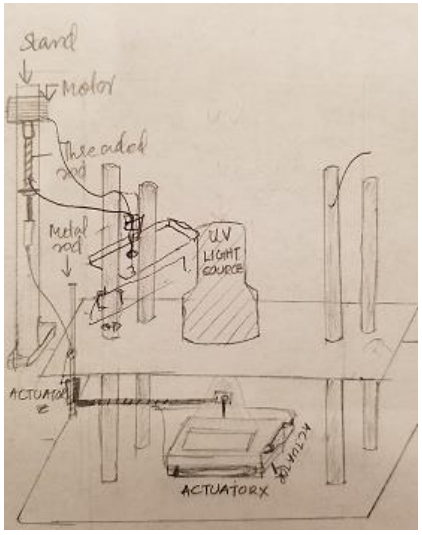


Figure 5.1 : DESIGNED SYSTEM FOR PHOTO_SENSITIVE MATERIALS

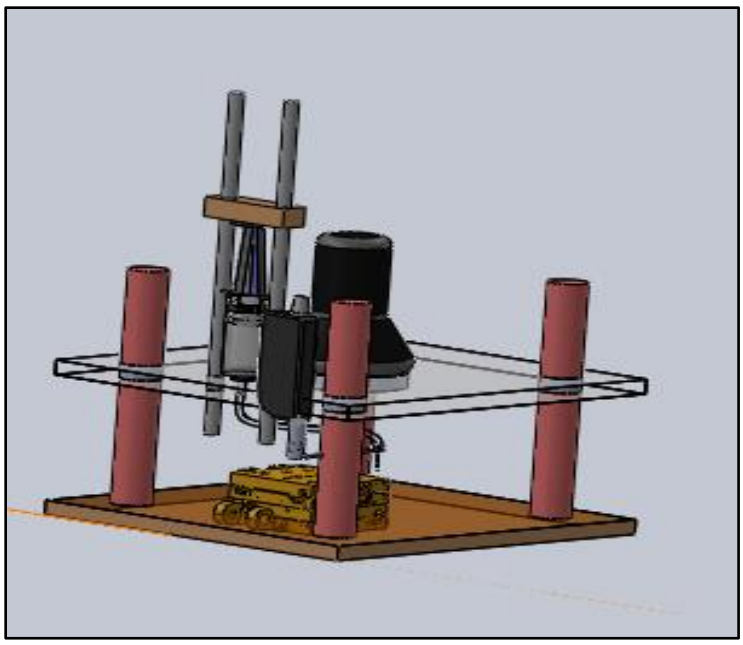


Figure 5.2: SolidWorks model

CHAPTER 6

CONCLUSION

The goal of this study was to compare the morphological and biological characteristics of the results from both electrospun and electromelt techniques. There are three objectives for this study: The first objective was to develop fibrous structures using electrospun process and their morphological characteristics. The second objective was to develop scaffolds using electromelt process and their morphological characteristics. The third objective is the comparative analysis of the results from both techniques.

The results from the electrospun system were analyzed morphologically and biologically. Morphological analysis was conducted by using SEM to analyze the fiber diameter and pore size. Both topographical datas were not uniform and not aligned. Whereas the biological analysis /*in vitro* cell analysis showed that the cells grew and multiplied in the structures. Cell adhesion and Cell proliferation was also observed . Cell viability test was also conducted. As the cell analysis was successful, the structures from the electrospun technique can be used for applications but aligned fibers are known to enhance and guide the cell growth in the desired orientation.

The electromelt system was developed with the suitable selection of the parameters and ambience . Scaffolds with different architecture were produced: Rectangular and Circular architectures. Rectangular first layer was developed in two different ways: thin fiber diameter and thick fiber diameter. Even though the thick fiber diameter is desired. Several trials were conducted for all the parameter values to be in synchronism with the system performance. Also the second layer was developed on top of the first layer, forming a structural scaffold resembling the

rectangular mesh was formed. Similarly, circle's first layer was formed and second layer was also developed on top it, to attain the circular structure.

These structures were then analyzed using SEM and profiler for the morphological analysis and the fluorescence microscope was used for the cell analysis. SEM images revealed that the fiber diameter and pore size was uniform throughout with the desired values as it was intended to be. Profiler gives the outline about the height and the surface roughness, for some samples, the height wasn't uniform but that doesn't affect the scaffold much. *In vitro* cell analysis was conducted and turned out to be successful. Cells grew abundantly, and the cell adhesion and cell proliferation are very good. Cell viability test was also conducted. Hence the electromelt system was a success and the scaffolds produced are a potential solution for the biomedical applications like, tissue engineering, drug delivery etc. Thus, electromelt technique proved to be better than the electrospun technique.

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APPENDIX

A. NEWPORT ACTUATOR SYSTEM

A.1 SYSTEM SPECIFICATIONS

All the tabs of the ESP 301software are discussed below:

Enable/Disable tab: Can enable and disable the motion of the axis. Green colour indicates the motion is allowed and Red colour indicates the disabling of the motion.

Jog tab: Can move the axis without the help of the program for a certain distance, by pressing the arrow buttons shown or also by entering the value in the space provided.

Trajectory tab: This tab also provides more specific information regarding the velocity and servo control parameters if needed.

Stop tab: This tab can abruptly stop the movement of the axis which was in motion.

Home tab: As discussed above , states the defined home position of the axis, and the speed at which the axis would navigate to the home.

Cycle tab: This tab would provide details if the movement occurs in a defined cycle.

Position tab: This tab is another important one and used very frequently in my thesis. This tab displays the current position of the axis where it is, either it is stable or in motion. Currently, it is showing the position of the third axis to be at 18.999mm.

Programming modes: The motion controller has two basic programming modes: LOCAL and COMMAND/REMOTE modes. In the LOCAL mode, the parameters can be changed using the front panel of the controller and without the computer or any other external source. Also, only a subset of the commands would be available for this mode as not all commands can be entered using the front panel. Typical parameters such as velocity, acceleration, home etc can be achieved

in this mode. In the COMMAND/REMOTE mode ,the ESP 301 enables an input buffer from the computer , from where the series of input commands are downloaded before any further action. After receiving the inout commands, they are analyzed and interpreted. If the syntax is valid, it proceeds with the execution.

Command syntax: The Newport actuators can be programmed very simply using the command syntax but also depends on the complexity of the structure to be produced. Commands consists of a series of two ASCII characters preceded by the axis number and followed by the appropriate parameters. The conversion to the ASCII characters should be done according to the respective communication protocols.

Blank spaces: If a command does not require parameter “xx” and/or parameter “nn”, that field may be skipped by leaving a blank character (space).

For example, BO1, 3WS, and AB are all valid commands.

If a command requires multiple parameters in the third field, all these parameters must be comma delimited.

For example, 1HN1,2 is a valid command. Restrain the use of blank spaces as it can consume more memory.

Command terminator: The controller interprets the commands sequentially and hence the commands on the same line are analyzed more faster than the second line. Henceforth, multiple commands can be issued on a single command line by separating the commands by a semi-colon (;). The maximum number of commands in a single line is 80.

For example, 3MO; 3PA10.0; 3WS; 3MF is a valid command line.

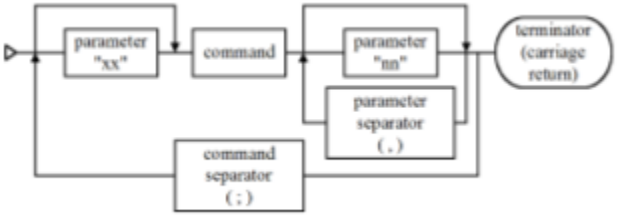

Figure A.1: COMMAND SYNTAX FLOW CHART

Communication protocols: For the three different interfaces used to communicate with the computer and the motion controller, each of them their own specific communication protocol which should be administered for efficient communication.

In case of RS 232, the parameters should be properly configured such as baud rate, parity type, number of stop bits and hand shake type, for both the devices. It is fixed at 8 data bits, no parity and 1 stop bit. To prevent the buffer overflow, handshake protocol is maintained. A CTS/RTS protocol which uses CTS (clear to send signal) before sending the commands and RTS (request to send) is enabled by the computer.

The USB communication protocol uses 921600 baud rate, 8 data bits, N parity and 1 stop bit.

The IEEE488 communication protocol is implemented which can use the standard IEEE 488.2 command sets. Like the handshake protocol in RS232, IEEE 488 uses service request signal (SRQ) by using RQ command.

A.2 PROGRAMS

a. RECTANGLE WITH 10 MICRONS SPACING (30 X 5)

1 EP

1 PA0; 1 WS ; 2 PA0; 2 WS
1 PA30; 1 WS ; 2 PA0.01; 2 WS
1 PA0; 1 WS ; 2 PA0.02; 2 WS
1 PA30; 1 WS ; 2 PA0.03; 2 WS
1 PA0; 1 WS ; 2 PA0.04; 2 WS
1 PA30; 1 WS ; 2 PA0.05; 2 WS
1 PA0; 1 WS ; 2 PA0.06; 2 WS
1 PA30; 1 WS ; 2 PA0.07; 2 WS
1 PA0; 1 WS ; 2 PA0.08; 2 WS
1 PA30; 1 WS ; 2 PA0.09; 2 WS
1 PA0; 1 WS ; 2 PA0.1; 2 WS
1 PA30; 1 WS ; 2 PA0.11; 2 WS
1 PA0; 1 WS ; 2 PA0.12; 2 WS
1 PA30; 1 WS ; 2 PA0.13; 2 WS
1 PA0; 1 WS ; 2 PA0.14; 2 WS
1 PA30; 1 WS ; 2 PA0.15; 2 WS
1 PA0; 1 WS ; 2 PA0.16; 2 WS
1 PA30; 1 WS ; 2 PA0.17; 2 WS
1 PA0; 1 WS ; 2 PA0.18; 2 WS
1 PA30; 1 WS ; 2 PA0.19; 2 WS
1 PA0; 1 WS ; 2 PA0.2; 2 WS
1 PA30; 1 WS ; 2 PA0.21; 2 WS
1 PA0; 1 WS ; 2 PA0.22; 2 WS
1 PA30; 1 WS ; 2 PA0.23; 2 WS
1 PA0; 1 WS ; 2 PA0.24; 2 WS
1 PA30; 1 WS ; 2 PA0.25; 2 WS
1 PA0; 1 WS ; 2 PA0.26; 2 WS
1 PA30; 1 WS ; 2 PA0.27; 2 WS
1 PA0; 1 WS ; 2 PA0.28; 2 WS
1 PA30; 1 WS ; 2 PA0.29; 2 WS
1 PA0; 1 WS ; 2 PA0.3; 2 WS
1 PA30; 1 WS ; 2 PA0.31; 2 WS
1 PA0; 1 WS ; 2 PA0.32; 2 WS
1 PA30; 1 WS ; 2 PA0.33; 2 WS
1 PA0; 1 WS ; 2 PA0.34; 2 WS
1 PA30; 1 WS ; 2 PA0.35; 2 WS

1 PA0; 1 WS ; 2 PA0.36; 2 WS
1 PA30; 1 WS ; 2 PA0.37; 2 WS
1 PA0; 1 WS ; 2 PA0.38; 2 WS
1 PA30; 1 WS ; 2 PA0.39; 2 WS
1 PA0; 1 WS ; 2 PA0.4; 2 WS
1 PA30; 1 WS ; 2 PA0.41; 2 WS
1 PA0; 1 WS ; 2 PA0.42; 2 WS
1 PA30; 1 WS ; 2 PA0.43; 2 WS
1 PA0; 1 WS ; 2 PA0.44; 2 WS
1 PA30; 1 WS ; 2 PA0.45; 2 WS
1 PA0; 1 WS ; 2 PA0.46; 2 WS
1 PA30; 1 WS ; 2 PA0.47; 2 WS
1 PA0; 1 WS ; 2 PA0.48; 2 WS
1 PA30; 1 WS ; 2 PA0.49; 2 WS
1 PA0; 1 WS ; 2 PA0.5; 2 WS
1 PA30; 1 WS ; 2 PA0.51; 2 WS
1 PA0; 1 WS ; 2 PA0.52; 2 WS
1 PA30; 1 WS ; 2 PA0.53; 2 WS
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1 PA30; 1 WS ; 2 PA4.99; 2 WS
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QP

b. RECTANGLE WITH 50 MICRONS (7 X 20)

3 EP

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2WS

1 PA0.05; 1 WS ;2 PA0; 2 WS
1 PA0.1; 1 WS ;2 PA20; 2 WS
1 PA0.15; 1 WS ;2 PA0; 2 WS
1 PA0.2; 1 WS ;2 PA20; 2 WS
1 PA0.25; 1 WS ;2 PA0; 2 WS
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1 PA1.45; 1 WS ;2 PA0; 2 WS
1 PA1.5; 1 WS ;2 PA20; 2 WS
1 PA1.55; 1 WS ;2 PA0; 2 WS
1 PA1.6; 1 WS ;2 PA20; 2 WS
1 PA1.65; 1 WS ;2 PA0; 2 WS
1 PA1.7; 1 WS ;2 PA20; 2 WS
1 PA1.75; 1 WS ;2 PA0; 2 WS
1 PA1.8; 1 WS ;2 PA20; 2 WS
1 PA1.85; 1 WS ;2 PA0; 2 WS
1 PA1.9; 1 WS ;2 PA20; 2 WS
1 PA1.95; 1 WS ;2 PA0; 2 WS
1 PA2; 1 WS ;2 PA20; 2 WS
1 PA2.05; 1 WS ;2 PA0; 2 WS
1 PA2.1; 1 WS ;2 PA20; 2 WS
1 PA2.15; 1 WS ;2 PA0; 2 WS

1 PA2.2; 1 WS ;2 PA20; 2 WS
1 PA2.25; 1 WS ;2 PA0; 2 WS
1 PA2.3; 1 WS ;2 PA20; 2 WS
1 PA2.35; 1 WS ;2 PA0; 2 WS
1 PA2.4; 1 WS ;2 PA20; 2 WS
1 PA2.45; 1 WS ;2 PA0; 2 WS
1 PA2.5; 1 WS ;2 PA20; 2 WS
1 PA2.55; 1 WS ;2 PA0; 2 WS
1 PA2.6; 1 WS ;2 PA20; 2 WS
1 PA2.65; 1 WS ;2 PA0; 2 WS
1 PA2.7; 1 WS ;2 PA20; 2 WS
1 PA2.75; 1 WS ;2 PA0; 2 WS
1 PA2.8; 1 WS ;2 PA20; 2 WS
1 PA2.85; 1 WS ;2 PA0; 2 WS
1 PA2.9; 1 WS ;2 PA20; 2 WS
1 PA2.95; 1 WS ;2 PA0; 2 WS
1 PA3; 1 WS ;2 PA20; 2 WS
1 PA3.05; 1 WS ;2 PA0; 2 WS
1 PA3.1; 1 WS ;2 PA20; 2 WS
1 PA3.15; 1 WS ;2 PA0; 2 WS
1 PA3.2; 1 WS ;2 PA20; 2 WS
1 PA3.25; 1 WS ;2 PA0; 2 WS
1 PA3.3; 1 WS ;2 PA20; 2 WS
1 PA3.35; 1 WS ;2 PA0; 2 WS
1 PA3.4; 1 WS ;2 PA20; 2 WS
1 PA3.45; 1 WS ;2 PA0; 2 WS
1 PA3.5; 1 WS ;2 PA20; 2 WS
1 PA3.55; 1 WS ;2 PA0; 2 WS
1 PA3.6; 1 WS ;2 PA20; 2 WS
1 PA3.65; 1 WS ;2 PA0; 2 WS
1 PA3.7; 1 WS ;2 PA20; 2 WS
1 PA3.75; 1 WS ;2 PA0; 2 WS
1 PA3.8; 1 WS ;2 PA20; 2 WS
1 PA3.85; 1 WS ;2 PA0; 2 WS
1 PA3.9; 1 WS ;2 PA20; 2 WS
1 PA3.95; 1 WS ;2 PA0; 2 WS
1 PA4; 1 WS ;2 PA20; 2 WS
1 PA4.05; 1 WS ;2 PA0; 2 WS
1 PA4.1; 1 WS ;2 PA20; 2 WS
1 PA4.15; 1 WS ;2 PA0; 2 WS
1 PA4.2; 1 WS ;2 PA20; 2 WS
1 PA4.25; 1 WS ;2 PA0; 2 WS
1 PA4.3; 1 WS ;2 PA20; 2 WS

1 PA4.35; 1 WS ;2 PA0; 2 WS
1 PA4.4; 1 WS ;2 PA20; 2 WS
1 PA4.45; 1 WS ;2 PA0; 2 WS
1 PA4.5; 1 WS ;2 PA20; 2 WS
1 PA4.55; 1 WS ;2 PA0; 2 WS
1 PA4.6; 1 WS ;2 PA20; 2 WS
1 PA4.65; 1 WS ;2 PA0; 2 WS
1 PA4.7; 1 WS ;2 PA20; 2 WS
1 PA4.75; 1 WS ;2 PA0; 2 WS
1 PA4.8; 1 WS ;2 PA20; 2 WS
1 PA4.85; 1 WS ;2 PA0; 2 WS
1 PA4.9; 1 WS ;2 PA20; 2 WS
1 PA4.95; 1 WS ;2 PA0; 2 WS
1 PA5; 1 WS ;2 PA20; 2 WS
1 PA5.05; 1 WS ;2 PA0; 2 WS
1 PA5.1; 1 WS ;2 PA20; 2 WS
1 PA5.15; 1 WS ;2 PA0; 2 WS
1 PA5.2; 1 WS ;2 PA20; 2 WS
1 PA5.25; 1 WS ;2 PA0; 2 WS
1 PA5.3; 1 WS ;2 PA20; 2 WS
1 PA5.35; 1 WS ;2 PA0; 2 WS
1 PA5.4; 1 WS ;2 PA20; 2 WS
1 PA5.45; 1 WS ;2 PA0; 2 WS
1 PA5.5; 1 WS ;2 PA20; 2 WS
1 PA5.55; 1 WS ;2 PA0; 2 WS
1 PA5.6; 1 WS ;2 PA20; 2 WS
1 PA5.65; 1 WS ;2 PA0; 2 WS
1 PA5.7; 1 WS ;2 PA20; 2 WS
1 PA5.75; 1 WS ;2 PA0; 2 WS
1 PA5.8; 1 WS ;2 PA20; 2 WS
1 PA5.85; 1 WS ;2 PA0; 2 WS
1 PA5.9; 1 WS ;2 PA20; 2 WS
1 PA5.95; 1 WS ;2 PA0; 2 WS
1 PA6; 1 WS ;2 PA20; 2 WS
1 PA6.05; 1 WS ;2 PA0; 2 WS
1 PA6.1; 1 WS ;2 PA20; 2 WS
1 PA6.15; 1 WS ;2 PA0; 2 WS
1 PA6.2; 1 WS ;2 PA20; 2 WS
1 PA6.25; 1 WS ;2 PA0; 2 WS
1 PA6.3; 1 WS ;2 PA20; 2 WS
1 PA6.35; 1 WS ;2 PA0; 2 WS
1 PA6.4; 1 WS ;2 PA20; 2 WS
1 PA6.45; 1 WS ;2 PA0; 2 WS

1 PA6.5; 1 WS ;2 PA20; 2 WS
1 PA6.55; 1 WS ;2 PA0; 2 WS
1 PA6.6; 1 WS ;2 PA20; 2 WS
1 PA6.65; 1 WS ;2 PA0; 2 WS
1 PA6.7; 1 WS ;2 PA20; 2 WS
1 PA6.75; 1 WS ;2 PA0; 2 WS
1 PA6.8; 1 WS ;2 PA20; 2 WS
1 PA6.85; 1 WS ;2 PA0; 2 WS
1 PA6.9; 1 WS ;2 PA20; 2 WS
1 PA6.95; 1 WS ;2 PA0; 2 WS
1 PA7; 1 WS ;2 PA20; 2 WS
QP

c. RECTANGLE WITH 100 MICRONS SPACING (7 X 20)

1 PA0; 1 WS;2 PA7; 2 WS
1 PA0.1; 1 WS;2 PA0; 2 WS
1 PA0.2; 1 WS;2 PA7; 2 WS
1 PA0.3; 1 WS;2 PA0; 2 WS
1 PA0.4; 1 WS;2 PA7; 2 WS
1 PA0.5; 1 WS;2 PA0; 2 WS
1 PA0.6; 1 WS;2 PA7; 2 WS
1 PA0.7; 1 WS;2 PA0; 2 WS
1 PA0.8; 1 WS;2 PA7; 2 WS
1 PA0.9; 1 WS;2 PA0; 2 WS
1 PA1; 1 WS;2 PA7; 2 WS
1 PA1.1; 1 WS;2 PA0; 2 WS
1 PA1.2; 1 WS;2 PA7; 2 WS
1 PA1.3; 1 WS;2 PA0; 2 WS
1 PA1.4; 1 WS;2 PA7; 2 WS
1 PA1.5; 1 WS;2 PA0; 2 WS
1 PA1.6; 1 WS;2 PA7; 2 WS
1 PA1.7; 1 WS;2 PA0; 2 WS
1 PA1.8; 1 WS;2 PA7; 2 WS
1 PA1.9; 1 WS;2 PA0; 2 WS
1 PA2; 1 WS;2 PA7; 2 WS
1 PA2.1; 1 WS;2 PA0; 2 WS
1 PA2.2; 1 WS;2 PA7; 2 WS
1 PA2.3; 1 WS;2 PA0; 2 WS
1 PA2.4; 1 WS;2 PA7; 2 WS
1 PA2.5; 1 WS;2 PA0; 2 WS
1 PA2.6; 1 WS;2 PA7; 2 WS
1 PA2.7; 1 WS;2 PA0; 2 WS

1 PA2.8; 1 WS;2 PA7; 2 WS
1 PA2.9; 1 WS;2 PA0; 2 WS
1 PA3; 1 WS;2 PA7; 2 WS
1 PA3.1; 1 WS;2 PA0; 2 WS
1 PA3.2; 1 WS;2 PA7; 2 WS
1 PA3.3; 1 WS;2 PA0; 2 WS
1 PA3.4; 1 WS;2 PA7; 2 WS
1 PA3.5; 1 WS;2 PA0; 2 WS
1 PA3.6; 1 WS;2 PA7; 2 WS
1 PA3.7; 1 WS;2 PA0; 2 WS
1 PA3.8; 1 WS;2 PA7; 2 WS
1 PA3.9; 1 WS;2 PA0; 2 WS
1 PA4; 1 WS;2 PA7; 2 WS
1 PA4.1; 1 WS;2 PA0; 2 WS
1 PA4.2; 1 WS;2 PA7; 2 WS
1 PA4.3; 1 WS;2 PA0; 2 WS
1 PA4.4; 1 WS;2 PA7; 2 WS
1 PA4.5; 1 WS;2 PA0; 2 WS
1 PA4.6; 1 WS;2 PA7; 2 WS
1 PA4.7; 1 WS;2 PA0; 2 WS
1 PA4.8; 1 WS;2 PA7; 2 WS
1 PA4.9; 1 WS;2 PA0; 2 WS
1 PA5; 1 WS;2 PA7; 2 WS
1 PA5.1; 1 WS;2 PA0; 2 WS
1 PA5.2; 1 WS;2 PA7; 2 WS
1 PA5.3; 1 WS;2 PA0; 2 WS
1 PA5.4; 1 WS;2 PA7; 2 WS
1 PA5.5; 1 WS;2 PA0; 2 WS
1 PA5.6; 1 WS;2 PA7; 2 WS
1 PA5.7; 1 WS;2 PA0; 2 WS
1 PA5.8; 1 WS;2 PA7; 2 WS
1 PA5.9; 1 WS;2 PA0; 2 WS
1 PA6; 1 WS;2 PA7; 2 WS
1 PA6.1; 1 WS;2 PA0; 2 WS
1 PA6.2; 1 WS;2 PA7; 2 WS
1 PA6.3; 1 WS;2 PA0; 2 WS
1 PA6.4; 1 WS;2 PA7; 2 WS
1 PA6.5; 1 WS;2 PA0; 2 WS
1 PA6.6; 1 WS;2 PA7; 2 WS
1 PA6.7; 1 WS;2 PA0; 2 WS
1 PA6.8; 1 WS;2 PA7; 2 WS
1 PA6.9; 1 WS;2 PA0; 2 WS
1 PA7; 1 WS;2 PA7; 2 WS

1 PA7.1; 1 WS;2 PA0; 2 WS
1 PA7.2; 1 WS;2 PA7; 2 WS
1 PA7.3; 1 WS;2 PA0; 2 WS
1 PA7.4; 1 WS;2 PA7; 2 WS
1 PA7.5; 1 WS;2 PA0; 2 WS
1 PA7.6; 1 WS;2 PA7; 2 WS
1 PA7.7; 1 WS;2 PA0; 2 WS
1 PA7.8; 1 WS;2 PA7; 2 WS
1 PA7.9; 1 WS;2 PA0; 2 WS
1 PA8; 1 WS;2 PA7; 2 WS
1 PA8.1; 1 WS;2 PA0; 2 WS
1 PA8.2; 1 WS;2 PA7; 2 WS
1 PA8.3; 1 WS;2 PA0; 2 WS
1 PA8.4; 1 WS;2 PA7; 2 WS
1 PA8.5; 1 WS;2 PA0; 2 WS
1 PA8.6; 1 WS;2 PA7; 2 WS
1 PA8.7; 1 WS;2 PA0; 2 WS
1 PA8.8; 1 WS;2 PA7; 2 WS
1 PA8.9; 1 WS;2 PA0; 2 WS
1 PA9; 1 WS;2 PA7; 2 WS
1 PA9.1; 1 WS;2 PA0; 2 WS
1 PA9.2; 1 WS;2 PA7; 2 WS
1 PA9.3; 1 WS;2 PA0; 2 WS
1 PA9.4; 1 WS;2 PA7; 2 WS
1 PA9.5; 1 WS;2 PA0; 2 WS
1 PA9.6; 1 WS;2 PA7; 2 WS
1 PA9.7; 1 WS;2 PA0; 2 WS
1 PA9.8; 1 WS;2 PA7; 2 WS
1 PA9.9; 1 WS;2 PA0; 2 WS
1 PA10; 1 WS;2 PA7; 2 WS
1 PA10.1; 1 WS;2 PA0; 2 WS
1 PA10.2; 1 WS;2 PA7; 2 WS
1 PA10.3; 1 WS;2 PA0; 2 WS
1 PA10.4; 1 WS;2 PA7; 2 WS
1 PA10.5; 1 WS;2 PA0; 2 WS
1 PA10.6; 1 WS;2 PA7; 2 WS
1 PA10.7; 1 WS;2 PA0; 2 WS
1 PA10.8; 1 WS;2 PA7; 2 WS
1 PA10.9; 1 WS;2 PA0; 2 WS
1 PA11; 1 WS;2 PA7; 2 WS
1 PA11.1; 1 WS;2 PA0; 2 WS
1 PA11.2; 1 WS;2 PA7; 2 WS
1 PA11.3; 1 WS;2 PA0; 2 WS

1 PA11.4; 1 WS;2 PA7; 2 WS
1 PA11.5; 1 WS;2 PA0; 2 WS
1 PA11.6; 1 WS;2 PA7; 2 WS
1 PA11.7; 1 WS;2 PA0; 2 WS
1 PA11.8; 1 WS;2 PA7; 2 WS
1 PA11.9; 1 WS;2 PA0; 2 WS
1 PA12; 1 WS;2 PA7; 2 WS
1 PA12.1; 1 WS;2 PA0; 2 WS
1 PA12.2; 1 WS;2 PA7; 2 WS
1 PA12.3; 1 WS;2 PA0; 2 WS
1 PA12.4; 1 WS;2 PA7; 2 WS
1 PA12.5; 1 WS;2 PA0; 2 WS
1 PA12.6; 1 WS;2 PA7; 2 WS
1 PA12.7; 1 WS;2 PA0; 2 WS
1 PA12.8; 1 WS;2 PA7; 2 WS
1 PA12.9; 1 WS;2 PA0; 2 WS
1 PA13; 1 WS;2 PA7; 2 WS
1 PA13.1; 1 WS;2 PA0; 2 WS
1 PA13.2; 1 WS;2 PA7; 2 WS
1 PA13.3; 1 WS;2 PA0; 2 WS
1 PA13.4; 1 WS;2 PA7; 2 WS
1 PA13.5; 1 WS;2 PA0; 2 WS
1 PA13.6; 1 WS;2 PA7; 2 WS
1 PA13.7; 1 WS;2 PA0; 2 WS
1 PA13.8; 1 WS;2 PA7; 2 WS
1 PA13.9; 1 WS;2 PA0; 2 WS
1 PA14; 1 WS;2 PA7; 2 WS
1 PA14.1; 1 WS;2 PA0; 2 WS
1 PA14.2; 1 WS;2 PA7; 2 WS
1 PA14.3; 1 WS;2 PA0; 2 WS
1 PA14.4; 1 WS;2 PA7; 2 WS
1 PA14.5; 1 WS;2 PA0; 2 WS
1 PA14.6; 1 WS;2 PA7; 2 WS
1 PA14.7; 1 WS;2 PA0; 2 WS
1 PA14.8; 1 WS;2 PA7; 2 WS
1 PA14.9; 1 WS;2 PA0; 2 WS
1 PA15; 1 WS;2 PA7; 2 WS
1 PA15.1; 1 WS;2 PA0; 2 WS
1 PA15.2; 1 WS;2 PA7; 2 WS
1 PA15.3; 1 WS;2 PA0; 2 WS
1 PA15.4; 1 WS;2 PA7; 2 WS
1 PA15.5; 1 WS;2 PA0; 2 WS
1 PA15.6; 1 WS;2 PA7; 2 WS

1 PA15.7; 1 WS;2 PA0; 2 WS
1 PA15.8; 1 WS;2 PA7; 2 WS
1 PA15.9; 1 WS;2 PA0; 2 WS
1 PA16; 1 WS;2 PA7; 2 WS
1 PA16.1; 1 WS;2 PA0; 2 WS
1 PA16.2; 1 WS;2 PA7; 2 WS
1 PA16.3; 1 WS;2 PA0; 2 WS
1 PA16.4; 1 WS;2 PA7; 2 WS
1 PA16.5; 1 WS;2 PA0; 2 WS
1 PA16.6; 1 WS;2 PA7; 2 WS
1 PA16.7; 1 WS;2 PA0; 2 WS
1 PA16.8; 1 WS;2 PA7; 2 WS
1 PA16.9; 1 WS;2 PA0; 2 WS
1 PA17; 1 WS;2 PA7; 2 WS
1 PA17.1; 1 WS;2 PA0; 2 WS
1 PA17.2; 1 WS;2 PA7; 2 WS
1 PA17.3; 1 WS;2 PA0; 2 WS
1 PA17.4; 1 WS;2 PA7; 2 WS
1 PA17.5; 1 WS;2 PA0; 2 WS
1 PA17.6; 1 WS;2 PA7; 2 WS
1 PA17.7; 1 WS;2 PA0; 2 WS
1 PA17.8; 1 WS;2 PA7; 2 WS
1 PA17.9; 1 WS;2 PA0; 2 WS
1 PA18; 1 WS;2 PA7; 2 WS
1 PA18.1; 1 WS;2 PA0; 2 WS
1 PA18.2; 1 WS;2 PA7; 2 WS
1 PA18.3; 1 WS;2 PA0; 2 WS
1 PA18.4; 1 WS;2 PA7; 2 WS
1 PA18.5; 1 WS;2 PA0; 2 WS
1 PA18.6; 1 WS;2 PA7; 2 WS
1 PA18.7; 1 WS;2 PA0; 2 WS
1 PA18.8; 1 WS;2 PA7; 2 WS
1 PA18.9; 1 WS;2 PA0; 2 WS
1 PA19; 1 WS;2 PA7; 2 WS
1 PA19.1; 1 WS;2 PA0; 2 WS
1 PA19.2; 1 WS;2 PA7; 2 WS
1 PA19.3; 1 WS;2 PA0; 2 WS
1 PA19.4; 1 WS;2 PA7; 2 WS
1 PA19.5; 1 WS;2 PA0; 2 WS
1 PA19.6; 1 WS;2 PA7; 2 WS
1 PA19.7; 1 WS;2 PA0; 2 WS
1 PA19.8; 1 WS;2 PA7; 2 WS
1 PA19.9; 1 WS;2 PA0; 2 WS

1 PA20; 1 WS;2 PA7; 2 WS
QP

d. RECTANGLE WITH 500 MICRONS SPACING (7 X 20)

1 PA0; 1 WS; 2 PA20; 2 WS
1 PA0.5; 1 WS; 2 PA0; 2 WS
1 PA1; 1 WS; 2 PA20; 2 WS
1 PA1.5; 1 WS; 2 PA0; 2 WS
1 PA2; 1 WS; 2 PA20; 2 WS
1 PA2.5; 1 WS; 2 PA0; 2 WS
1 PA3; 1 WS; 2 PA20; 2 WS
1 PA3.5; 1 WS; 2 PA0; 2 WS
1 PA4; 1 WS; 2 PA20; 2 WS
1 PA4.5; 1 WS; 2 PA0; 2 WS
1 PA5; 1 WS; 2 PA20; 2 WS
1 PA5.5; 1 WS; 2 PA0; 2 WS
1 PA6; 1 WS; 2 PA20; 2 WS
1 PA6.5; 1 WS; 2 PA0; 2 WS
1 PA7; 1 WS; 2 PA20; 2 WS
1 PA 0; 1 WS; 2 PA0; 2 WS
QP

e. RECTANGLE WITH 1000 MICRONS SPACING (7 X 20)

2EP
1 PA0; 1 WS;2 PA7; 2 WS
1 PA0.1; 1 WS;2 PA0; 2 WS
1 PA0.2; 1 WS;2 PA7; 2 WS
1 PA0.3; 1 WS;2 PA0; 2 WS
1 PA0.4; 1 WS;2 PA7; 2 WS
1 PA0.5; 1 WS;2 PA0; 2 WS
1 PA0.6; 1 WS;2 PA7; 2 WS
1 PA0.7; 1 WS;2 PA0; 2 WS
1 PA0.8; 1 WS;2 PA7; 2 WS
1 PA0.9; 1 WS;2 PA0; 2 WS
1 PA1; 1 WS;2 PA7; 2 WS
1 PA1.1; 1 WS;2 PA0; 2 WS
1 PA1.2; 1 WS;2 PA7; 2 WS
1 PA1.3; 1 WS;2 PA0; 2 WS
1 PA1.4; 1 WS;2 PA7; 2 WS
1 PA1.5; 1 WS;2 PA0; 2 WS
1 PA1.6; 1 WS;2 PA7; 2 WS
1 PA1.7; 1 WS;2 PA0; 2 WS
1 PA1.8; 1 WS;2 PA7; 2 WS

1 PA1.9; 1 WS;2 PA0; 2 WS
1 PA2; 1 WS;2 PA7; 2 WS
1 PA2.1; 1 WS;2 PA0; 2 WS
1 PA2.2; 1 WS;2 PA7; 2 WS
1 PA2.3; 1 WS;2 PA0; 2 WS
1 PA2.4; 1 WS;2 PA7; 2 WS
1 PA2.5; 1 WS;2 PA0; 2 WS
1 PA2.6; 1 WS;2 PA7; 2 WS
1 PA2.7; 1 WS;2 PA0; 2 WS
1 PA2.8; 1 WS;2 PA7; 2 WS
1 PA2.9; 1 WS;2 PA0; 2 WS
1 PA3; 1 WS;2 PA7; 2 WS
1 PA3.1; 1 WS;2 PA0; 2 WS
1 PA3.2; 1 WS;2 PA7; 2 WS
1 PA3.3; 1 WS;2 PA0; 2 WS
1 PA3.4; 1 WS;2 PA7; 2 WS
1 PA3.5; 1 WS;2 PA0; 2 WS
1 PA3.6; 1 WS;2 PA7; 2 WS
1 PA3.7; 1 WS;2 PA0; 2 WS
1 PA3.8; 1 WS;2 PA7; 2 WS
1 PA3.9; 1 WS;2 PA0; 2 WS
1 PA4; 1 WS;2 PA7; 2 WS
1 PA4.1; 1 WS;2 PA0; 2 WS
1 PA4.2; 1 WS;2 PA7; 2 WS
1 PA4.3; 1 WS;2 PA0; 2 WS
1 PA4.4; 1 WS;2 PA7; 2 WS
1 PA4.5; 1 WS;2 PA0; 2 WS
1 PA4.6; 1 WS;2 PA7; 2 WS
1 PA4.7; 1 WS;2 PA0; 2 WS
1 PA4.8; 1 WS;2 PA7; 2 WS
1 PA4.9; 1 WS;2 PA0; 2 WS
1 PA5; 1 WS;2 PA7; 2 WS
1 PA5.1; 1 WS;2 PA0; 2 WS
1 PA5.2; 1 WS;2 PA7; 2 WS
1 PA5.3; 1 WS;2 PA0; 2 WS
1 PA5.4; 1 WS;2 PA7; 2 WS
1 PA5.5; 1 WS;2 PA0; 2 WS
1 PA5.6; 1 WS;2 PA7; 2 WS
1 PA5.7; 1 WS;2 PA0; 2 WS
1 PA5.8; 1 WS;2 PA7; 2 WS
1 PA5.9; 1 WS;2 PA0; 2 WS
1 PA6; 1 WS;2 PA7; 2 WS
1 PA6.1; 1 WS;2 PA0; 2 WS
1 PA6.2; 1 WS;2 PA7; 2 WS
1 PA6.3; 1 WS;2 PA0; 2 WS
1 PA6.4; 1 WS;2 PA7; 2 WS

1 PA6.5; 1 WS;2 PA0; 2 WS
1 PA6.6; 1 WS;2 PA7; 2 WS
1 PA6.7; 1 WS;2 PA0; 2 WS
1 PA6.8; 1 WS;2 PA7; 2 WS
1 PA6.9; 1 WS;2 PA0; 2 WS
1 PA7; 1 WS;2 PA7; 2 WS
1 PA7.1; 1 WS;2 PA0; 2 WS
1 PA7.2; 1 WS;2 PA7; 2 WS
1 PA7.3; 1 WS;2 PA0; 2 WS
1 PA7.4; 1 WS;2 PA7; 2 WS
1 PA7.5; 1 WS;2 PA0; 2 WS
1 PA7.6; 1 WS;2 PA7; 2 WS
1 PA7.7; 1 WS;2 PA0; 2 WS
1 PA7.8; 1 WS;2 PA7; 2 WS
1 PA7.9; 1 WS;2 PA0; 2 WS
1 PA8; 1 WS;2 PA7; 2 WS
1 PA8.1; 1 WS;2 PA0; 2 WS
1 PA8.2; 1 WS;2 PA7; 2 WS
1 PA8.3; 1 WS;2 PA0; 2 WS
1 PA8.4; 1 WS;2 PA7; 2 WS
1 PA8.5; 1 WS;2 PA0; 2 WS
1 PA8.6; 1 WS;2 PA7; 2 WS
1 PA8.7; 1 WS;2 PA0; 2 WS
1 PA8.8; 1 WS;2 PA7; 2 WS
1 PA8.9; 1 WS;2 PA0; 2 WS
1 PA9; 1 WS;2 PA7; 2 WS
1 PA9.1; 1 WS;2 PA0; 2 WS
1 PA9.2; 1 WS;2 PA7; 2 WS
1 PA9.3; 1 WS;2 PA0; 2 WS
1 PA9.4; 1 WS;2 PA7; 2 WS
1 PA9.5; 1 WS;2 PA0; 2 WS
1 PA9.6; 1 WS;2 PA7; 2 WS
1 PA9.7; 1 WS;2 PA0; 2 WS
1 PA9.8; 1 WS;2 PA7; 2 WS
1 PA9.9; 1 WS;2 PA0; 2 WS
1 PA10; 1 WS;2 PA7; 2 WS
1 PA10.1; 1 WS;2 PA0; 2 WS
1 PA10.2; 1 WS;2 PA7; 2 WS
1 PA10.3; 1 WS;2 PA0; 2 WS
1 PA10.4; 1 WS;2 PA7; 2 WS
1 PA10.5; 1 WS;2 PA0; 2 WS
1 PA10.6; 1 WS;2 PA7; 2 WS
1 PA10.7; 1 WS;2 PA0; 2 WS
1 PA10.8; 1 WS;2 PA7; 2 WS
1 PA10.9; 1 WS;2 PA0; 2 WS
1 PA11; 1 WS;2 PA7; 2 WS

1 PA11.1; 1 WS;2 PA0; 2 WS
1 PA11.2; 1 WS;2 PA7; 2 WS
1 PA11.3; 1 WS;2 PA0; 2 WS
1 PA11.4; 1 WS;2 PA7; 2 WS
1 PA11.5; 1 WS;2 PA0; 2 WS
1 PA11.6; 1 WS;2 PA7; 2 WS
1 PA11.7; 1 WS;2 PA0; 2 WS
1 PA11.8; 1 WS;2 PA7; 2 WS
1 PA11.9; 1 WS;2 PA0; 2 WS
1 PA12; 1 WS;2 PA7; 2 WS
1 PA12.1; 1 WS;2 PA0; 2 WS
1 PA12.2; 1 WS;2 PA7; 2 WS
1 PA12.3; 1 WS;2 PA0; 2 WS
1 PA12.4; 1 WS;2 PA7; 2 WS
1 PA12.5; 1 WS;2 PA0; 2 WS
1 PA12.6; 1 WS;2 PA7; 2 WS
1 PA12.7; 1 WS;2 PA0; 2 WS
1 PA12.8; 1 WS;2 PA7; 2 WS
1 PA12.9; 1 WS;2 PA0; 2 WS
1 PA13; 1 WS;2 PA7; 2 WS
1 PA13.1; 1 WS;2 PA0; 2 WS
1 PA13.2; 1 WS;2 PA7; 2 WS
1 PA13.3; 1 WS;2 PA0; 2 WS
1 PA13.4; 1 WS;2 PA7; 2 WS
1 PA13.5; 1 WS;2 PA0; 2 WS
1 PA13.6; 1 WS;2 PA7; 2 WS
1 PA13.7; 1 WS;2 PA0; 2 WS
1 PA13.8; 1 WS;2 PA7; 2 WS
1 PA13.9; 1 WS;2 PA0; 2 WS
1 PA14; 1 WS;2 PA7; 2 WS
1 PA14.1; 1 WS;2 PA0; 2 WS
1 PA14.2; 1 WS;2 PA7; 2 WS
1 PA14.3; 1 WS;2 PA0; 2 WS
1 PA14.4; 1 WS;2 PA7; 2 WS
1 PA14.5; 1 WS;2 PA0; 2 WS
1 PA14.6; 1 WS;2 PA7; 2 WS
1 PA14.7; 1 WS;2 PA0; 2 WS
1 PA14.8; 1 WS;2 PA7; 2 WS
1 PA14.9; 1 WS;2 PA0; 2 WS
1 PA15; 1 WS;2 PA7; 2 WS
1 PA15.1; 1 WS;2 PA0; 2 WS
1 PA15.2; 1 WS;2 PA7; 2 WS
1 PA15.3; 1 WS;2 PA0; 2 WS
1 PA15.4; 1 WS;2 PA7; 2 WS
1 PA15.5; 1 WS;2 PA0; 2 WS
1 PA15.6; 1 WS;2 PA7; 2 WS

1 PA15.7; 1 WS;2 PA0; 2 WS
1 PA15.8; 1 WS;2 PA7; 2 WS
1 PA15.9; 1 WS;2 PA0; 2 WS
1 PA16; 1 WS;2 PA7; 2 WS
1 PA16.1; 1 WS;2 PA0; 2 WS
1 PA16.2; 1 WS;2 PA7; 2 WS
1 PA16.3; 1 WS;2 PA0; 2 WS
1 PA16.4; 1 WS;2 PA7; 2 WS
1 PA16.5; 1 WS;2 PA0; 2 WS
1 PA16.6; 1 WS;2 PA7; 2 WS
1 PA16.7; 1 WS;2 PA0; 2 WS
1 PA16.8; 1 WS;2 PA7; 2 WS
1 PA16.9; 1 WS;2 PA0; 2 WS
1 PA17; 1 WS;2 PA7; 2 WS
1 PA17.1; 1 WS;2 PA0; 2 WS
1 PA17.2; 1 WS;2 PA7; 2 WS
1 PA17.3; 1 WS;2 PA0; 2 WS
1 PA17.4; 1 WS;2 PA7; 2 WS
1 PA17.5; 1 WS;2 PA0; 2 WS
1 PA17.6; 1 WS;2 PA7; 2 WS
1 PA17.7; 1 WS;2 PA0; 2 WS
1 PA17.8; 1 WS;2 PA7; 2 WS
1 PA17.9; 1 WS;2 PA0; 2 WS
1 PA18; 1 WS;2 PA7; 2 WS
1 PA18.1; 1 WS;2 PA0; 2 WS
1 PA18.2; 1 WS;2 PA7; 2 WS
1 PA18.3; 1 WS;2 PA0; 2 WS
1 PA18.4; 1 WS;2 PA7; 2 WS
1 PA18.5; 1 WS;2 PA0; 2 WS
1 PA18.6; 1 WS;2 PA7; 2 WS
1 PA18.7; 1 WS;2 PA0; 2 WS
1 PA18.8; 1 WS;2 PA7; 2 WS
1 PA18.9; 1 WS;2 PA0; 2 WS
1 PA19; 1 WS;2 PA7; 2 WS
1 PA19.1; 1 WS;2 PA0; 2 WS
1 PA19.2; 1 WS;2 PA7; 2 WS
1 PA19.3; 1 WS;2 PA0; 2 WS
1 PA19.4; 1 WS;2 PA7; 2 WS
1 PA19.5; 1 WS;2 PA0; 2 WS
1 PA19.6; 1 WS;2 PA7; 2 WS
1 PA19.7; 1 WS;2 PA0; 2 WS
1 PA19.8; 1 WS;2 PA7; 2 WS
1 PA19.9; 1 WS;2 PA0; 2 WS
1 PA20; 1 WS;2 PA7; 2 WS
QP

f. RECTANGULAR MESH 500 MICRONS SPACING (7X20)

3EP
3 PA 23; 3 WS
1 PA0; 1 WS; 2 PA20; 2 WS
1 PA0.5; 1 WS; 2 PA0; 2 WS
1 PA1; 1 WS; 2 PA20; 2 WS
1 PA1.5; 1 WS; 2 PA0; 2 WS
1 PA2; 1 WS; 2 PA20; 2 WS
1 PA2.5; 1 WS; 2 PA0; 2 WS
1 PA3; 1 WS; 2 PA20; 2 WS
1 PA3.5; 1 WS; 2 PA0; 2 WS
1 PA4; 1 WS; 2 PA20; 2 WS
1 PA4.5; 1 WS; 2 PA0; 2 WS
1 PA5; 1 WS; 2 PA20; 2 WS
1 PA5.5; 1 WS; 2 PA0; 2 WS
1 PA6; 1 WS; 2 PA20; 2 WS
1 PA6.5; 1 WS; 2 PA0; 2 WS
1 PA7; 1 WS; 2 PA20; 2 WS
1 PA 0; 1 WS; 2 PA0; 2 WS
3 PA 22.5; 3 WS
1 PA0; 1 WS; 2 PA0; 2 WS
1 PA7; 1 WS; 2 PA0.5; 2 WS
1 PA0; 1 WS; 2 PA1; 2 WS
1 PA7; 1 WS; 2 PA1.5; 2 WS
1 PA0; 1 WS; 2 PA2; 2 WS
1 PA7; 1 WS; 2 PA2.5; 2 WS
1 PA0; 1 WS; 2 PA3; 2 WS
1 PA7; 1 WS; 2 PA3.5; 2 WS
1 PA0; 1 WS; 2 PA4; 2 WS
1 PA7; 1 WS; 2 PA4.5; 2 WS
1 PA0; 1 WS; 2 PA5; 2 WS
1 PA7; 1 WS; 2 PA5.5; 2 WS
1 PA0; 1 WS; 2 PA6; 2 WS
1 PA7; 1 WS; 2 PA6.5; 2 WS
1 PA0; 1 WS; 2 PA7; 2 WS
1 PA7; 1 WS; 2 PA7.5; 2 WS
1 PA0; 1 WS; 2 PA8; 2 WS
1 PA7; 1 WS; 2 PA8.5; 2 WS
1 PA0; 1 WS; 2 PA9; 2 WS
1 PA7; 1 WS; 2 PA9.5; 2 WS
1 PA0; 1 WS; 2 PA10; 2 WS
1 PA7; 1 WS; 2 PA10.5; 2 WS

1 PA0; 1 WS; 2 PA11; 2 WS
1 PA7; 1 WS; 2 PA11.5; 2 WS
1 PA0; 1 WS; 2 PA12; 2 WS
1 PA7; 1 WS; 2 PA12.5; 2 WS
1 PA0; 1 WS; 2 PA13; 2 WS
1 PA7; 1 WS; 2 PA13.5; 2 WS
1 PA0; 1 WS; 2 PA14; 2 WS
1 PA7; 1 WS; 2 PA14.5; 2 WS
1 PA0; 1 WS; 2 PA15; 2 WS
1 PA7; 1 WS; 2 PA15.5; 2 WS
1 PA0; 1 WS; 2 PA16; 2 WS
1 PA7; 1 WS; 2 PA16.5; 2 WS
1 PA0; 1 WS; 2 PA17; 2 WS
1 PA7; 1 WS; 2 PA17.5; 2 WS
1 PA0; 1 WS; 2 PA18; 2 WS
1 PA7; 1 WS; 2 PA18.5; 2 WS
1 PA0; 1 WS; 2 PA19; 2 WS
1 PA7; 1 WS; 2 PA19.5; 2 WS
1 PA 0; 1 WS; 2 PA20; 2 WS
1 PA0; 1 WS; 2 PA0; 2 WS
QP

g. CIRCLE FIRST LAYER WITH 20 MICRONS SPACING

1 PA0; 1 WS;2 PA5; 2 WS
1 PA0.01; 1 WS;2 PA5.31; 2 WS; 2 PA4.68;2 WS
1 PA0.03; 1 WS;2 PA4.45; 2 WS; 2 PA5.54;2 WS
1 PA0.05; 1 WS;2 PA5.7; 2 WS; 2 PA4.29;2 WS
1 PA0.07; 1 WS;2 PA4.16; 2 WS; 2 PA5.83;2 WS
1 PA0.09; 1 WS;2 PA5.94; 2 WS; 2 PA4.05;2 WS
1 PA0.11; 1 WS;2 PA3.95; 2 WS; 2 PA6.04;2 WS
1 PA0.13; 1 WS;2 PA6.13; 2 WS; 2 PA3.86;2 WS
1 PA0.15; 1 WS;2 PA3.78; 2 WS; 2 PA6.21;2 WS
1 PA0.17; 1 WS;2 PA6.29; 2 WS; 2 PA3.7;2 WS
1 PA0.19; 1 WS;2 PA3.63; 2 WS; 2 PA6.36;2 WS
1 PA0.21; 1 WS;2 PA6.43; 2 WS; 2 PA3.56;2 WS
1 PA0.23; 1 WS;2 PA3.5; 2 WS; 2 PA6.49;2 WS
1 PA0.25; 1 WS;2 PA6.56; 2 WS; 2 PA3.43;2 WS
1 PA0.27; 1 WS;2 PA3.37; 2 WS; 2 PA6.62;2 WS
1 PA0.29; 1 WS;2 PA6.67; 2 WS; 2 PA3.32;2 WS
1 PA0.31; 1 WS;2 PA3.26; 2 WS; 2 PA6.73;2 WS
1 PA0.33; 1 WS;2 PA6.78; 2 WS; 2 PA3.21;2 WS

1 PA0.35; 1 WS;2 PA3.16; 2 WS; 2 PA6.83;2 WS
1 PA0.37; 1 WS;2 PA6.88; 2 WS; 2 PA3.11;2 WS
1 PA0.39; 1 WS;2 PA3.06; 2 WS; 2 PA6.93;2 WS
1 PA0.41; 1 WS;2 PA6.98; 2 WS; 2 PA3.01;2 WS
1 PA0.43; 1 WS;2 PA2.97; 2 WS; 2 PA7.02;2 WS
1 PA0.45; 1 WS;2 PA7.07; 2 WS; 2 PA2.92;2 WS
1 PA0.47; 1 WS;2 PA2.88; 2 WS; 2 PA7.11;2 WS
1 PA0.49; 1 WS;2 PA7.15; 2 WS; 2 PA2.84;2 WS
1 PA0.51; 1 WS;2 PA2.8; 2 WS; 2 PA7.19;2 WS
1 PA0.53; 1 WS;2 PA7.24; 2 WS; 2 PA2.75;2 WS
1 PA0.55; 1 WS;2 PA2.72; 2 WS; 2 PA7.27;2 WS
1 PA0.57; 1 WS;2 PA7.31; 2 WS; 2 PA2.68;2 WS
1 PA0.59; 1 WS;2 PA2.64; 2 WS; 2 PA7.35;2 WS
1 PA0.61; 1 WS;2 PA7.39; 2 WS; 2 PA2.6;2 WS
1 PA0.63; 1 WS;2 PA2.57; 2 WS; 2 PA7.42;2 WS
1 PA0.65; 1 WS;2 PA7.46; 2 WS; 2 PA2.53;2 WS
1 PA0.67; 1 WS;2 PA2.49; 2 WS; 2 PA7.5;2 WS
1 PA0.69; 1 WS;2 PA7.53; 2 WS; 2 PA2.46;2 WS
1 PA0.71; 1 WS;2 PA2.43; 2 WS; 2 PA7.56;2 WS
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1 PA0.85; 1 WS;2 PA7.78; 2 WS; 2 PA2.21;2 WS
1 PA0.87; 1 WS;2 PA2.18; 2 WS; 2 PA7.81;2 WS
1 PA0.89; 1 WS;2 PA7.84; 2 WS; 2 PA2.15;2 WS
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1 PA0.93; 1 WS;2 PA7.9; 2 WS; 2 PA2.09;2 WS
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QP

h. CIRCLE MESH LAYER WITH 500 MICRONS SPACING (10 X 10)

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1 PA8.5; 1 WS; 2 PA0.93; 2 WS; 2 PA13.06; 2 WS
1 PA9; 1 WS; 2 PA12.74; 2 WS; 2 PA1.25; 2 WS
1 PA9.5; 1 WS; 2 PA1.63; 2 WS; 2 PA12.36; 2 WS
1 PA10; 1 WS; 2 PA11.89; 2 WS; 2 PA2.1; 2 WS
3 PA 19.99; 3 WS
2 PA 0; 2 WS; 1 PA11.89; 1 WS ; 1 PA2.1; 1 WS
2 PA 0.5; 2 WS; 1 PA1.63; 1 WS ; 1 PA12.36; 1 WS
2 PA 1; 2 WS; 1 PA12.74; 1 WS ; 1 PA1.25; 1 WS
2 PA 1.5; 2 WS; 1 PA0.93; 1 WS ; 1 PA13.06; 1 WS
2 PA 2; 2 WS; 1 PA13.32; 1 WS ; 1 PA0.67; 1 WS
2 PA 2.5; 2 WS; 1 PA0.46; 1 WS ; 1 PA13.53; 1 WS
2 PA 3; 2 WS; 1 PA13.7; 1 WS ; 1 PA0.29; 1 WS
2 PA 3.5; 2 WS; 1 PA0.16; 1 WS ; 1 PA13.83; 1 WS

2 PA 4; 2 WS; 1 PA13.92; 1 WS ; 1 PA0.07; 1 WS
2 PA 4.5; 2 WS; 1 PA0.01; 1 WS ; 1 PA13.98; 1 WS
2 PA 5; 2 WS; 1 PA14; 1 WS ; 1 PA0; 1 WS
2 PA 5.5; 2 WS; 1 PA0.01; 1 WS ; 1 PA13.98; 1 WS
2 PA 6; 2 WS; 1 PA13.92; 1 WS ; 1 PA0.07; 1 WS
2 PA 6.5; 2 WS; 1 PA0.16; 1 WS ; 1 PA13.83; 1 WS
2 PA 7; 2 WS; 1 PA13.7; 1 WS ; 1 PA0.29; 1 WS
2 PA 7.5; 2 WS; 1 PA0.46; 1 WS ; 1 PA13.53; 1 WS
2 PA 8; 2 WS; 1 PA13.32; 1 WS ; 1 PA0.67; 1 WS
2 PA 8.5; 2 WS; 1 PA0.93; 1 WS ; 1 PA13.06; 1 WS
2 PA 9; 2 WS; 1 PA12.74; 1 WS ; 1 PA1.25; 1 WS
2 PA 9.5; 2 WS; 1 PA1.63; 1 WS ; 1 PA12.36; 1 WS
2 PA 10; 2 WS; 1 PA11.89; 1 WS ; 1 PA2.1; 1 WS
QP

i. CIRCLE MESH STRUCTURE WITH 10 MICRONS SPACING

7EP
2PA 7;2 WS;1PA 0;1 WS;1PA 1;1 WS
2PA10;2WS;2PA4;2WS;1PA2;1WS
2PA3;2WS;2PA11;2WS;1PA3;1WS
2PA11.6;2WS;2PA2.4;2WS;1PA4;1WS
2PA2.10;2WS;2PA11.89;2WS;1PA5;1WS
2PA12;2WS;2PA2;2WS;1PA6;1WS
2PA2.10;2WS;2PA11.89;2WS;1PA7;1WS
2PA11.6;2WS;2PA2.4;2WS;1PA8;1WS
2PA3;2WS;2PA11;2WS;1PA9;1WS
2PA10;2WS;2PA4;2WS;1PA10;1WS
2PA7;2WS
2PA2;2WS;1PA5;1WS;2PA3;2WS
1PA8;1WS;1PA2;1WS;2PA4;2WS
1PA1;1WS;1PA9;1WS;2PA5;2WS
1PA9.6;1WS;1PA0.4;1WS;2PA6;2WS
1PA0.1;1WS;1PA9.9;1WS;2PA7;2WS
1PA10;1WS;1PA0;1WS;2PA8;2WS
1PA0.1;1WS;1PA9.9;1WS;2PA9;2WS
1PA9.6;1WS;1PA0.4;1WS;2PA10;2WS
1PA1;1WS;1PA9;1WS;2PA11;2WS
1PA8;1WS;1PA2;1WS;2PA12;2WS
QP