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ABSTRACT

ELECTROSPINNING OF BIPHASIC BIOPOLYMER AND POLYMER: A FEASIBILITY AND CHARACTERIZATION STUDY

by
Sherry Hsiu-Ying Wang

Electrospinning of polymeric materials have been experimented to achieve nanoscaled diameters. The approaches to the current study are to combine a natural material such as collagen with synthetic materials and determine if the interactions between the materials can be used in the electrospinning process. Collagen is a material of choice due to its biocompatibility. When collagen is combined with polymer, Polyethylene Oxide (PEO), the fiber diameters ranged from 150nm to one micron. The fibers produced have shown drastic phase separation. The next study involved using a duel solvent system to electrospin fibers. The polymer of choice is Poly (l-lactic acid) (PLLA) combining with collagen. This approach has given fibers with diameter ranging from 650nm to over a micron. This method of electrospinning is the least successful due to the poor solvent miscibility and evaporation. The third approach to electrospin PLLA with collagen is through the use of a single solvent Trifluoroacetic acid (TFA). This is a common solvent for collagen and PLLA and it is very volatile. The diameter of fibers produced through this process is around 350nm to 500nm. This method showed the most promise in producing excellent fiber mats. When thermal analyses are performed the results indicated rapid densification and reorientation of PLLA. This is determined to be interactions that are occurring between collagen and PLLA resulting in rapid enthalpic recovery of PLLA.

ELECTROSPINNING OF BIPHASIC BIOPOLYMER AND POLYMER: A FEASIBILITY AND CHARACTERIZATION STUDY

by
Sherry Hsiu-Ying Wang

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Master of Science in Biomedical Engineering

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APPROVAL PAGE

ELECTROSPINNING OF BIPHASIC BIOPOLYMER AND POLYMER: A FEASIBILITY AND CHARACTERIZATION STUDY

Sherry Hsiu-Ying Wang

1/19/05

Dr. Michael Jaffe — Date
Research Professor, Biomedical Engineering, NJIT

1/19/05

Dr. George Collins — Date
Adjunct Instructor, Biomedical Engineering, NJIT

1/20/05

Dr. Treena Arinzeh — Date
Assistant Professor, Biomedical Engineering, NJIT

BIOGRAPHICAL SKETCH

Author: Sherry Hsiu-Ying Wang

Degree: Master of Science

Date: January 2005

Undergraduate and Graduate Education:

- Master of Science of Biomedical Engineering,
  New Jersey Institute of Technology, Newark, NJ, 2005
- Bachelor of Science of Biomedical Engineering,
  Rutgers University, Piscataway, NJ, 2002
- Bachelor of Arts of Music,
  Rutgers University, New Brunswick, NJ, 2002

Major: Biomedical Engineering

To my dearest ones

We have climbed through some insurmountable hills

Here at the top of one hill I have spotted the next hill

Let's go!

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CHAPTER 1

INTRODUCTION

1.1 Prologue

As the importance of biomedical technologies are becoming more prominent, attention has been placed heavily on continuing discovery and development of future technologies. The possibility of using materials in replacing damaged components of the human body have been a practice for thousands of years going back to the fifth Egyptian Dynasty. The oldest known artificial limb, dating from 300 B.C., was a copper and wood leg unearthed at Capri, Italy in 1858 [1]. As time advances and because of the turmoil of war, came the need for more artificial replacements. These replacements are deemed essential and necessary to improving quality of life. In this country artificial replacements became a growing industry during the time of the Civil War where the demand was pushed for advancements and innovations in the biomaterial field. Thousands of total joint replacements are being done that have returned normal function to patients young and old. The once deadly conditions of atherosclerosis can now be alleviated by the insertion of stents or vascular grafts. These are just some examples of biomaterials in action today. With emergence of new and more powerful technologies the field of biomaterials has become diverse, where it is no longer limited to just artificial replacements for extremities, but has gone into sophisticated areas such as internal tissue replacements. The ability to replace or induce growth of new skin tissue through the aid of artificial replacements is no longer a concept but a reality.

applications in stem cell research. The structural design of artificial matrices or scaffolds that mimic the supra molecular structure and biological functions of the extracellular matrix is a key issue in tissue engineering and the development of artificial organs [2]. Because of the nature of the stem cells, it is optimal to provide an environment that is similar to the cell in size to stimulate cell growth. The cells favor a highly porous microstructure with interconnected pores and a large surface area for adhesion and differentiation [3]. In most cases a nanoscaled environment is preferred to satisfy the conditions described. One example of tissue regeneration process is that of bone regeneration. It has been discovered that the optimal growth of cells are in scaffolding with pore sizes between 100 and 350 μm and porosities of more than 90% [4]. Working with such small scales, development of nanotechnology is necessary to enhance stem cell and tissue engineering research.

The idea of electrospinning was not a new concept especially in the polymer fiber industry. The advantages of nanofibers are its high surface area to volume ratio, flexibility in surface functionalities, and excellent mechanical performance such as stiffness and tensile strength [5]. There are many other methods of producing fibers in the fiber industry that have been attempted to produce nanofibers. However, methods such as drawing, template synthesis, phase separation, self-assembly, have many limitations in producing the desired size of fibers. Electrospinning as a result is considered to be the most logical method to pursue in producing consistent quality nanofibers.

1.2 Objective

The objective of this research is to produce composite electrospun fiber of collagen and polymer. The electrospun fibers can be part of the tissue engineering approach for cell growth and differentiation in vitro and specific tissue formation. In addition, the electrospun fibers can serve as controlled drug delivery devices. An important aspect of the scaffolding is in the materials being used. The scaffolding should be of natural, biocompatible material that will not interfere with cell growth and aggregation while seeded onto the scaffolds.

The polymer selection for the study is based on their individual unique properties. Collagen as a biopolymer is the main polymer of interest in the current study. Polyethylene oxide is chosen for its hydrophilic properties. Poly(l-lactic acid) is chosen for its hydrophobic properties. Both polymers are known to be excellent biodegradable polymers that have various applications in the biomaterials industry. The solvent selection is very important in the electrospinning process. The degree of volatility is a major factor in producing small diameter fibers. The solvents used in this study ranged from the least volatile, lactic acid, to trifluoroacetic acid.

During this study three different solution forming techniques have been developed for a comparative approach to electrospin collagen and a polymer. The process for making the scaffolds varies in the different types of polymers and solvents used.

- Polyethylene oxide (PEO) and collagen using D.I. water as solvent for the non-volatile solvent electrospinning technique

- Poly (l-lactic acid) (PLLA) and collagen in chloroform and lactic acid for biphasic solvent electrospinning technique
- Poly (l-lactic acid) (PLLA) and collagen in trifluoroacetic acid for biphasic component with volatile solvent electrospinning technique

CHAPTER 2

BACKGROUND

2.1 Collagen

Collagen is the most abundant protein in the human body. There are roughly 19 types of collagen discovered with Type I being the most common. Type I collagen is the major fibrillar collagen that account for 25% of the dry protein found in mammals. Different types of collagen have been categorized by their function. The fibrillar collagens type I, II, III, V, and XI consists of a central uninterrupted triple-helical region of about 1000 amino acids [6]. The second functional category is the non-fibrillar type IV collagen, which forms antiparallel sheet-like structures that are the principal components of the basement membrane in the skin. The third functional category place collagen type IX, XII, XIV, XVI as fibril associated collagens where the triple helical structures are interrupted and in various lengths [7]. Many medical applications involving Type I collagen have been successful. However, even Type I collagen within itself has many different properties depending on the location from which the collagen is derived. Ranging from the most sophisticated implantable devices to smoothing wrinkles, collagen has a solid presence.

2.1.1 Chemical Structure of Collagen

Collagen is a protein with the most unique structure. It is an amino acid consisting of about 33% glycine and 25% proline and hydroxyproline. The intrinsic viscosity of collagen is about 1000ml/g and 1200ml/g [8]. The molecular length is about 300nm by rotary shadowing. It has a translational diffusion coefficient of about 0.85 x 10^{-7} cm^2/sec with the ability to form fibrilar elements with a macroperiod of between 65 to 67nm [9,10]. Rotational diffusion coefficient for type I collagen has been found to be 1082 sec^{-1} [11]. The molecular weight of collagen is determined to be 285,000; which reflects the presence of crosslinks between molecules or physical associations [2].

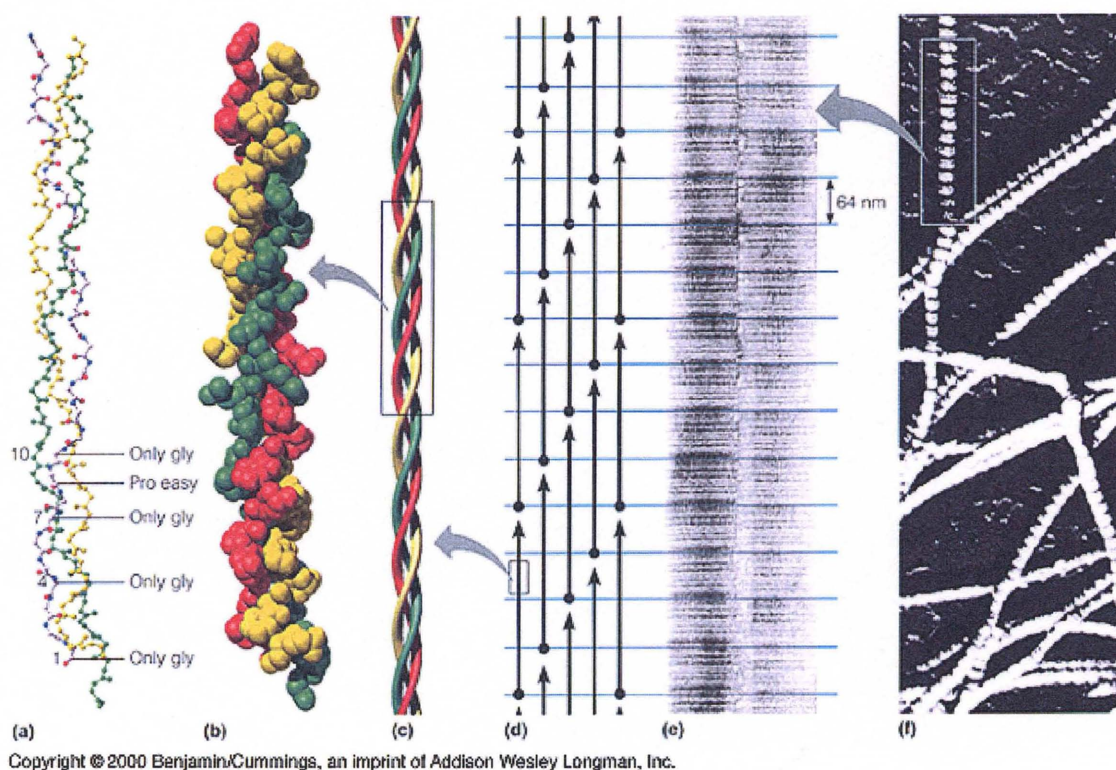

Figure 2.1.1a Structure of collagen a) molecular structure, b) triple helical structure, c) molecular chain distribution, d) collagen fibril banding pattern, f) image of collagen fibril [12].

The molecular sequences of collagen are composed of approximately 1,000 amino acids in the form of Gly-X-Y with small nonhelical ends before and after the sequences. With the Gly-X-Y sequences of collagen they form fibrous networks that prevents premature mechanical failure of most tissues. The triple helical structures that are unique to collagen pack laterally into a quarter-stagger structure in tissues to form characteristic D-periodic fibrils. The fibrils range in diameter from about 20nm in cornea to more than 100 nm in tendon. The collagen fibrils in tendons are packed into bundles that are aligned along the tendon axis. In skin, types I and type III collagen fibrils form a nonwoven network that aligns with the direction of force. In the cartilage, type II collagen fibrils form oriented networks that are parallel to the surface and perpendicular alignment in the deeper zones relative to the surface layer [6]. The different orientations of collagen offer an array of mechanical properties to different tissue types.

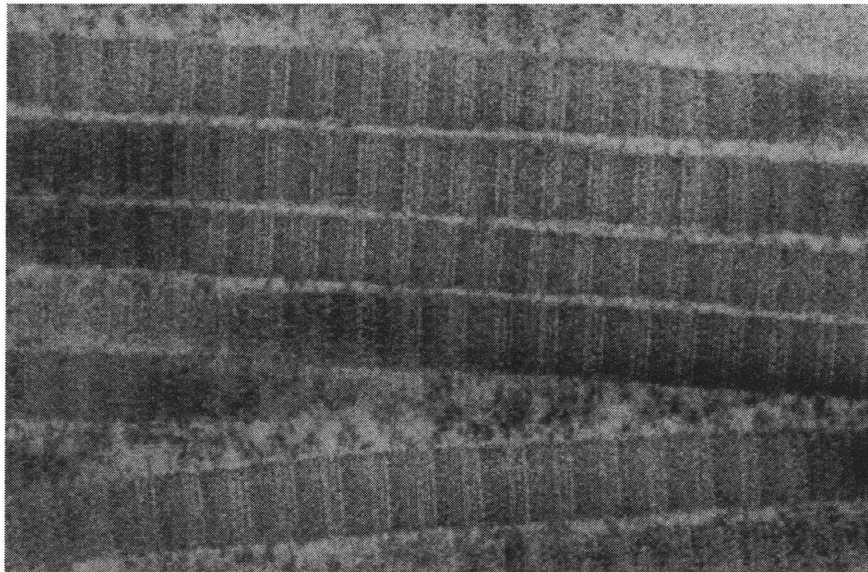

Figure 2.1.1b Banding pattern of collagen fibrils under SEM.

2.1.2 Mechanical Properties of Collagen

Collagen is one of the most important stress-carrying protein structures of the mammalian body. These proteins collectively form connective tissues that function to maintain shape of tissues. The mechanical aspects of collagen is usually observed and measured through the stress and strain relationship of the tendon fibrils. The stress and strain relationship of fibrillar Type I collagen varies from tissue to tissue but the ultimate tensile stress is correlated with the diameter of the fibril and fiber. The classic stress and strain curve of collagen can be divided into 3 distinct regions where each region represents a state of fibril arrangement. (Figure 2.1.2a) The region of small strains "toe region" is the removal of microscopic crimps in the collagen fibril [13]. At this stage the light microscope can observe the uncrimping. However, when at larger strains, the heel and linear region, the light microscope can no longer allow for visible observation of the changes in the structure. The changes are, therefore, at the submicron range.

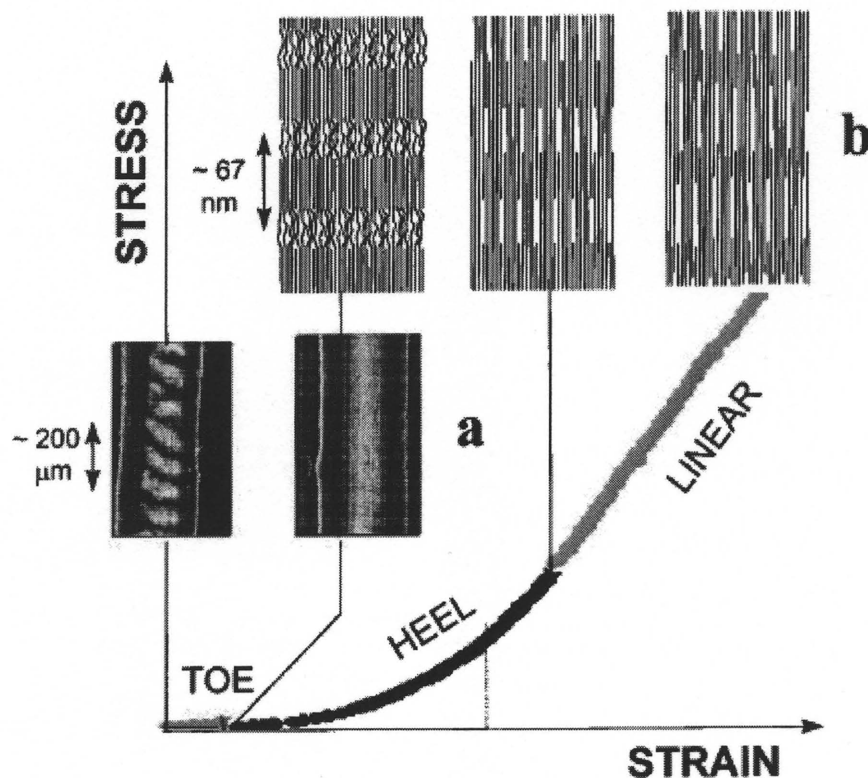

FIGURE 2.1.2a Stress and strain curve of collagen rat-tail tendon [12].

In the heel region the collagen fibrils appear to align in parallel this phenomenon is interpreted as a reduction of the disorder in the lateral molecular packing within fibrils, resulting from the straightening of kinks in the collagen molecules [14]. Experimentation using X-ray scattering technique to study the structural changes occurring in the heel region of the stress and strain curve is conducted to observe the changes [15]. The result showed that the intensity of the diffused equatorial scattering of the X-ray is due to the lateral arrangement of the collagen molecules inside the fibrils and it increases with the strain [16,17]

The linear region of the stress and strain curve indicated that the kinks in collagen are straightened where the load is directly loaded onto the collagen fibrils and no further extension is possible [8]. It is believed that the triple-helices or the cross-links between the helices are being stretched and gliding with the neighboring molecules. In an experiment conducted using synchrotorn radiation diffraction to investigate this phenomenon, the results indicated that gliding of neighboring molecules indeed occur in the linear region [18]. A separate study conducted on the mechanical properties of collagen fibers of diameter from 50 to 100 mm yielded ultimate tensile strength of 40 MPa with strain up to 10% [19]. These properties reflect the stiffness of the collagen triple helix, which has been estimated to have stiffness as high as 4 GPa, based on solution properties [20].

2.1.3 Immunogenicity of Collagen

Collagen as a safe and effective biomaterial for in-vivo use has been approved for use in many different medical applications. Since collagen is a material that is abundant in the human body it rarely causes any immunological or toxicological responses when implanted. However, some concern has risen over the years where autoimmune responses have been exhibited in some patients. Cases of rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, and polymyositis appeared in some patients after collagen injections while the patients exhibit no previous symptoms before the injections [2]. Although such statements are made for injectable collagen content there are no direct correlations of collagen induced autoimmune diseases.

With recent concern over bovine spongiform encephalopathy and the cross contamination into humans causing degeneration in the central nervous system has give rise to doubts in the safety of collagen related products. However, contamination of collagen related medical devices are of no danger to patients.

2.1.4 Applications of Collagen

In many instances collagen has been the biomaterial of choice due to its biocompatibility and minimal immunological responses. Collagen as a biomaterial has always been processed into a powder form or into slurries for further usage. Other forms that collagen can be processed into include hydrogel or as a porous matrix after freeze-drying. As a connective tissue, collagen consists of fibers that are organized into protein networks ranging from twenty to several hundred nanometers in diameter [21]. Collagen networks act to resist high strain deformation and in the process transmit forces, dissipate energy, and prevent premature tissue mechanical failure. Due to this aspect, collagen has always been sought after for ligament repair and replacement. Many different research studies have been conducted to manufacture ligament from collagen to achieve the same flexibility and strength as natural ligaments. However in most cases it proves to be difficult to match the natural strength.

Collagen in the cardiovascular graft industry serves as a natural sealant for woven grafts. Although finely woven there are pores in the grafts that can cause serious leakages once implanted. Utilizing collagen as a coating substance allows for complete sealing of the graft and yet is bioresorable where the collagen is gradually replaced by the tissues of the patient.

2.1.5 Electrospinning of Collagen

The feasibility of electrospinning just collagen has been proven. Collagen that is electrospun is shown to have retained all its characteristics such as the quarter staggered banding pattern that is an identification for collagen. It has been discovered that the structural properties of electrospun collagen varied with the tissue of origin (Type I from skin vs Type I from placenta), the isotype (Type I vs Type III), and the concentration of the collagen spinning solution, which was used to spin the fibers [22]. A further refinement in the electrospinning technique is continued where the ground source is a continuously rotating drum designed for in collecting aligned electrospun collagen fibers.

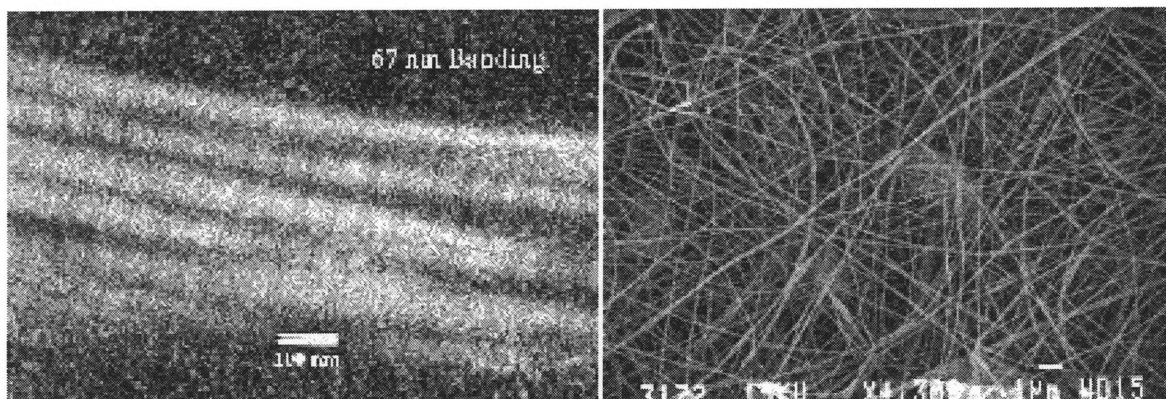

Figure 2.1.5.a Collagen electrospun fiber with banding and collagen electrospun mat [22].

Other researchers have been successful in electrospinning collagen with PEO polymer that is non-toxic and non-denaturing. This process uses a weak acid solution in dissolving collagen into solution. In both cases the fibers that are produced are ranged from a diameter of around 100 to 150nm [22,23]. The nonwoven mats have the potential application in wound healing, tissue engineering, and as hemostatic agents.

2.2 Polyethylene Oxide PEO

Polyethylene oxide (PEO) is a crystalline, non-ionic, hydrophilic polymer. The chemical structure of PEO is identical to that of polyethylene glycol (PEG), the PEO is distinguished by significantly higher molecular weights relative to PEG. The molecular weight of PEO ranges between 100,000 and 7 million daltons. PEO has a melting temperature of about 74°C and a glass transitions temperature of -54°C [24]. Water-soluble polymers fall into two main categories: polyelectrolytes and neutral polymers that are soluble in aqueous media because of the formation of hydrogen bonds with water [25,26]. PEO is modeled as a flexible linear polymer such as a polymer. However, PEO is not a simple polymer. It can exist in different numbers of distinct and introconverting configurations characterized by their dimensions and by their interaction free energies with water, other monomers and solutes such as proteins [25].

In biomedical applications PEO has generated special interest due to its good biocompatibility and low toxicity. Scaffolding in tissue engineering applications must direct the arrangement of cells in an appropriate three-dimentional configuration and present molecular signals in appropriate spatial and temporal manner so that the individual cells will form the desired tissue structures and do so in a way that can be carried out reproducibly, economically, and on a large scale. PEO is most commonly used to create protein-resistant surfaces [27]. However, surface modifications remain an area of active interest and research where the polymer can mimic the hydrophilic, non-adhesive oligosaccharides protruding from the cell surfaces [28].

2.3 Poly (L-lactic acid) PLLA

Enantiomerically pure PLA is a semicrystalline polymer with a glass transition temperature (T_g) of about 55°C and melting point (T_m) of about 180°C [29]. Solubility of lactic acid based polymers is highly dependent on the molar mass, degree of crystallinity, and other comonomer units present in the polymer. Good solvents for enantiomerically pure poly(lactide) are, for example, chlorinated or fluorinated organic solvents, dioxane, dioxolane, and furane. Typical non-solvents for lactic acid based polymers are water, alcohols (e.g. methanol, ethanol, propylene glycol), and unsubstituted hydrocarbons (e.g. hexane, heptane) [29]. The miscibility of PLLA with other polymers allows for flexibility in manipulating the properties of PLLA. Blending of different polymers with PLLA can change the degradation rate, permeability characteristics, drug release profiles, and thermal and mechanical properties. As a general rule of thumb the polymers chosen for blending should be miscible with PLLA to avoid phase separation. However, immiscible blends are useful when rubber toughening of PLLA is desired [29].

Mechanical properties of PLLA can be varied to a wide extent which is dependent on the application. Semicrystalline PLLA is preferred to the amorphous polymer when higher mechanical properties are desired. Semicrystalline PLLA has an approximate tensile modulus of 3 GPa, tensile strength of 50-70 MPa, flexural modulus of 5 GPa, flexural strength of 100 MPa, and an elongation at break of about 4% [30.31,32,33]. It has been shown that tensile strength and modulus of PLLA increases by a factor of 2 when the weight-average molar mass is raised from 50 to 100 kDa [34]. Further increase

in molar mass to 300 kDa has no influence on the properties of the polymers in any significant way but the degree of crystallinity does change [29].

Aromatic polyesters are known to undergo thermohydrolysis due to the ability to absorb water [35]. Thermal stability of lactic acid based polymers is poor at elevated temperatures such as PLLA. Semicrystalline poly(L-lactic acid) tends to increase its weight by water uptake with only a few percents [36]. The amorphous parts of the polyesters have been noticed to undergo hydrolysis before the crystalline regions because of a higher rate of water uptake. The first stage of the hydrolytic degradation is accordingly located to the amorphous regions where the molecule fragments, that are tying the crystal blocks together by entaglement, are hydrolyzed. The remaining undegraded chain segments therefore obtain more space and mobility, which lead to reorganizations of the polymer chains and an increased crystallinity [37].

PLLA of high molar mass has sufficient strength for use as load bearing materials in medical applications, but the material degrades slowly because of the reinforcing crystalline domains [38,39].

2.4 Electrospinning

2.4.1.History

The term "electrospinning" is derived from "electrostatic spinning" which is first used by Formhals in 1934 in the United States Patents [40]. The experimental setup proposed by Formhals utilized the electric field that is generated to draw the polymer solution into filaments. Two electrodes bearing electrical charges of opposite polarity are set up having one electrode inside the polymer solution while the other is situated a short distance away as the collector. The behavior observed has shown that the electrospinning process depends on the potential difference depended on the properties of the spinning solution, such as polymer molecular weight and viscosity. When the distance between the spinneret and the collecting device was short, spun fibers tended to stick to the collecting devices as well as to each other, due to incomplete solvent evaporation [41].

Vonnegut and Neubauer in 1952 refined the process where they are able to produce streams of highly electrified uniform droplets of about 0.1 mm in diameter [42]. A glass capillary tube is filled with liquid with electrified wire is powered to 5-10 kV. The liquid is charged and drawn out of the capillary tube. Drozin in 1955 investigated the process proposed by Vonnegut and Neubauer. He found that for certain liquids and under proper conditions, the liquid was issued from the capillary as a highly dispersed aerosol consisting of droplets with a relatively uniform size. He also captured different stages of the dispersion [43]. A patent appeared in 1966 for production of ultra thin and light weight non-woven fabrics using electrical spinning [44]. This system incorporates a conveyer belt that serves as collector for the electrospun polymer fibers. Simons

discovered that viscosity of the polymer solution could influence the diameter of the fiber and the consistency of the fibers. The fibers from more viscous solutions produce continuous fiber while less viscous solutions yield thinner and shorter fibers. In 1971, Baumgarten was able to electrospin acrylic fibers with diameters in the range of 0.05-1.1 microns [45]. His method of electrospinning utilizes a stainless steel capillary tube with a constant solution feed by a pump that can control the speed in which the solution is being extruded. A high voltage current is attached to the capillary tube that charges the solutions. The grounding is a metal mesh on which the fibers are grounded and collected.

In recent times, the emergence of nanotechnology has pushed electrospinning forward in producing ultrafine fibers that are down to the nanoscale. However, with many precedence of the electrospinning concept, methods, and devices in existence the understanding for it is limited.

2.4.2 Concept and Theory

The basic concept of electrospinning involves of an electrically charged jet of polymer solution that is being drawn toward a grounded source. Three major components fundamental to the electrospinning method include: high voltage supply, tubing for solution extrusion, and metal collecting plate with proper grounding. The applied high voltage on the polymer solution will induce mutual charge repulsion and the attraction of the surface charges to the ground cause a force directly opposite to the surface tension. As the intensity of the electrical charge increase the hemispherical surface of the fluid tip elongates to form a conical shape know as the Taylor cone [46]. Once a critical point is reached the electrostatic force overcomes the surface tension and the charged jet of the

fluid is ejected from the tip of the Taylor cone (Figure 2.4.2a). The polymer that is discharged travels to the ground in an unstable, whipping motion. This instability allows for the elongation of the polymer solution. It is important that from the time the fluid jet leaves the Taylor cone to the point of collection, that the solvent should evaporate leaving only the polymer on the grounding plate.

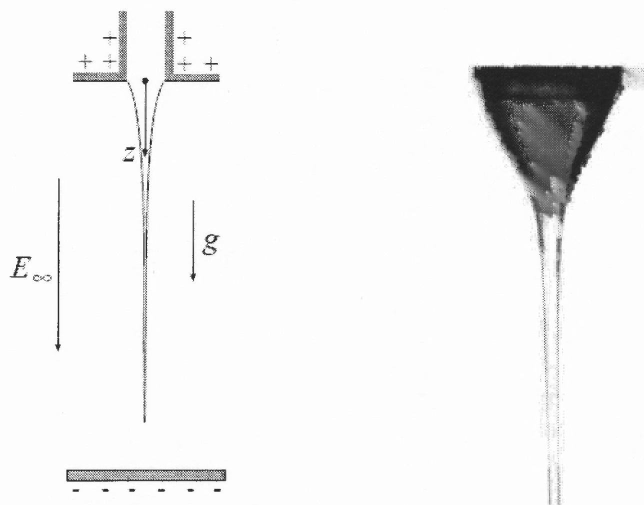

Figure 2.4.2a The schematics of charges in the Taylor Cone [46].

Electrospinning seems to be a straightforward method of producing ultrafine fibers from any solution. However, there are many parameters that govern the formation of fibers through this process. These parameters include: (a) the solution properties such as viscosity, elasticity, conductivity, and surface tension, (b) governing variables such as hydrostatic pressure in the capillary tube, electric potential at the caplillary tip, and the distance between the tip and the collecting plate, and (c) ambient parameters such as solution temperature, humidity, and air velocity in the electrospinning chamber [46]. One of the most significant parameters influencing the fiber diameter is the solution viscosity. A higher viscosity results in a larger fiber diameter [38,45,48]. When a solid polymer is

dissolved in a solvent, the solution viscosity is proportional to the polymer concentration. Thus, the higher the polymer concentration the larger the resulting nanofiber diameters will be. Also the applied voltage can affect the diameter of the fibers. A higher applied voltage ejects more fluid in a jet, resulting in a smaller fiber diameter. The distance between the capillary tip to the ground source can dictate the fiber diameter. As the distances closes the fiber diameter increases, at the same instance the time allowed for solvent evaporation also decreases. This could lead to a potential problem of film formation instead of fiber formation or formation of beading in the fibers.

A common problem encountered during electrospinnig is beading (Figure 4.2.2b). It is found that at higher polymer concentrations the beading formation is significantly reduced [49]. Although, raising the polymer concentrations reduces the amount of bead formation it does not eliminate beading from occurring. In some instances the bead formation at higher polymer concentrations are of larger size compare to the lower polymer solution beading. Reducing the surface tension will alleviate the formation of beads.

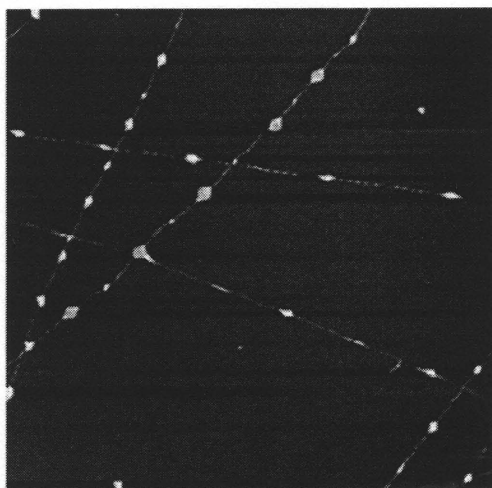

Figure 4.2.2b Examples of beading on the fibers [49].

It is part of the electrospinning system that the fibers are deposited in a random, non-woven fashion. However, attempts have been made to collecting the fibers in an aligned fashion or into spools. Thus far it has prove to be difficult to single out fibers and align them in any uniform way. Different methods proposed and attempted have had some degree of success include a high speed, rotating drum (Figure 2.4.2c) that attempt to wind the fiber as it is extruded. The difficulty in having the rotating drum to collect aligned fibers is the whipping motion that occurs at a high rate of speed. The drum has to match the deposition speed of the fibers to continuously collect fibers in aligned fashion.

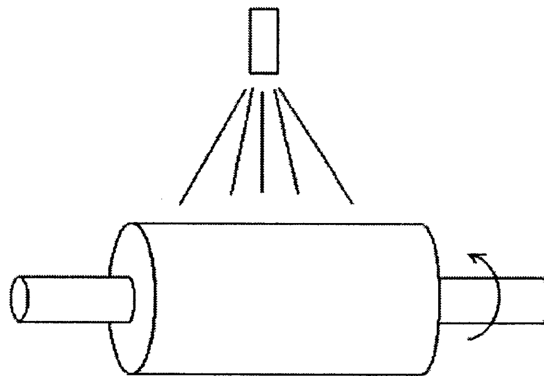

Figure 2.4.2c Schematic of a rotating drum as ground source and fiber collector.

Others have proposed auxiliary electrodes and electrical fields to align the fibers. The idea is to introduce additional electrical fields that can direct the stream of the jet and limit the whipping motion. The limitation to this method is the amount of additional applied field that can be introduced to the system without interfering with the primary grounding source that is collecting the aligned fibers. Having additional applied fields can cause the jet to be directed away from the main collector plate.

An alternative method of electrospinning that is suggested eliminated the use of an extruder. The polymer of interest is contained in a bath with an electrode charging the polymer solution (Figure 2.4.2d) [50]. The ground is place above the polymer solution bath that draws the solution upwards and forming fibers. This method resulted in the formation of high volume of fibers in the shortest time.

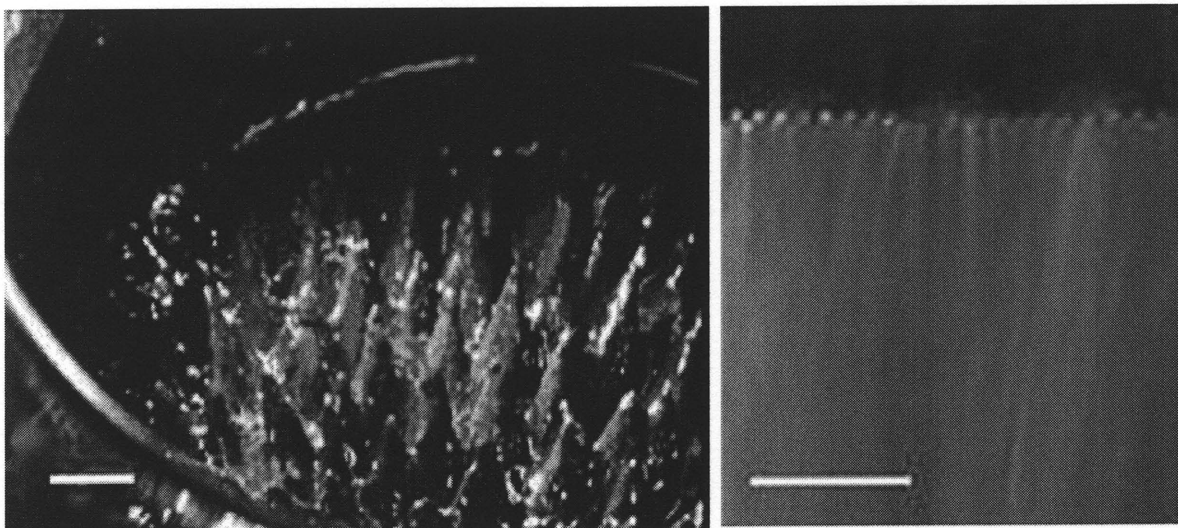

Figure 2.4.2d The formations of spikes as the polymer solution is being pulled toward the ground; the formations of fibers off the ground is shown on the saw tooth [50].

2.4.3 Applications of Electrospun fibers

The uses of electrospun fibers can be divided into several areas. The main area of application based on the number of US patents filed, is in the medical prosthesis. The key issue in medical prosthesis or tissue engineering and the development of artificial organs is in the structural design of artificial matrices or scaffolds that mimic the supramolecular structure and biological functions of the extracellular matrix (ECM). Extracellular matrix usually forms a composite-like structure of proteoglycans and fibrous proteins such as

collagens. The collagen fibrous structures are organized into the mesoscopic three-dimensional fiber network composed of nanoscale multifibrils of collagens. Therefore, a significant guideline for designing the artificial ECM or scaffold maybe for formation of nanoscale building blocks such as nanofibers and its appropriate spatial organizations on the mesoscopic scale. From this viewpoint, electrospinnig has provided a basis for the fabrication of unique matrices and scaffolds for tissue engineering.

Medical uses for electrospun polymer fibers are not limited to scaffoldings for tissue engineering applications. Wound dressings composed of electrospun fibers can also provide enhanced wound healing and scar reduction. It is proposed that the fibers be electrospun directly onto the wound and be able to provide a protective layer from the environment while promoteing healing. At the same time medications can be incorporated into the polymer solution that can be used not only for conventional wound healing but can be used as controlled drug delivery devices internally.

Other applications such as filtration can greatly benefit from the reduced diameter of the fibers. Fibrous materials used for filter media provide advantages of high filtration efficiency and low air resistance. The filtration efficiency is dependent on the fineness of the fibers, the reduction in diameter can greatly enhance filtration performance. This filtration system can also be applied to clothing where protection from aerosols can be possible due to the great surface area of the nanofibers that are capable of the neutralization of chemical agents and without impedance of the air and water vapor permeability to the clothing.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Bovine Collagen Tendon Derivation and Purification

Raw bovine tendon is packaged into a plastic sleeve each about 1000 grams and frozen before further processing. The sleeve is sliced with a deli slicer and the thin slices are than ground using a meat grinder. Potassium phosphate monobasic solution buffer solution is prepared by adding 41.25 grams of KH_2PO_4 to 8.4 liters of distilled water. NaOH in the amount of 1.77 grams is added to the solution in order to maintain the pH at 6.15±0.15. The ground tendons are placed in the solution.

The buffer prepared bovine tendon is then placed under enzyme treatment of Ficin for 1 hour. The enzyme treatment bath is composed of 10 grams of Ficin dissolved in the previously prepared buffer solution and heated to 37°C. The temperature is maintained while the tendon is in solution.

After 1 hour of Ficin treatment deactivation is necessary to prevent further denaturation of the tendon. The deactivation solution is prepared by adding 84 grams of NH_4NO_3 and 10 grams of $NaClO_2$. The pH of the solution must be strictly monitored and that is should be kept between pH of 6 and 7. The tendons are hand washed and squeezed to eliminate the enzyme solution from the tendon before placing into the deactivation bath. The tendons must remain in the deactivation bath for 1 hour for full deactivation. After 1 hour 3 batches of distilled water of 3 liters each are used to wash the tendons and

eliminate the remaining traces of the deactivation bath. Each washing must last no less than 15 minutes.

Alkalai treatment is essential to further cleanse tendons of impurities. The alkalai treatment bath is prepared by adding 1400 grams of anhydrous sodium sulfate and 350 grams of sodium hydroxide to 6.8 liters of distilled water. The temperature of the bath is kept at 25°C for 42 hours.

Deactivating the alkalai treatment requires 3 baths of sodium sulfate washes. First bath requires 400 grams of Anhydrous Sodium Sulfate and washing for 15 minutes. Second and third bath is with 600 gram of Anhydrous Sodium Sulfate and washing for 15 minutes each. Three acid baths pH of 4.6 is prepared by adding H_2SO_4 into water.

The remaining treatment of isopropanol is intended to dry the tendons by driving out excess solution taken in by the tendons. The tendons are soaked in 100% isopropanol for 2 hours at 60°C and followed by a second bath of 100% isopropanol for 1 hour at 60°C. The tendons are taken out of the treatment and hand squeezed and teased into fine fibers. The teased fibers are then placed in 45°C oven and dry overnight. The tendon that is harvested is type I bovine tendon collagen and elastin.

3.1.2 Collagen Suspension

Collagen suspension is made from Type I bovine collagen tendon fiber that is made by the process mentioned above. Different concentrations of collagen suspension are made according to weight percentages. Two different weight concentrations of collagen suspensions are produced and used for this study.

Taking 16 grams of dry collagen tendon fibers and combineing with 2 ml of lactic acid and 2 liters of D.I. water makes a suspension of 1.6 wt %. The collagen tendon fibers are blended in the blender for several short bursts of no more than 3 seconds for further refinement of the suspension. A long blending time will tend to heat up the suspension and subsequently denature the collagen. This must be avoided to preserve the properties of collagen. The material is removed from the blender and stored in the refrigerator for 15 min. This process is repeated two more times and the finished suspension is centrifuged. The purposed of the centrifuge is to remove the air bubbles that are trapped in the suspension during the blending process. The suspensions are kept in refrigeration until the time of use. The 2.0 wt % collagen suspension is prepared the same way with 2 ml of lactic acid and 1 liter of D.I. water.

3.1.3 Polymers and Solvents

The polymers and solvents used during this study are listed below:

- Poly(ethylene oxide) from Aldrich [M_v 2,000,000; M_v 600,000; M_v 300,000]
- Poly(l-lactic acid) from Purasorb®
- Deionized water from Ricca Chemical Company
- Chloroform, 99% from Sigma
- Dichloromethane, A.C.S reagent (methylene chloride) from Sigma-Aldrich
- Trifluoroacetic acid, 99% from Acros Organics [M_w 114,02]

3.2 Methods

3.2.1 Electrospinning set up

The electrospinning set up used is based on the basic concept of electrospinning. Stainless steel needles of diameters: 18 gauge and 20 gauge are used. A 10ml plastic syringe is used as the container and extruder of the polymer solution. A syringe pump with speed control is placed in isolation to prevent creating an additional electrical field that can serve as a grounding source for the polymer solutions. A hydraulic system was developed where a duel syringe filled with water serves as the hydraulics between the syringe pump and the piston of the syringe. A high voltage supplier is connected to the syringe through an alligator clip. A metal plate is used as the ground and collector of polymer fibers. Connecting the ground wire back to the power supply accomplishes the grounding of the metal plate.

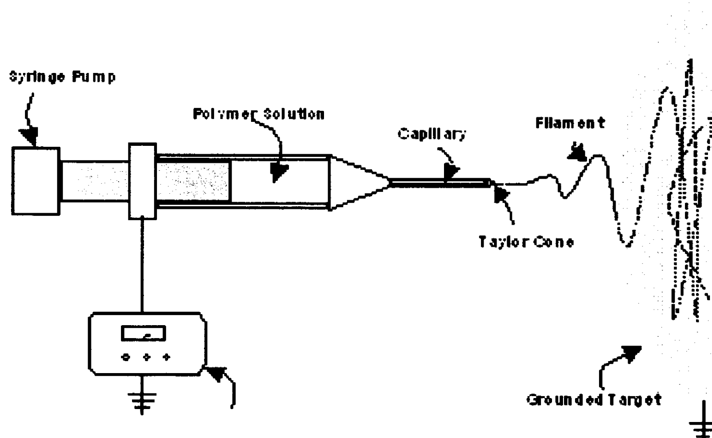

Figure 3.2.1a Electrospinning set-up.

3.2.2 Scanning Electron Microscope

For analysis of the diameter and morphology of the electrospun fiber and mats the Scanning Electron Microscope (SEM) is the tool of choice for such application. The ability of SEM to image micron and nano scaled materials is based on the emission and absorption of electrons under the electron beam. The surface may or may not be polished and etched, but it must be electrically conductive; a very thin metallic surface coating must be applied to nonconductive materials.

Electron microscopes were developed due to the limitations of light microscopes. Which are limited by the physics of light to 500x or 1000x magnifications and a resolution of 0.2 micrometers. In the early 1930's this theoretical limit had been reached and there was a scientific desire to see the fine details of the interior structures of organic cells (nucleus, mitochondria, etc.). This required 10,000x plus magnification which was just not possible using a light microscope. The first Scanning Electron Microscope (SEM) debuted in 1942 with the first commercial instruments around 1965. Its late development was due to the electronics involved in "scanning" the beam of electrons across the sample.

The concept and inner workings of SEM are not very much different from the light microscope. The "Virtual Source" (Figure 3.2.2a) at the top represents the electron gun, producing a stream of electrons.

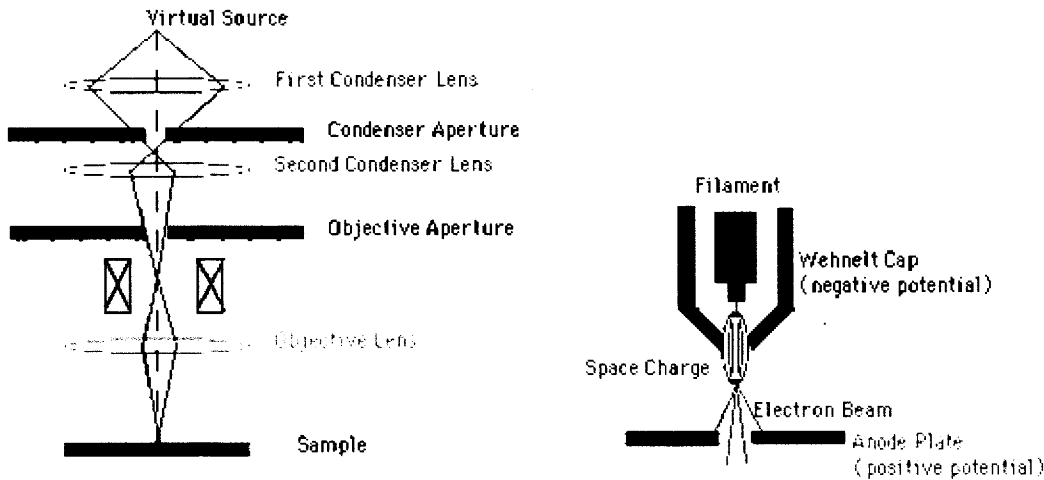

Figure 3.2.2a The schematics to the inner workings of SEM with electron gun details.

The stream is condensed by the first condenser lens (usually controlled by the coarse probe current knob). This lens is used to both form the beam and limit the amount of current in the beam. It works in conjunction with the condenser aperture to eliminate the high-angle electrons from the beam. The beam is then constricted by the condenser aperture eliminating some high-angle electrons. The second condenser lens forms the electrons into a thin, tight, coherent beam and is usually controlled by the fine probe current knob. A user selectable objective aperture further eliminates high-angle electrons from the beam. A set of coils then scan or sweep the beam in a raster (like a television), dwelling on points for a period of time determined by the scan speed (usually in the microsecond range). The final lens, the objective, focuses the scanning beam onto the part of the specimen desired. When the beam strikes the sample (and dwells for a few microseconds) interactions occur inside the sample and are detected with various instruments. Before the beam moves to its next dwell point these instruments count the

number of interactions and display a pixel on a CRT whose intensity is determined by this number (the more reactions the brighter the pixel). This process is repeated until the raster scan is finished and then repeated, the entire pattern can be scanned 30 times per second.

3.2.3 Differential Scanning Calorimetry

The Differential Scanning Calorimetry (DSC) measures the amount of energy (heat) absorbed or released by a sample as it is heated, cooled, or held at a constant temperature. Typical applications include determination of melting point temperature and the heat of melting; measurement of the glass transition temperature; curing and crystallization studies; and identification of phase transformations. The DSC works by having a pair of cells placed in a thermostated chamber. The sample cell is filled with the fiber mat and the other is left blank as the reference. The two cells are heated with a constant power input to their heaters during a scan. Any temperature difference between the two cells is monitored with a feedback system so as to increase (or decrease) the sample cell's power input. Since the masses and volumes of the two cells are matched, the power added or subtracted by the cell feedback system is a direct measure of the difference between the heat capacity of the sample and reference solutions. The cell feedback power is the raw data, expressed in units of cal/min. By knowing the scan rate (typically 1 K/min) and the sample concentration, these units are converted to cal/mol-K (or cal/g-K). Another system that can be employed in the DSC system is by measuring the temperature difference between the cells instead of the power output by the cells. The

instrument constantly monitors the change in heat flow (W/g) in relation to changes in temperature to determine T_g and T_m.

3.2.4 Thermogravimetric analysis

The Thermogravimetric Analysis (TGA) allows for precise measurement of the weight change of a solid as it is heated at a controlled rate can be used to determine the amount of chemically attached water or the organic content of an otherwise inorganic substance. This analysis can be valuable if used in conjunction with methods such as DSC in order to not only investigate a weight loss due to degradation or decomposition upon thermal changes, but also to distinguish what chemical structures are given off.

3.2.5 Solution Preparation

Polyethylene oxide and collagen solution is prepared by weight percentages. For a 6 wt % solution, 3 grams of collagen suspension and 3 grams of PEO is mixed with 94 grams of D.I. Water. Different concentrations of this combination are prepared with the same method.

Poly (l-lactic acid) in chloroform and collagen (tendon collagen) in lactic acid solution is prepared separately. PLLA in chloroform is maintained at 2 wt % for all solutions. The 2 wt % is prepared with 1 grams of PLLA in 49 grams of chloroform. Collagen in lactic acid solutions are prepared in three different concentrations. The concentrations are as follows: 1 gram collagen in 49 gram of lactic acid, 1 gram of collagen in 19 grams of lactic acid, and 1 grams of collagen in 9 grams of lactic acid. The two solutions are combined by adding the collagen solution by weight into the PLLA solution.

Poly (l-lactic acid) and collagen (tendon collagen) in trifluoroacetic acid solution is prepared by keeping the amounts of TFA and PLLA as constant. Combining 2 grams of PLLA with 1 grams of collagen in 10 ml of TFA produce a solution with approximately 5 wt % of collagen in the solution. Subsequently the 8 wt% and 10 wt % collagen solution is prepared by increasing the amount of collagen added from 1 gram to 1.5 gram and to 2.0 grams.

CHAPTER 4

RESULTS

4.1 Collagen and Polyethylene Oxide with D.I. Water

Electrospinning of collagen and Polyethylene Oxide (PEO) in D.I water has often produced water droplets and film structure as seen in figure 4.1.1. The formation of water droplets on the grounding plate is contributed by poor evaporation rate of water in the electrospinning application. Formation of film structure is also due to water droplets. The fine spray of the polymer solution onto the ground plate is such that each droplet is positioned so that it builds on top of each other. When the solution subsequently dried a film is produced. The fanning out or the flake like images that are captured through the polarized light microscope in figure 4.1.1 has indicated as such.

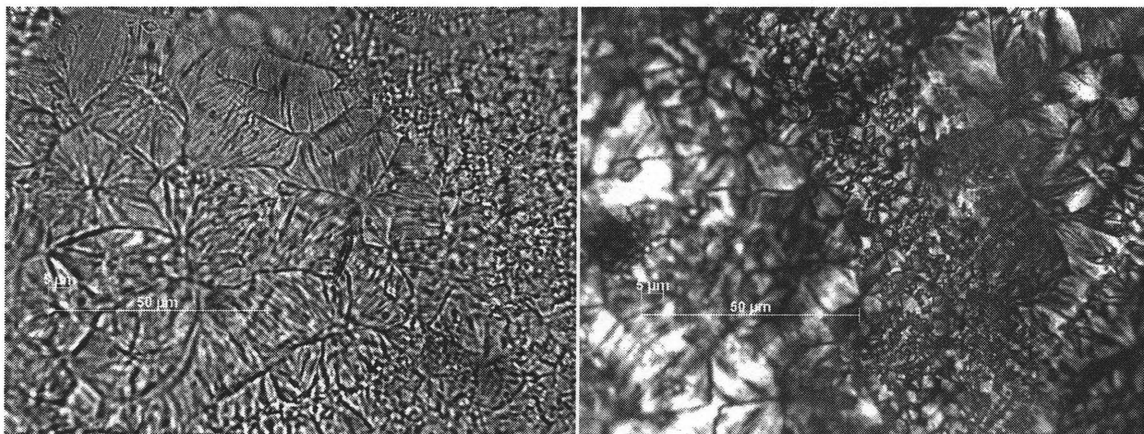

Figure 4.1.1 PEO and collagen film captured under polarized microscope.

On rare occasions when fibers are formed the mats produced are delicate and difficult to handle. The mats that are analyzed under SEM displayed a dense bundle of fibers that appeared fairly aligned and uniform under lower magnifications (Figure 4.1.2).

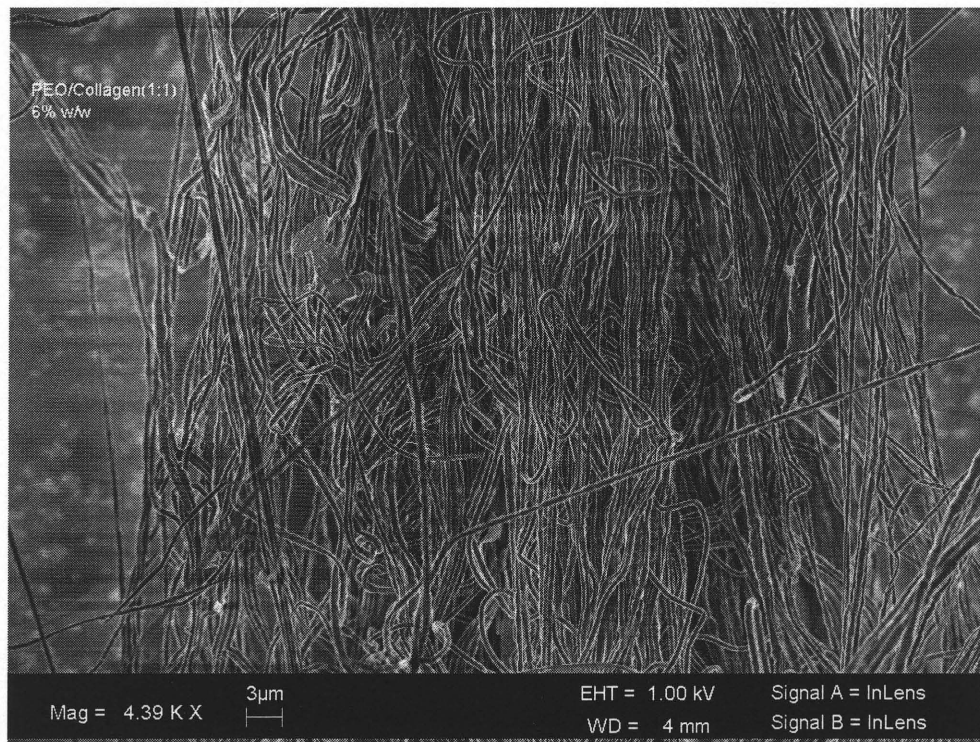

Figure 4.1.2 PEO and collagen fibers under SEM.

Figure 4.1.3 is an image capture by SEM showing fiber formation of PEO and collagen solution. The fibers are in the range of 151nm to 1µm. The solution is of equal concentration of PEO and collagen. In this image fibers are clearly displayed without any visible film formation.

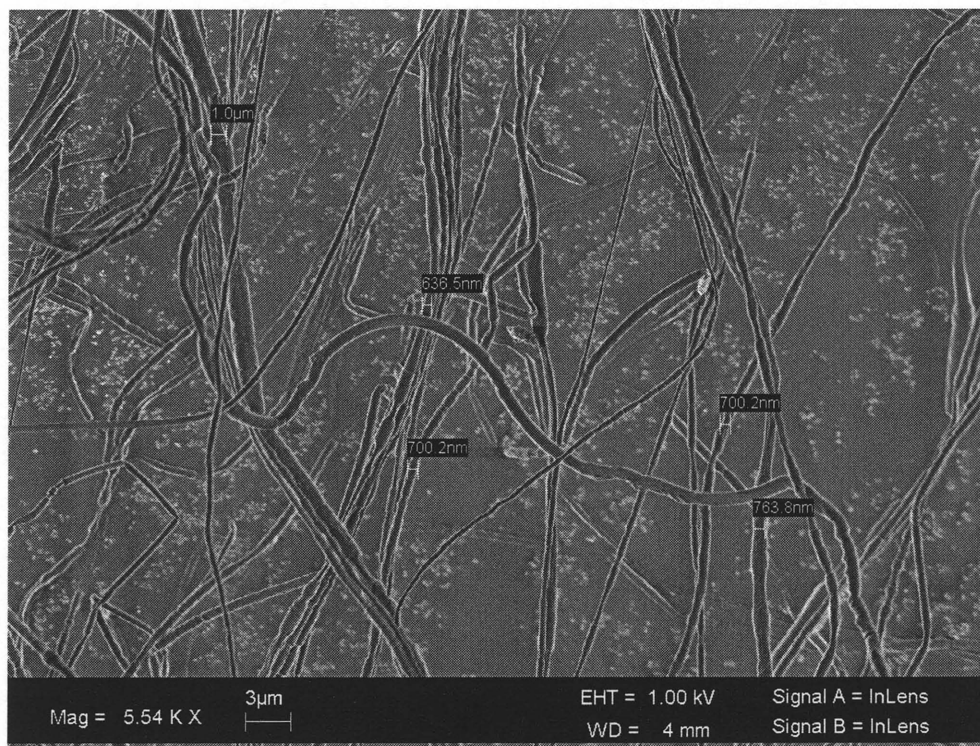

Figure 4.1.3 Area with less density of fibers in the PEO and collagen mat.

Overall collagen and PEO in D.I. Water combinations is a difficult method in producing consistent and uniform fibers. At higher magnifications as seen in figure 4.1.4, the evidence of gross phase separation is very apparent. The fibers appeared to be linkages of collagen by PEO. PEO in figure 4.1.4 appeared as a casing holding collagen sections together where the thinner sections of the fibers are PEO only sections and the thicker sections contain collagen. To confirm that collagen is not a continuous fiber in this instance the mats are placed in a water bath. PEO would readily dissolved in the water bath while collagen, as a water insoluble protein, should remain. The result is a complete dissolution of the fiber mats. This complete dissolution of the mats indicated that collagen is not in a continuous fiber form while being electrospun in this technique.

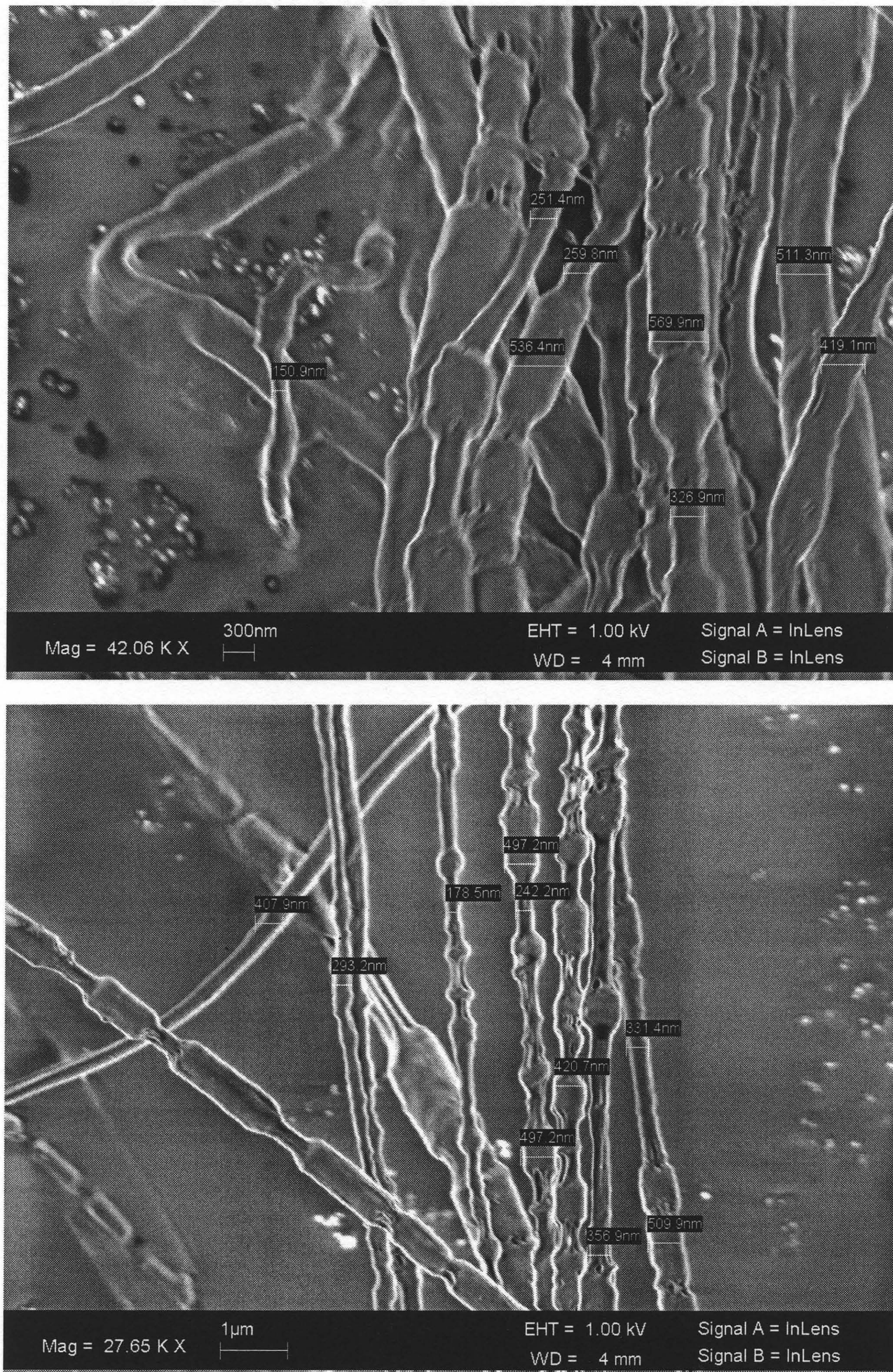

Figure 4.1.4 Evident phase separation of the PEO and collagen fibers.

4.2 Collagen and PLLA with chloroform and lactic acid solvents

PLLA and collagen dissolved in chloroform and lactic acid produced unconventional fiber networks. The fiber diameter is of large size above 1 micron as seen in figure 4.2.1. The unconventional mat formation stems from the unique method of fiber deposition onto the ground during the electrospinning process. It is observed that the fibers do not adhere on the mat in one plane. They grow on top of each layer giving a "cotton candy like" effect. The resulting mat is a puffy network of fibers that can be compressed into a flat mat. Very often at higher concentrations the mat produced is sticky with thick fibers being formed. The sticky substance is the lactic acid being coated onto the fibers. Ideally lactic acid should evaporate with chloroform, however, lactic acid is a non-volatile solvent compare to other solvents used for the electorspinning processes. As a result the fibers are coated with the lactic acid that remains.

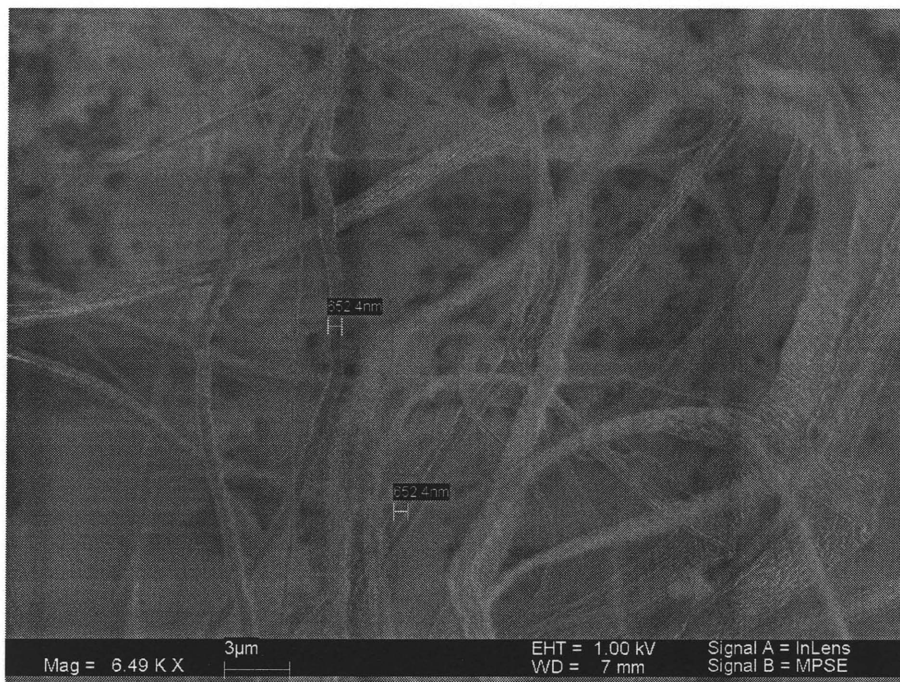

Figure 4.2.1 Collagen and PLLA in biphasic solvents.

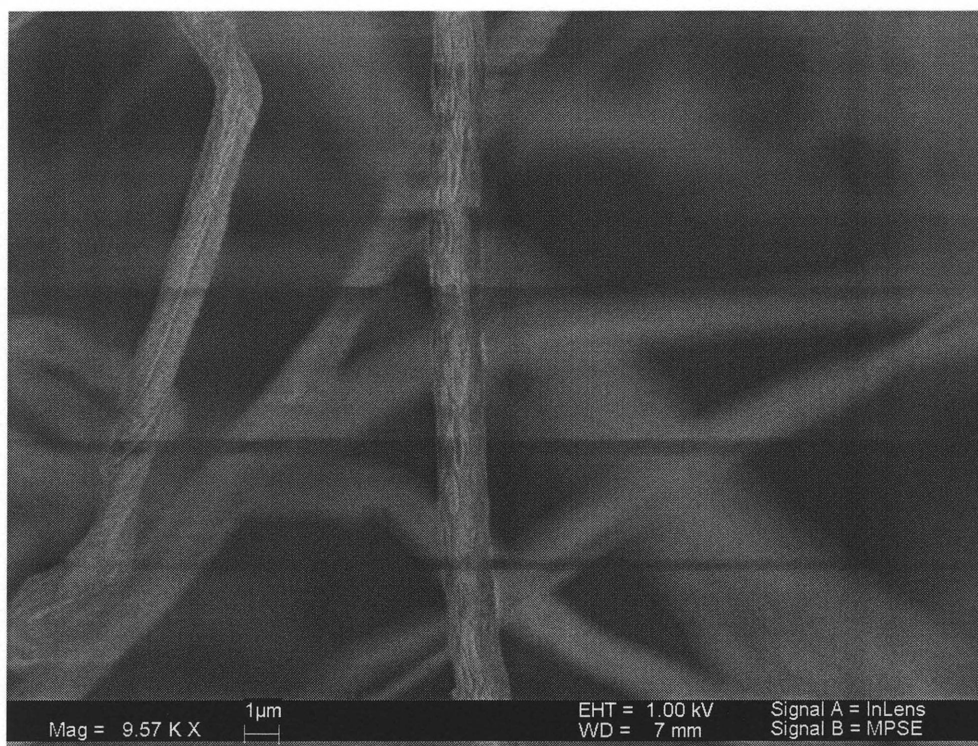

Figure 4.2.2 Micron sized fibers produced with uniform diameter.

The fiber diameter is large ranging from 600nm to over 1µm (Figure 4.2.1 & Figure 4.2.2). The solvent is not able to evaporate properly. This interferes with the whipping motion of the unstable jet and limits the amount that the solution is being drawn. This results in the large diameter.

4.3 Collagen and PLLA with trifluoroacetic acid

Collagen and PLLA in TFA mats produced mats with fiber of constant diameters (Figure 4.3.1 & Figure 4.3.2). In figure 4.3.1 the thin bundle of fibers in mat area of less density has shown to have diameter consistently at a little over 300nm. The mats produced do not contain droplet structure. In other dense areas of the mats (Figure 4.3.2) the images captured also showed the consistency in the fiber diameters.

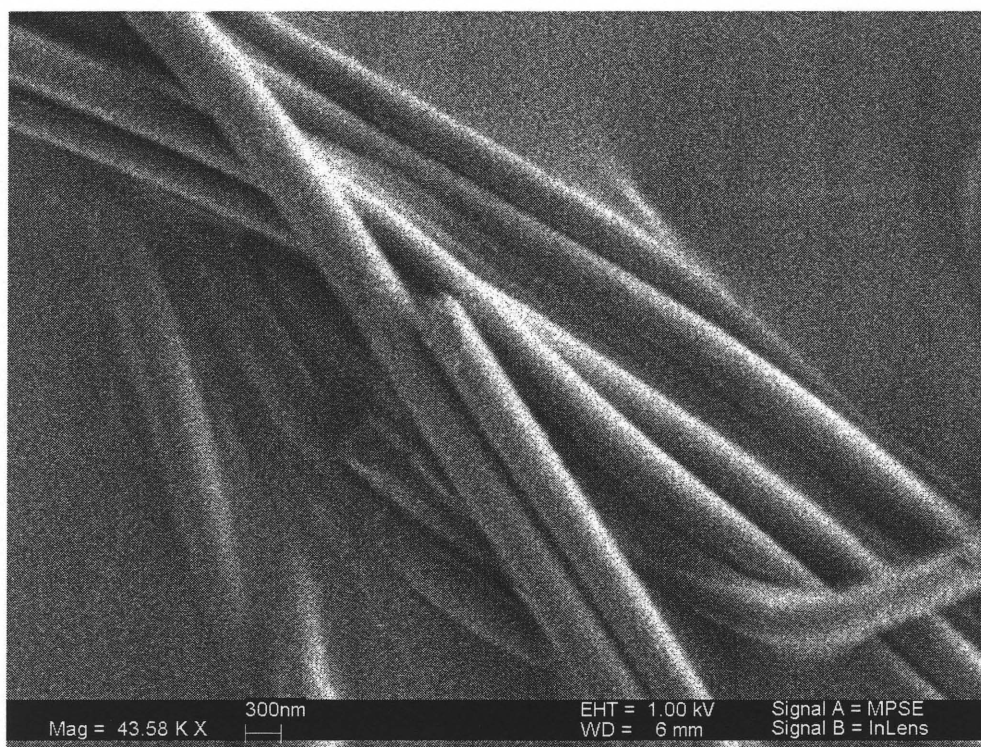

Figure 4.3.1 Collagen and PLLA in TFA fibers under SEM.

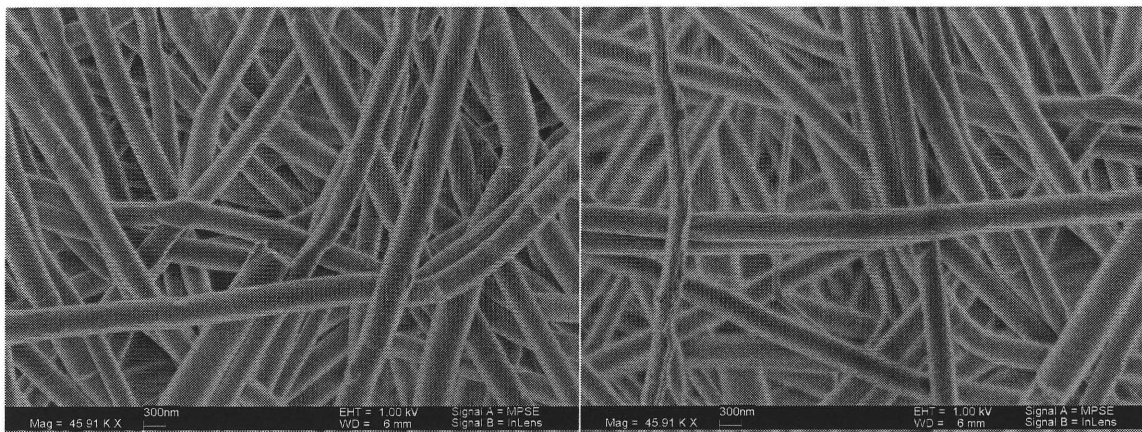

Figure 4.3.2 Scaffolds of collagen and PLLA electrospun with higher density.

It is observed that solutions placed under ambient temperature for more than 42 hours are unable to yield fiber mats. The solution that is electrospun will not yield fibers instead the material deposited on the ground is that of a powder. A visual inspection of the mat that is produced is indiscernible between solution ages. However, upon handling it is apparent that the mat of new solutions yield fibrous mats but the aged solution mats crumble into powder. This could be the direct effect of continuing hydrolysis of collagen and PLLA by the TFA while they remain in solution. This phenomenon can also be observed in mats that are not placed under vacuum to eliminate TFA that did not evaporated during the electrospinning process. The mats that are stored immediately after electrospinning without the vacuuming process became brittle after 3 days of storage. Fractures of the mats are evident when the mats are handled. Therefore, it is critical that the solvent be eliminated as much as possible to ensure the life span of the scaffold.

As the collagen and PLLA in TFA fibers are produced the question of phase separation in the fibers is considered. In the case of collagen and PEO electrospinning, phase separation is very apparent as seen in figure 4.1.4, but the mat in figures 4.3.1 &

4.3.2 is not. Looking closely at figure 4.3.4 some banding patterns can be seen and be interpreted as the presence of phase separation of materials. Areas pointed out by arrows are indications of phase separation in the fibers. After discovering these banding patterns it is believed that these fibers are similar to the PEO and collagen fibers (Figure 4.1.3). Another image is captured using different contrasts (Figure 4.3.4), the separations in some areas are more apparent. The appearances of thinning in the fiber diameter have appeared, shown by arrows in figure 4.3.4.

Figure 4.3.3 Banding pattern on the collagen and PLLA in TFA fibers.

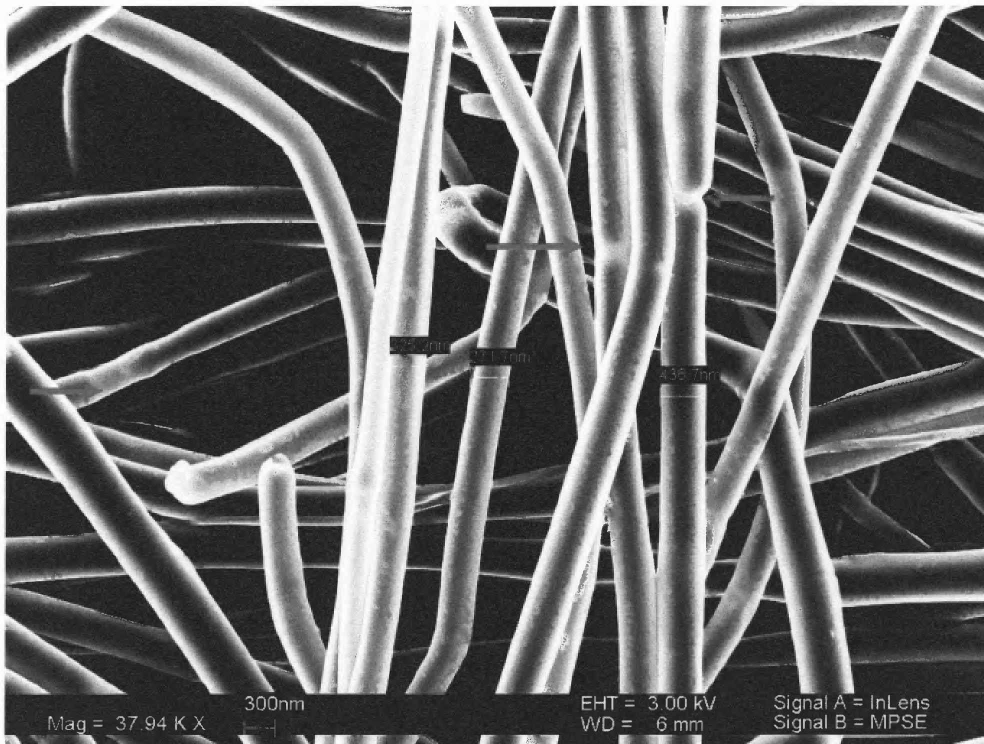

Figure 4.3.4 Phase separation in fibers.

Following on the thought that the fibers contained phase separated materials and thus yield fibers that does not have uniform amounts of collagen and PLLA, a further investigation is followed to determine the location and the content of the collagen and PLLA. It is known that collagen is not soluble in chloroform or methylene chloride but PLLA can be readily dissolved in either solvent. A 3cm x 3cm electrospun mat of only PLLA and another 3cm x 3cm electrospun mat of collagen and PLLA is prepared. Both mats are placed in their individual bath of chloroform to observe and confirm the presence of collagen. Within the first 3 second of immersion the PLLA only mat completely dissolved while the mat containing collagen remained even after 24 hours in the chloroform bath.

This method is repeated on the collagen and PLLA mat and observed under microscope to determine the locations of collagen in the fibers. The mat is placed onto microscope slides and slowly washed with chloroform that is introduced onto the slide through a syringe. The result is that PLLA quickly dissolved away leaving collagen in place. Figure 4.3.5 is an image captured by polarized light microscope showing fibrous structures of collagen with PLLA dissolved away. Another image taken from denser areas of electrospun mat also showed that the fiber formation is retained (Figure 4.3.6). Certain areas rich in collagen retain the fiber diameter and other areas that are PLLA rich the diameter of the fiber reduced. This indicates that collagen is present in the fiber and without PLLA it is still able to maintain the structural integrity of the mat.

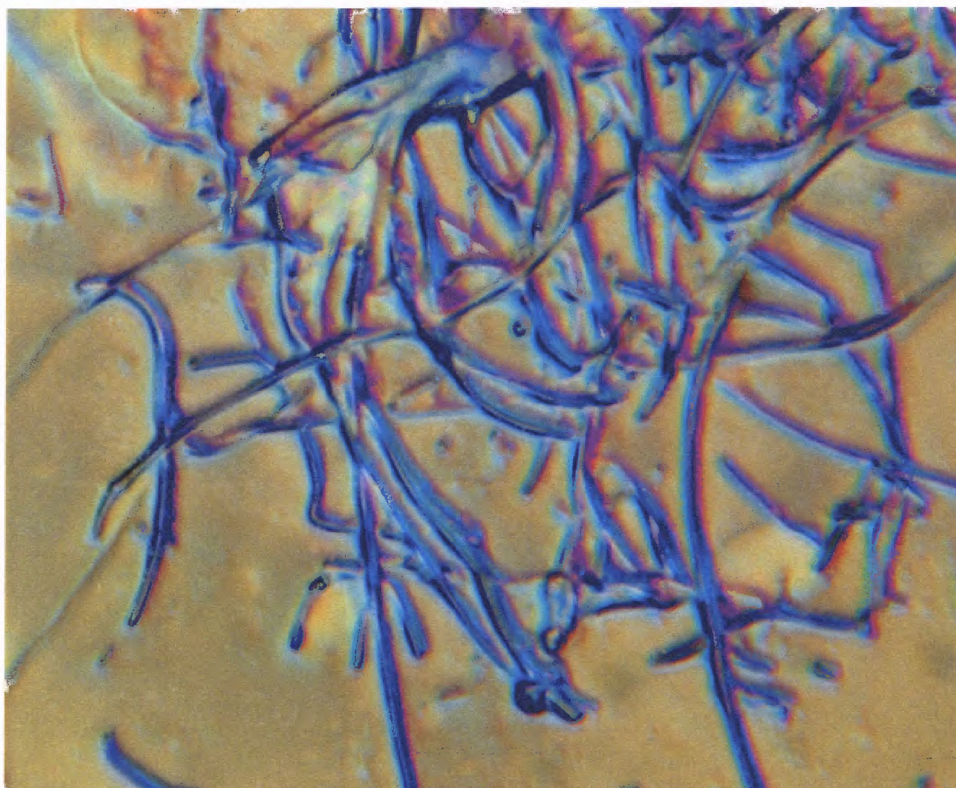

Figure 4.3.5 Fibrous structure retained by collagen after PLLA is leeched out.

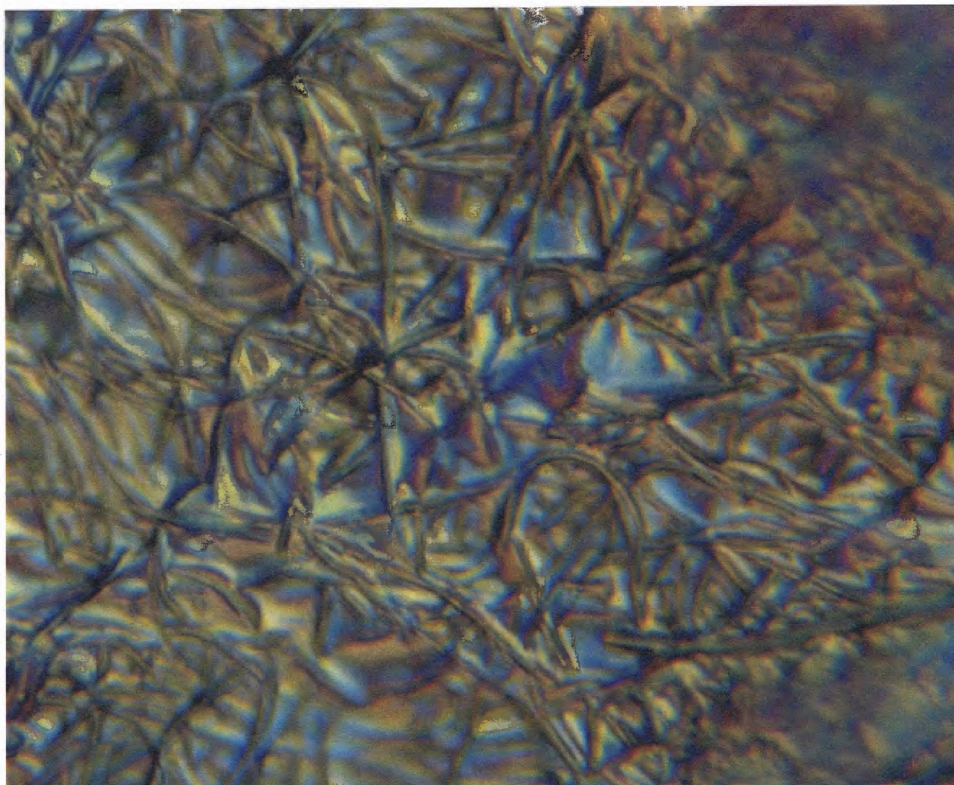

Figure 4.3.6 Denser collagen mat after PLLA is leeched out.

To determine that the electrospinning process did not interfere or destroy the raw material DSC analysis is carried out. DSC analysis is conducted on: collagen tendon fibers, PLLA, PLLA electropun mats, and collagen & PLLA electrospun mats. When the collagen tendons ran under DSC curious features appeared. In figure 4.3.7 the DSC curve for collagen tendon that is analyzed has a broad and large peak that occurred at about 130°C. This is uncharacteristic of collagen denaturation. It is inferred that the curve is the result of moisture in the tendons. The collagen tendons retain this moisture during the purification process.

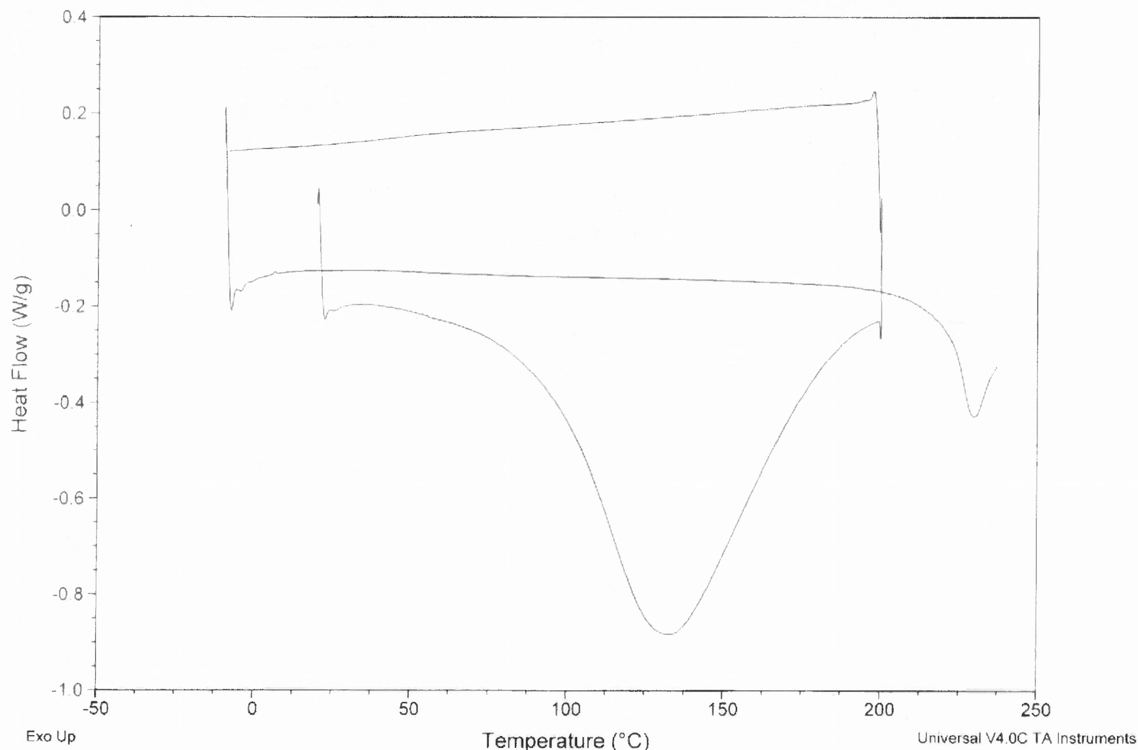

Figure 4.3.7 DSC of collagen tendon fibers.

DSC analysis heating cycle is modified to isotherm at 100°C to allow the water to evaporate. Results indicated that the broad curve has reduced in intensity by comparing figure 4.3.8 & 4.3.9. In figure 4.3.6 the tendons are placed under isotherm for 1 minute while in figure 4.3.9 the tendons are placed under isotherm for 2 minutes. The change in heat flow between the two figures is approximately 0.7 W/g. This dramatic reduction in heat flow prompted a more systematic approach to determine the point in which the broad peak is eliminated.

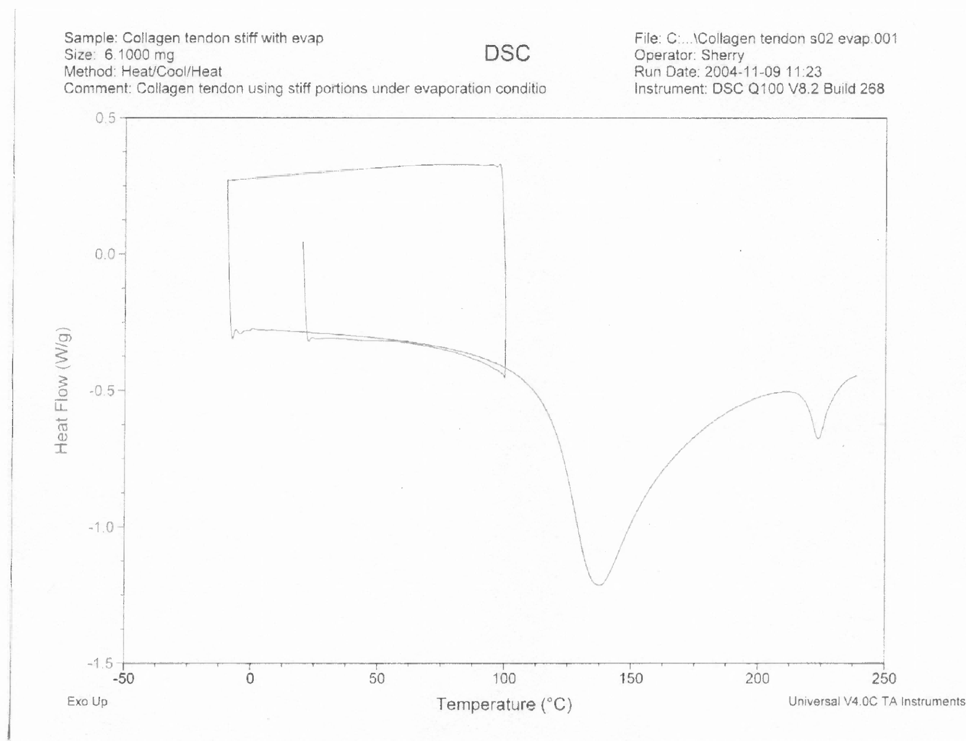

Figure 4.3.8 DSC with isotherm at 100°C testing the effect of evaporation on the tendons.

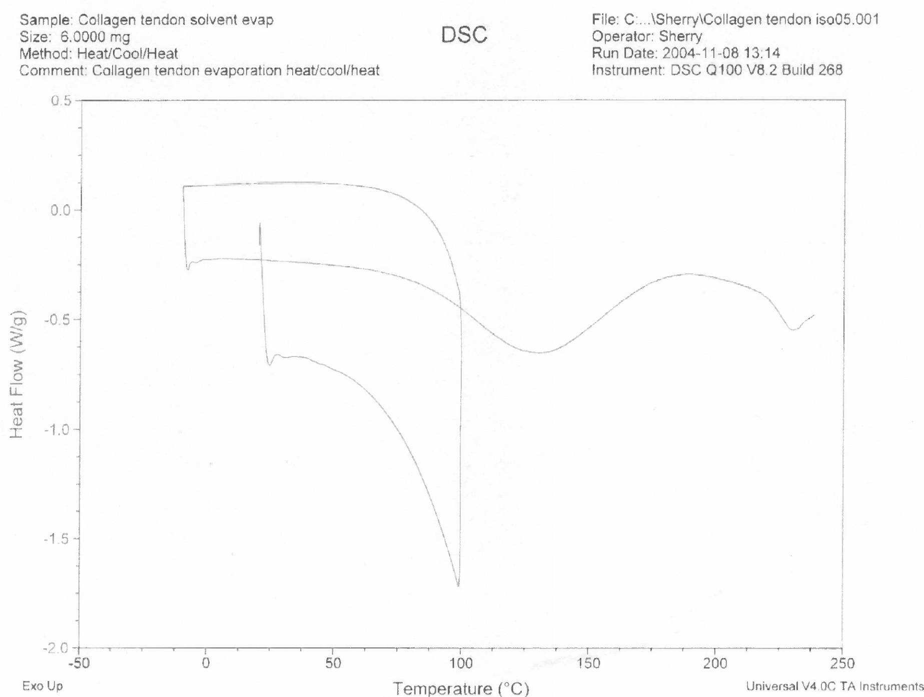

Figure 4.6.9 DSC of isotherm at 100°C for 2 minutes showing peak intensity reduction.

Further DSC analysis of tendon where the tendons are placed under isotherm for 1 minute, 2 minutes, and 3 minutes. The results indicated once again that large, broad peak begin to reduce in intensity and eventually the peak disappeared all together (Figure 4.3.10). A TGA analysis is followed up to confirm the moisture loss in the tendons (Figure 4.3.11). The moisture loss is approximately 15% of the original weight as seen in figure 4.3.11. Confirming that the board peak is contributed by moisture loss the denaturation temperature of collagen can be determined. The denaturation temperature of collagen is determined to be around 225°C and it can be seen in all collagen tendon analysis.

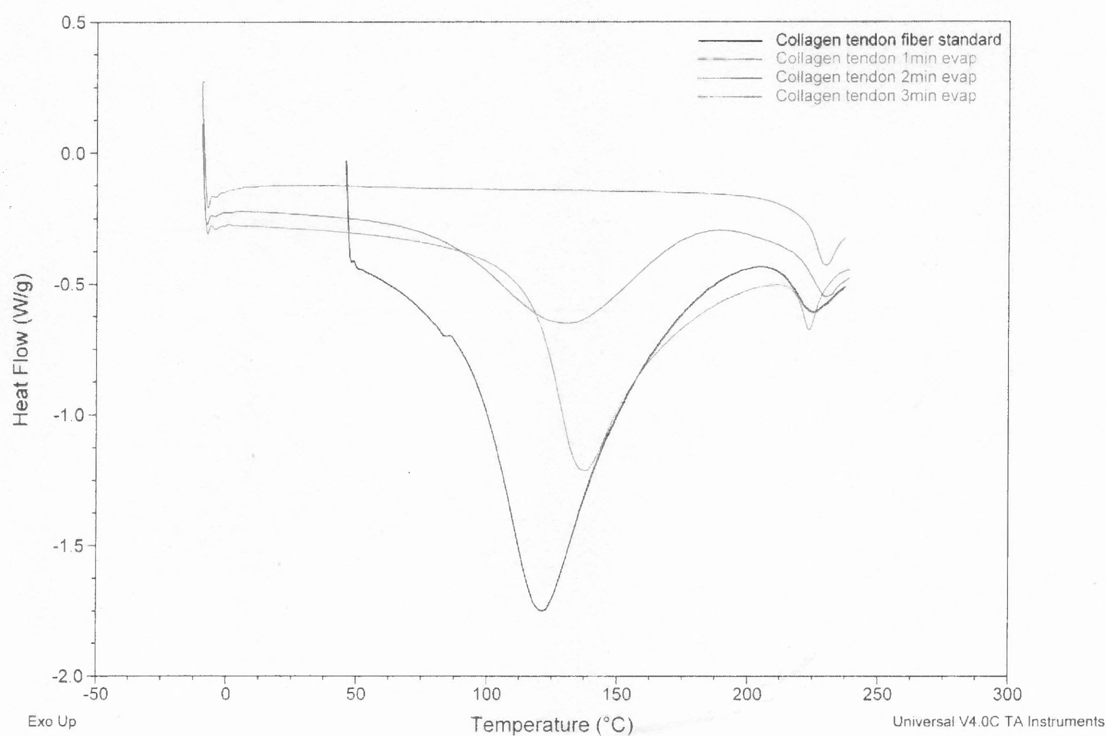

Figure 4.3.10 The collective DSC of 4 runs with isotherm from 0 minutes to 3 minutes.

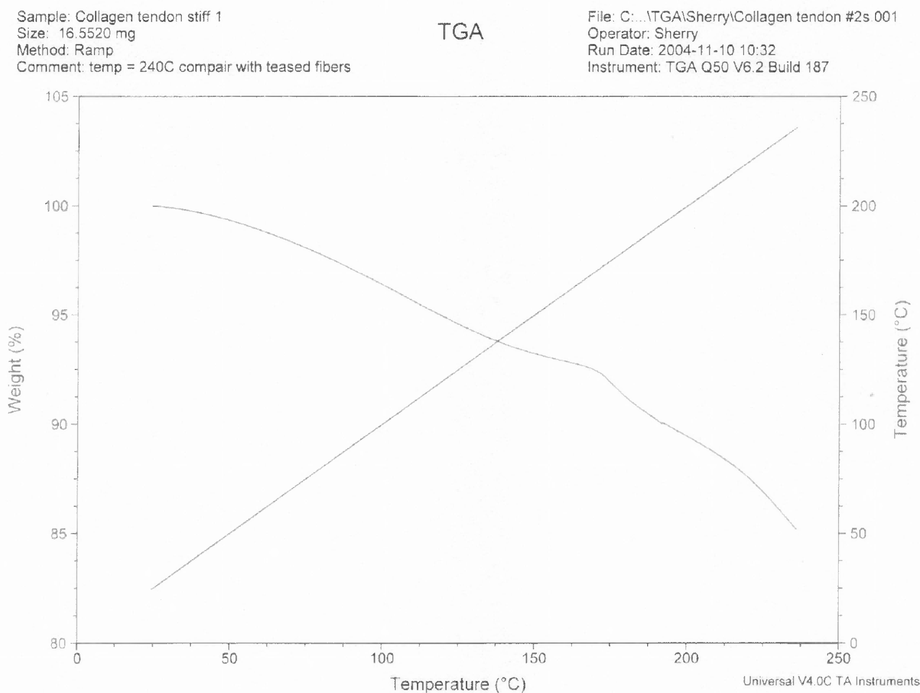

Figure 4.3.11 TGA showing the moisture lose on the collagen tendons.

The PLLA DSC analysis yielded the classic T_m and T_g curves. The T_g of the PLLA tested is around 55°C and the T_m is around 180°C (Figure 4.3.12). The analysis for PLLA and collagen mats yielded the features that are seen in the individual materials (Figure 4.3.13). The T_g and T_m peaks of PLLA have consistently appeared at the temperature around 55°C and 180°C as seen in figure 4.3.13. Figure 4.3.13 is an overlay graph of 3 consecutive DSC analyses on PLLA and collagen electrospun mats. When comparing figure 4.3.12 and figure 4.3.13 the peak that indicated collagen denaturation temperature is not present in figure 4.3.12 but in figure 4.3.13 the denaturation peak is present. This is an excellent indication that collagen also retained its original properties after the electrospinning process.

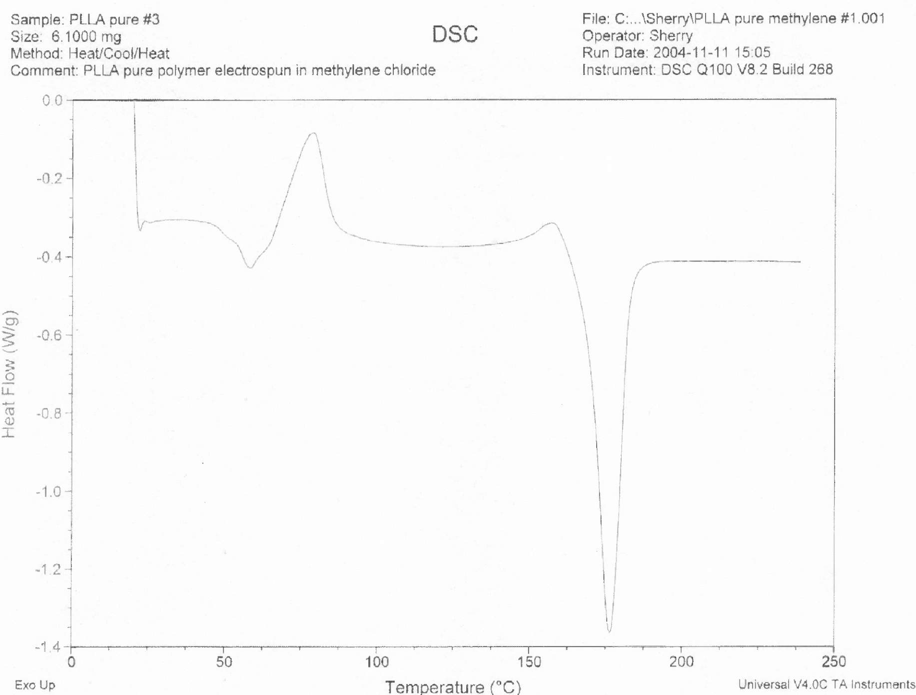

Figure 4.3.12 Second heat cycle of PLLA mat.

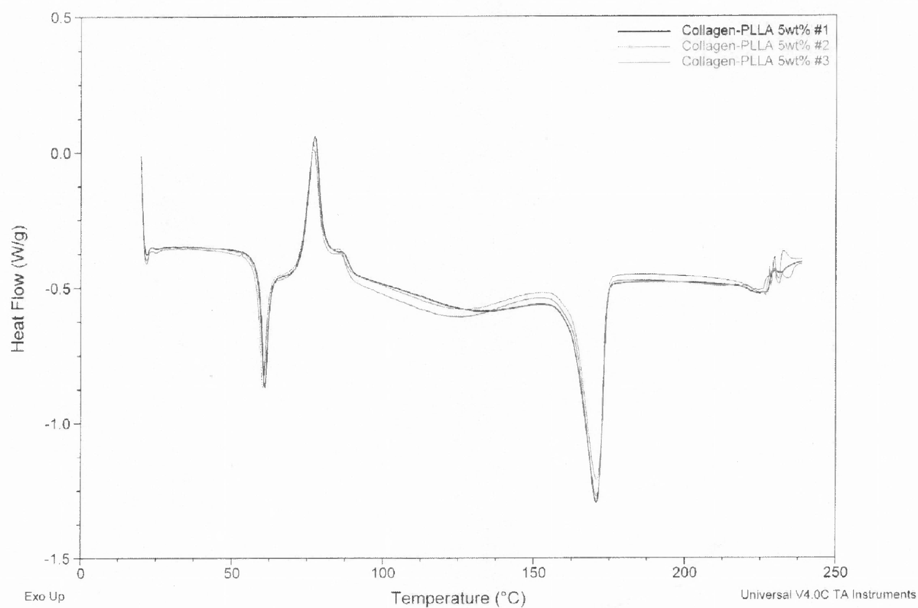

Figure 4.3.13 First heat cycle of DSC on the 5 wt % Collagen & PLLA mat.

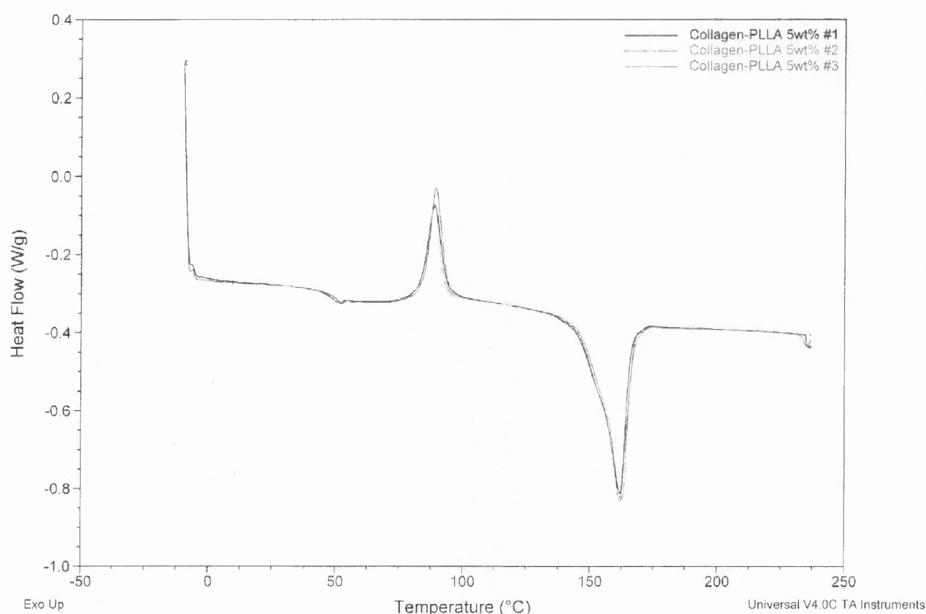

Figure 4.3.14 Second heat cycle of DSC on the 5 wt % Collagen & PLLA mat.

TGA are conducted on the composite mats with the results seen in figure 4.3.15. The most weight loss in the mats is around 8%, which compared to the collagen TGA weight loss of 15% seen in figure 4.3.11 is significantly less. Figure 4.3.12 is an overlay of all TGA data on the composite mats in comparison to collagen tendons. The purpose of this overlay is to show that the moisture that is once present in the tendons are not present after the electrospinning process. The solvent evaporation is sufficient in carrying the moisture off during electrospinning.

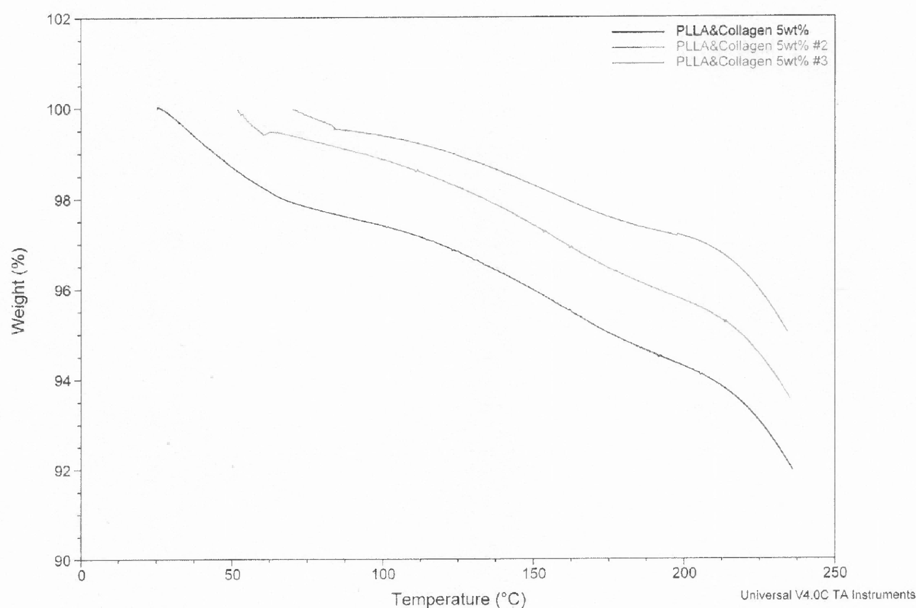

Figure 4.3.15 TGA of 5 wt % Collagen and PLLA mat.

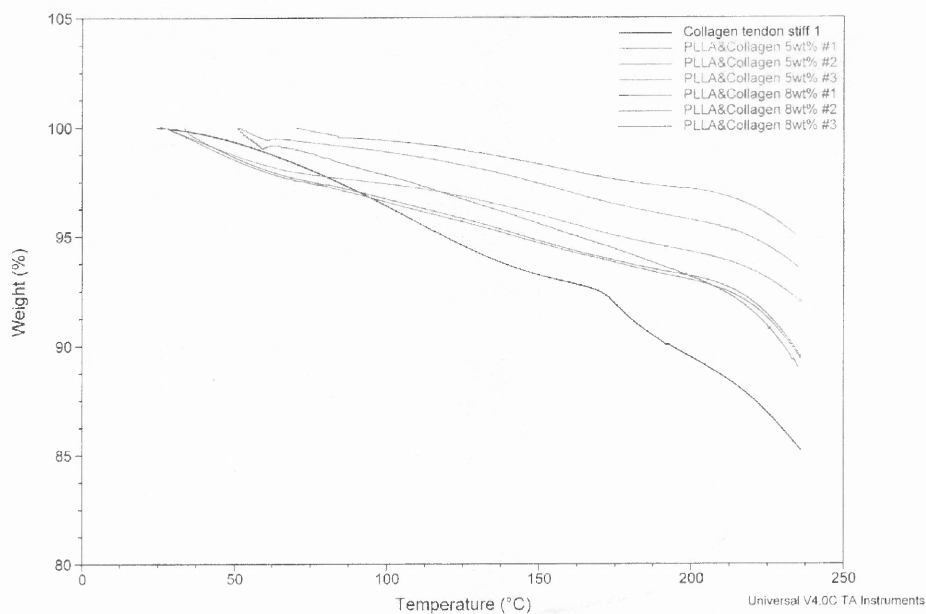

Figure 4.3.16 Combination of 5 wt% and 8 wt % TGA with the collagen tendon TGA comparing weight lose.

An interesting aspect has appeared on the DSC analysis of the composite mats. The sharp peak that appeared at the T_g of PLLA suggested that something is occurring at that peak. Normal PLLA DSC curve will have the T_g where there is change in heat capacity such as in figure 4.3.12. The sharp endotherm peak appears consistently in the DSC of electrospun composite mat analysis. Typically this type of behavior is indicative of enthalpic recovery, which an indicator of densification of the non-crystalline phase. The densification usually thakes place over long periods of time. The easiest way to find out if electrospinning is the cause of the enthalpic recovery on PLLA is to perform DSC aging analysis on pure PLLA that has not been electrospun. The PLLA pure polymer and the PLLA electrospun mat without collagen were analyzed. The results indicated that there is no visible difference or the appearance of the enthalpic peak in the curves.

Since there are no visible differences in the curves the other conclusion is tested. The method employed to determine the origin of the enthalpic recovery peak are carried out by having the pure PLLA polymer and the electrospun PLLA mat isotherm at 40°C for 3 hours. By having the materials isotherm for extended periods gives the polymer chance to organize and orient itself to try to force the enthalpic peak to appear. However, the enthalpic peak did not appear (Figure 4.3.17 & 4.3.18).

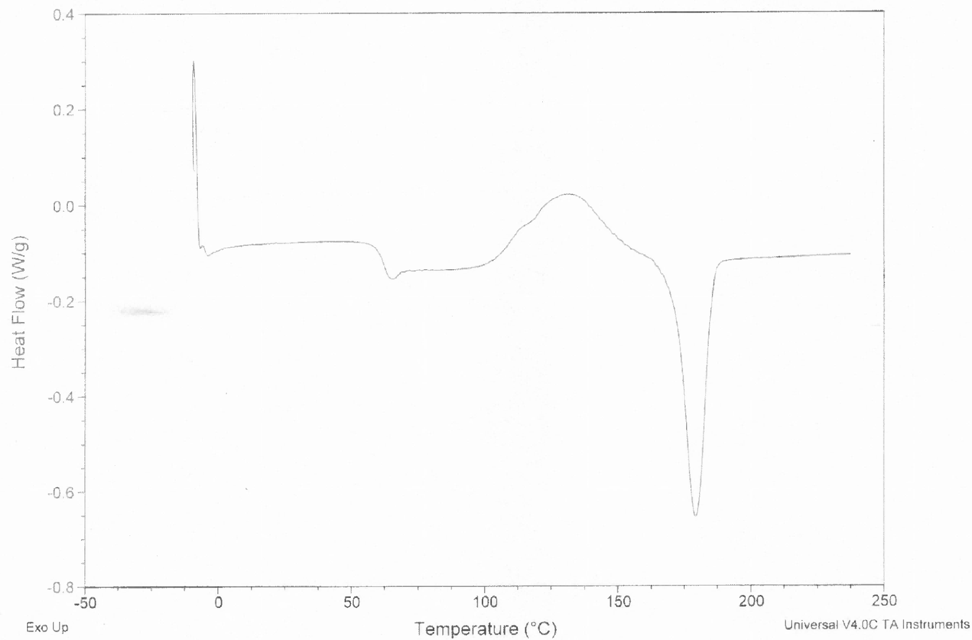

Figure 4.3.17 First heat cycle of PLLA pure polymer after isotherm at 40°C for 3 hours.

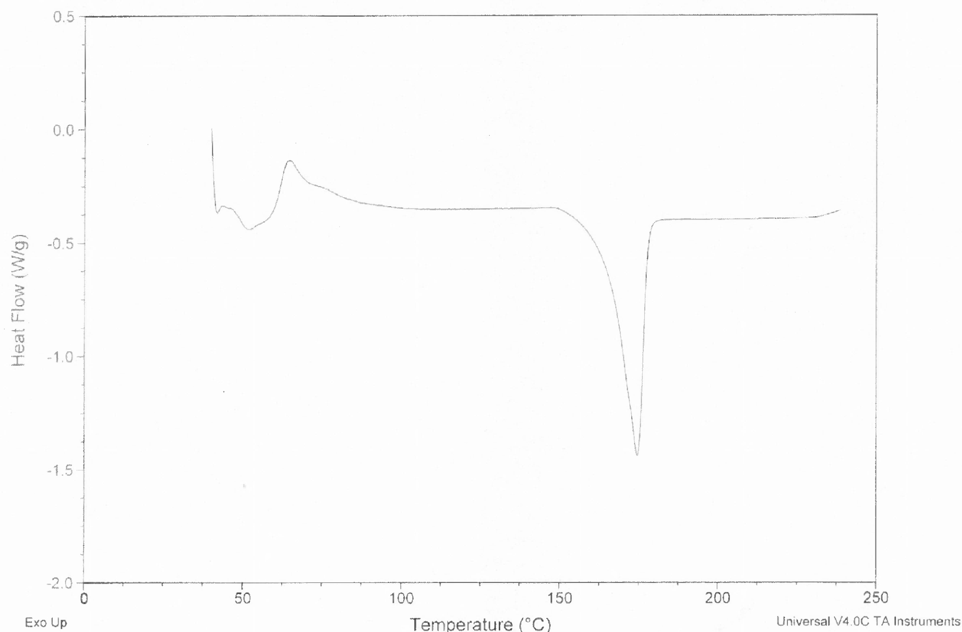

Figure 4.3.18 First heat cycle of PLLA electrospun mat after isotherm at 40°C for 3 hours

This indicats that PLLA did interact with collagen in a manner has driven molecular recrystallization to occur at exceedingly accelerated rate. The time it takes for enthalpic peak to appear in polymers usually takes long periods of time sometimes as long as several months. The enthalpic peak for the collagen and PLLA electrospun fibers appear immediately on spinning. Enthalpic recovery is not the consequence solely of electrospinning because figure 4.3.17 and figure 4.3.18 both did not display any enthalpic recovery in the polymer. The comparison of the figures points to collagen as the key element in densification of the PLLA polymer.

CHAPTER 5

DISCUSSION

The potential of combining a biopolymer and synthetic polymer in electrospinning is mostly unexplored. Various other applications utilizing a biopolymer such as collagen with a synthetic material has been available in the medical fields. Electrospinning as a technique can be considered as one of the most economical and effective applications in producing consistent micron and nano diameter fibers. The Virginia Commonwealth University research group has set precedence in electrospinning collagen where they showed the feasibility of producing fibers with 100nm diameter [21]. With this in mind the idea of adding modifications to the collagen electrospun fibers to give it more unique characteristics is the next step in improving the versatility and compatibility to biomaterial processes.

Collagen is the type of polymer that requires strict processing techniques to enhance its workability in different applications. In this current study collagen as a suspension where the main component is water proves to be difficult to work with. The high water content of the collagen and PEO electrospinning and the further usage of water as the solvent in the electrospinning process, the fibers that can be produced are scarce and difficult to handle. Although, it is possible to electrospin collagen with PEO in high water content solutions, the group at the Emory University and the results presented here show that the hydrophilic nature of PEO will not be the best choice in certain in-vivo applications [22]. PEO as a polymer is readily dissolved in water with elevated temperature. For in-vivo applications that require the polymer to remain for extended

periods, PEO and collagen composites will not be the ideal choice. However, for applications where quick dissolution of the polymer material is required PEO would be the material of choice. In the scaffolding applications PEO would be a poor choice, which is also due to the hydrophilic nature of PEO.

Because of its hydrophobic nature PLLA is chosen as the next material to test. PLLA has already shown that it is a viable biodegradable polymer that has seen many successes in the biomaterial industry. Electrospinning applications utilizing PLLA also have been extensively studied and tested both in-vitro and in-vivo. The bioerosion rates have also been closely studied. It would serve as more ideal scaffolding material where it will retain the fibrous structure in aqueous environments for extended periods of time for the cells to adhere and aggregate. Combining collagen with PLLA in this instance would require the use of solvent that are capable of rendering the two solid materials into liquid form and at the same time will not destroy the materials. The solvent selections for PLLA is not a difficult choice based on the various studies that have been done with good successes. Collagen and solvent selection is more difficult to determine. The key issue is that collagen must be dissolved into liquid form and still retain its characteristics and properties. The initial choice of dissolving each material that is going to be electrospun in their individual solvents the purpose is to retain their original properties. The volatility of lactic acid is low in comparison to chloroform but in this study the assumption is made that chloroform will be able to enhance the evaporation rate of lactic acid during the electrospinning process. However, this assumption is incorrect where chloroform that is used is insufficient in carrying the lactic acid and evaporating it during the electrospinning process. Although, fibers can be produced through this method, the

results are poor. Successful runs of electrospinning through this method can only be achieved with small amounts of collagen in lactic acid. This type of biphasic solvent electrospinning might be of great application if the problem of the solvent miscibility can be solved. At the current time this approach to electrospinning collagen and PLLA is suspended.

Trifluoroacetic acid as a highly volatile solvent is ideal for electrospinning applications. It is first tested in spinning PLLA with much success in producing small diameter fibers. Tendon collagen is then added to the mixture and is able to mix nicely in with PLLA and be electrospun. The results that are obtained is that of fairly uniform diameter fibers in non-woven mats that has retained both properties of the materials. These properties are confirmed through various analysis using DSC and TGA and observed under SEM. The most significant findings in these fibers is the possibility of interactions of collagen and PLLA which normally would not have been possible due to the immiscibility of the materials. The appearance of the enthalpic recovery peak in the DSC has suggested that electrospinnng of PLLA with collagen has resulted in a interaction that has facilitated densification of non-crystalline PLLA. Since this enthalpic recovery does not appear when PLLA and TFA is electrospun, the enthalpic recovery is either of the interaction between PLLA and collagen on the complex interaction of PLLA; collagen and the electrospinning process itself. It can be speculated that the rapid densification occurs because of an orientation in the PLLA phase. It is important to note that the T_g and T_m of PLLA have consistently appeared as well as the denaturation of collagen. Enthalpic recovery is a physical aging or structural recovery in amorphous and semi-crystalline polymers. Usually this phenomenon occurs in polymers only after

several months in room temperature. Through the combinations with collagen this can be observed in less than two days. This signifies that collagen has interacted with PLLA during the process and has affected the structural properties in a significant way in relatively short periods of time. The possibility of interaction between two immiscible material has brought new possibility in biopolymer and polymer blends. The significance is in the fact that the physical and mechanical properties of PLLA through interactions with collagen have been modified.

The discovery of this modification to PLLA through collagen interactions has set precedence to future biopolymer and polymer blends. It is apparent that in the combination that is tested in this study that the original characteristics of the materials are not destroyed but enhanced. The possibilities of combining existing polymers with biopolymers and inducing changes in properties will allow for possible new polymer blending to occur.

CHAPTER 7

CONCLUSION

From the process of electrospinning collagen with PEO in D.I. water the formation of films is the predominant result. Obtaining fiber structures during this process only occurs on occasion. Fibers that are produced have large diameters with apparent phase separation. The explanation for the gross phase separation occurring during this process is directly related to the immiscibility of the polymers. The phase separated fibers served as a basis for comparison for the subsequent studies.

The electrospinning of collagen and PLLA in lactic acid and chloroform is an attempt in alleviating the immiscibility issue that occurred in the previous study. By dissolving the materials in the solvent that each is most suited the electrospinning solution can be more uniform in material distribution. The volatility of lactic acid is very low compared to chloroform but it is believed that chloroform is able to carry lactic acid and evaporate during electrospinning. This assumption is incorrect where chloroform is unable to achieve the desired effect of carrying lactic acid and evaporate. The result indicated that with small quantities of collagen in lactic acid in the electrospinning solution fibers are produced but at higher concentrations thick fibers of PLLA are produced but the fibers are coated with collagen in lactic acid. The phase separation is very apparent in solution where two layers of solutions can be clearly seen.

From observing the shortcomings in the previous two studies solvent selection is of utmost importance. By using trifluoroacetic acid the problems of solvent miscibility is solved. By using only one volatile solvent in the process, solvent elimination is no longer

a problem. Another advantage of using trifluoroacetic acid is that both collagen and PLLA can be readily dissolved. The resulting fibers are small and relatively uniform in diameter. Interesting findings revealed by DSC analysis on the electrospun mats has pointed to possible influence of collagen on PLLA resulting in PLLA densification and orientation in accelerated rate. This enthalpic recovery of PLLA is thought to be solely under the influence of collagen and not of the electrospinning process. Since PLLA pure polymer is electrospun in TFA and yet did not show the enthalpic recovery peak that are seen in all collagen and PLLA mats. The significance of this finding has suggested interactions between a biopolymer and a synthetic polymer to be possible.

CHAPTER 7

FUTURE WORK

Applications of the non-woven mats with the collagen and PLLA blends in the tissue engineering field is the next step toward a more complete study of the feasibility of the bicomponent electrospinning techniques. In addition a more detailed imaging analysis must be carried out to determine the surface morphology of the fibers as well as porosity determinations on the mats. The porosity of the mats is significant in enhancing cell viability once seeded onto the mats. The amounts of pores will determine the possibility of penetration of the cells through out the mats beneath the surface. Although the DSC and TGA analysis has shown that the characteristics of the collagen and PLLA are retained but further analysis into the enthalpic recovery phenomenon should be continued to determine the magnitude of interaction of collagen and PLLA. Base on the possibility and feasibility of creating biopolymer and polymer blends other such combinations should be attempted for varieties of possibility of enhancements in properties in the materials.

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