Electrospinning or electrostatic fiber spinning is an emerging technology that requires electric fields for the formation of non-woven materials that can be used in clothing, high-performance filters, separation membranes, scaffolds in tissue engineering, reinforcement in composite materials, templates for the preparation of functional nanotubes, and many others applications [1]. Polycaprolactone, a bioresorbable polymer, was electrospun into nanofibers in the form of nonwoven scaffold structures for tissue engineering. Such tissue-engineered structures will be valuable in the repair and replacement of diseased liver tissue.

In this paper, preliminary results are presented using the electrospinning technique as a new nano-fibers process. We investigated some of the variables affecting the behavior of the electrical fluid jet during the electrospinning process (fluid properties and operating parameters). By controlling the spinning parameters, fibers of polymers as small 140 - 500 nm size diameters can be obtained using this technique.

One of the objectives of this research is to construct the operating diagrams or phase diagrams that can give a better understanding of the variables affecting the behavior of the electrified fluid jet during electrospinning process. These diagrams can link the processing conditions (operating parameters) and polymer fluid properties to the structure and properties of the final product, electrospun nanofibers, to use this information to correlate yarns orientation by electrospinning, and to increase the productivity of the alternative process so that it may be scale up to a large production scale. The ultimate goal of this research will be supercritical carbon dioxide assisted electrospinning process. A modified Suprex PrepMaster supercritical fluid extractor will be used to run the electrospinning experiments [2].

INTRODUCTION

Current research has pointed to the use of electrospun scaffolds for novel tissue engineering. Electrospinning enables a semi-nonwoven scaffold to be constructed from nanofibers. This process allows for the process of spinning to be skipped altogether. The polymer can be spun into a scaffold directly from a solution. Not only can the solution be spun directly, but also the fibers are smaller in diameter than can be achieved in any other process [3].

This process is breaking through in the field of tissue engineering to make nanofibers. The smaller diameters help make the scaffold have numerous smaller pores. Throughout the field of tissue engineering, the pore size has been determined to impact the success of cell proliferation more than any other factor. With nanofibers, the electrospun scaffolds are more compact when
compared to any other scaffold that has been fabricated from a nonwoven process. This means, the smaller pore size allows for more cell proliferation [3].

Many researchers have shown that the smaller diameters help in cell growth. One study made note of the irregular surfaces of the fibers themselves. This is from the irregularity of the electrospinning process in general. It is next to impossible to repeat an identical electrospun scaffold [3]. What the smaller diameters and pore sizes do is to allow for the cells to penetrate the surface of the scaffold and grow on the inside of the scaffold in a more 3-D manner [4]. This is ideal for the complete encapsulation of the polymer scaffold. When the PCL is absorbed by the natural mechanisms of the body, a full form of the cell growth will be left behind. Surface growth is very common in many tissue-engineering ventures. This can be a positive and a negative, depending upon the objectives of the study. Any growth indicates a degree of biocompatibility and affinity for the types of cells that are being cultured. Total encapsulation shows the ultimate level of cell affinity and that the structure is one that will facilitate the growth wanted. Surface growth indicates that cell affinity has been achieved, but if tissue growth is the goal, then more research is needed.

Other studies have examined the use of electrospun meshes for tissue engineering use. Many have found the nanofibers produced to be helpful in the field and allow cells to easily develop adhesions to the surfaces. The smaller interstices developed in the matrices with the nanofibers provide a more hospitable environment and is more conducive for cell growth. These smaller interstices are what make electrospinning a novel process in the field of tissue engineering. Previously, using a nonwoven mesh and even conventional textile materials (weaves, knits, and braids) were characterized by the pore size. What helps the proliferation of the cells is the porosity of the material [3].

APPLICATIONS AND PREVIOUS RESEARCH

Patients with liver disease have little options. Many previous studies have attempted to use tissue-engineering techniques to develop synthetic liver tissue. These attempts have resulted in many different approaches. Across the board, the most successful approach has been to use a nonwoven mesh to encourage hepatocyte proliferation. Other studies have found polycaprolactone for tissue engineering. Mammary cells, stem cells, cardiomyocytes, and keratinocytes have all used PCL for tissue engineering studies [4-7]. Pore size effects tissue engineering scaffolds greatly. In order to develop cell-to-cell functionality the pore size needs to be the optimal size so the cells will grow in the pores instead of along the fibers. Once the extracellular matrices (ECMs) are formed along the fibers, the scaffold will be able to resorb and ECMs will be able to stand-alone. The meshes are developed with nanofibers that should be able to support the cell cultures with the best porosity [3].

Tissue engineering has been known to employ scaffold support systems to guide cellular growth. Cellular regeneration needs the scaffolds to guide the growth and with the optimal pore size and three-dimensional (3-D) shape, extracellular matrices can be achieved. Cell proliferation and adhesion are dependent on the specifications of the scaffolds, especially the porosity. The pore size is a vital aspect of guiding the cell growth and differentiation. Pore size can be controlled in many different ways, including the specifications of the fabrication process, phase separation, emulsion/freezing, solvent selection and membrane lamination. When using a bioreabsorbable polymer, the cells will then be able to stand-alone as the polymer eventually degrades. These tissue structures are on the forefront of the biotechnology field and will revolutionize organ transplantation [5]. When a patient is diagnosed with liver disease, there are
limited treatment options. Different drug treatments are available for certain types of liver disease, including primary biliary cirrhosis and hepatitis C. However, liver transplantation is needed for most severe conditions of liver disease. Using electrospinning techniques, nanofibers can be formed in nonwoven meshes. These meshes have pore sizes much smaller than conventional techniques of textile fabrication and should be more able to develop extra-cellular matrices. In keeping the main objective in mind, developing meshes to support hepatocyte growth, we embark on this study [3].

**Polymers**

Polycaprolactone has been used in early studies for use in the field of tissue engineering. Also, PCL fibers have been proven effective for cell proliferation, especially under the cultures of MCF-7 mammary carcinoma [5]. The cultures of the mammary cells had a slow growth during the first few days, however when compared to the control groups, the PCL does improve the cell growth kinetic rates over time. Yet another study shows the use of PCL for tissue engineering. PCL has become very beneficial in the development of tissue structures for bone growth. Previously, poly(DL-lactide-co-glycolide) (PLGA) has been used for developing scaffolds for bone cell growth. In particular, bone marrow was harvested from rats and used in this study [4]. Mesenchymal stem cells, from the bone marrow, were used upon PCL electrospun scaffolds. The PLGA used previously had only shown limited topical growth and 40% shrinkage in length [4]. The usefulness of the PCL can be attributed to the higher glass transition temperature (-70°C to -60°C) [3].

Overall, PCL has been proven effective for the use in tissue engineering settings. The biocompatibility with the body has been proven. The fact the polymer is bioresorbable helps with numerous tissue engineering factors. With bioresorbable polymers, the fibers provide a back support for the cell growth. After time in the body, the fibers essentially dissolve and leave the cell growth (sometimes in tissue form) in a pure form within the body. Another positive of using polymer, like PCL, is that the body will be more apt to accept the fibers and not cause a potentially devastating immune response cascade. PCL has not been used at much as poly-L-lactic acid (PLLA), but their similarity helps with many aspects and its lower glass transition temperature has potential to allow for even more applications [3].

**Pore Size**

The amount of space in the mesh versus the amount of open space is what determines the success of the tissue engineering. For standard polyethylene terephthalate (PET) with a typical collagen coating, high porosity results in large aggregates forming. The cells will typically grow along the branches of the fibers and cling to the familiarity of the collagen. What the engineer wants to see happen are the cells branching further out and forming aggregates in the open spaces, or pores of the mesh. When cells form aggregates without clinging to a scaffold, in the pore, they are creating cell-to-cell functionality and more tissue-like growths. A particular study shows this occurring with trophoblast cells (ED27 cells) [8].

What many researchers have witnessed is a phenomenon with the pore size of specific matrices. There seems to be no specific standard for the optimal cell growth. However, this property of the scaffold has major ramifications on the cell proliferation and differentiation. Observations have concluded that porosity tends to be cell specific and fiber specific. When a match has been made between what fiber is most compatible with a specific cell type, the match will typically positive results and the formation of aggregates. In order to see the cell-to-cell
functionality, the pore size must be perfect. Smaller pore sizes tend to yield more cell growth, but it stays mainly along the fibers. Larger pore sizes will yield the formation of aggregates, but not always. The advent of nanofibers lends researchers to believe that the pores will be even smaller and the circumferences of the fibers so much smaller, the cells will find a more compatible environment [3].

PCL and skin grafts (human osteoblasts, keratinocytes, and fibroblasts)

One study in particular shows the use of PCL for skin grafts as a mix of collagen and PCL in a composite film. The objective was to monitor the degradation of the collagen in a cultured environment and monitor the cell adhesion over several days. The study followed the behavior of several variations of the mixture of PCL and collagen. The resorption rate of about one year of the PCL shows promise for the use for developing skin grafts for burn victims. The PCL phase also provides the necessary stability for a graft of this nature. Unique for this study, is the ability to control the cell growth and adhesion by the amount of collagen in the initial mixture with PCL. Solutions with a higher PCL content show a cellular matrix with a higher cell adhesion. This also suggests there can be a way to manipulate the PCL/collagen solution within the scaffolds to develop a drug delivery system to continue the healing of the wound site. The resorption of the PCL over a year’s time also aids in the healing process of the wound site and makes the wound more apt to heal effectively [3, 6].

PCL and contractile cardiac grafts

Electrospun PCL solutions were used to develop a nonwoven mesh upon which cultures of rat ventricles cells grew. The PCL was used in a similar manner to the current research we have conducted. A 10% by weight solution was made using PCL (Aldrich, Mw = 80,000) in chloroform (1): methanol (1) solution. The cardiomyocytes were taken from newborn rats and used in the study to examine the potential of a myocardial patch for patients who have suffered extensive infarction. The meshes were suspended on wire rings, which acted as a passive support system. The scaffolds were initially coated with collagen to further support the cell growth. What this study shows is the potential in cell growth under a similar electrospun meshes. After a 14-day culture, the study shows the cardiomyocytes were shown to have retained their character and have been overrun by fibroblasts. The cell growth was very promising and warrants further investigation [3].

The scaffolds themselves were shown to be soft enough to support the growth the fragile cell type and yield to their growth, while remaining stable enough for handling and supporting the cell system. The porosity of the scaffolds proved to be of the optimal pore size. The claim made by this particular study is that the PCL scaffolds’ topography resembled very that of an extracellular matrix. The wire ring was beneficial for the meshes in that it provided extra tension to keep the mesh in tact during the two weeks of cultures and enable the mesh to retain its shape throughout [3].

What is very encouraging about this study is that the cells were able to penetrate the 10 µm of thickness of the mesh. As the cardiac region is a particularly muscular, the researchers have hypothesized there is possibility to develop layers of muscle by stacking different synthetic patches [3, 7].
I. MATERIALS AND PROCEDURES

I.1. Materials

We have investigated polycaprolactone (Mw = 60,000 g/mol) purchased from Aldrich with a melting point of 60 °C and a density of 1.145 g/cm³. All solvents (acetone, chloroform and methanol) were stored and used as received. Scanning electron microscopy (SEM) was conducted in Analytical Instrumentation Facility at North Carolina State University (NCSU) using the Hitachi S-3200N. Polymer solutions were stored at room temperature and also the electrospinning experiments were performed at room temperature in air. The hepatocyte cultures were delivered to the Transplant Lab in the Microbiology Research Building (Medical School) at University of Chapel Hill, NC.

I.2. Electrospinning setup and Procedures

All experiments on electrospinning were carried out with a homemade laboratory apparatus designed and constructed by researchers at NCSU, College of Textiles. The apparatus has similar basic major pieces of equipment (plates, syringe pump and voltage supply) used in previous works [9-11]. A schematic illustration of the experimental setup is shown in Figure 1. The electrospun nano-fibers were collected on the lower (bottom) plate that is covered with aluminum foil that served as a cathode. The polymer collected on the aluminum foil (Al) is used for later microscopy analysis. All scaffolds were collected and transferred to the bottom of a culture dish as shown in Figure 2. Scanning electron microscopy (SEM) was used to determine the fiber diameters after coating the polymer samples with a layer (150 Å thick) of gold-palladium (Au/Pd).

II. PRELIMINARY RESULTS AND DISCUSSION

II.1. Preparation of samples

After collecting 11 samples from the chloroform (3): methanol (1) solution (10% by weight), samples were prepared and transferred to the Transplant Lab for hepatocyte cultures. The collection of the meshes was on tin foil and the polymer solution seemed to stick to the tin foil. Thus, the samples, which were about 50 mm in diameter in concentric circles, were cut into circles 35 mm in diameter to fit into the Petri dishes for cell cultures. Because of the fragile nature of the meshes and the apparent relationship between the meshes and the foil, the samples were cut with the foil still attached. All samples were placed in the dishes, with the foil on the bottom of the dish. The samples were labeled and hand-delivered to University of Chapel Hill-Medical School, Chapel Hill, NC.

II.2. SEM of samples

The SEM micrographs show the accumulation of the layers during the electrospinning process. The depth of the polymer layers also vary significantly between the samples left under the capillary for different amounts of collection time. Figure 3B shows the depth the scaffolds. However, many of the SEM micrographs show an accumulation of beading. While the beading is evident, there is also a very large differentiation of fiber diameter. While some larger fibers show a diameter of almost 800 nm, there are many others that have diameters about 200-250 nm. The pore size is also very important for the scaffold. However, each sample seems to exhibit different properties when it comes to porosity. Figure 4 shows a mixture of larger and smaller pore sizes, which was one of the goals in constructing the scaffolds. Figure 4 also shows the
irregularity between different fibers. Some fibers along the middle layers seem to have fused to other fibers and form permanent interstices.

**Supercritical Carbon Dioxide (CO₂) Assisted Electrospinning Process**

The use of supercritical fluids (carbon dioxide) in combination with electrospinning presents an unprecedented opportunity to develop new materials and processes for a wide range of areas. Fluids near and above the critical point can be used to form materials with interesting and controllable properties, including biologically active protein micro-particles; biocompatible polymer micro-spheres, for drug delivery applications, ceramic precursor powders and heat-resistant polymer fibers; and also to reduce the melt-viscosity of polymers sufficiently to allow fibers to be electrostatically pulled from an undissolved bulk polymer sample [13]. Therefore, the use of supercritical CO₂ assisted electrospinning process will start a new research area for a novel technology for the formation of nanofibers at a pilot plant scale and then to a commercial scale as polymer melts rather than polymer solutions could be used thereby increasing production throughput, minimizing the use of harmful organic solvents and reducing the power requirements of the electrospinning process when compared to conventional extrusion processes (melt spinning, dry or wet spinning).

**CONCLUSIONS AND REMARKS**

This paper summarizes preliminary results obtained during the electrospinning process. Laboratory apparatus was designed, constructed and tested with a model polymer (PCL). Range of key process variables has been identified and methods implemented for their quantifications. The preliminary SEM micrographs of electrospun fibers for PCL (10 wt.%) in chloroform (3): methanol (1) solution obtained show fiber diameters ranged from 140 to 500 nm. Also, it was observed a relationship between the fiber diameters and the amount of time spent under the capillary during the collection. The fiber diameters became smaller as the collection time under the capillary increased. What was also noticed was the fact that the fibers became more aligned as the time under the capillary increased as seen in sample 3. The more time spent under the capillary was able to make the fibers smaller in diameter and also make them more aligned instead of as randomized as seen in the other samples.

Future work will be performed on process analysis, increased productivity, and optimization of processing operating regimes (phase diagrams) to produce fibers of high orientation, well defined and controlled sized and great mechanical properties.

**REFERENCES**

Figure 1: Schematic diagram of the experimental homemade setup of the vertical parallel-plate electrospun fibers [12].
Figure 2. This figure shows how the samples were collected on tin foil. In order to transfer the samples, which were adhered to the aluminum foil, they were cut in circles from the foil and placed in the bottom of a culture dish.

Figure 3. These two SEM pictures are of sample 2. It was left under the capillary during whipping motion for 5 minutes. In B, the depth of the scaffold can be seen. Because this sample was left for the duration of 5 minutes, many PCL layers were able to accumulate.
Figure 4. Sample 3: This sample also shows the depth of the layers and minimal beading. Sample 3 was a sample that was sent to UNC-CH Medical School for further testing. The fiber diameters formed are on average between 150 - 200 nm and in some cases vary with each layer.