ELECTROSPUN NANOFIBERS FOR PROGRAMMABLE DRUG DELIVERY SYSTEM SEQUENTIALLY TARGETING INFLAMMATION AND INFECTION

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ABSTRACT

A high amount of trauma on the battlefield gives rise to critical blood loss and large, open wound areas that risk infection. Due to the limitations of addressing wounds and injuries during times of war, conflict, or emergency, it would be highly desirable to have available simple, portable thin film systems that can be used to wrap or apply to a wound directly to eliminate infection and promote wound healing. Electrospinning, as a simple and robust way, developed in the last several decades, has been a heat spot for fabricating thin and multi-functional film.

In this project, for the first step, we aim to develop versatile and durable electrospun matrix system based on nature-derived polymers, which means that it would be not toxic or poisonous to wound and injury areas. Meanwhile, we also aim to engineer its core-shell structured fiber morphology so as to achieve desirable release kinetics of multi-drugs such as anti-inflammatory and anti-bacterial agents on demand. In this case, the sequence of multi-drugs releasing can be well controlled and the drugs can be released equally during a long period of time.

Electrospinning parameters to fabricate biocompatible core-shell structured fibers were investigated and optimized. Effect of methanol post treatment was also investigated by means of Fourier transform infrared spectroscopy, differential scanning calorimetry and Instron tensile test. Material properties of core portion were controlled and reinforced
significantly by judiciously selecting counterpart bio-additives. In the future work, the drug release profiles of newly developed core-shell electrospun scaffolds will be investigated via high performance liquid chromatography and plate reader.
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CHAPTER I
INTRODUCTION AND LITERATURE REVIEW

A high amount of trauma on the battlefield gives rise to critical blood loss and large, open wound areas that risk infection.\cite{1,2} Wound dressing and wound healing are vital for saving the valuable lives of thousands of soldiers. Different types of wounds need different approaches to be healed based on the understanding and incorporating of basic wound healing principles. Meanwhile, suitable types of wound dressing with desirable materials and processing methods could help tissues to recover from trauma efficiently. In this case, this introduction will be elaborated from three aspects as follow: (1) Wounds, in terms of their types and healing principles. (2) Current solutions to wound dressing regarding advantages and disadvantages. (3) Materials that are suitable for wound dressing in this project.

1.1 Wound

A wound is a condition of dysfunctional structure\textsuperscript{3} of the skin which is the result of injuries of cutting, puncturing or tearing, or from contusion caused by blunt force. The former are typically open wounds where the latter are normally closed wounds.

1.1.1 Different kinds of wounds

1.1.1.1 Classification of wounds based on the time scale

Based on the repaired time scale, wounds can be classified as acute or chronic wounds.

1.1.1.1.1 Acute wounds
Acute wounds, usually lasting 8–12 weeks, are types of wounds that deal with temporary tissue injuries, which means these types of wounds will leave small scars and can be healed completely after treatment. Acute wounds are mainly caused by mechanical factors from outside. For example, (1) abrasions and tears generated by contact of the friction between hard objects and human skin. (2) Wounds penetrated through the surface of the skin from a variety of sharp sources such as knives and speedy objects such as bullet wounds, or even some surgical wounds due to typical surgical incisions. Also, radiation, electricity, corrosive chemicals and thermal sources could cause other types of acute wounds, varying significantly by the exposure time, burning intensity, and source temperature. Normally these types of acute wounds are more complicated regarding the associated trauma.

Figure 1-1 Typical acute wound caused by mechanical factors such as impact by sharp sources (from the poster of Huston hospital, allowed to be cited here).

1.2.1.1.2 Chronic wounds

Chronic wounds, usually lasting longer than 12 weeks and potentially recurring intermittently, are types of wounds that need continuous treatment and tend to leave large areas of scars due to several repetitive tissue damage. For example, (1) persistent
infections caused by pathogenic bacteria and microorganisms induced by poor air condition, improper wound treatment, and some other environmental related factors. (2) Complications accompanied from diseases that can destroy the human immune system gradually, such as Diabetes, mellitus, or AIDS. Normally chronic wounds are more difficult to cure because they deal with damage to the human tissues based on the disruption of the inherence of wound healing process that will be introduced later.

Figure 1- 2 Schematic of chronic wound normally caused by infections of bacteria or microorganism (from the front page of Kirk V. Dahl, MD, allowed to be cited here).

1.2.1.1.3 Classification of wounds based on the other aspects

Other than the classification by time scale as acute and chronic wounds, wounds can also be classified according to how many layers of skin are infected and how large the affected skin surface is. For example, (1) a superficial wound is a wound caused by unpenetrated injuries that can only affect the skin surface. (2) Partial thickness wound is a wound involving both the skin surface and the further layers into derma, which consist of the micro blood vessels. (3) Full thickness wound is a wound that happens when the injuries are so
serious that they penetrate beneath the dermal layers into subcutaneous fat or even deeper tissues.

Occasionally, wounds can be mixed with both acute and chronic factors. Ferreira et al. reported such wounds that are hard to heal as ‘complex wounds’. In terms of their investigation, the identifications of complex wounds can be summarized as: (a) massive shedding of skin, hair, and relevant glands, (b) infections that result in tissue loss, (c) tissue death with circulation impairment.

1.2.1 Wound healing principles

Typically, there are four steps for wound healing as shown in figure 1-3. The first step of healing, which is inflammation, occurred immediately after damage happened. In this step, fibrinogen transferred into fibrin to lead to coagulation, which stops bleeding. The migration step happened simultaneously with the inflammatory stage. At this step, blood neutrophils were followed by phagocytes and entered the wound medium to penetrate inside the dead cells to eat up these dead cells. At the proliferation step, the wound was covered completely with the epithelium. In this step, new live cells moved toward the wound and proliferated massively to replace the dead cells. In the final stage called the maturation step, the fibroblasts covered the surface of the wounds to generate a new skin, which is also called the tissue-remodeling step.
1.2.2 Wound dressing

1.2.2.1 Classification of Dressings

Wound dressings can be classified in diverse approaches (1) depending on their functions in the wound such as debridement, antibacterial or adherence, etc.,\textsuperscript{12} (2) type of material employed to fabricate the dressing such as synthetic polymers or naturally(?) derived materials,\textsuperscript{13} (3) the appearance of the dressing such as film, ointment or gel, etc..\textsuperscript{14} Much of work has been done related to the classification of wound dressings.\textsuperscript{15}

Based on its history, wound dressings could be defined as traditional dressings such as cotton wool, and modern and advanced dressings such as electrospun film or hydrogel film. Based on the contact mechanism, wound dressings can be classified as primary, secondary,
and island types. (1) Primary dressings are those wound dressings that directly contact the wound surface, on the contrary, (2) secondary dressings are those wound dressings that contact the wound surface indirectly on top of the primary dressing. (3) Island dressings, as the name suggests, are surrounded by an adhesive margin area with a central medical effective area.

Currently, compared with traditional wound dressings, modern wound dressings with more moist and biocompatible wound surface treatment attract more attention, and normally modern wound dressings could have multiple functions taking both mechanical (film, scaffold) and medical (drug carrier) needs into account.

1.2.2.2 Traditional wound dressings

For a long time, cotton wool bandages and gauzes have been used as traditional wound dressings. When using such kinds of traditional wound dressings as primary or secondary wound dressings, they can efficiently (1) support and protect the wounded human tissue by surrounding the wound area with multiple layers and (2) absorb exudate leaked from the wound during the anti-inflammatory step, in terms of the principles of wound healing. And to a certain extent, such traditional dressings can provide protection from the threat of dangerous environmental bacteria.

However, unlike modern wound dressings, traditional dressings have obvious disadvantages that impede them from being the standard in the wound dressing field. (1) these traditional dressings are dry without providing a moist wound environment and actually such kinds of moist wound environments are key to deliver drugs and heal the wound. (2) The protection from bacteria could be less efficient when traditional dressings are immersed by either wound exudate or external fluids. (3) When dealing with chronic
wounds, the area wrapped with traditional dressings could adhere to the dressings and become painful and uncomfortable when removed. 18 (4) Traditional wound dressing is inconvenienced since the wound needs to be wrapped by thick dressings and normally such traditional dressings need to be changed multiple times to permit wound ‘breathing’ which is less economical.

1.2.2.3 Modern Wound Dressings

Based on the development of traditional wound dressings, modern dressings utilized the advantages of traditional dressing and meanwhile it has unique moist wound environment characteristics. The advantage for such moist wound condition with modern wound dressings could be referred to the biocompatibility when healing wounds. According to the materials (hydrocolloids, hydrogels) or physical structured formation (gels, thin films and foam sheets) of the wound dressings, typical Species of the modern dressings are introduced here as below.

1.2.2.3.1 Hydrocolloid Dressings

Currently, hydrocolloid dressings are the dressings used most widely. Typical film or sheet shaped forms are applied to these dressings and normally they are made of colloid materials (e.g. agents that can form gel, typically carboxymethylcellulose (CMC), gelatin and pectin) combined with other materials such as elastomers and adhesives.

Hydrocolloid dressings have advantages of (1) different permeability of vapor. More specifically, initially within the static state, hydrocolloid dressings are impermeable to water vapor, however, once the state changes into gel formation since the wound exudate is absorbed, they will become significantly more permeable to water and air as the gel forms. 19 (2) As they do not cause pain upon removal, they are particularly useful in
paediatric wound care for management of both acute and chronic wounds. Some possible mechanisms involved in hydrocolloids’ ability to reduce pain have been discussed.

However, since hydrocolloid dressings generally have an occlusive outer cover that prevents water vapor exchange between the wound and its surroundings, it can be disadvantageous for infected wounds that require a certain amount of oxygen to heal rapidly. Another disadvantage applies to dressings containing fibers that are deposited in the wound and often have to be removed during dressing change.

Figure 1-4 A typical hydrocolloid dressing (cited from commercial 3M Healthcare) combining moisture vapor permeability with absorbency and conformability, meanwhile the transparent appearance gives the ability to check the wound directly.
1.2.2.3.2 Semi-Permeable Adhesive Film Dressings

Film dressings, originally made from nylon derivatives, have been used in the medical field for a very long time. The film dressings have advantages of (1) transparency, conform to contours (due to their elastic and flexible nature) such as elbows, knees and sacral areas, (2) do not require additional taping, however, they also have disadvantages such as (1) limited ability to absorb sufficient quantities of wound exudates, which results in the accumulation of excess exudates beneath the dressing. This leads to skin maceration and bacterial proliferation and the risk of infection, and therefore requires regular changing as well as irrigation of the wound with saline, making them unsuitable as wound dressings. (2) Too thin to be packed into deep or cavity wounds and only suitable for relatively shallow wounds.

1.2.2.3.3 Biological Dressings

Biological dressings are those dressings consisting of active biomaterials derived from natural materials such as collagen, chitosan and elastin, etc. and sometimes referred to as ‘bioactive dressings’. Biomaterials have several advantages: biodegradability, biocompatibility, and play an active part in normal wound healing. In some cases they may be incorporated with active compounds such as antimicrobials and growth factors for delivery to the wound site. Collagen is a natural constituent of connective tissue and a major structural protein of any organ. Its structural, physical, chemical, biological and immunological properties have been discussed widely in the literature. It stimulates formation of fibroblasts and accelerates the migration of endothelial cells upon contact with damaged tissue. The matrix can be medicated, thus serving as a reservoir for drug delivery. The use of collagen matrices for delivery of different classes of antibiotic drugs
have been discussed extensively. Chitosan is known to accelerate granulation during the proliferative stage of wound healing, and its wound healing application has been reviewed.

1.2 Silk
Historically, silk has been used for thousands of years in the form of threads in textiles as a well described natural fiber produced by the silkworm, Bombyx Mori. The silk fibroin is a highly insoluble protein containing up to 90% of the amino acids glycine, alanine, and serine leading to antiparallel β-pleated sheet formation in the fibers. The schematic of the structure of the silk fibroin is in Figure 1-4. Recent interest in the use of regenerated silk fibroin (RSF) solutions in biomedical applications and in biotechnological films is attributed to the unique mechanical properties of these fibers as well as their biocompatibility.

Although since it is a natural polymer, B. mori silk fibroin fibers have a broad range of molecular weight distribution, it has an average molecular weight near 390,000(g/mol), meanwhile, silk fibers are coated with a family of hydrophilic proteins called sericins, which have molecular weight ranging from 20,000-310,000(g/mol). However, researchers found that sericin proteins are one type of main impurities encapsulating silk fibroin, and physiologically sericin has been proved to have the ability to cause immunogenic response in vivo. Therefore, during silk purification, alkali such as sodium carbonate is used in the first degummed step to remove sericin.
Figure 1-5 Schematic of the structure of silk compositions (cited from Dr. Younjin Min with permission.)

Figure 1-5 shows structural hierarchy of Bombyx mori silkworm silk (protein block copolymers). The thread is composed of small crystalline b-sheet rich subunits (Alanine-rich), which are embedded into an amorphous structure (Glycine-rich). The beta-sheets are formed by self-assembly through hydrogen bonds as well as van der Waals interactions.\textsuperscript{34}

1.3 Polyethylene oxide (PEO)

Polyethylene oxide (PEO) is a branch of polymers polymerized by ethylene oxide with a wide molecular weight range from 300 g/mol to above 10,000,000 g/mol. The chemical structure of PEO is shown in figure 1-6, which consists of multiple repetitive unit -CH2-CH2-O-. These polymers have low toxicity to human tissues and are traditionally used as a lubricating coating for various surfaces in aqueous and non-aqueous environments.\textsuperscript{35} Since PEO is well documented in a variety of applications relevant to bioengineering and biomaterials as a biocompatible polymer,\textsuperscript{36, 37} its solution is used as an encapsulation shell solution in this project due to its biocompatibility to mimic the function of sericin.
1.4 Collagen

Similar to silk, many polymers, including collagen\textsuperscript{38,39}, gelatin\textsuperscript{40}, Chitosan\textsuperscript{41,42,43} as naturally derived polymers and poly(\(\varepsilon\)-caprolactone)\textsuperscript{44}, Polylactic acid\textsuperscript{45} as synthetic polymers, have been studied worldwide and successfully processed as tissue engineering scaffolds. It is noteworthy that collagen as one of nature derived polymers widespread in animals’ bodies to connect tissues, has been proved to have plenty of excellent properties as an advanced material for fabricating tissue scaffolds such as biocompatibility in the human body, potential in utilization as medical carriers due to its biodegradability and low antigenicity.\textsuperscript{46}

Normally the molecular weight of collagen is near 300kDA, which is near the range of molecular weight of silk. In terms of its structure, collagen fibrils are made by multiple collagen fiber bundles, which consist of triple helix as shown in Figure 1-7 referred from Shoulders et al.\textsuperscript{47}
Figure 1- 7 Schematic of reversible synthetic cycle of collagen materials including collagen fibers and triple helix, which are the major component of skin. Oxidation of lysine side chains leads to the spontaneous formation of hydroxylsyl pyridinoline and lysyl pyridinoline cross-links. (This image is referred from Shoulders et al. \(^{47}\) with permission.)

1.5 Drug release

With proper methods, desirable release kinetics of multi-drugs such as anti-inflammatory and anti-bacterial agents could be controlled on demand. Uhrich et al.\(^{48}\) reported that controllable drug release could be established via polymeric system based on the nontoxic polymers such as poly(ethylene glycol) block copolymers, poly(lactic acid), poly(glycolic acid) and their copolymers. Naturally, the anti-inflammatory should release faster than anti-bacterial drugs accordingly corresponding to the mechanism of wound healing.

1.6 Objectives

To my knowledge, this is the first research working on the programmable drug delivery system aiming at Inflammation and Infection with electrospun regenerated silk fibers using core-shell coaxial electrospinning.

Compared with those traditional and modern dressings, silk/PEO electrosopun film dressing system in this project can be defined as biological semi-permeable adhesive film dressings, in this case, it has advantages of flexibility and easily conforms to contours because of elasticity, biodegradability, biocompatibility, and playing an active part in normal wound healing.

Meanwhile, processing with core-shell coaxial electrospinning techniques, it also has its
unique advantages. For example, (1) it is not only wound dressing, but can also be utilized as a drug carrier and, more importantly, deliver dual drugs simultaneously with desirable dual drug release profile. (2) Due to the high aspect ratio of electespun film, more drug can be carried onto thinner film, which means the materials used in this system are equipped more efficiently. (3) By changing the parameters during the electrospinning process, fiber morphology of the film can be better controlled and so as the fiber orientation, which could in turn fabricate film dressings that have both desirable mechanical properties and drug dosage.
CHAPTER II

ELECTROSPUN NANOFIBERS FOR PROGRAMMABLE DRUG DELIVERY
SYSTEM SEQUENTIALLY TARGETING INFLAMMATION AND INFECTION

2.1 Introduction

The main purpose of this research is to study the incorporation of multiple naturally derived polymer systems as the material sources of wound dressing bandage. The bandage, fabricated from an electrospinning device, consists of nanofiber films made by naturally derived polymer and commercial biocompatible polymer, which are programmed in core-shell structured morphological form.

Such core-shell structured nanofiber film system fabricated by an/the electrospinning process, will lead to a programmable dual drug release effect once the two types of drugs, which are anti-inflammatory and anti-bacterial, are dissolved in different polymers of core and shell parts, respectively. In this project, the following focused objectives will be studied:

(1) The determination of various electrospinning parameters during core-shell electrospinning to generate uniformed electrospun film.

(2) Modification of the naturally derived polymer system to fit with the human skin tissue from the aspect of mechanical properties.

(3) The evolution of fraction of swelling and weight loss of the electrospun mat after mimic vitro environmental incubation.
(4) The evolution of mechanical properties with multiple time points after mimic vitro environmental incubation.

(5) The evolution of phase change inside of the polymeric scaffold.

2.2 Materials

The given materials below will be used in this research. Their chemical properties are listed here.

2.2.1 Polyethylene oxide (PEO)

Polyethylene oxide (PEO), purchased from Sigma-Aldrich, is in powder morphology and its average molecular weight is 900,000(g/mol) with 65\(^\circ\)C melting temperature and -67\(^\circ\)C glass transition temperature.

2.2.2 B. mori silkworm silk

Cocoons of B. mori silkworm silk were purchased from Aurora Silk, Co. all the cocoons used in the experiments are well documented and carefully selected with basically similar appearance by looking. The average molecular weight of the silk is near 390kDA.

2.2.3 Collagen

The type 1 collagen used in this project to modify silk properties is kindly provided by DSM, Co. This collagen is in powder form and derived from calf skin. The molecular weight of type I collagen is about 300,000(g/mol).

2.2.4 Other main chemicals

All the chemicals below are purchased from Sigma-Aldrich Co.

Sodium carbonate (Na\(_2\)CO\(_3\)) (99.9%)

Lithium Bromide (LiBr) (99.9%)

Polyethylene glycol (PEG) with average molecular weight of 8,000(g/mol)
2.3 Experimental section

2.3.1 Preparation of regenerated \textit{B. mori} silk fibroin solutions

The 8wt \% \textit{B. mori} silk solution was prepared as the protocol described by Kaplan et al.\textsuperscript{49} Cocoons were boiled for 30 min in an aqueous solution of 0.02 M Na\textsubscript{2}CO\textsubscript{3} to extract the glue-like sericin proteins, and then rinsed thoroughly with water 3 times to wash out the residual Na\textsubscript{2}CO\textsubscript{3}. The extracted silk was then torn into small pieces and dissolved in 9.3 M LiBr solution at 60\textdegree{}C for 4 hours. After that, the yielded solution was dialyzed in water using dialysis tube (Thermo Scientific, MWCO 3500) with proper dimensions. After that, the dialysate was centrifuged twice at 4\textdegree{}C for 20 min at 9000rpm and later 10wt\% PEG solution was used to concentrate the solution with dialysis tube (Thermo Scientific, MWCO 3500). The final concentration of aqueous silk solution was 8.0-9.0wt \%, which was determined by weighing the remaining solid after drying.

2.3.2 Preparation of spinning solutions

A 3.0wt \% PEO solution was prepared under gentle rotatory mixing with a rotary mixer for at least 24 hours at room temperature in order to obtain a homogeneous solution. A Silk/PEO blends was prepared by adding 3.0wt \% PEO (900,000 g/mol) into 8.0wt \% silk aqueous solution at a volume ratio of 1: 6 generating 7.3wt \% silk/PEO solutions. To avoid the premature formation of \textbeta{}-sheet structure during blending of the two solutions, the solutions were mixed gently with the rotary mixer at low rpm for at least 24 hours.

2.3.3 Preparation of modified spinning solutions

A 3.0wt \% PEO solution was prepared under gentle rotary mixing with a rotary mixer for at least 24 hours at room temperature in order to obtain a homogeneous solution. A
Silk/PEO blend was prepared by adding 3.0wt % PEO (900,000 g/mol) into 8.0wt % silk aqueous solution at a volume ratio of 1: 6 generating 7.3wt % silk/PEO solutions. Before that, a collagen aqueous solution was prepared by gently heating in 60°C for 10 minutes and added into the silk/PEO blend. To avoid the premature formation of β-sheet structure during blending, the solution was mixed gently with the rotary mixer at low rpm for at least 24 hours.

2.3.4 Uniaxial electrospinning

After the preparation of the spin solution, the effects of each electrospinning parameters such as voltage, flow rate and tip-collector distance are investigated primarily. Meanwhile, during uniaxial electrospinning, the preliminary optimal values for each electrospinning parameter are determined to give initial set points for the coaxial electrospinning experiments.

2.3.5 Coaxial Electrospinning

The coaxial electrospinneret, which allows coaxial extrusion of two fluids simultaneously, is shown in Figure 2-1. The design of coaxial electrospinneret is aimed to keep the fluids separate before exiting the nozzle and Figure 2-1 shows the photos of coaxial spinneret used in this experiment. Similar designs have been reported independently by Sun et al., Li and Xia, Loscertales et al., Wang et al. and Zhang et al. The electrospinneret is made of two stainless steel concentric tubes in which the core (silk) and shell (PEO) fluids are pumped from two separate syringes to the spinneret. The inner tube has an inner diameter of 1.47 mm and an outer diameter of 0.95 mm, while the outer tube has an inner diameter of 2.42 mm and an outer diameter of 2.78 mm. The diameter ratio of the inner
and outer tubes is roughly 2:5.

Figure 2-1 Coaxial electrospinneret: (a) The integral view (b) The integral view (c) The separated view

Figure 2-1 shows (a) the integral view of the coaxial electrospinneret. (b) The top view of the concentric inner and outer tubes. (c) The separated view of inner and outer tubes. The coaxial electrospinneret is designed by Dr. Younjin Min, which is made by 360 stainless steel with high salt solution resistance. Also it is assembled manually so that it can be easily cleaned.

A horizontal coaxial electrospinning setup was used in this project as shown in figure 2-2. Briefly, the setup consisted of two syringe pumps (Harvard Apparatus 11plus) that were used to deliver the core and shell fluids to the spinneret independently at constant flow rates and a square grounded stainless steel plate covered with Aluminum foil (20*20cm). An electrical potential up to 30kV was applied to the nozzle by a high-voltage power supply (EMCO) with a display of current voltage. The voltage, flow rates of both core and shell fluids, and the distance between the spinneret and the collector were adjusted to obtain a
stable Taylor cone and a continuous stream. Both the inner and outer tubes were charged to the same electrical potential. A chamber that covers all electrospinning devices is equipped incorporating with the connection to the continuous compressed air so as to control the relative humidity in the chamber during extremely high ambient humidity conditions.

Figure 2-2 Schematic of the coaxial electrospinning setup

Figure 2-2 shows the schematic of the coaxial electrospinning. Two syringe pumps push the fluids in two syringes simultaneously into a coaxial electrospinneret. A high voltage is applied between the tip of the spinneret and the collector.

Based on the purpose of the electrospun products during the different experiments, a variety of electrospinning times are applied, from 1 minute to 3 hours. For microscope imaging, samples are electrospun onto the surface of a glass slide for 1 minute to have a
clear view of the morphology of individual fibers, while for PBS incubation and mechanical tensile test, 3 hours electrospinning is processed to get relatively thicker films. Normally during long time electrospinning, the position of plate collector is constantly changed every 30 minutes to obtain electrospun film with even thickness.

2.3.6 Post treatment
Before utilizing the as-spun film for PBS incubation, the samples need to be treated with methanol to induce beta-sheet structure to silk part to prevent dissolving completely. This is called post treatment. To specify the method of this treatment, initially the sample film peeled from the collector was cut into small pieces to get several samples by the determined dimensions. After that, two methods of methanol treatment were used in this project. The samples were treated by (1) 100% methanol solution immersion, or (2) 80% methanol vapor at 30°C for 2 hours. The difference between these two methods will be revealed later.

2.3.7 Experiments to investigate the methanol treatment methods
During this project, the samples with different methanol treatment methods exhibited different properties from both thermal analysis and mechanical test. In this case, the difference among different methanol treatment methods was studied. Basically, the methanol concentration, the direct immersion or the indirect vapor diffusion and the treating time are primarily investigated by only changing one type of variable each time.

2.3.8 Vitro incubation
The as-spun film was cut into small pieces to get several samples by the determined dimensions. And then after methanol treatment, the samples were incubated into 37°C, pH7.4 buffer solution in a temperature controlled rotary mixer. After that, samples were
removed gradually at given time points, for example, 30 minutes, 2 hours, 4 hours, etc. Meanwhile, weight of each sample before and after was recorded accordingly to measure the weight loss.

2.3.9 Mechanical test

The as-spun samples, methanol treated samples and PBS incubated samples were tested on instron 5567 to compare their mechanical properties.

Each sample was mounted between holders at a distance of near 3 cm. The tensile test was conducted at a rate of 1 mm/min at room temperature. Young’s modulus (in MPa), ultimate tensile strength (in MPa) and strain at break (mm/mm) were calculated from the stress-strain data. To clarify Young’s modulus, it was calculated from the slope of each stress-strain curve in its elastic deformation region represented as initial linear region of the stress-strain curve. Pictures of the instron machine and its set up during tests are shown in Figure 2-3 (a) and (b).

![Figure 2-3](image)

Figure 2- 3 (a) instron 5567 machine utilized to tested sample mechanical properties in this project, (b) sample mounted onto the instron machine.
2.4 Characterization

2.4.1 Spinnability characterization

Viscosities of solutions were tested from Bohlin Gemini $^{\text{HR nano}}$ Rheometer in room temperature.

2.4.2 Morphological characterization

The images illustrating the fiber morphology were obtained by both optical microscope and scanning electron microscopy (Model JEOL SEM 7401).

The diameters of electrospun fibers were measured from the images captured from Nikon optical microscope.

2.4.3 Thermal analysis

TA instrument Q2000 differential scanning calorimetry (DSC) was conducted to investigate the phase difference between each sample with different condition.

2.4.4 FTIR

The Thermo Scientific Nicolet 380 FTIR spectrometer was used to measure the FTIR spectroscopy of each sample with different condition.

2.4.5 Mechanical test

The thickness of the samples was measured by micrometer and the weight of each sample was weighed by micro balance.

The mechanical properties of samples are tested with instron 5567 machine with sample dimensions of 40mm long, 7mm wide and near 0.08mm thick. All tests were performed at ambient temperature and a crosshead speed of 1 mm/min was applied.

2.5 Results and Discussions

2.5.1 Electrospinning parameters determination
2.5.1.1 Viscosity of solutions

Figure 2-4 Apparent viscosity vs shear rate for the corresponding materials. Figure 2-4 shows the viscosities of silk/PEO blends in water. The volume ratio of blends is 6:1. The viscosities of pure 3wt%PEO solution, 12wt% and 14wt%silk solutions are also added into the graph as a comparison. The viscosity data is obtained from Bohlin Gemini HR nano Rheometer tested in room temperature. From the data, the viscosity of pure silk solution was much lower than the other solutions, whereas the viscosity of pure aqueous solutions of PEO is higher than the other solutions. For the electrospinnable solutions with higher viscosities, it is harder to elongate to fiber filament when travelling between tip and collector, which in turn increased the final diameter for the electrospun fibers on the collector.55,56 The viscosity data is coincident with the preliminary experimental data from
Jin et al.$^{57}$

For the uniaxial electrospinning, RSF solution cannot be electrospun without adding PEO solution to make a blend; no fibers were formed because the viscosity and surface tension of the solution were not high enough to maintain a stable drop at the end of the capillary tip. However, higher concentrations of silk in water to increase the viscosity of the solution resulted in gel formation. Jin et al. did the electrospinning of RSF/PEO blend and summarized a similar result.$^{57}$ The stable drop at the end of the capillary tip could only be achieved once the PEO was added to the silk solution. Therefore, PEO was added into RSF solution to increase its electrospinnability for uniaxial electrospinning.

2.5.1.2 Flow rate effect
Figure 2-5 Electrospun fiber diameters vs flow rate for the corresponding solutions.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Flow rate range Q</th>
<th>Voltage V</th>
<th>TCD H</th>
</tr>
</thead>
<tbody>
<tr>
<td>8wt%silk/3wt%PEO blend</td>
<td>0.010mL/min – 0.060mL/min</td>
<td>15kV</td>
<td>6.0”</td>
</tr>
<tr>
<td>3wt%PEO</td>
<td>0.010mL/min – 0.060mL/min</td>
<td>15kV</td>
<td>6.0”</td>
</tr>
</tbody>
</table>

Table 2-1 Experimental conditions for flow rate test

Figure 2-5 shows the trends of Increase in diameters of electrospun fibers with increasing feed rate tested with 8wt%silk/3wt%PEO blend at 15kV and 6.0” tip-collector distance and 3wt%PEO at 15kV and 6.0” tip-collector distance. The error bars indicate the width of the diameter profile edges. Initially, when the flow rate was lower than 0.02mL/min, since the
flow rate was too low, the jet was unstable and sprayed immediately when coming out from tip, which made the 3wt%PEO opposite trend.

The reason for this effect is that a lower flow rate ejects less fluid in a stream\textsuperscript{58, 59}, and the flow rate influences the volume of drop suspended at the tip of the spinneret directly, which plays a significant role on the shape of the Taylor cone. The maintenance of the cone shape at the tip during electrospinning is important to obtain a continuous stream and as a result, to generate the uniformed electrospun fibers. Empirically, the optimal flow rate should range from 0.020-0.040mL/min to keep a well-shaped Taylor cone.

2.5.1.3 Voltage effect

Figure 2-6 shows the trend of decrease in diameters of electrospun fibers with increasing voltage tested with 8wt%silk/3%PEO blend at constant flow rate of 0.020mL/min and 6.0” tip-collector distance and 3%PEO at constant flow rate of 0.040mL/min and 6.0” tip-collector distance. The error bars indicate the width of the diameter profile edges.
Figure 2- 6 Electrospun fiber diameters vs applied high voltage between tip and collector for the corresponding solutions.

<table>
<thead>
<tr>
<th>Materials</th>
<th>Voltage range V</th>
<th>Flow rate Q</th>
<th>TCD H</th>
</tr>
</thead>
<tbody>
<tr>
<td>8wt% silk/3wt% PEO blend</td>
<td>6kV – 24kV</td>
<td>0.020mL/min</td>
<td>6.0”</td>
</tr>
<tr>
<td>3wt% PEO</td>
<td>6kV – 30kV</td>
<td>0.040mL/min</td>
<td>6.0”</td>
</tr>
</tbody>
</table>

Table 2- 2 Experimental conditions for voltage test

Generally, increasing applied voltage results in a smaller fiber diameter. However, different trends could also be found in previous work from other groups with a variety of explanations. Although, the arguments on the influence of voltage on the diameter of electrospun fiber are still controversial, one explanation is that with increasing electric potential, which is represented by voltage, the polymer fluid is discharged with a greater
electrostatic repulsion that causes it to undergo higher levels of drawing stress, which results in the smaller diameters of electrospun fibers. However, this trend was not so dramatic compared with the other parameters, and this voltage effect on fiber diameter also disappeared particularly when the polymer concentration was low. According to past works, higher voltage was reported to induce not only smaller diameter but also higher diameter. Applied voltage may affect some factors such as mass of polymer fed out from the tip of the needle, elongation level of a jet by an electrical force, morphology of a jet (a single or multiple jets), etc. A balance among these factors may determine a final diameter of electrospun fibers.61

2.5.1.4 Tip-collector distance effect
Figure 2-7 Electrospun fiber diameters vs distance between tip and collector for corresponding solutions.

<table>
<thead>
<tr>
<th>Materials</th>
<th>TCD range H</th>
<th>Voltage V</th>
<th>Flow rate Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>8wt%silk/3wt%PEO blend</td>
<td>4” - 8”</td>
<td>15kV</td>
<td>0.020mL/min</td>
</tr>
<tr>
<td>3wt%PEO</td>
<td>4” - 8”</td>
<td>15kV</td>
<td>0.030mL/min</td>
</tr>
</tbody>
</table>

Table 2-3 Experimental conditions for tip-collector distance test.

Figure 2-7 shows the trend of decrease in diameters of electrospun fibers with increasing distance tested with 8wt%silk/3wt%PEO blend at 15kV and constant flow rate of
0.02mL/min and 3wt%PEO at 15kV and constant flow rate of 0.03mL/min. The error bars indicate the width of the diameter profile edges.

Typically, increasing the Distance from nozzle to collector tends to decrease the average diameter of the electrospun fibers since the jet elongation time increases so as to enable a smaller diameter of the electrospun fibers.\textsuperscript{58-60} Meanwhile, the inadequate tip-collector distance may cause a wet grounded area on the collector and form a bead-like morphology of electrospun fibers, which can be attributed to the insufficient drying time at short distances during the electrospinning before the stream reaching the collector.\textsuperscript{62} To obtain an even mat without wet central area, the distance should be optimized up to 8 inches based on the different flow rate, and the voltage should be slightly adjusted to keep the Taylor cone during the electrospinning process.

2.5.1.5 Concentration effect

Figure 2-8 Left: SEM image showing the slightly bead-like formation of 6wt% silk/3wt% PEO blend. Middle: SEM image showing the uniformed and smooth formation of 8wt% silk/3wt% PEO blend. Right: SEM image showing the ribbon-like formation of 12wt% silk/3wt% PEO blend.

Figure 2-8 from left to right show the SEM images with 25 seconds silver sputter coating. The SEM operating details are in the scale bars. The left image in Figure 2-8 shows the
image of 8wt%silk/3wt%PEO blend, the middle one shows the image of 12wt%silk/3wt%PEO blend and the right one shows the image of 14wt%silk/3wt%PEO blend. All the blends have a volume ratio of 1:6.

It is clear that the morphology of the electrospun fibers changed gradually from beaded fibers to uniformed fibers, and then to ribbon-like fibers with the increase of the concentration of spinning solutions. For 8wt%silk/3wt% PEO blend, the ratio of bead-like fibers increases among the uniformed fibers and for 12wt%silk/3wt%PEO blend, the uniformed nanofibers are generated. When the concentration of blends keep increasing, for 14wt%silk/3wt%PEO blend, the ribbon-like fibers are observed. A similar trend could also be found in the research of H. Cao et al.63

The reason for this trend could be explained from two aspects. Although the adding of PEO eliminated the gel formation compared with individual RSF solution electrospinning, the increase of the concentration still contribute to the increase of viscosity. When the concentration of the RSF/PEO blend is too high, which means the viscosity of the blend is too high to make an unstable Taylor cone when electrospinning, which in turn leads to uneven and ribbon-like electrospun fibers. Also due to the high viscosity of the solution, it is not easy for water to evaporate from the fiber surface in the spin distance between the tip and collector, so fibers have to solidify on the aluminum foil, which is attributed to the flat and ribbon shaped formation.64, 65

2.5.2 Core and shell fraction determination

2.5.2.1 Diameter of core and shell quantification
The fraction of core and shell part after electrospinning is the first concern because in this project, core and shell are utilized to carry different types of drugs where the fraction of core and shell will determine the release profile of the drugs.

Figure 2-9 and 2-10 are images captured by optical microscope showing the diameters of core-shell electrospun fibers and diameter distribution data of corresponding images. After gently washing to remove PEO shell part, the diameter of fibers decreased from near 800nm to near 600nm which could be referred as the fraction of core part, and the difference should be the fraction of shell part. The specific average diameter values could be found in table 2-4.

<table>
<thead>
<tr>
<th>Diameter quantification</th>
<th>Average diameter/nm</th>
<th>SDTEV/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td>574.1</td>
<td>88.7</td>
</tr>
<tr>
<td>Shell</td>
<td>796.3</td>
<td>145.5</td>
</tr>
</tbody>
</table>

Table 2-4 Diameters of core and shell parts, respectively

Figure 2-9 Left: Optical microscope image of core-shell structured fibers; Right: corresponding fiber diameter distribution.
Figure 2-10 Left: Optical microscope image of only core part of fibers after washing for 4 hours, PEO shell part was washed away; Right: corresponding fiber diameter distribution.

2.5.2.2 Scaffolds’ Weight loss and thickness change

Scaffold’s weight loss and thickness change after certain periods of PBS incubation are measured as another approach to reveal the fraction of core and shell parts since the weight loss and thickness change are mainly attributed to the dissolution of PEO shell part into PBS incubation solution.
Figure 2-11 shows the weight loss and thickness change profile with the evolution of PBS incubation time. The weight lost dramatically in the first 5 hours to above 25% and gradually stabilized at the level a little higher than 30%. On the other hand, the thickness decreased significantly in the beginning and approached to near 0.02mm finally. Weight loss is basically coincident with the core and shell ratios, while thickness seems decrease too much because the thickness change of the electrospun film is not only come from the PEO dissolution, but also attribute to the great shrink of film itself during PBS incubation.

2.5.3 The influence of different methanol treatment on our electrospun film

2.5.3.1 Methanol immersing test
During the post treatment incorporating with methanol, the silk core part transformed into hydrophobic beta-sheet structure, which prevented the film from dissolving completely. Meanwhile, by means of different methanol treating methods and conditions, the effect on sample properties could vary a lot.

Figure 2-12 Tensile stress over tensile strain of 10 minutes methanol immersing treated samples. Black line represents 100% methanol treated samples, red line represents 85% methanol treated samples, and green line represents 70% methanol treated samples.

Figure 2-12 shows the effect of concentration of methanol on samples during methanol immersing test. From the comparison of stress-strain curves in figure 2-12 we can see that the elastic modulus gradually increased with the decrease of methanol concentration, which means the ratio of beta-sheet structure increased with the decrease of methanol concentration. The reason why such phenomena happened could be related to the different
functions of water and methanol molecules. During immersion, (1) the smaller water molecules travel into the free space between the random coils of silk to make it swollen. (2) After swollen, the bigger methanol molecules could move into silk coils to break the remained interactions amino acid groups to rearrange beta-sheet structure. In this case, with decrease of methanol concentration, the ratio of water molecules increased to accelerate the silk coil swollen, hence speed up the pace of generating beta-sheet by methanol.

2.5.3.2 Methanol vapor treatment vs. methanol immersing treatment

Directly immersing samples into methanol solution helps generating beta-sheet to prevent samples from dissolving completely during the PBS incubation, however, by directly immersing into methanol, the samples exhibit quite stiffer properties which violates the purpose of application of wound bandage, thus a new method of methanol treatment, methanol vapor treatment, is utilized to improve this drawback.

![Figure 2-13 Difference between two methanol treatment methods.](image-url)
Figure 2-13 shows the difference between methanol immersing treatment and methanol vapor treatment. From the comparison of stress-strain curves in Figure 2-13 we can see that differ from the samples immersed in methanol, the samples treated with methanol vapor exhibit a longer elongation and a smaller modulus and ultimate tensile strength, illustrating that samples treated by methanol vapor relatively softer and more ductile than samples treated by directly immersing into methanol solution. This effect may attribute to the vapor penetrating speed. Obviously, the penetrating speed during methanol vapor treatment is way lower than the speed of methanol immersion, which slows down the generation of beta-sheet structure.

2.5.3.3 Structure shift from FTIR test

The mechanical properties out of the instron data in figure 2-12 and 2-13 can partially reveal the beta-sheet transformation during the methanol treatment, however, to investigate the phase transition and structure change more directly, a stronger characterization method in terms of structure determination is required. Therefore the FTIR test is processed. The FTIR here is based on the absorption differences of variety types of amide bonds including alpha-helix, beta-sheets and tyrrside chains, etc., where the mainly reported peak of the alpha-helix conformation appear at 1655 cm\(^{-1}\) (amide I) and beta-sheet conformation appear at 1616-1637 cm\(^{-1}\) (amide I).\(^{66}\)
Figure 2-14 Difference of two methanol treatment methods in terms of beta-sheets transformation in FTIR.

Figure 2-14 shows the secondary structure changes of proteins from FTIR. It is clear that for methanol vapor and methanol immersion treatments, an obvious transition from alpha-helix to beta-sheet occurred, however, by comparing the peak areas of two methods, we can see that with methanol immersion treatment, such a transition is stronger than that with methanol vapor treatment. And this result is coincident with Figure 2-13 that methanol
vapor treatment could transform fewer beta-sheets, leading to a softer mechanical property after treatment.

Figure 2-15 Beta-sheet transformation rate vs. Methanol concentration.

Figure 2-15 shows the comparison of structure changes of proteins between different methanol concentrations from FTIR. By comparing the peak areas of each methanol concentration, it is clear that the transition rate increased with the decrease of the methanol concentration, which is coincident with the result from figure 2-12. And notice that 80% methanol vapor treated samples have a relatively medium amount of transformed beta-sheet structure, leading us to use this condition later as the final methanol treatment method.

2.5.3.4 Estimation of Crystallinity by DSC
Differential scanning calorimetry (DSC), developed by E.S. Watson and M.J. O'Neill in 1962, is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. When applying to the polymers, thermal transitions could be detected by comparing the heat flow difference between the samples and the reference.

The heat of crystallization (ΔH) is one of the main thermal transitions of polymers that people are normally concerned with since the heat of crystallization (ΔH) of the given sample can be utilized to estimate the fraction of sample crystallinity. Figure 2-16 shows Enthalpy change profile of samples with different methanol treated concentration and the specific data summary of ΔH of each concentrated methanol vapor treatments, which is derived from the normalized DSC thermograms by sample mass, shown in table 2-5 as below. Generally, the higher ratio of the crystallinity of beta-sheet, the more heat flow is required for decomposition of silk. 280°C indicates the silk degradation process, from which we can see that 60°C indicates the melting of PEO, the more the PEO component is, the larger the peak area is.
Figure 2-16 Comparison of crystallinity of different methanol vapor concentration by DSC.

<table>
<thead>
<tr>
<th>Enthalpy of the peak near 280°C</th>
<th>60% M-V</th>
<th>80% M-V</th>
<th>Water-V</th>
<th>100% M-V</th>
<th>As-Spun</th>
</tr>
</thead>
<tbody>
<tr>
<td>45.58 J/g</td>
<td>46.95 J/g</td>
<td>43.21 J/g</td>
<td>42.02 J/g</td>
<td>26.31 J/g</td>
<td></td>
</tr>
</tbody>
</table>

Table 2-5 Enthalpy difference of silk degradation peak near 2800°C, which indicates the beta-sheet fraction of each concentrated methanol vapor treatments.

2.5.4 Collagen as a reinforcement into silk/PEO system

2.5.4.1 Weight fraction of each components
Since the very beginning of this project, despite the two main components, silk regenerated solution and PEO solution, are determined, the investigation about the addition of the third component into the system and the weight fractions of each component to improve the spinnability, the fiber morphology and the mechanical properties never stopped. Table 2-6 below shows the final weight fraction of each component after blending Type I collagen into the system as a mechanical reinforcement. The reason why collagen is added into the core part is that before incorporating with collagen, the core-shell system after a certain period of PBS incubation, specifically the silk core part since the hydrophilic PEO shell part will dissolve during incubation, shows very weak mechanical properties and is quite brittle when the samples are completely dried. In this case, a material with better mechanical properties and, more importantly, biocompatibility is needed as a third component, hence Type I collagen is introduced. Since the solubility of collagen in water is not so good, multiple experiments to determine the concentration of collagen into silk solution are processed to find out the optimized concentration of collagen. Finally, the weight fraction of collagen as 4.65% is settled for the comparison between solubility of collagen and the reinforcement effect.

<table>
<thead>
<tr>
<th>Components</th>
<th>Weight w/mg</th>
<th>Weight ratio n/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Core</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I collagen</td>
<td>12</td>
<td>4.65</td>
</tr>
<tr>
<td>silk</td>
<td>231</td>
<td>89.53</td>
</tr>
<tr>
<td>PEO</td>
<td>15</td>
<td>5.81</td>
</tr>
<tr>
<td>Shell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEO</td>
<td>210</td>
<td>100%</td>
</tr>
</tbody>
</table>
Table 2-6 Weight fraction of each component of the electrospun solution after involving collagen as a reinforcement.

2.5.4.2 mechanical test

Because we are aiming to apply this core-shell system into wound bandage, we need desirable mechanical properties of this system that near the range of that of the human skin. Therefore, a great quantity of work has been done on an instron machine dealing with the mechanical tests of a variety of electrospun samples on different conditions. Figure 2-17 shows the stress-strain curves of some of the samples out of the data of instron tensile test, which represents two pairs of comparisons to show the effect of reinforcement by addition of collagen into silk/PEO system. To clarify how the data of the tensile test was analyzed, three main properties are considered in the first place, which are (1) elastic modulus calculated from the slope of the initial linear region of the stress-strain curve, (2) ultimate tensile strength (UTS) or shortened as tensile strength or ultimate strength, which is the highest stress that the sample could reach during tensile test before breaking, and (3) the elongation rate referred from the final strain at breaking point. Meanwhile, some minor properties such as toughness and yield point are also taken into account. The specific data value of each factor are listed in Table 2-7.

Initially, before adding collagen component into silk/PEO system, we found that the mechanical properties are quite unsatisfactorily low from that of human skin as our target value.
Figure 2-17 Comparison between the mechanical properties of the electrospun membranes with or without addition of collagen component in terms of instron data. The dash lines indicate the samples without collagen while the solid lines indicate the samples with collagen. The black lines represent the samples in as-spun condition whereas the red lines represent the samples after methanol treatment and 0.5 hour 37°C PBS incubation.
Table 2-7 Data value derived from the stress-strain curves of instron test as shown in Figure 2-17.

<table>
<thead>
<tr>
<th></th>
<th>Modulus</th>
<th>Ultimate Tensile strength (UTS)</th>
<th>Elongation rate</th>
<th>Toughness</th>
</tr>
</thead>
<tbody>
<tr>
<td>As-spun samples</td>
<td>25.072±8.597 MPa</td>
<td>1.299±0.077 MPa</td>
<td>29.1±3.2%</td>
<td>0.364±0.052 J/m³</td>
</tr>
<tr>
<td>0.5h PBS incubated samples after MeOH vapor treatment and test in semi-wet condition</td>
<td>83.932±6.769 MPa</td>
<td>3.426±0.279 MPa</td>
<td>31.2±0.7%</td>
<td>0.727±0.024 J/m³</td>
</tr>
<tr>
<td>Previous as-spun samples without collagen</td>
<td>72.087±1.733 MPa</td>
<td>0.762±0.111 MPa</td>
<td>12.2±1.0%</td>
<td>0.072±0.009 J/m³</td>
</tr>
<tr>
<td>Previous 0.5h incubated samples without Collagen, In semi-dry condition</td>
<td>7.285±0.706 MPa</td>
<td>1.136±0.028 MPa</td>
<td>68.9±2.3%</td>
<td>0.265±0.045 J/m³</td>
</tr>
<tr>
<td>Human skin</td>
<td>15-150 MPa</td>
<td>5-30 MPa</td>
<td>35-115%</td>
<td></td>
</tr>
</tbody>
</table>
From Table 2-7, the samples of (collagen/silk)/PEO electrospun mat has a modulus of 83.932±6.769 MPa and UTS of 3.426±0.279 MPa with an ultimate tensile strain of 31.2±0.7% when tested in semi-dry conditions, whereas the silk/PEO electrospun mat has 7.285±0.706 MPa, 1.136±0.028 MPa and 68.9±2.3% for each three values. Both modulus and UTS increased significantly.

Therefore, it indicates that the induction of collagen into this system can surely improve the mechanical properties, thus forming a stronger and tougher structure as a benefit.

On the other hand, the tensile modulus of the composite (collagen/silk)/PEO mat fits well with the data value of human skin, whereas the value of UTS and ultimate strain of the scaffolds are low when compared with that of human skin.

But these weakness don't decrease its potential when considering the purpose of the bandage, because such material would eventually be immobilized at a wound site, which means high load suffering and high elongation situations are quite rare. Meanwhile, it seems more suitable and desirable that the tensile modulus should be similar to that of human skin, making it harder to move when used as a skin bandage, thus giving more resilience and resistance.
CHAPTER III

CONCLUSION

In this project, electrospinning setup was assembled. The effects of each electrospinning parameter such as voltage, flow rate and tip-collector distance were investigated primarily, and based on the result of electrospinning parameter investigation, the optimal condition for core-shell electrospinning was determined.

Based on the parameters optimized preliminarily, core-shell structured fibers were successfully fabricated. By controlling the electrospinning time, electrospun film with desirable thickness was obtained to be processed to different test.

The morphologies of core and shell part were studied by microscope imaging. Basically the fibers were conducted uniformly and continuously for both core and shell part.

The average diameters of both core and shell part were measured by microscope images with the assistance of ImageJ software. The average diameter for shell part is near 800 nm while the average diameter for core part is near 600 nm. And the standard deviation is acceptable also indicating that the electrospun fibers are uniform.

The weight loss evolution over PBS incubation basically coincided with the core and shell ratios, while thickness seems decrease too much because the thickness change of the electrospun film not only comes from the PEO dissolution, but also attributes to the great shrink of film itself during PBS incubation.
The experiments to study the influence of different methanol treatment on the electrospun film system in this project helped finally determining the methanol treatment method for further study. Based on the results of instron test, FITR and DSC, 80% methanol vapor treated samples have a relatively medium amount of transformed beta-sheet structure, leading us to use this condition later as the final methanol treatment method.

Before incorporating with collagen, the core-shell system after a certain period of PBS incubation shows very weak mechanical properties and is quite brittle when the samples are completely dried. In this case, collagen as a material with better mechanical properties and, more importantly, biocompatibility is introduced into the system.

The introduction of collagen into this system can surely improve the mechanical properties from the results of comparison between the mechanical properties of the electrospun membranes with or without addition of a collagen component in terms of instron tensile test, thus forming a stronger and tougher structure as a benefit.

In future work, a drum collector will be utilized to fabricate electrospun scaffold with even better mechanical properties, and also simultaneously the drug release profile will be investigated based on the current thick core-shell electrospun scaffold. Each part will carry different types of drug relative to the function of anti-inflammatory or anti-bacteria based on the mechanism of wound healing.
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