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JUDUL : DEVELOPMENT OF POLYMERIC DRUG DELIVERY SYSTEM USING ELECTROSPUN NANOFIBERS FOR CONTROLLED RELEASE OF MULTIPLE PROTEINS

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DEVELOPMENT OF POLYMERIC DRUG DELIVERY SYSTEM USING ELECTROSPUN NANOFIBERS FOR CONTROLLED RELEASE OF MULTIPLE PROTEINS

MOHD AMIRUDDIN FADZLI BIN MOHAMAD ZAINI

A thesis submitted in fulfillment
of the requirements for the award of the Degree of
Bachelor of Chemical Engineering (Biotechnology)

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DECEMBER 2010

I declare that this thesis entitled “Development of Polymeric Drug Delivery System Using Electrospun Nanofibers for Controlled Release of Multiple Proteins” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.”

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To my parents, Mohamad Zaini Saari and Tengku Sharifah Siti Sara Tengku Syed Abdul Kadir for understanding my dream and passion, and for letting me chase it.

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You can do anything you want in the world if you challenge yourselves to overcome the difficulties and to fight against the impossibilities. You need to be brave and have courage to achieve your dreams. And you managed to get through it, it would be your best satisfaction in life.

Many thanks!

ABSTRACT

The purpose of the research is to develop a novel type of drug delivery carrier with the capability of encapsulating multiple drugs and release the drugs in controlled manner. Nanotechnology as a delivery platform offers very promising applications in drug delivery. Polymeric drug delivery devices were successfully developed via electrospinning technique using PCL, PLLA and PLLA-CL biodegradable polymers. Coaxial electrospinning configurations was used to encapsulate three proteins which are BSA, lysozyme and IgG into the electrospun nanofibers. Using the configuration, two separate polymers solutions flowed through two different capillaries which are coaxial with a smaller capillary inside a larger capillary. A number of processing parameters were investigated during the electrospinning process which are formulation of drug loading, the type of polymer and drug and the concentration of polymer and drug. The morphology of the electrospun nanofibers was analyzed using Scanning Electron Microscopy (SEM). The mechanical property was analyzed by using tensile strength and UV-Spectrophotometer was used to study the proteins release profile. The results showed that the incorporated proteins could be controlled release by adjusting the process parameters and the proteins structure and bioactivity are maintained. Polymeric drug delivery device is able deliver a pre-determined amount of drug over specific amount of time in a predictable manner. It can improve the effectiveness of drug therapy by increasing the therapeutic activity and reducing the number of drug administrations.

ABSTRAK

Tujuan kajian ini adalah untuk membina sistem penghantaran dadah asli yang mampu menggabungkan berbilang dadah dan membebaskan dadah tersebut dalam cara yang terkawal. Teknologi nano sebagai lantasan penghantaran menawarkan aplikasi yang menjanjikan dalam penghantaran dadah. Sistem penghantaran dadah berpolimer telah berjaya dibina melalui teknik *electrospinning* menggunakan polimer biodegradasi PCL, PLLA dan PLLACL. Konfigurasi *electrospinning* sepaksi digunakan untuk menggabungkan tiga protein iaitu BSA, lysozyme dan IgG ke dalam fiber nano. Menggunakan konfigurasi ini, dua larutan polimer yang terpisah dialirkan melalui dua kapilari yang diatur sepaksi dengan kapilari kecil di dalam kapilari besar. Beberapa parameter proses dikaji semasa proses *electrospinning* iaitu keadaan molekul dadah dalam medium *electrospinning*, formulasi muatan dadah, jenis kajian pembebasan, konfigurasi dadah serta jenis dadah. Parameter yang dinilai dalam kajian ini ialah jenis polimer Morfologi fiber nano yang terhasil melalui *electrospinning* dianalisis menggunakan SEM. Sifat-sifat mekanikal dianalisis menggunakan kekuatan tensil manakala *UV-Vis spectrophotometer* digunakan untuk mengkaji profil pembebasan protein. Hasil dapatan kajian menunjukkan protein yang digabungkan boleh dibebaskan secara terkawal dengan mengubahsuai parameter yang dinilai. Struktur protein dan aktivitibio adalah dikekalkan. Sistem penghantaran dadah berpolimer adalah mampu untuk menghantar dadah dalam kadar yang dijangka dalam keadaan yang terkawal. Sistem ini mampu menambah baik terapi dadah dengan meningkatkan aktiviti terapeutik dan mengurangkan jumlah pentadbiran dadah.

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LIST OF SYMBOLS/ABBREVIATIONS

%	Percent
°C	A scale and unit of measurement for temperature
µL	Microliter
µm	Micrometer
BSA	Bovine serum albumin
cm	Centimeter
CO_2	Carbon dioxide
DC	Direct current
DMF	Dimethylformamide
ECM	Extracellular matrix
PBS	Phosphate buffer saline
FE-SEM	Field emission scanning electron microscope
FTIR	Fourier transform infrared spectroscopy
G	Needle gauge
HCl	Hydrochloric acid
HFIP	1,1,1,3,3,3-hexafluor-2-propanol
IgG	Immunoglobulins
kDa	Kilo Dalton
kV	Kilovolts
M	Molar
mL	Milliliter
mL/h	Volumetric flow rate
mm/min	Measurement of flow
h	Hour
mol/L	Molar concentration
MW	Molecular weight
nm	Nanometer

PCL	Poly(ε-caprolactone)
Ph	Measure of the acidity or basicity of an aqueous solution
PLLA	Poly(L-lactic acid)
PLLACL	Poly(L-lactide-co-ε-caprolactone)
PMMA	Poly(methylmethacrylate)
PVC	Poly(vinyl chloride)
rpm	Rotation per minute
SEM	Scanning electron microscope
TEM	Transmission electron microscope
THF	Tetrahydrofuran
UV	Ultra violet
wt %	Mass fraction
w/v %	Mass concentration

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Lamprecht (2009) describes the use of nanoparticulate drug delivery systems as one important aspect in the newly developing field of nanomedicine to allow innovative therapeutic approaches. Nanotechnology as a delivery device has a very promising application in drug delivery as it has advantages to enhance drug transport across biological barriers and deliver the drug at selective or targeted tissue or organ. As the principle of drug targeted is to reduce the total amount of drug administration, ongoing efforts have been made over the past decade to develop systems or drug carriers that are capable of delivering the active molecules specifically to the target organs to increase the therapeutic efficacy. The site-specific delivery systems will allow an effective drug concentration to be maintained for a longer time interval and decrease the side effects.

According to Kim and Pack (2006), a wide variety of new, more potent and specific therapeutics are being created in advances in biotechnology. A drug delivery system is designed to provide a therapeutic agent in the needed amount, at the right time and to the proper location in the body in a manner that optimizes the efficacy, increases compliance and minimizes side effects. Due to common problems in drug delivery such as low solubility, high potency and poor stability, it can impact the efficacy and potential

of the drug itself. Thus, there is a corresponding need for safer and more effective methods and devices for drug delivery.

Gaur and Bhatia (2008) argues that drug resistance at the target level due to physiological barriers or cellular mechanisms can be encountered using nanoparticle drug delivery systems. Many drugs have a poor solubility and bioavailability. The efficacy of different drugs such as chemotherapeutic agents is limited by dose-dependent side effects. Anticancer drugs, which have large volume of distribution are toxic to both normal and cancer cells and precise drug release into highly specified target requires the delivery systems to become much smaller than their targets. With the use of nanotechnology, targeting drug molecules to the site of action will reduce the drug side effects and optimizing its therapeutic effects. The small particle size can penetrate across different barriers through small capillaries into individual cells. Nanoparticles can be prepared to encapsulate the drugs to improve its solubility, stability, absorption and protecting the drug from premature inactivation during delivery.

Controlled release drug delivery systems are developed to address difficulties in the conventional drug delivery systems by employing devices that encapsulate drug and release it at controlled rates for relatively long periods of time. The advantages of employing such system are drug release rates can be tailored to the needs of a specific application, provide protection of drugs and increase patients' comfort and compliance by substituting frequent doses with infrequent injection (Kim and Pack, 2006).

Su *et al.* (2009) explains that drug delivery systems have numerous advantages compared with conventional dosage forms such as improving therapeutics effect, reduce toxicity and improve patients' compliance by delivering drugs at a controlled rate over a period of time to the site of action. Electrospun fibers either non-degradable or biodegradable materials can be used as delivery matrices as drugs mainly released and driven by concentration gradient.

Controlled drug release at a defined rate over a definite period of time is possible with biocompatible delivery matrices. Biodegradable polymers are used as drug delivery device to deliver therapeutic agents as they can be easily designed for releasing the therapeutic agents in controlled manner. Nanofiber mats are applied as drug carriers in the drug delivery system due to their high functional characteristics as it relies on the principle that a dissolution rate of a particulate drug increases with increasing surface area of both the drug and the corresponding carrier. The pharmaceutical dosage can be designed as rapid, immediate, delayed or modified dissolution depending on the polymer carrier used (Bhardwaj and Kundu, 2010).

During the past few decades, many researchers have developed drug delivery systems and most of the developed drug delivery systems are commercially available. Combination therapies in which multiple drugs of different therapeutic effects are used have been used in many clinical treatments to improve the outcomes. To produce effective combination therapies, well-organized multi-release systems must be established by investigating new modes and systems for regulated multiple-drug delivery in a single formulation by precise control of the release order, timing, dose and duration for individual drugs. Several dual-drug delivery systems using polymer micelles, hydrogel, hydrogel/polymer micelle composite, alignate beads embedded silk fibroin scaffold and other materials have been reported to date (Okuda *et al.*, 2010). The release rates of individual drugs can be controlled independently by changing the compositions of matrices or environmental factors using these methods but the release timing and order control remained problematic.

Okuda *et al.* (2010) discusses that the timed release of a single drug was accomplished using multilayered particulate formulations by developing a time-controlled single drug delivery system using sigmoidal release system, pulsatile release tablet and time-controlled explosion system to indicate the effectiveness of multilayered formulation for controlling the drugs release order and timing. To date, no multidrug drug delivery systems that can comprehensively control the administration order, dose, period and timing for individual drugs were established. A versatile and general

methodology for drug carrier design that enables pre-setting of the release profile of all drugs component is required to establish time-programmed multidrug drug delivery systems with a single formulation.

Electrospinning is a technique that can easily fabricate nanofiber and microfiber meshes from different types of polymer. Due to their unique features such as high surface-to-volume ratio, morphological design flexibility and extracellular matrices structure-like, nanofibers are used as scaffolds for drug delivery and tissue engineering. Low molecular weight drugs and biomolecules such as proteins and nucleic acids can be encapsulated into the electrospun fibers (Xu *et al.*, 2008).

Ramakrishna *et al.* (2005) states that nanotechnology refers to the science and engineering concerning materials, structures and devices which at least one of the dimensions is 100 nm or less. A nanofiber is a nanomaterial in view of its diameter. It is a porous structure with high surface area and large surface-to-volume ratio. The morphology of the fiber like pore size and shape can be changed by adjusting the operational parameters compared to the conventional porous structures.

There are various ways of fabricating nanofibers such as drawing, template synthesis, phase separation, self-assembly and electrospinning. The most versatile method of producing nanofibers with relatively high production is electrospinning. Electrospinning is a process that creates nanofibers through an electrically charged jet of polymer solution or polymer melt. It also provides the simplest approach to nanofibers with both solid and hallow interiors that are exceptionally long in length, uniform in diameter and diversified in composition.

Unlike other methods, the formation of nanofibers via electrospinning is based on uniaxial stretching or elongation of a viscoelastic jet derived from a polymer solution or melt. The usage of electrostatic repulsion between surface charges to continually reduce the diameter of viscoelastic jet. Electrospinning is better suited for producing

fibers with much thinner diameters since the elongation can be accomplished through the application of external electric field.

Important features of electrospinning are suitable solvent to dissolve the polymer, suitable vapor pressure of the solvent, viscosity and surface tension of the solvent must neither be too high nor too small, adequate power supply to overcome the viscosity and surface tension of the polymer solution and distance between pipette and grounded surface should be large enough for the solvent to evaporate. The advantages of fabricating nanofibers from electrospinning are cost effective, long in length, continuous production; high production rate and the process can be scaled up while the disadvantage is jet instability.

According to Kenawy *et al.* (2002), electrospinning is a straightforward method of producing fibrous polymer mats and are useful for many applications in medicine such as wound dressings and scaffolds for tissue engineering. A single fiber is generated in the electrospinning process and the mat is created from the single fiber rather than finely splayed fibers. The ability to vary the fiber diameter by changing the solution concentration and the liquid surface tension and the ability to incorporate therapeutic compounds into the nanofiber during electrospinning provide a promising prospect in preparing polymer systems for controlled drug delivery. Materials derived from polymer blends will not be an admixture of two different fibers. Instead, the fibers will contain two different fibers which in principle is an unique means of controlling release rates.

Supporting the statement, Luu *et al.* (2003) elaborates that the natural acaffolds of most tissues in the extracellular matrix (ECM) whose structure and morphology contribute to the properties and functions of each organs. According to Ramakrishna *et al.* (2006), the ECM is a complex composite of fibrous proteins such as collagen and fibronectin, glycoproteins, proteoglycans, soluble proteins such as growth factors and other bioactive molecules that support cell adhesion and growth. This device is also expected to interact well with the cell by adhering to the cell and proliferate the cell well. The ECM contributes to the rigidity and tensile strength of bone, the resilience of

cartilage, the flexibility and hydrostatic strength of blood vessels and the skin elasticity. The properties of this matrix include a largely porous structure with a wide pore size distribution. Using electrospinning with polymer solutions, nanostructured fibers scaffolds are fabricated. Electrospinning is a variation of the electrospray process which occurs when the surface tension force of a polymer solution is overcomed by an applied electrical force and tiny droplets of fibers are extruded from the solution. The nonwoven fibers will then forming a large and interconnected porous network that is ideal for drug delivery.

Medicated ultrafine fibers can be prepared by electrospinning a mixture solution of drug and a polymer. Polymeric drug delivery systems have advantages over the conventional dosage forms. They are expected to be promising in future biomedical applications especially in post-operative local chemotherapy due to their advantages in improving therapeutic effect, reducing toxicity and enhance patients' compliance. Electrospinning is better suited for producing nanofibers with much thinner diameters since the elongation can be accomplished through the application of external electric field. The advantages of fabricating nanofibers using electrospinning method are cost effective, long in length, continuous production, high production rate can be scaled up. Polymer-based drug delivery systems does not only optimize the therapeutic properties of the drug but making it safer, effective and reliable compared to the conventional drug-loaded capsules or tablets consumed orally or through direct injection into the body.

1.2 Problem Statement

The use of proteins and peptides as therapeutic agents is growing due to developments in biotechnology. However, the delivery of these therapeutic proteins is limited by their fragile structure and frequent monitoring is required (Sinha and Trehan, 2003). Releasing a protein without denaturation when the polymer is degraded is challenging. When protein is released over time, protein instability problems may occur and result in incomplete release even when the polymer has been degraded. Previous

studies shown that the methods proposed to prevent incomplete release are often unsuccessful for many proteins (Giteau *et al.*, 2008). To ensure the bioactivity of these proteins is retained, a mild procedure is needed using electrospinning. A device with inherent property similar to the extracellular matrices (ECM) of tissue and organs is proposed. Therefore, biodegradable nanoparticles such as nanofibers are required to encapsulate the therapeutic proteins to prevent proteolysis and to obtain sustained delivery and maintained therapeutic efficacy due to their nanofibrous network to tissue engineering scaffolds. Polymeric drug delivery systems are able to improve therapeutic efficacy by releasing protein at a controlled rate over a period of time (Ramakrishna *et al.*, 2006).

1.3 Research Objectives

In this study, there are three objectives aligned to achieve the purpose of developing polymeric drug delivery system using electrospun nanofibers for controlled release of multiple proteins. The objectives are to develop a polymeric drug delivery system using electrospun nanofibers, to characterize the electrospun nanofibers incorporated with multiple proteins and to study the release profile of the multiple proteins.

1.4 Scope

The study covers the production of electrospun nanofibers using electrospinning process, the characterization of the electrospun nanofibers incorporated with multiple proteins and the study of multiple proteins release profile by varying the parameters. The scope of the study is categorized to experimental design and parameters evaluation.

The experimental design of this study is based on the electrospinning setups and mechanisms. Parameter evaluation is identified after considering the type of polymer as limitation.

Table 1.1: Identified parameter evaluation for the scope of study

Category	Scope	Specification	Justification
Experimental design	State of drug molecule in electrospinning medium	Coaxial electrospinning	Produce core/shell nanofibrous polymer to deliver drugs in sustained manner and more adaptive for incorporating proteins
	Formulation of drug loading	Resevoir type structure	Drug enclosed in polymer matrix forming ‘core-shell’ structure and preserved from aggressive environments
	Type of release study	In-vitro release study	Determine the total content of the drug by immersing the drug-loaded fiber meshes with PBS solution at pH 7.4 and incubate at 37 °C to simulate human skin
	Drug configuration	Three months	Retaining the bioavailability and bioactivity of the drug at longer period of time
	Type of drugs/proteins	BSA, lysozyme and IgG	Therapeutic proteins
Parameter	Type of polymers	PCL, PLLA and	Biodegradable polymers

evaluation		co-polymer PLLACL	have nanofibrous skeletal structure that is similar to the extracellular matrix (ECM) present in living tissue

The summary of electrospinning processing parameters used throughout the fabrication of drug-loaded electrospun fiber meshes using electrospinning technique is as below.

Table 1.2: Summary of electrospinning processing parameters

Processing Parameter	Value/Range	Specification
Polymer concentration	10 wt%, 16 w/v % and 10 wt% respectively	PCL, PLLA and PLLACL
Drug/protein concentration	5 wt%	Multiple proteins mixture (BSA, lysozyme and IgG)
Voltage supply	10-18 kV	-
Shell solution flow rate	0.5-1.6 mL/h	-
Core solution flow rate	0.05-0.3 mL/h	-
Distance from needle tip (nozzle) to collector	10-14 cm	-
Humidity	54-70%	-
Needle diameter	18-27 G	-

1.5 Rationale and Significance

Ramakrishna *et al.* (2006) claims that both synthetic and natural polymers can be produced as nanofibers with diameter ranging from tens to hundreds of nanometers with controlled morphology and function. The potential of these electrospun nanofibers in

healthcare application is promising, for example as vector to deliver drugs and therapeutics. Electrospun fiber mat provide the advantage of increased drug release due to the increased surface area. According to Jiang *et al.* (2005), the interconnected, three-dimensional porous structure and enormous surface area of electrospun nanofibers prepared from biodegradable polymers have great potential in tissue engineering, drug delivery and gene therapy. This is due to their biodegradability and fiber-forming properties.

By designing drug delivery systems that are able to provide a therapeutic agent in the needed amount, at the right time, to the proper location in the body, in a manner that optimizes efficacy, increases compliance and minimizes side effects, controlled release drug delivery systems are able to address many of the difficulties in the conventional method of drug administration. The drug delivery systems were responsible for $47 billion in sales in 2002 and the drug delivery market is expected to grow to $67 billion by 2006 (Kim and Pack, 2006).

Controlled drug delivery systems are developed to address the difficulties in the conventional drug administration. By using nanofibers membrane encapsulated with drug as device, the drug can be released at controlled rates for a long period of time. The advantages of employing such systems are the drug release rates can be designed to the needs of a specific application. Apart from that, controlled drug delivery systems may provide drug protections especially proteins that are easily destroyed by the body. Controlled drug delivery systems can also increase patient comfort and compliance by substituting frequent doses (daily injectibles) with infrequent injection (once a month injection or less).

Soluble polymers are among materials preferred to be made as drug carriers. Using biodegradable polymers as the polymer material in the polymeric drug delivery device has the potential of degrading the device at slow rate and is a promising method for after post-operative local chemotherapy treatment. Researchers have discovered the potential of polymeric materials as drug carriers in the drug delivery system as it is easy

to prepare and has the ability to control its physical and chemical properties. Polymeric drug delivery system is effective in enhancing drug targeting specificity, lowering systemic drug toxicity, improving treatment absorption rates and providing protection for therapeutics against biodegradation.

The significance of this study is the development of polymeric drug delivery device using electrospun nanofibers for controlled release of multiple proteins. It is found that using coaxial nozzle configuration in electrospinning, water-soluble therapeutic proteins can be encapsulated into biodegradable non-woven polymer fibers resulted in subsequent controlled release compared with other methods. Encapsulation of protein using electrospun nanofibers has the advantages of being facile, high loading capacity and efficiency, mild preparation condition and steady release characteristics (Jiang *et al*., 2005).

CHAPTER 2

LITERATURE REVIEW

2.1 Electrospinning Process

The combined use of two techniques which are electrospraying and spinning is made use in a technique called electrospinning (Agarwald *et al.,* 2008). Sill *et al.* (2008) elaborates that electrospinning utilizes a high voltage source to inject charge of certain polarity into a polymer solution or melt, which is then accelerated toward a collector of opposite polarity. As the electrostatic attraction between the two oppositely charged liquid and collector and the electrostatic repulsions between like charges from a rounded meniscus to a cone known as Taylor cone. A fiber jet is ejected from the Taylor cone as the electric field strength exceeds the surface tension of the liquid. The fiber jet travels through the atmosphere allowing the solvent to evaporate leading to the deposition of solid fibers on the collector. Fibers produced using this process has diameters on the order of few micrometers down to tens of nanometers. The development of nanofibers has led to interest for its potential application in filtration, protective clothing and biological applications such as tissue engineering scaffolds and drug delivery devices.

A typical electrospinning setup consist of capillary through which the liquid to be electrospunned is forced, a high voltage source with positive or negative polarity, which injects charge into the liquid and a grounded collector. A syringe pump is

typically used to force the liquid through a small-diameter capillary forming a pendant drop at the tip. An electrode from the highly voltage source is directly attached to the capillary if a metal needle is used. The voltage source is turned on and charge is injected into the polymer solution. Increasing the electric field strength causes the repulsive interactions between like charges in the liquid and the attractive forces between the oppositely charged liquid and a collector begin to exert forces on the liquid, elongating the pendant drop at the tip of the capillary. As the electric field strength is increased further, a point will be reached at which the electrostatic forces balance out the surface of the liquid leading to the formation of Taylor cone. When the applied voltage is increased beyond this point, a fiber jet will be ejected and accelerated toward the grounded collector. While the fiber jet is accelerated through the atmosphere toward the collector, a chaotic bending instability occurred and increasing the transit time and the path length to the collector and aiding in the fiber thinning and solvent evaporation processes. Bending instability is due to repulsive interactions between like charges found in the polymer jet (Yarin *et al.*, 2001). Solid polymer fibers are deposited onto a ground collector.

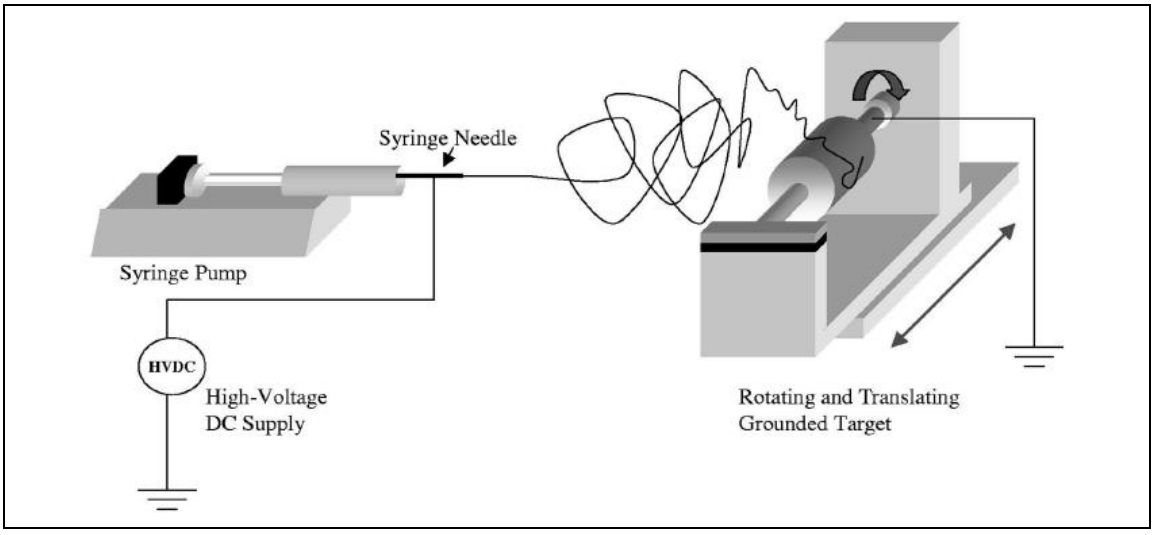


Figure 2.1: Basic electrospinning setup

Li and Xia (2004) explains that electrospinning consists of three major components: a high-voltage power supply, a spinneret or a metallic needle and a grounded collector. Direct current (DC) power supplies are used for electrospinning. The

spinneret is connected to a syringe in which the polymer solution or melt is hosted. With the use of a syringe pump, the solution is fed through the spinneret at a constant and controllable rate. When a high voltage usually in the range of 1 to 30 kV is applied, the pendent drop of polymer solution at the nozzle of the spinneret will become highly electrified and the induced charges are evenly distributed over the surface. As a result, the drop will experience two major types of electrostatic forces: the electrostatic repulsion between the surface charges and the Coulombic force exerted by the external electric field. Under the action of these electrostatic interactions, the liquid drop will be distorted into a conical object known as the Taylor cone.

Once the strength of electric field has surpassed a threshold value, the electrostatic forces can overcome the surface tension of the polymer solution forcing the ejection of a liquid jet from the nozzle. The electrified jet is continuously elongated and the solvent is evaporated, its diameter can be greatly reduced from hundreds of micrometer to as small as tens of nanometers. Attracted by the ground collector placed under the spinneret, the charged fiber is deposited as a randomly oriented, nonwoven mat.

2.2 Processing Parameters

According to Li and Xia (2004), although the setup for electrospinning is simple, the spinning mechanism is complicated. The morphology and diameter of electrospun fibers are dependent on a number of processing parameters. The morphology and diameter of electrospun nanofibers are dependent on a number of processing parameters that include the intrinsic properties (the type of polymer, the confirmation of polymer chain, viscosity or concentration, elasticity, electrical conductivity, polarity and surface tension of the solvent) and operational conditions (strength of applied electrical field, the distance between spinneret and collector and the feeding rate of the polymer solution). In addition to these variables, the humidity and temperature of the surroundings play an

important role in determining the morphology and diameter of the electrospun nanofibers.

Sill *et al.* (2008) explains that these parameters are grouped in order of relative impact to the electrospinning process which are applied voltage, polymer flow rate and capillary-collector distance. All these parameters can influence the formation of bead defects. Huang *et al.* (2003) adds that many parameters can influence the transform of polymer solutions into nanofibers through electrospinning. These parameters include the solution properties (viscosity, elasticity, conductivity and surface tension), governing variables (hydrostatic pressure in the capillary tube, electrical potential at the capillary tip and distance between the tip and the ground collector) and ambient parameters (solution temperature, humidity and air velocity in the electrospinning chamber).

2.2.1 Polymer Concentration

Sill *et al.* (2008) states that polymer concentration determines the spinnability of a solution. The solution must have high enough polymer concentration for chain entanglements to occur. However, the solution cannot be either too dilute or too concentrated. The polymer concentration influences both the viscosity and surface tension of the solution. If the solution is too dilute, the polymer fiber will break up into droplets before reaching the collector due to the effect of surface tension. If the solution is too concentrated, then fibers cannot be formed due to the high viscosity which makes it difficult to control the solution flow rate through the capillary. An optimum range of polymer concentration exists in which fibers can be electrospun when all other parameters are held constant.

. Increase in concentration and molecular weight increase the fiber shell density thus resulting in a higher controlled release barrier. Chakraborty *et al.* (2009) adds the increase in polymer concentration can delay the release of drugs such as paclitaxel and tetracycline hydrochloride. Another factor to consider is the possible changes in the fiber

diameter as a result of alterations in concentration and molecular weight which can be a contributing factor affecting the drug release kinetics. The strength of the polymer-drug interaction is another variable that influences the extent of drug release. Hydrophobixity, change density and degradability are characteristics of polymer carrier that play role in its interaction with the drug of interest.

2.2.2 Voltage Supply

High voltage will induced charges on the solution and together with the external electric field will initiate the electrospinning process when the electrostatic force in the solution overcomes the surface tension of the solution. Both high negative or positive voltage of more than 6kV is able to cause the solution drop at the tip of the needle to distort into the shape of a Taylor cone during jet initiation. Depending on the federate of the solution, a higher voltage may be acquired so that the Taylor cone is stable (Ramakrishna *et al.*, 2005). Zhong *et al.* (2002) argues that if the applied voltage is higher, the greater amount of charges will cause the jet to accelerate faster and more volume of the solution will be drawn from the tip of the needle.

2.2.3 Shell/Core Solution Flow Rate

According to Ramakrishna *et al.* (2005), the flow rate will determine the amount of solution available for electrospinning. For a given voltage, there is a corresponding feed rate if a stable Taylor cone is to be maintained. When the feed rate is increased, there is a corresponding increase in the fiber diameter or beads size. This is due to greater volume of solution that is ejected from the needle tip. Yuan *et al.* (2004) argues that a lower feed rate is more desirable as the solvent will have more time for evaporation. The jet will take a long time to dry due to the greater volume of solution drawn from the needle tip.

2.2.4 Distance between Tip and Collector

Varying the distance between the tip and the collector will have a direct influence in flight time and electric field strength. For fibers to form, the electrospinning jet must be allowed time for most of the solvents to be evaporated. When the distance between the tip and the collector is reduced, the jet will have shorter distance to travel before it reaches the collector plate. The electric field strength will increase at the same time and this will increase the acceleration of the jet to the collector. As a result, there may not have enough time for solvents to evaporate when it reach the collector. When the distance is too low, excess solvents may cause the fibers to merge when they contact to form junctions resulting in intra layer bonding (Ramakrishna *et al*, 2005).

2.2.5 Humidity

The humidity of the electrospinning environment may have an influence in the polymer solution during electrospinning. At high humidity, it is likely that water condenses on the fiber surface when electrospinning is carried out under normal atmosphere. As a result, this may have an influence on the fiber morphology especially polymers dissolved in volatile solvents (Megelski *et al.*, 2002). Polysulfone (PS) is dissolved tetrahydrofuran (THF) and is electrospun. The result showed that at humidity of less than 50%, the fiber surfaces are smooth. An increase in the humidity during electrospinning will cause circular pores to form on the fiber surfaces. The sizes of the circular pores increases with the increasing humidity until they coalescence to form large, non-uniform shaped structures. The depth of the pore increases with increasing humidity as determined by atomic force microscopy. Above certain humidity, the depth of pores, its diameters and numbers start to saturate (Casper *et al.*, 2004).

During electrospinning, water vapor may condense on the jet surface due to jet surface cooling as a result of rapid evaporation of the volatile solvent. Pores are created when both water and solvent eventually evaporate. Pores seen on electrospun fibers mat

due to the dynamic condition of the electrospinning jet as compared to static condition (Megelski *et al.*, 2002). The humidity of the environment will also determine the rate of solvent evaporation of the solvent in the solution. At a very low humidity, a volatile solvent may dries very rapidly. The solvent evaporation may be faster than the removal of the solvent from the needle tip. As a result, the electrospinning process may only be carried out for a few minutes before the needle tip is clogged (Ramakrishna *et al.*, 2006).

The effect of humidity on electrostatic charges on non-conducting surfaces is widely studied. Studies on glass particles transported in a grounded copper pipe had found that at higher relative humidity (>76%), there were no charges on the particles. With decreasing humidity, there is an increased in the amount of charge on the particle (Ramakrishna *et al.*, 2006). It is also suggested that the high humidity can help the discharge of the electrospun fibers (Li and Xia, 2004).

2.2.6 Needle Diameter

Mo *et al.* (2004) states that the internal diameter of the needle of the pipette orifice has a certain effect on the electrospinning process. A smaller internal diameter was found to reduce the clogging as well as the amount of beads on the electrospun fibers. The reduction in the clogging could be due to less exposure of the solution to the atmosphere during electrospinning. Decrease in the internal diameter of the orifice was also found to cause a reduction in the diameter of the electrospun fibers. When the size of the droplet at the tip of the orifice is decreased, the surface tension of the droplet increases. Zhao *et al.* (2004) argues that if the diameter of the orifice is too small, it may not be possible to extrude a droplet of solution at the tip of the orifice.

2.3 State of Drug Molecule in Electrospinning Medium

The emergence of coaxial electrospinning has allowed the development of many new designs of functional nanotechnological materials (Chakraborty *et al.*, 2009). Coaxial electrospinning is a simple and rapid technique to produce micro or nanotubes, drug or protein loaded nanofibers and hybrid 'core-shell' nanofibrous materials. The greatest advantage of coaxial electrospinning is its versatility in the type (hydrophobic or hydrophilic) and size (ranging from 100 nm to 300 μm) of fibers it can produced. Monoaxial electrospun fibers have been reported to be able to incorporate and release antibiotics, drugs and proteins in a sustained manner. However, the distribution and release of drugs from the fibers are poorly controlled. Moreover, growth factors and cytokines embedded in polymer matrixes also suffer from significant decrease in bioactivity. Coaxial electrospun fibers as delivery system for tissue engineering offer better drug stability, more complete drug encapsulation and tighter control of release kinetics as compared to monoaxial fibers.

Coaxial electrospinning overcomes technical limitations of monoaxial electrospinning by its core-shell design allowing cytokines and growth factors to be dissolved in aqueous solution for encapsulation. Encapsulation lysozyme and platelet derived growth factor-bb released from core-shell nanofibers have maintained high bioactivity over a period of 1 month. The core-shell design allows better control over the release kinetics of the drug of interest due to an increased number of variable parameters. Charges in the shell and core material properties via variation in molecular weight, polymer type and addition of porogen can fine-tune the release profile (Chakraborty *et al.*, 2009).

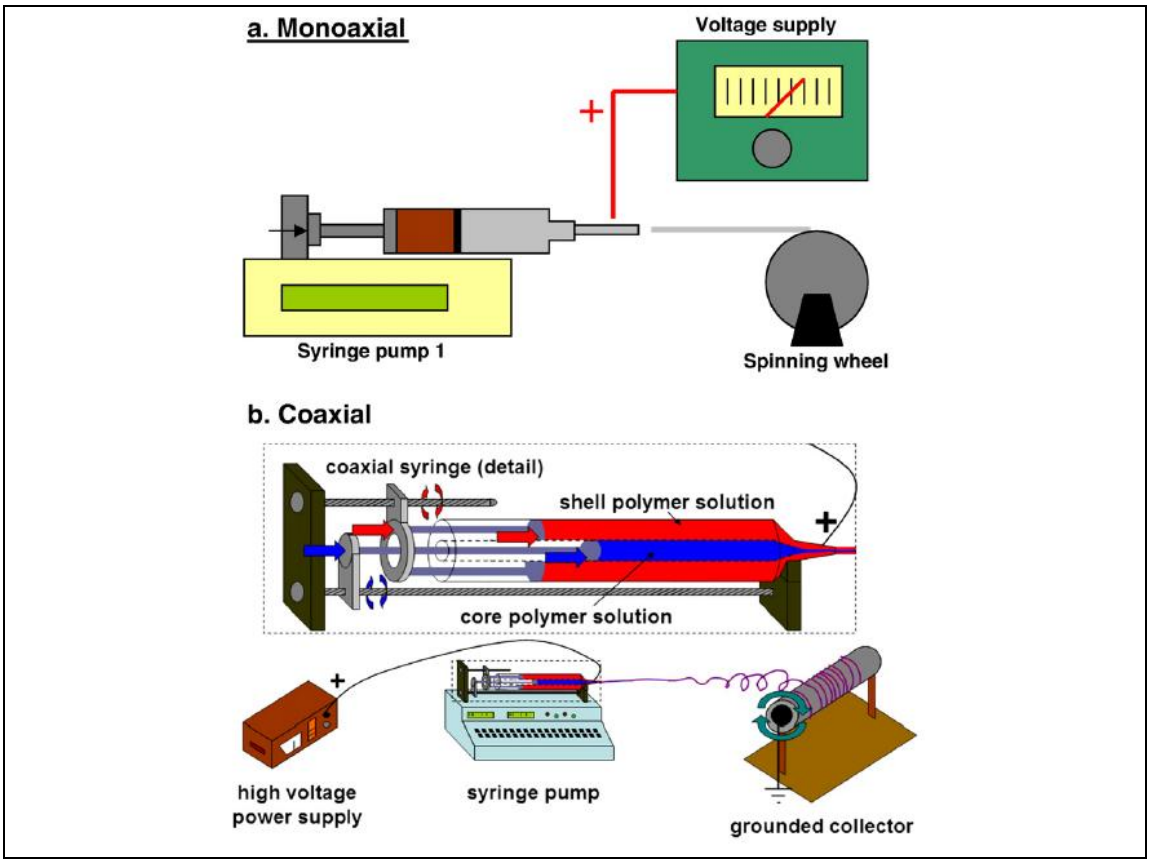


Figure 2.2: Monoaxial and coaxial electrospinning setup

Chakraborty *et al.* (2009) also states that in coaxial electrospinning, two needles of different gauge size are arranged coaxially to disperse two different solutions concurrently. Depending on the solvents used, the two solutions can either mix or phase-separate at the needle. Similar to monoaxial electrospinning, electrostatic force induced by the high charging potential shears the core-shell droplet into polymeric coaxial fibers. Sill *et al.* (2008) supports the statement by stating that while electrospinning polymer blends is often desirable in order to achieved the desired combination of properties, it may not be possible using a single needle configuration if the polymers of interest are not soluble in the common solvent. It is necessary to use a side-by-side configuration. Using this configuration, two separate polymer solutions flow through two different capillaries which are set side-by-side. Gupta and Wilkes (2003) used a side-by-side configuration to electrospin biocomponent systems out of poly(vinyl chloride)/segmented polyurethane and poly(vinyl chloride)/poly(vinylidiene fluoride). They observed that the solution conductivity plays a more important role in the ability to

form a single fiber jet under a strong electric field in the side-by-side configuration. The conductivity of the PVC solution was significantly higher than either of the two solutions and two distinct Taylor cones, one from each solution were formed when subjected to a strong electric field.

A relative new nozzle configuration is the coaxial configurfation which allows for the simultaneous coaxial electrospinning of two different polymer solutions. In this configuration, two separate polymer solutions flow through two different capillaries which are coaxial with a smaller capillary inside a larger capillary. This technique has received great interest due to its potential in drug delivery applications. Using this nozzle configuration, a smaller fiber can be encapsulated in a larger fiber leading to a core-shell morphology (Sill *et al.*, 2008). In a drug delivery application, Zhang *et al.* (2006) demonstrates encapsulation of a modal protein, fluorescein osothiocyanate conjugated bovine serum albumin along with poly(ethylene glycol) (PEG) in poly(ε-caprolactone) (PCL) fibers using coaxial configuration and were able to demonstrate that the resulting core-shell system mitigated the initial burst release associated with release from polymer/drug blends and had a longer sustained release.

Core-shell electrospun nanofibers are designed to concentrate the drug in the core of the fibers as opposed to the randomly distributing the drug throughout the fiber matrix. Using poly(ε-caprolactone) and poly(methylmethacrylate) (PMMA) as carriers and Rhodamine 610 dye as a model drug, Srikar *et al.* (2008) investigates the effect of varying the polymer type (PCL and PMMA), polymer concentration (11, 13 and 15 wt%) and molecular weight (120, 350 and 996 kDa). As expected, increase in polymer concentration and molecular weight both reduce the release rate of Rhodamine dye from the fiber.

2.4 Formulation of Drug Loading

Drugs loaded into the polymeric delivery systems have been formulated in two basic designs: matrices or reservoirs. In the matrix-type structure, a therapeutic agent is homogenously dispersed throughout a polymer matrix. The rate of drug release by diffusion through the polymer matrix normally decreases with time since the agent has a longer distance to travel and requires a longer diffusion time to release. In reservoir-type structure, the drug is enclosed in the polymer matrix forming the core-shell structure. If the polymer shell is uniform and thick enough compared to the core, the diffusion rate of the therapeutic agent will be stable throughout the life of the delivery system (Xu *et al.*, 2008).

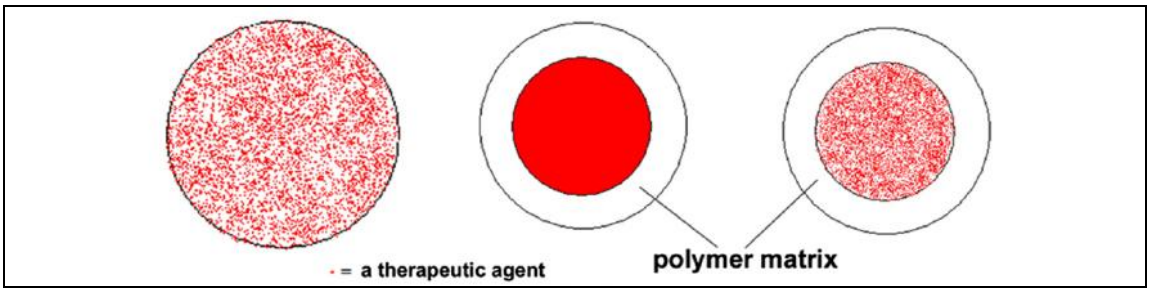


Figure 2.3: Matrix-type and reservoir-type structure for drug loading

As the drug and carrier materials can be mixed together for nanofibers electrospinning, the drug likely modes in the resulting nanostructured products are drug as particles attached to the carrier surface, both drug and carrier are nanofiber-form hence the end product will be the two kinds of nanofibers interlaced together, the blend of drug and carrier materials integrated into fibers containing both components and the carrier material is electrospun in a turbular form in which the drug particles are encapsulated (Huang *et al.*, 2003).

Sill *et al.* (2008) states that due to the ability to fabricate scaffolds containing pores on the nanometer size scale, high drug loadings are allowed and mass transfer limitations are overcomed in the drug delivery system. The polymeric drug delivery systems are able to meet the requirements of barrier function and preventing abdominal

adhesion. Taek *et al*. (2007) adds that the electrospinning process enables a diverse range of drugs to be directly encapsulated with the bulk phase of nanoscale fibers by dissolving or dispersing them in the organic solvent used for electrospinning.

2.5 Type of Release Study

According to Su *et al.* (2009), PLLACL nanofibrous mats loaded with different weight ratios of BSA were suspended in PBS (pH 7.4) solution in a sealed 12-well plates. The electrospun fiber meshes were each soaked in 3.0 mL of PBS (pH 7.4). Fibrous mats were incubated under static sonditions at 37 °C in the presence of 5% carbon dioxide. At various time points, 1.5 mL of supernatant is retrieved from the wells and an equal volume of fresh medium was replaced. The concentration of each retrieved BSA solution is determined by measuring the absorbance at 280 nm using UV-Vis spectrophotometer.

Jiang *et al.* (2005) reports that 120 mg of the electrospun scaffolds are placed in 2 ml of 0.05 M, pH 7.4 phosphate buffer saline (PBS). The test was performed in a 37 °C incubator-shaker at 50 rpm. At appropriate intervals, 1 ml of the supernatant is removed and replenished with an identical volume of fresh buffer. The protein concentrations are determined by a BCA protein microassay. Each sample is analyzed in triplicate.

The circular pieces of lysozyme loaded fibrous mesh were placed in triplicate in a 12 well tissue culture plate and immersed in 2 mL of 33 mM phosphate buffer saline (PBS, pH 7.4, 0.02% NaN_3) solution at 37 °C in a humidified 5% CO_2 environmental incubator. At pre-determined time intervals, 1 mL of released medium was collected and replaced with an equal volume of fresh buffer medium. The amount of lysozyme in the collected solution was measured using a micro-BCA protein assay kit (Taek *et al.*, 2007).

All in vitro drug release experiments were conducted in release medium (500 µg/mL, proteinase K, 50 mM Tris-HCl, pH 8.6). To attach the basement guide of the multilayered nanofiber mesh closely to the bottom of glass dish containing the release medium, the mesh sheets were pressed lightly with tripodic plastic rods (5 mm diameter) during the release experiment, which inhibits the release medium from affecting the degradation of basement meshes. 10 mL of release medium is added. The glass dishes are incubated at 37 °C with shaking speed of 70 rpm. At the predetermined time intervals, 9 mL of released medium is collected and the same volume of fresh medium was added. The drug concentrations in the collected release medium are determined by measuring the UV absorbance at appropriate wavelengths (Okuda *et al.*, 2010.

According to Xu *et al.* (2008), the released Dox in the buffer solution was monitored by a UV-Vis spectrophotometer at the wavelength of 483.5 nm. The drug loaded fiber is incubated at 37 °C in 20 mL of phosphate buffer saline (PBS, pH 7.4). At the required incubation time, the sample is transferred to 20 mL of fresh buffer solution and the released Dox in the original buffer solution is determined. The detected UV absorbance of Dox was converted to its concentration according to the calibration curve of Dox in the same buffer. Then, the accumulative weight and the relative percentage of the released Dox were calculated as a function of incubation time. The total content of Dox is determined as follows. The three original Dox-loaded fiber mats are placed into separate vials filled with 20 mL of 0.05 mol/L Tris-HCl buffer solution (pH 8.6) containing 50 µg/mL of proteinase K at 37 °C. After several hours, the fiber mats will degraded into small chippings, indicating that the Dox had been completely released in to the buffer solution. The resultant solutions are monitored at the wavelength of 483.5 nm. The concentrations of Dox in the release solutions are determined according to the calibration curve of Dox in the same buffer. The total content of Dox in the fibers is calculated from the average of the three fiber mats.

Release studies are carried out in PBS buffer solution pH 7.4. Samples of the nanofiber nonwovens (2 cm×2 cm) are put in 15 mL plastic tubes and 2 mL release medium is added. The tubes are constantly moved at a speed of 30 rpm at 37 °C. At

certain time points, 200 µL of the release medium is removed and replaced with fresh buffer solution. The amount of released Cytochrome C is determined via BCA assay. 50 µL of the samples is mixed with 170 µL BCA reagent in 96-well plates. After 18 hours at ambient temperature, the absorption at 560 nm is measured using a plate reader. All measurements are carried out in triplicate (Maretschek *et al.*, 2008).

2.6 Drug Configuration

In diffusion controlled systems, the carrier usually retains its structural integrity even after the drug is depeted (Ravivarapu *et al.*, 2006). Polymer degradation may take place throughout the drug release process, during only a part of the drug release time or only after the delivery system is exhausted. The rate of diffusion in such systems is controlled by the following factors: solubility of the drug in surrounding medium (including aqueous and polymer solubility), concentration gradient across the delivery system, drug loading, morphological characteristics (porosity and surface area), hydrophilicity/hydrophobicity of the system, chemical interaction between drug and polymer, polymer characteristics (molecular weight) and external stimulus (pH and ionic strength).

Rate of diffusion from a biodegradable system with large physical dimension can be very high from colloidal or very small particulate carriers that have enormous surface area because they have shorter distance to diffuse. Microspheres of etoposide prepared by oil/oil suspension and solvent evaporation technique using polylactide (PLA) of molecular weight 50 000 Da are divided into size ranges of less than 75 µm, 75 to 180 µm and 180 to 425 µm by passing through series of standard sieves and their drug release is evaluated. Particles that are less than 75 µm showed faster release rates compared with larger size fractions. The difference in the rate is attributed to the difference in the surface area. Alterations in drug release rates could be retained by simple mixing of different size fractions of microspheres (Ravivarapu *et al.*, 2006).

Ravivarapu *et al.* (2006) also states that a biodegradable delivery system where drug release rate can be controlled by the initial drug loading could be designed. The rate of diffusion will be higher for drugs with higher aqueous and polymer solubility, as well as for those not chemically interacting with the polymer. Higher drug loading will mean higher amounts of drug present on the surface or proximal to the surface that will lead to higher initial release. The rate of pore formation can be higher on drug depletion because the drug-polymer ratio is higher.

Molecular weight of polymer offers an attractive opportunity to design biodegradable delivery systems with tailored drug release rates. This is evident from leuprolide acetate-loaded microspheres where the microspheres were prepared using polymers (50:50) with molecular weights of 28.3 and 8.6 kDa showed different porosity and associated specific surface areas. Particles prepared from lower-molecular-weight polymer were very porous and of lower bulk density and higher specific surface area even though their mean particle sizes were comparable. As expected, in vitro release of leuprolide is rapid with approximately 60% release within the first 24 hours. Microspheres from 28.3 kDa polymer are nonporous and only about 3% of the entrapped drug is released within the same time frame (Ravivarapu *et al.*, 2006).

According to Ravivarapu *et al.* (2006), two different approaches are evaluated for obtaining quicker onset of therapeutic action as well as avoiding possible acute toxicity. In the first approach, polymers are premixed in various ratios (3:1, 4:1 and 5:1) of the 28.3 kDa and 8.6 kDa polymers and microspheres are made. In the second approach, microspheres are prepared from these two molecular weight PLGAs separately and are physically mixed in a 3:1 ratio. As compared with the 28.3 kDa microspheres, microspheres prepared with the 8.3 kDa polymers obtained with both the preceding approaches yielded a faster onset of therapeutic action, validating the feasibility of these approaches in designing delivery systems with required release characteristics.

Biodegradable polymers such as PLGA or PLA contain terminal carboxyl groups which may interact with drugs and alter their degradation rate and hence release kinetics. Neutralization reactions between these carboxyl groups and basic drugs may minimize the autocatalytic effect of the acidic chain and reduce the polymer degradation rate. In contrast, these drugs may act as base catalysts and enhance polymer degradation by cleaving the ester bonds. Such ionic interactions should be paid attention during the design of delivery systems. Makoto *et al*. (1998) investigates the relationship of the ionic property of drugs to their release profiles from the PLGA matrix. In the case of acidic and neutral drugs, their weaker ionic interaction with the terminal carboxylic group resulted in precipitation of drugs as crystals in the matrix within a day after immersion in the phosphate-buffered solution (PBS). This has transformed the rods into drug dispersed matrix and hence matrix erosion was not affected by the drug. The solubility in the hydrated matrix is the primary rate-limiting factor for drugs that show weaker ionic interaction with the polymer matrix.

Schmidt and Lamprecht (2009) discusses that among the influencing factors on the extent of drug loading are method of preparation, additives (stabilizers and bioadhesives), nature of drug and polymer, solubilities and pH. Formulation variables can be modulated to increase the drug loading in nanoparticles. Depending on the preparation process and the physiochemical properties of the drug molecule and the carrier, the drug entrapment can be either by inclusion within the carrier and by surface adsorption onto the carrier. Polymerization of monomers requires the drug molecule solubility in the macromolecular material while porous nanoparticles may entrap the drug molecule by adsorption either onto the surface or within the macromolecular network. Entrapment within the nanocapsules core implies the drug molecule solubility in the oil phase used during preparation. The drug to polymer ratio can be as large as 500:1 in nanocapsules (inner core made of the drug itself) when the ratio is usually under 10% in nanospheres. Electrical charges on the drug molecule and carrier may influence the loading capacity.

A pharmaceutical formulation faces various stability challenges during preparation, storage and after administration, before the drug included can be delivered to the targeted site of action. Depending on its chemistry and morphology, a polymer will absorb some water on storage in a humid atmosphere. Absorbed moisture can initiate degradation and a change in physiochemical properties, which can affect the performance in vivo. Storage conditions are critical to the shelf life of a polymeric nanoparticulate formulation. The presence of oligomers, residual monomer or remaining polymerization catalysts or solvents may impair the storage stability, catalyzing moisture absorption or degradation (Schmidt and Lamprecht, 2009). The incorporation of drug also effect the storage stability of a polymer matrix. The relative strength of water polymer bonds and the degree of crystallization of polymer matrix are other important factors. To maintain absolute physiochemical integrity of a degradable polymeric drug delivery device, storage an inert atmosphere is recommended.

Saez *et al.* (2002) states that commercialization of liquid nanoparticulate systems has not taken up partly due to problems in maintaining stability of suspensions for an acceptable shelf life. The colloidal suspension does not tend to separate just after preparation because submicronic particles sediment very slowly and the aggregation effect is counteracted by mixing tendencies of diffusion and convection. After several months of storage, aggregation can occur. Additionally, microbial growth, hydrolysis of the polymer, drug leakage and other component degradation in aqueous environment is possible. Schroeder *et al.* (1998) adds that when stored in acidic medium, nanoparticles are found to be stable for at least two months.

2.7 Type of Drugs/Proteins

According to Ravivarapu *et al.* (2006), in case of macromolecular drugs, a major portion of drug is relased by polymer degradation and erosion and a small portion is release by the diffusion mechanism. Polypeptides usually have limited solubility in the polymer which prevents their diffusion. The aqueous channel present in the delivery

system could be too narrow or tortuous for these macromolecules. The drug release is multi or triphasic which is characterized by higher initial release or burst release, a lag phase where minimal amount of drug is released and release of drug at a higher rate until depletion. This may mean immediate therapeutic activity physiologically after dosing or acute toxicity depending on the drug, no therapeutic activity corresponding to the varied length of the lag phase and sustained activity. The initial release is attributed to the release of drug that is adsorbed onto the surface or present close to the surface in the pores by diffusion. If the carrier system contains a nonporous structure, the initial release of drugs may be reduced. The degradation of polymer starts slowly but without losing its mass or structure during which the drug is immobile. After the degradation of the polymer reaches a critical level, it triggers erosion of the carrier structure and leads to continuous drug release.

The increase in the ionic strength of the release medium often induces a decrease of the release rates. The increased ionic strength may reduce the swelling of polymer matrix by reducing the diffusion of the protein from the microspheres (Bodmer *et al.*, 1992). The therapeutic efficacy of a drug delivery system is dependent upon its release characteristics at the target site. Prolonged release of incorporated drug in a controlled manner minimizes its frequent dosing, often warranted in conventional chemotherapy. In addition it reduces the chances of systemic toxicities associated with the protein. As with other reservoir-type systems, increase in encapsulated drug concentration leads to a higher diffusive driving force for drug release (Chakraborty *et al.*, 2009). Control of drug release by varying drug-polymer interactions can be an empirical process as the degree of interaction varies depending on the polymers and drugs type used.

2.8 Type of Polymers

There are a wide range of polymers used in electrospinning and are able to form fine nanofibers within the submicron range and used for varied application such as tissue engineering scaffolds and biomedical applications (Bhardwaj *et al.*, 2010). Naturally

occurring polymers normally exhibit better biocompatibility and low immunogenicity compared to synthetic polymers when used in biomedical applications. A strong reason for using natural polymers for electrospinning is their inherent capacity for binding cells since they carry specific protein sequence such as RGD (arginine/glycine/aspartic acid) (Pierschbacher and Ruoslahti, 1984). In recent years, electrospinning of proteins mainly from collagen, gelatin, elastin and silk fibroin has been reported (Li *et al.*, 2005). Scaffolds fabricated from natural polymers promise better clinical functionality.

Sill *et al.* (2008) argues that despite relative use of electrospinning, there are number of processing parameters that can affect the fibers formation and structure. Applied voltage, polymer flow rate and capillary to collector distance are grouped in order of relative impact to the electrospinning process. These three parameters can influence the formation of bead defects.

According to Ramakrishna *et al.* (2006), there are two types of synthetic polymers: the ethenic polymers and the condensation polymers. Ethenic polymers are formed by polymerizing monomers containing the carbon to carbon double bond group. The simplest monomer that contain this structure is the olefin ethylene, CH_2=CH_2. Polymerization involves the breaking of the double bond of the monomer and linking up with another monomer. The resultant polymer has a linear structure and is formed by polymerizing the carbon-carbon double group to give a highly cross-linked polymer. The general linear structure of ethenic polymers make it suitable for fiber formation. Important polymers from this class include polyethylene, vinyl chloride polymers and copolymers and polystyrene.

For condensation polymers, the monomers have at least two functional groups such as alcohol, amine or carboxlic acid group instead of a carbon-carbon double group. In condensation reaction, two units often not the same monomer structure reacts to form a polymer at the same time releasing a small molecule such as H_2O. The reaction is slow and the growth in molecular weight is gradual. However, not all condensation polymerization involves the liberation of small molecules. Active hydrogen in this case

is transferred from one molecule to the next instead. A typical example is polymerization of dialcohol and diisoyanate monomers to form polyurethane (Ramakrishna *et al.*, 2006).

In electrospinning, the submicron dimension of the electrospun fibers resemble that of natural extracellular matrix. There are great interests in the use of electrospun fibers in bioengineering area. One of the frequently used synthetic polymers for tissue scaffolds are the biodegradable aliphatic polyesters. These degradable polyesters are derived from three monomers: lactide, glycolide and caprolactone. Hydrolytic attack of the ester bond within the polymer is responsible for its degradation (Griffith, 2000). Poly-L-lactic acid for example is able to degrade to lactic acid which is a normal intermediate of carbohydrate metabolism.

The types of polymer viable for electrospinning can be classified by their hydrophilicity. Hydrophilic polymers (polysaccharides) or extracellular matrix protein (collagen and hyaluronic acid) have been processed into electrospun nanofibers by dissolving the polymers in water, strong acids or a mixture of water and polar organic solvents. Hydrophobic polymers such as poly(ε-caprolactone) or poly (lactic-co-glycolic acid) are dissolved in organic solvents (Chakraborty *et al.*, 2009).

Ravivarapu *et al.* (2006) explains that biodegradable polymers may be synthetic or natural in origin. Natural biodegradable polymers include human serum albumin, low-density lipoproteins, bovine serum albumin, gelatin, collagen, hemoglobin and polysaccharides. Use of the natural polymers is limited by difficulties in purification and large-scale manufacture. They are also known to cause immunogenic adverse reactions. Many synthetic biodegradable polymers possess some common characteristics: stability and compatibility with the drug molecule, biocompatible and biodegradable, ease of manufacture on a larger scale, amenability to sterilization and flexibility to yield multiple release profiles. Biodegradable polymers can be divided into water-soluble and water-insoluble polymers. Polymer biocompatibility and lack of toxicity are important considerations in the design of a drug delivery system designed for systemic application.

Poly,(caprolactone) (PCL) is a semu-crystalline polymer, rather hydrophobic with a high molecular weight. It may be ised in diffusion-controlled delivery systems. The main mode of degradation for caprolactone polymer is hydrolysis. The degradation proceeds first by diffusion of water into the material followed by random hydrolysis fragmentation of the material and finally more extensive hydrolysis accompanied by phagocytosis, diffusion and metabolism. The hydrolysis is affected by the size, hydrophilicity and crystallinity of the polymer and the environment pH and temperature (Kenawy *et al.*, 2007).

Ramakrishna *et al.* (2006) states that it is beneficial to obtain a structure that shows the properties of two or more polymers. This can be achieved either through polymerization of two different homopolymers to form a copolymer or by physical mixing of two or more polymers to form a blend. In copolymers, the covalent bonding between the mers is very strong. The individual mers cannot be separated without breaking the copolymer chain. There are generally two types of copolymers: random copolymers and block copolymers. In random copolymers, there is no sequence in the distribution between the two types of homopolymers. The random copolymers exhibit properties that is intermediate to those of corresponding homopolymers. In block copolymers, the repeating homopolymers exist in a long sequence within the polymer chain. The block copolymer may show property characteristics of each of the constituent homopolymer.

In blending, the polymers tend to separate into two or more distinct phases due to incompatibility. To improve compatibility and miscibility, interactive functional groups are introduced to the polymers so that the polymer chains would form stronger hydrogen-bonding with the advantage of improving the strength of the blend. Common functional groups include carboxylic and sulfonate groups. As there are no chemical reactions involved in polymer blendings, the links between the different polymers are not strong and leaching of one of the polymers may occur when submerged in a solvent (Ramakrishna *et al.*, 2006).

2.9 Morphology Characterization

The morphology of the electrospun nanofibers can be characterized by scanning electron microscope (SEM), field emission SEM (FE-SEM) and transmission electron microscope (TEM). Since nanofiber membranes have porous structure, morphological properties include pore geometry and density (Ramakrishna *et al.*, 2006).

2.9.1 Fiber Diameter

The diameter of an electrospun nanofiber can be examined under scanning electron microscope (SEM). The electron beam is accelerated by holding the tungsten filament at a large negative potential between 1 kV to 50 kV and whilst the specimen is grounded. When the electron beam impinges on the material surface, backscattered electrons (BE), secondary electron (SE) and X-rays escape from the material surface. In the display of SEM system, the SE is captured by the detector for producing the images. If the SEM is equipped with an X-ray detector, particular material on the surface can be mapped with SEM image. SEM sample preparation of electrospun nanofibers can be conducted. In the electrospinning process, polymer solution is stretched by electrical charge difference between the needle tip and the ground collector. While polymer jet is travelling to the collector, solvent is evaporated. After electrospinning, residual solvent may still exist on the nanofibers. Electrospun nanofibers are dried at least one night under vacuum condition. From a completely dried nanofiber membrane, an area of 1 cm x 1 cm is cut and attached by means of carbon tape to a copper stub. It is important at this juncture to ensure that direct adhesion of nanofibers is not recommended since adhesive of carbon tape may damage the nanofibers. This is especially so if the biodegradable polymer nanofibers are treated.

Since polymer nanofibers require conductive coating, gold was selected as coating material due to its ease to vapor deposit and on bombardment with high energy electrons it gives a high secondary yield. Polymer nanofibers whose diameter is 200 nm

~ 1000 nm are observed at around x15 000 magnification with 10 ~ 20 kV acceleration voltage under SEM. Basically, each diameter of 50 ~ 100 nanofibers is examined using image analyzer and average fiber diameter and fiber distribution are determined. If fiber observation is conducted at extremely high magnification above x15 000 magnification, fiber damage by energetic impinging of electrons takes place. It is known that a significant temperature rise (tens of degrees) occurs when a material surface is bombarded by an energetic electron beam (Campbell *et al.*, 2000). When ultrafine nanofibers with less than 200 mm diameter are observed, the accuracy of the measured value is doubtful. If biodegradable polymer nanofibers with poor heat resistance are observed, precaution must be taken tom prevent fiber damage.

In this regard, field emission SEM (FE-SEM) is highly recommended to observe electrospun nanofibers (Casper *et al.*, 2004). The feature of FE-SEM is that high resolution images can be obtained with low acceleration voltage. Another important concern in observing ultrafine nanofibers is the thickness of the conductive gold coating. The thickness of gold coating generally is around 25 nm. If the ultrafine nanofibers are examined under SEM, coating thickness interrupts the accuracy of diameter measurement. To avoid the coating influence, the nanofiber diameter is measured under transmission electron microscopy (TEM).

In a TEM, the electron source is generally tungsten filament heated with a low voltage source. The filament is held at a large negative potential and the electrons are accelerated towards specimen with less than 100 nm thick. Similar to SEM, X-ray escapes from material surface and the detected X-ray supplies the information of particular element of the sample. After the electron beam passes through the sample, transmitted beams accordingly passes through the other lenses and finally an image is produced. A metal mesh is subjected to coating and fine supporting polymer film is placed on the metal mesh. The carbon coating is then further applied to the metal mesh and nanofibers are are electrospun on the mesh. Gold coating is not necessary for a TEM sample. TEM observation is a useful methodology to accurately measure the diameter of ultrafine nanofibers (Ramakrishna *et al.*, 2006).

2.9.2 Organic Group Detection

For functional group detection on electrospun nanofibers, Fourier-Transform Infrared Spectroscopy (FTIR) is utilized (Campbell *et al.*, 2000). As infrared frequency corresponds to molecular frequency, infrared spectroscopy sensitively reflects molecular structure of material. Two cases (influence by spinning process and chemical or physical reaction after spinning) are found to investigate chemical functional groups which exist in electropsun nanofibers. As to the influence by the spinning process, the concern is how the chemical structure of polymer is influenced by electrospinning process. After making electrospun nanofibers, certain applications may require surface modification to attach chemical function on the nanofiber surface.

2.9.3 Mechanical Property

From the material size view point, the size effect on single fiber property should is investigated. The crystalline structure of a single fiber is affected by processing conditions even in a same diameter of two types of fibers when the fiber diameter shrinks from micron to nano size level. Tensile testing method is applied in terms of mechanical testing of nanofibrous membranes. A single fiber testing is limited to nanofibrous membrane testing due to limitation in preparing the sample and applying the loading to a tiny nanofiber. Three testing apparatus are available to measure tensile and bending properties of a single nanofiber: Cantilever technique, AFM-based nanoindentation system and nano tensile tester (Ramakrishna *et al.*, 2006).

Tensile properties of electrospun nanofibrous membranes by preparing the specimen in dumbbell and rectangular shape. Although rectangular shape specimen is easily prepared, there is a possibility to get the exaggerated testing value due to the stress concentration vicinity grip part. Dumbbell shape specimen precisely removes the stress concentration and the obtained testing values are cross to true properties. However, the preparation of dumbbell shape specimen of nanofiber membranes is costly

since sharp dumbbell shape blade must be used. Used tensile apparatus is conventional universal testing machine and testing speed is 10 mm/min (Bhattarai *et al.*, 2003).

Randomly-oriented nanofiber membrane is collected on flat plane while aligned nanofiber membrane is collected by the rotating drum (Huang *et al.*, 2004). With respect to aligned nanofiber membranes, tensile property in each drum rotating direction and transverse direction is discussed. As nanofibers are likely to align in drum rotating direction, tensile property of nanofiber specimen could indicate higher values. Drastic property difference is hardly seen between the two fiber orientations. This is attributed to the fiber alignment of collected nanofibers on the rotating drum. Even if nanofibers are collected with rotating drum, there is no certainty that the alignment of nanofibers can be undirectionally oriented and there is only subtle difference of tensile properties. In order to get more precise fiber alignment, the current fiber collection using rotating drum still needs further improvements.

2.10 In Vitro Release Study

Su *et al.* (2009) explains on BSA release profiles from mix electrospun PLLACL nanofibers with different BSA proportions. Experiments were performed in triplicate and error bars indicated on standard deviation. The release kinetics for mix electrospinning cases can be illustrated by two stages: an initial fast release before the inflections (stage I) followed by a constant release (stage II). In stage I, there were initial burst releases from mix electrospun mats. Then the release was ceased and the total released amount was 60–80% in stage II. Xu *et al.* (2005) had reported a water-soluble drug capsulated in an oily phase of chloroform solution of amphiphilic poly(ethylene glycol)-poly(L-lactic acid) (PEG–PLLA) diblock copolymer, and they found that the drug release behavior was related with the distribution of drug in the fiber mats. In the process of electrospinning, the ions were easily attracted at the surface of nanofibers, namely, BSA molecules were easily distributed on the surface of nanofibers.

During the release processes, BSA presented on the surface was dissolved in PBS solution. Thereafter, the inner presented BSA diffused in PBS in the later phases, but the quantitywas very tiny. The encapsulation efficiencies of BSA in PLLACL scaffolds electrospun from blend solution of PLLACL–BSA were found to be 78.5_ 4.6% for 5% BSA loaded and 80.2 _5.1% for 10% BSA loaded. However, the mats derived from coaxial electrospinning showed a relatively stable release behavior of BSA in Fig. 7. The method of coaxial electrospinning made the BSA encapsulated in the inner part of nanofibers during the processes of electrospinning. Some of the BSA also emigrated to the surface of PLLACL nanofibers, because the inner and outer BSA solution solutions could mix together completely. There were also initial stages in the curves of coaxial electrospun PLLACL/BSA nanofibrous mats. But they were quite different from those with the method of mix electrospinning. The profile of BSA released from coaxial electrospun nanofibrous mat present an initial stages of 10–20%, and after these sections, the release curves exhibited sustained behavior. The whole process of release lasted for 108 h till the total released amount was about 70%. It was obvious that the release was not complete, and the rest of the BSA may continue to release for longer time. In this case, the capsulation efficiency of BSA from coaxial electrospinning increased to 93.2% for 5% BSA loaded and 89.7% for 10% BSA loaded.

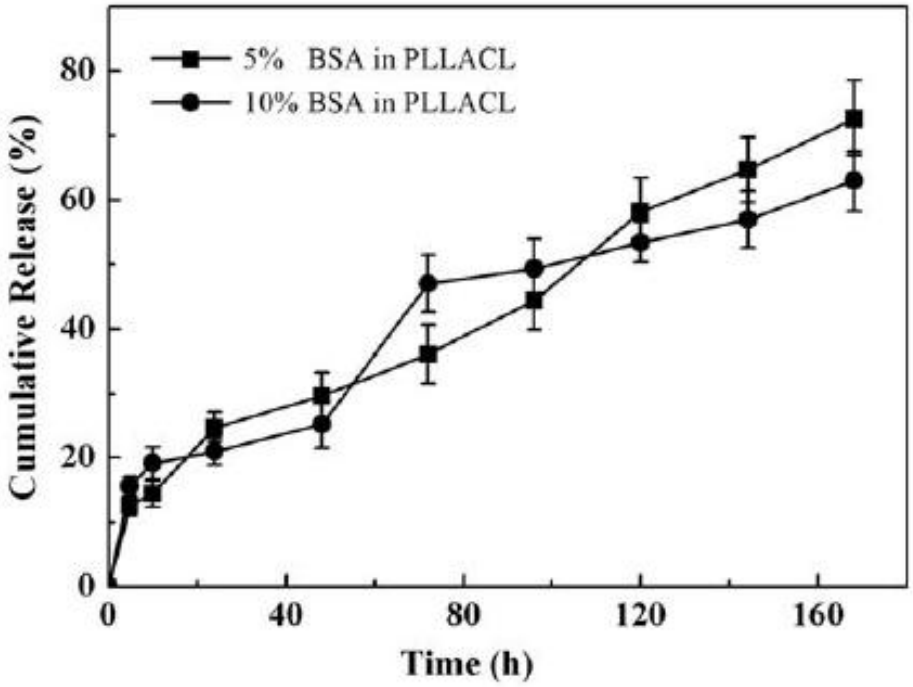


Figure 2.4: *In vitro* BSA release profiles from PLLACL nanofibers which are fabricated by coaxial electrospinning with different BSA proportions (Su *et al.*, 2009)

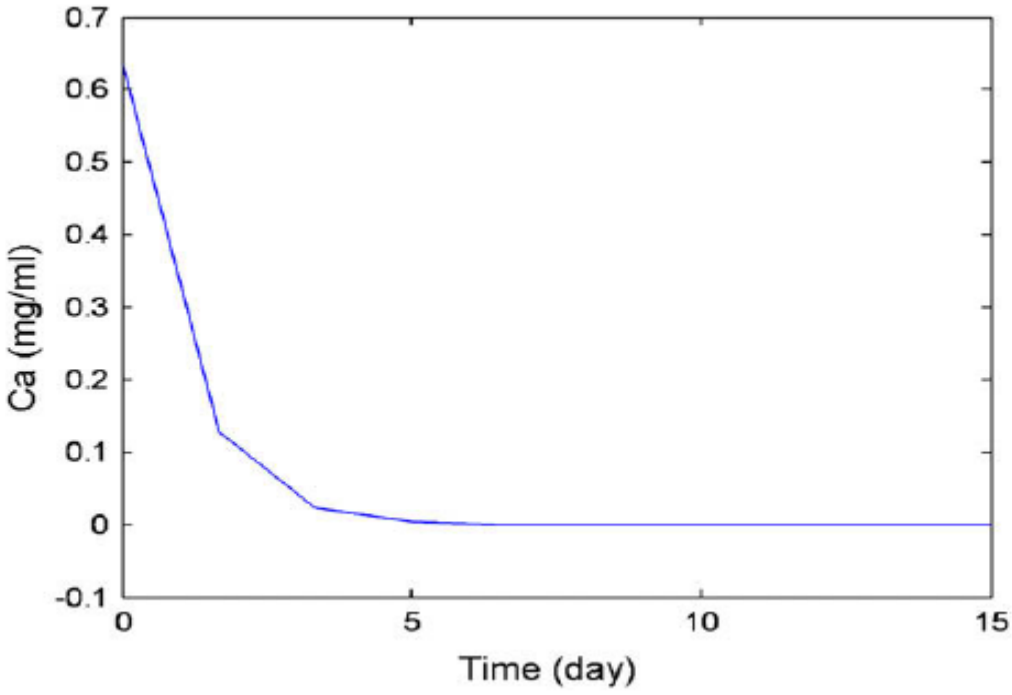


Figure 2.5: The relationship between average concentration of BSA in nanofibers and release time (Su *et al.*, 2009)

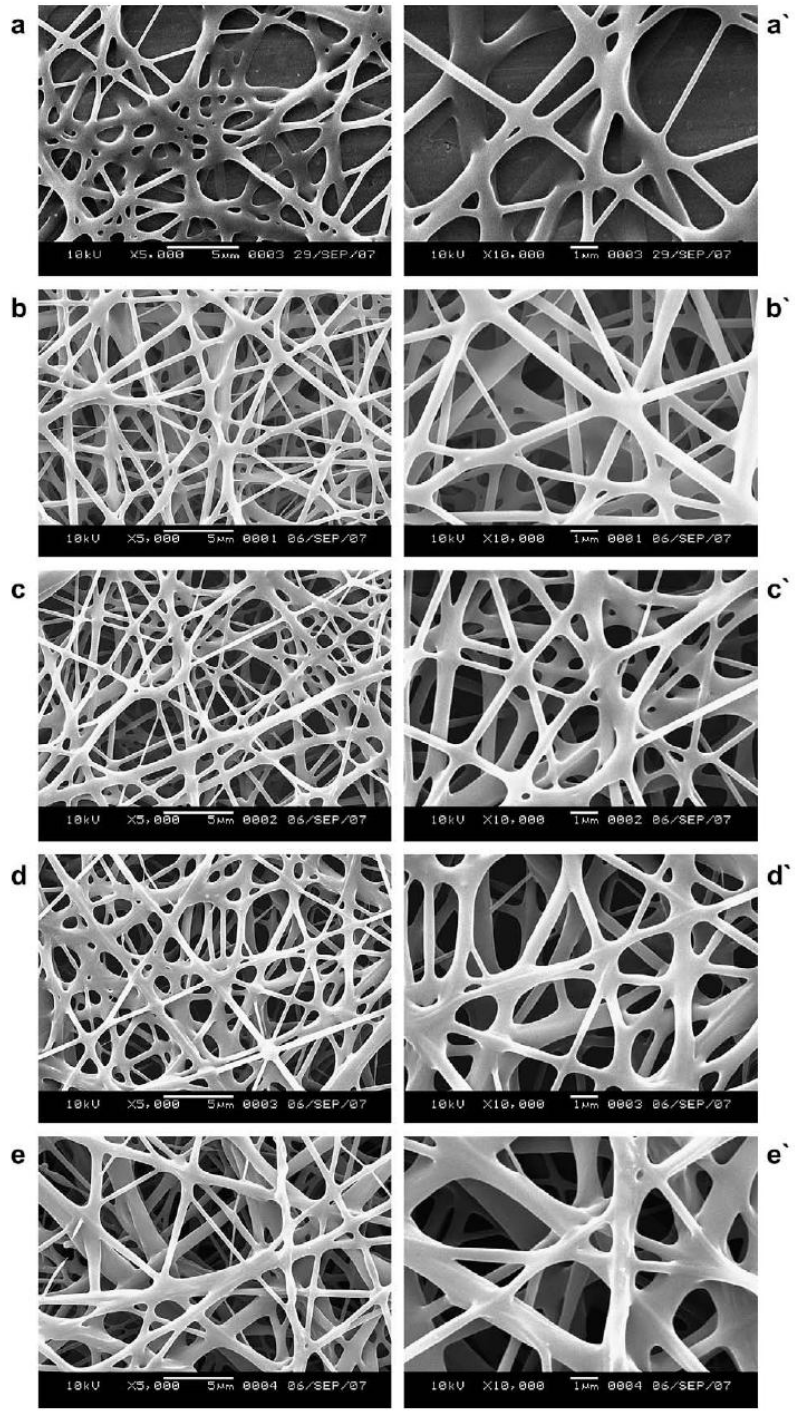


Figure 2.6: SEM images of the PLLACL/BSA nanofibers prepared generated from coaxial electrospinning with BSA contents of 5, 10, 15 and 20 wt% compared with PLLACL (Su *et al.*, 2009)

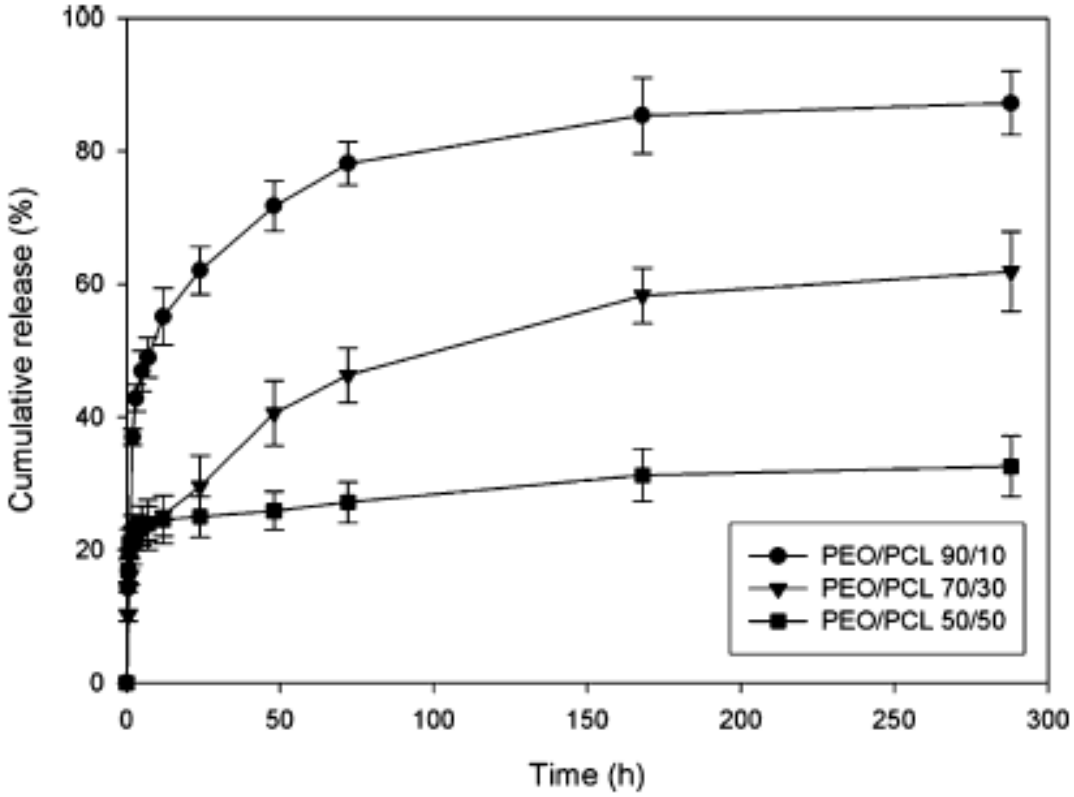


Figure 2.7: Lysozyme release profiles from electrospun PEO/PCL fibrous mesh with varying blend weight compositions (90/10, 70/30 and 50/50) (Kim *et al.*, 2007)

Figure 2.7 shows lysozyme release profiles from PEO/PCL blend meshes with different blend ratios. Accorking to Kim *et al.* (2007), it can be seen that lysozyme is released out more rapidly when the amount of PEO increases in the blend. For the 90/10 blend mesh, the cumulative lysozyme release percent reached to about 87% after 12 day incubation. For the 50/50 blend mesh, however, about 32% was released out during the same period. The extent of initial burst release was much higher for the fiber mesh containing higher PEO. The results suggest that the dissolution rate of PEO domains in the fiber structure controlled the release rate of lysozyme. Since lysozyme molecules were likely partitioned into the amorphous phase of PEO domains in the blend mixture, entrapped lysozyme species were released out through the aqueous fluid filled porous and interconnected channels that were created from gradual dissolution of phase separated PEO domains. Thus the incomplete release observed in the 70/30 blend mesh

was also caused by poor inter connectivity between the PEO domains, restricting the diffusion of lysozyme entrapped deep inside the fiber.

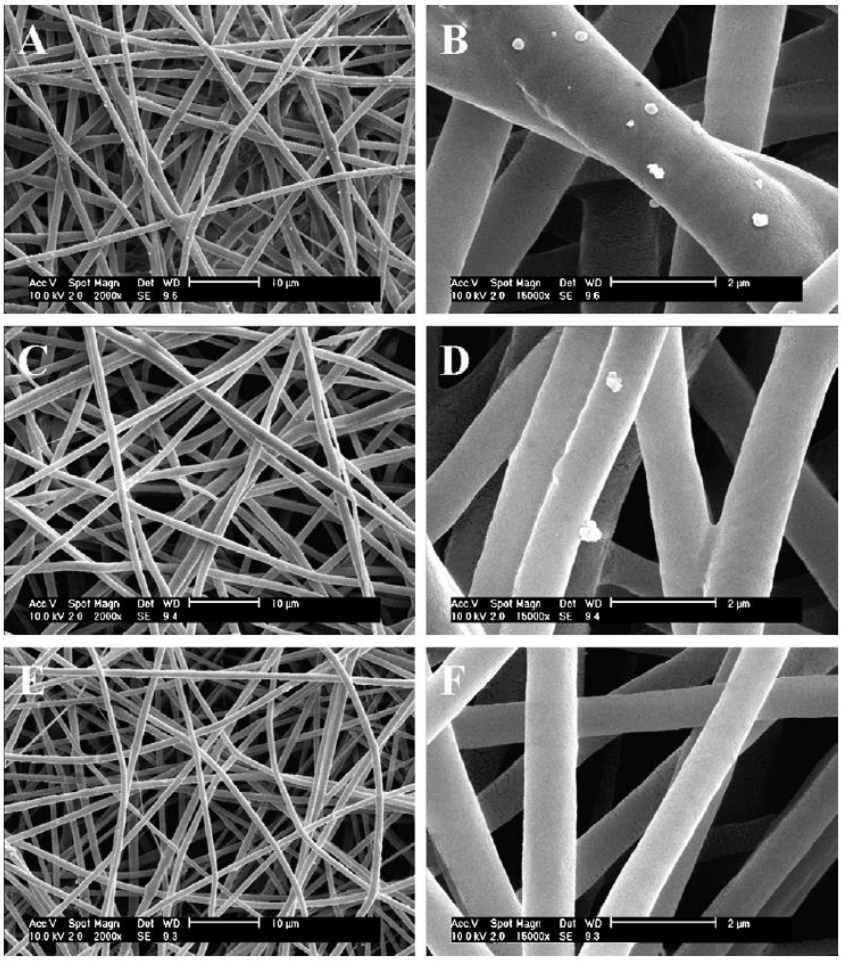


Figure 2.8: SEM images of PEO/PLLA, PEO/PCL and PEO/PLGA electrospun fibers at 70/30 blend weight composition (Kim *et al.*, 2007)

It should be noted that protein stability problems such as aggregation and non-specific adsorption additionally contributed to protein release behaviors from biodegradable devices to varying extents. In this sense, the observed lysozyme release patterns cannot be solely explained from the combined mechanism of polymer erosion and lysozyme diffusion. It can be seen that the released lysozyme fraction after 12 h incubation still retained about 90% of its catalytic activity compared to that of native lysozyme. This reveals that lysozyme survived during the electrospinning process

involving direct dissolution in a mixed organic solvent of DMSO and chloroform and rapid jet stream line ejection through a nozzle under high electric voltage conditions.

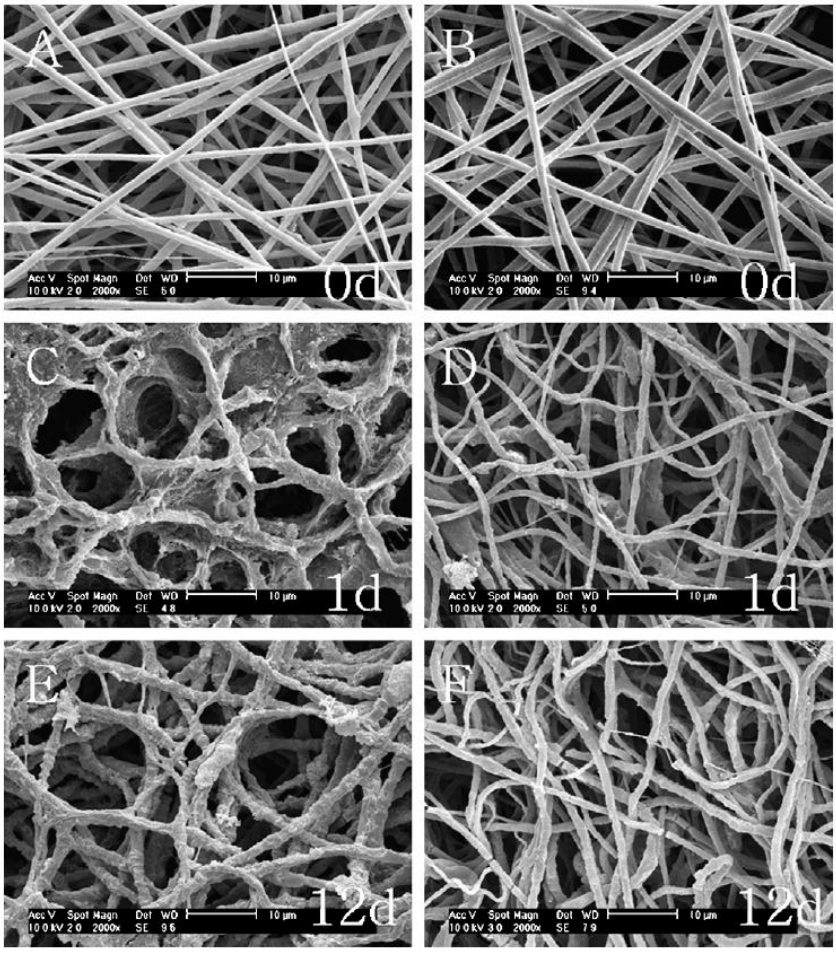


Figure 2.9: SEM images of morphological changes of 90/10 and 70/30 PEO/PCL electrospun blend fibrous mesh as a function of incubation time (Kim *et al.*, 2007)

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter will discuss the experimental design used to develop polymeric drug delivery system using electrospun nanofibers, the characterization of the electrospun nanofibers incorporated with multiple proteins and the multiple proteins release profiles from the protein-loaded electrospun nanofibers.

3.2 Materials

Poly(ε-caprolactone) (PCL, Mw: 80 000) was purchased from Aldrich (St. Louis, USA). Poly(L-lactic acid, Mw: 300 000) was obtained from Polysciences (Warington, PA). Copolymer poly(L-lactide-co-ε-caprolactone, 70:30 blend, Mw: 100 000) was supplied by Boehringer Ingelheim (Ingelheim, Germany). Albumin from bovine serum, lysozyme from chicken egg white and IgG from human serum technical grade were obtained from Sigma (St Louis, USA). All the chemicals used are of analytical grade and used without further purification.

3.3 Core and Shell Solution Preparation

0.25 g BSA and lysozyme in 5 mL distilled water respectively and diluting 0.25 mL of IgG in 5 mL distilled water. The core solution was obtained by mixing three three solutions in a bottled and stirred for 2 hours. The proteins mixture was stored for an overnight in a 4 °C refrigerator.

The shell solutions were obtained by dissolving 0.75 g PCL in 4.67 mL chloroform and 2.31 mL methanol (75:25 ratio), 0.9 g PLLA in 3.94 mL dichloromethane (DCM) and 1.69 mL dimethyformamide (DMF) (70:30) ratio and 0.9 g PLLACL in 1.6 mL 1,1,1,3,3,3-hexafluor-2-propanol (HFP). The polymer solutions were stirred for an overnight to ensure that the solutions have sufficient viscosity for electrospinning.

Figure 3.1: Preparation of PCL, PLLA and PLLACL polymer solutions

3.4 Coaxial Electrospinning Setup

The coaxial electrospinning setup used in this experiment was obtained from Dr. Subramaniam Sundarrajan of NUSNNI. In this setup, a 22 G needle (Becton Dickinson & Company, USA) is inserted inside a 18 G needle and the needles are attached together in the coaxial electrospinning. Each of the two coaxial electrospinning setup channels is connected to two syringes (Becton Dickinson & Company, USA) using Teflon tubes and the two syringes are connected to two syringes pumps (Kd Scientific, Singapore) to control the core and shell solutions flow rate.

Figure 3.2: Monoxial electrospinning setup. The coaxial electrospinning setup is developed by improvising the above setup using a device designed for coaxial electrospinning purpose

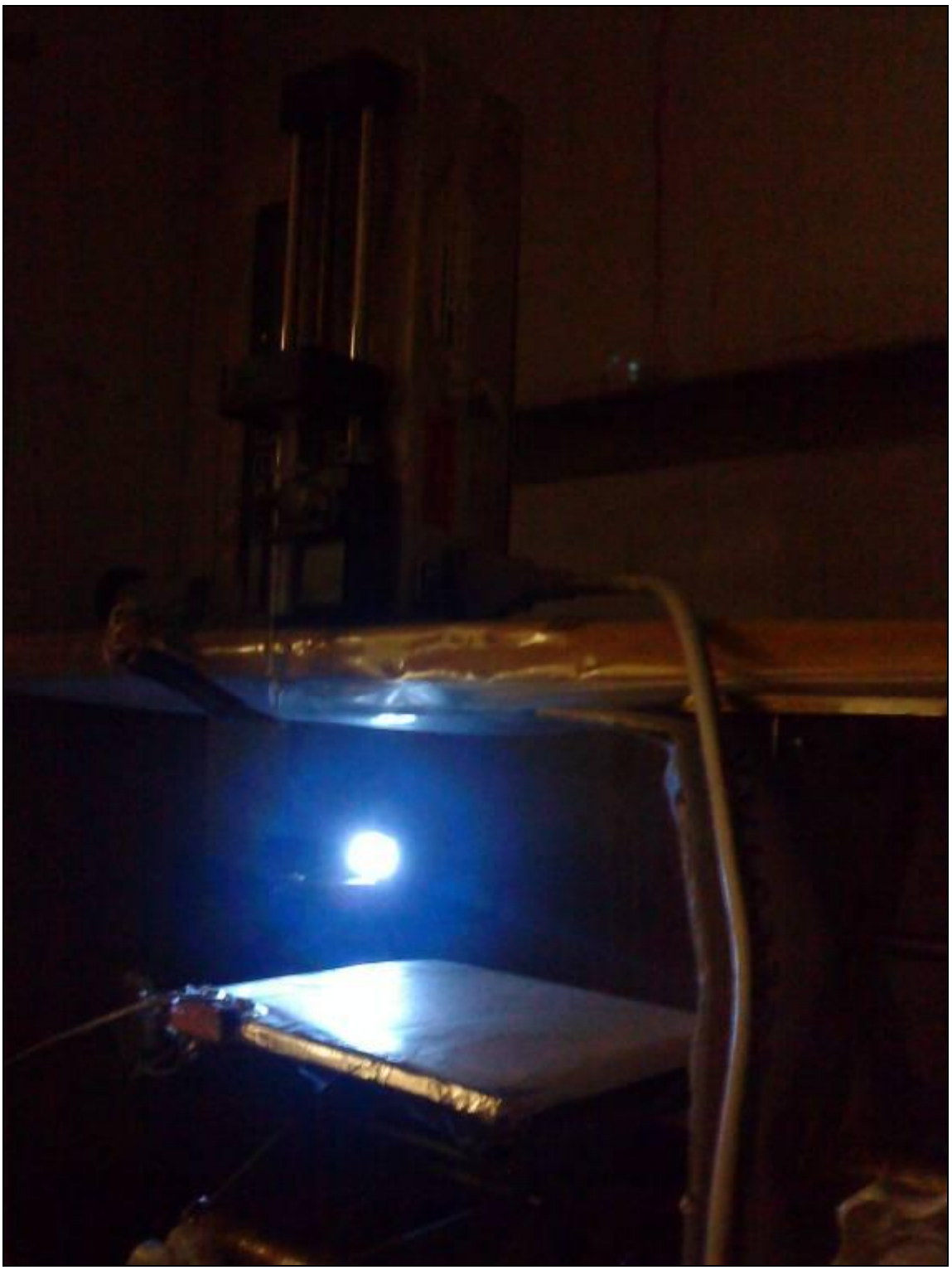


Figure 3.3: Deposition of electrospun nanofibers on the metal ground collector during electrospinning process. A bending instability occurred during this process attributing to the formation of multiple jets of the electrospun fibers

3.5 Electrospinning Process

A positive high-voltage supply is used to maintain the voltage in the range of 10 to 18 kV. The electrospun nanofibers are collected on a piece of aluminum foil covered on an electrical grounded metal plate which is placed at a distance of 10 to 14 cm below the tip of the coaxial electrospinning setup needle. The flow rate of the core and shell solutions are maintained at 0.05 mL/h to 0.3 mL/h and 0.5 mL/h to 1.6 mL/h respectively. The electrospinning was conducted under ambient conditions with humidity in the range of 54% to 70% humidity. The collected scaffolds are stored in vaccum oven overnight at room temperature to eliminate solvent residuals.

Figure 3.4: Basic electrospinning setup consisting of three major components- a high voltage power supply, a metallic needle (spinneret) amd a grounded collector

Figure 3.5: High voltage power generator to supply positive direct current to the electrospinning setup

Figure 3.6: Humidifier to control the humidity inside the electrospinning chamber

Figure 3.7: Vacuum oven to store the electrospun nanofibers overnight at room temperature

3.6 Characterization of Electrospun Fiber Meshes

The morphological observation of each electrospun nanofibers is carried out using a scanning electron microscope (SEM, JIOL Asia, Singapore) and transmission electron microscope (TEM, JIOL Asia, Singapore). From the SEM and TEM images, each fiber diameter is determined by using an image analyzer (Image J, developed by the

Figure 3.8: Carbon grid preparation for morphological analysis using TEM

Figure 3.9: Tensile strength testing to analyze tensile stress and tensile strain

National Institute of Health, USA). Tensile strength of the electrospun nanofibers with and without proteins mixture encapsulation is carried out by using mechanical analyzer (Instron, Singapore).

3.7 *In Vitro* Proteins Release

The circular pieces of proteins mixture loaded nanofiber meshes are placed in a 12-well tissue culture plate, in triplicate and immersed with 2 mL of phosphate buffer saline (PBS) solution (0.05 M, pH 7.4). The samples are incubated for 24 hours in an incubator. At pre-determined time intervals which is every 4 hours, 1 mL of the release medium is collected and replaced with an equal volume of fresh buffer medium. The release rate of the proteins mixture is determined by measuring the absorbance using UV-Vis spectrophotometer at 450 nm wavelength.

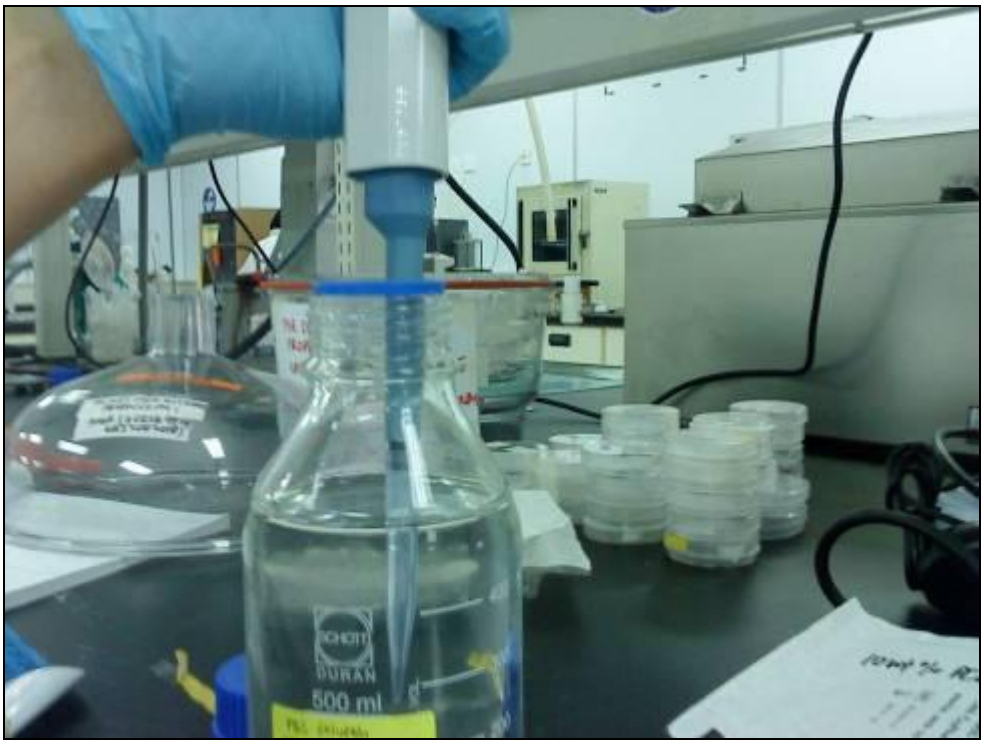


Figure 3.10: Preparation of phosphate buffer saline (PBS) solution (0.05 M, pH 7,4)

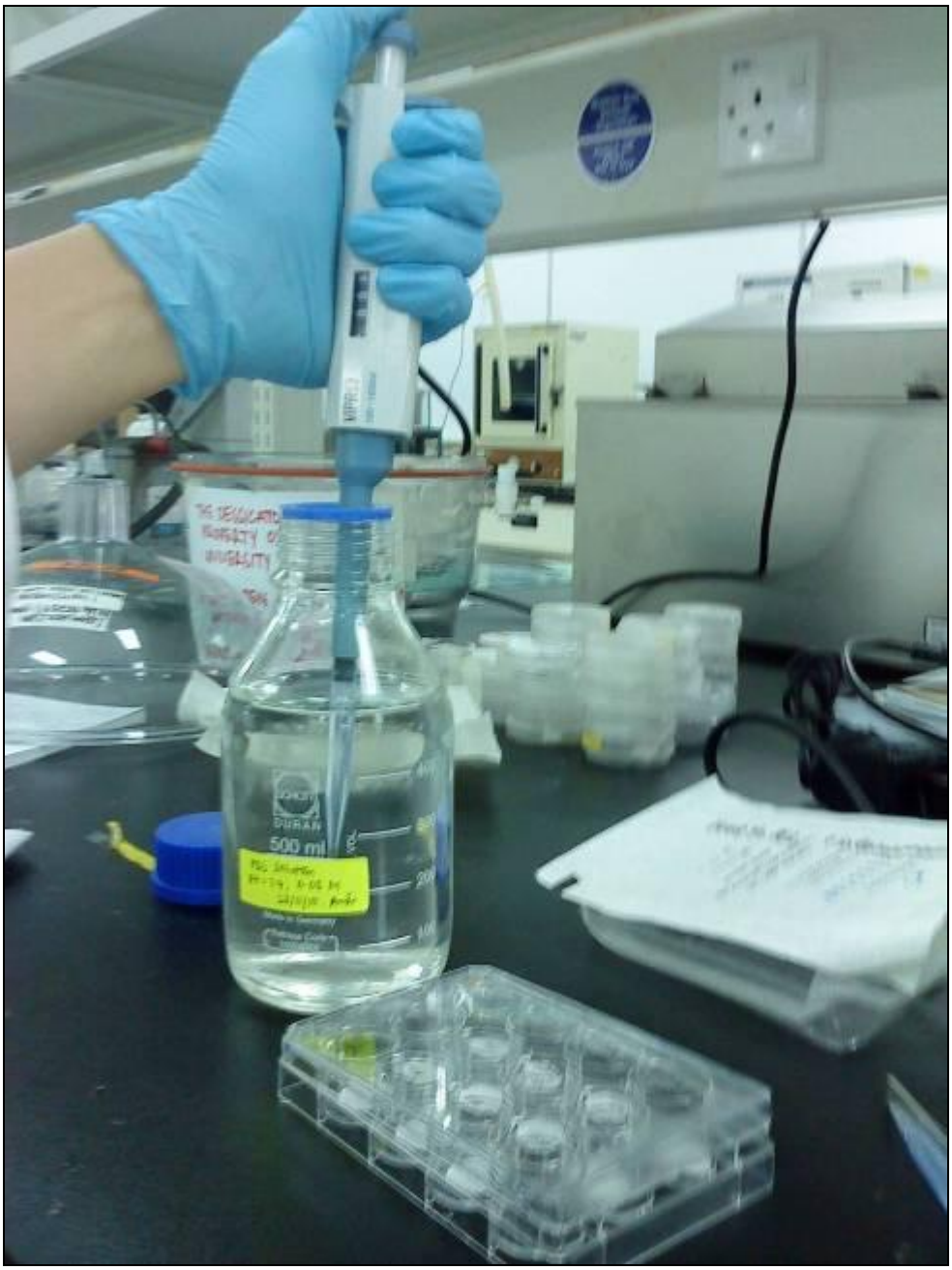


Figure 3.11: Immersing the proteins mixture loaded nanofiber meshes with PBS solution in a 12-well tissue culture plate

Figure 3.12: PCL electrospun nanofibers with proteins mixture loading immersed with 2 mL PBS solution before 24 hours incubation

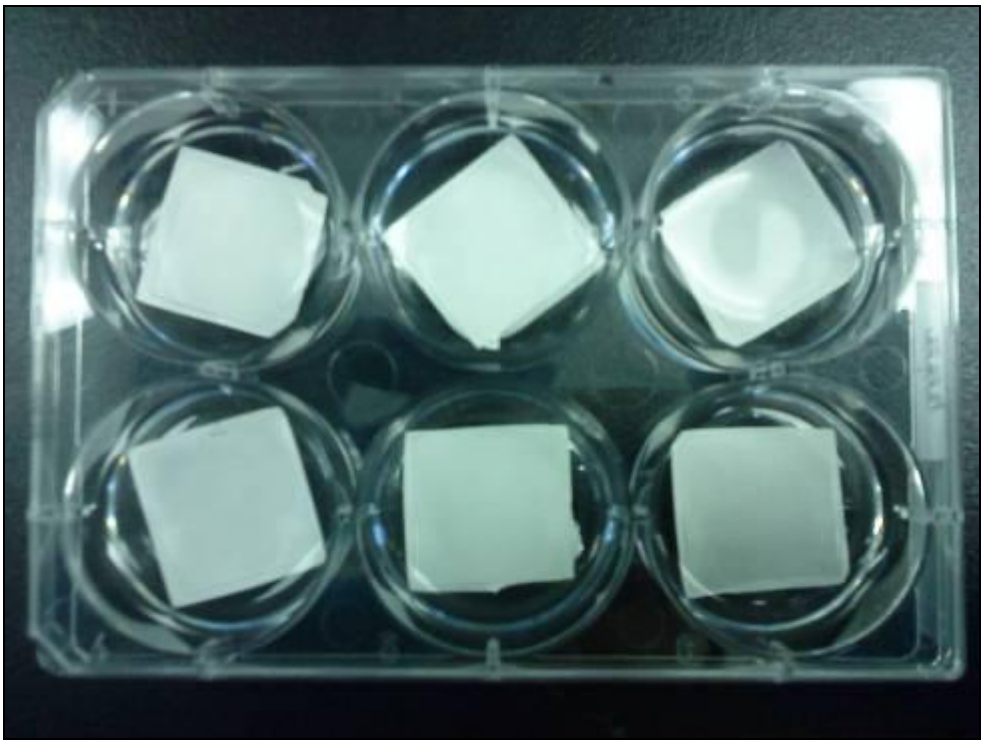


Figure 3.13: PLLA electrospun nanofibers with proteins mixture loading immersed with 2 mL PBS solution before 24 hours incubation

Figure 3.14: PLLACL electrospun nanofibers with proteins mixture loading immersed with 2 mL PBS solution before 24 hours incubation

Figure 3.15: The samples are incubated inside the incubater for 24 hours

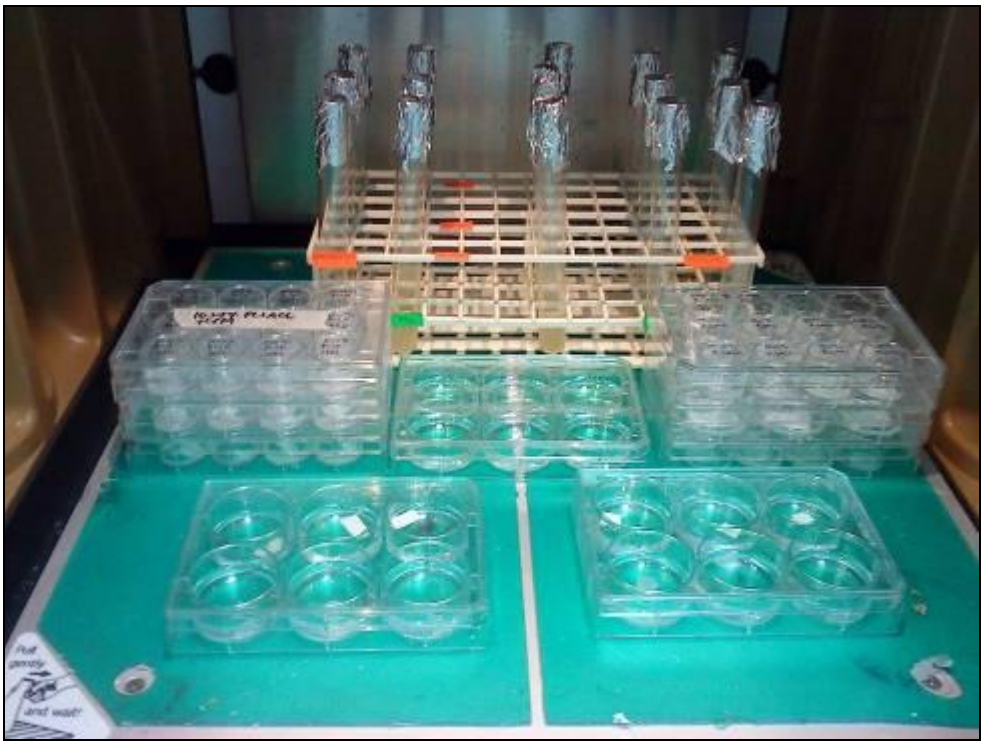


Figure 3.16: The samples are incubated inside the incubater for 24 hours

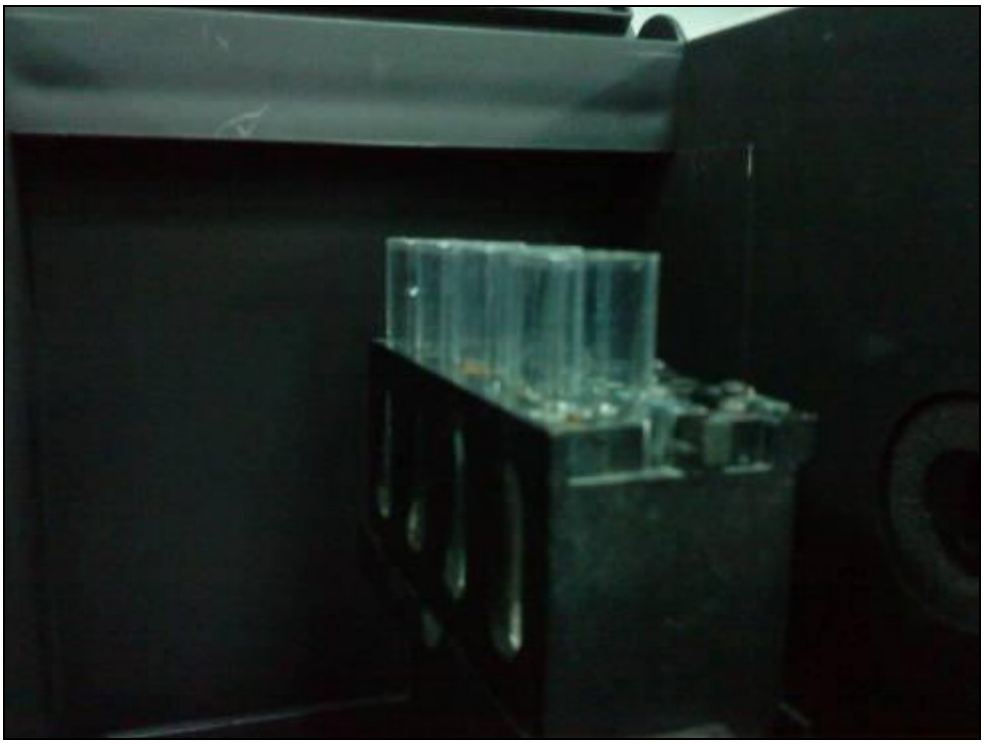


Figure 3.17: Analyzing the proteins release profile by measuring the absorbance at selected time intervals using a UV-Vis spectrophotometer

In summary the methodology to develop a polymeric drug delivery system using PCL, PLLA and PLLACL electrospun nanofibers for controlled release of multiple proteins can be summarized as below.

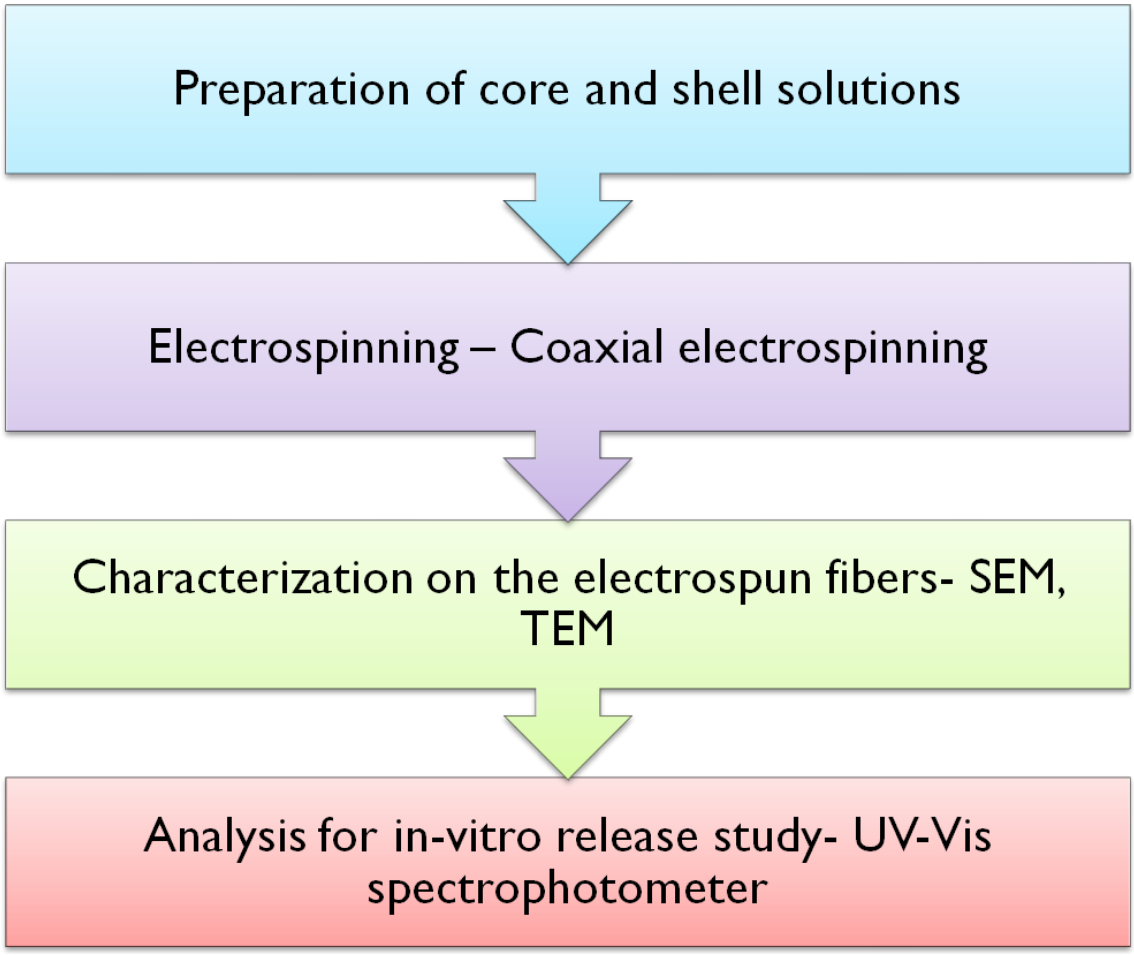
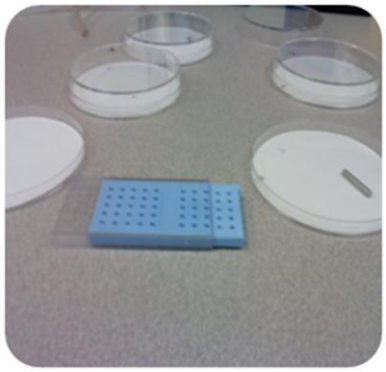


Figure 3.18: Methodology summary for developing polymeric drug delivery system using electrospun nanofibers

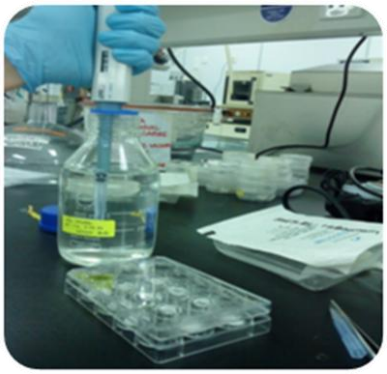
Preparation of core and shell solutions

Coaxial electrospinning



Characterization of electrospun nanofibers using SEM and TEM

Characterization of mechanical properties via tensile strength



Immersing with PBS solution

Incubate for 24 hours and measure absorbance

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Development of Polymeric Drug Delivery System Using PCL, PLLA and PLLACL Electrospun Nanofibers

PCL, PLLA and PLLACL were used as model polymers to shell structure of the electrospun nanofibers. BSA, lysozyme and IgG dissolve in distilled water were used as model core contents. PCL, PLLA and PLLACL are biocompatible polymers and display excellent fiber-forming properties. The stability of fragile bioagents can be enhanced by PCL, PLLA and PLLACL.

Three different biodegradable polymers (PCL, PLLA and PLLACL) were used as base materials for the production of proteins mixture (BSA, lysozyme and IgG) loaded fibrous meshes. PCL was dissolved in the solvent mixture of chloroform and methanol at the ratio of 75:25. PLLA was dissolve in the solvent mixture of dichloromethane (DCM) and dimethylformamide (DMF) at 70:30 ratio while PLLACL was dissolved in 1,1,1,3,3,3-hexafluor-2-propanol (HFIP). The results showed that ultrafine PCL, PLLA and PLLACL nanofibers could be electrospun at the concentrations between 10% to 18% (w/w).

Various processing parameters including polymer concentration, drug/protein concentration, voltage supply, shell/core solutions flow rate, distance between the needle tip to collector and needle diameter were responsible for determining the electrospun fiber meshes morphology. Electrical potential and polymer concentration are the key factors governing the fiber diameter, apparent density and porosity (Zong *et al.*, 2002). Overall morphology, degradation rate and matrix characteristics of the electrospun fiber meshes can be tailored by controlling electrospinning parameters and polymer blend composition (Kim *et al.*, 2007).

Each polymer is produced at different concentrations of 10% (w/w), 16% (w/v) and 10% (w/w) respectively for PCL, PLLA and PLLACL electrospun fibers to produce stable and regular fibrous structure. In electrospinning, the polymer solution jet solidifies when it is ejected from the needle tip to the ground collector with the solvents evaporation and the solidified jet turns into a nanofiber (Su *et al.*, 2009). The solvent evaporation occurs only when the following conditions are satisfied in electrospinning: the jet has micron or submicron-scaled diameter, the jet carries excess charges which are beyond the surface tension of electrospun polymer solution and the solvents evaporate under the influence of a strong magnetic field (Su *et al.*, 2009).

As all the polymer solutions were spinnable, the concentrations used were able to entangle the polymer chains and overcome the solution surface tension. The formation of stable Taylor cone during electrospinning process indicates that the voltage supply used was acceptable as the electrostatic force in the solution was generated to overcome the solution surface tension. The feed rate used for both core and shell solutions were sufficient to produce fine fibers even though small diameter beads were seen in the electrospun fibers. The distance used in the electrospinning process to produce the electrospun nanofibers were able to allow enough time for the solvents to evaporate when it reach the collector. As pore diameters on the electrospun fibers were not clearly seen, it can be said that the humidity used in the electrospinning process was acceptable.

In the configuration of the coaxial spinneret, two immiscible liquids are fed through two concentrically arranged needle (Jiang *et al.*, 2005). 18G and 22G needles inner diameter are used for the shell and core respectively. The flow of the solution is controlled by a syringe pump in the range of 0.5 to 1.6 mL/h for shell solution and 0.05 to 0.3 mL/h for core solution. The electric voltage used in the study is in the range of 10 to 18 kV. When a higher voltage is applied, uneven deposition on the ground collector is seen. Kim *et al.* (2007) suggests such phenomena might happen due to the large whipping motion.

PCL, PLLA and PLLACL biodegradable polymers were successfully electropsun using electrospinning technique. Using coaxial electrospinning configuration, multiple proteins consisting of BSA, lysozyme and IgG mixture were encapsuloated within the electrospun fiber meshes. Compared to the monoaxial electrospinning technique, coaxial electrospun fibers are able to distribute and release proteins in a sustained manner. The core-shell design allows bioagents such as BSA, lysozyme and IgG to dissolve in aqueous solution for encapsulation. Using reservoir-type structure, the core-shell structure ensures that the drug enclosed in the polymer matrix and the proteins is concentrated in the core of the fibers as opposed to the random distribution of the proteins in the fiber matrix. This will guarantee in better control over the release kinetics of the proteins.

PCL and PLLA which are homopolymers in this study while PLLACL is a block copolymer. PCL and PLLA are semi-crystalline polymers, rather hydrophobic with a high molecular weight. PLLACL as the block copolymer showed a property characteristic of the constituent homopolymers which are PCL and PLLA. Compared to blending polymers, the block copolymers, the covalent bonding between the mers are very strong while the absence of interactive functional groups caused the blending polymers to have a weak hydrogen bonding. PLLACL block copolymer has better strength and compatibility compared to the blending polymers. The ionic strength between proteins and the polymers will affect the release rate as the increase in the ionic strength of the release medium induces a decrease of the release rates. As proteins are

polypeptides, they have a limited solubility in the polymer that prevents their diffusion due to the narrow aqueous channel.

4.2 Morphology Study of Electrospun Nanofibers

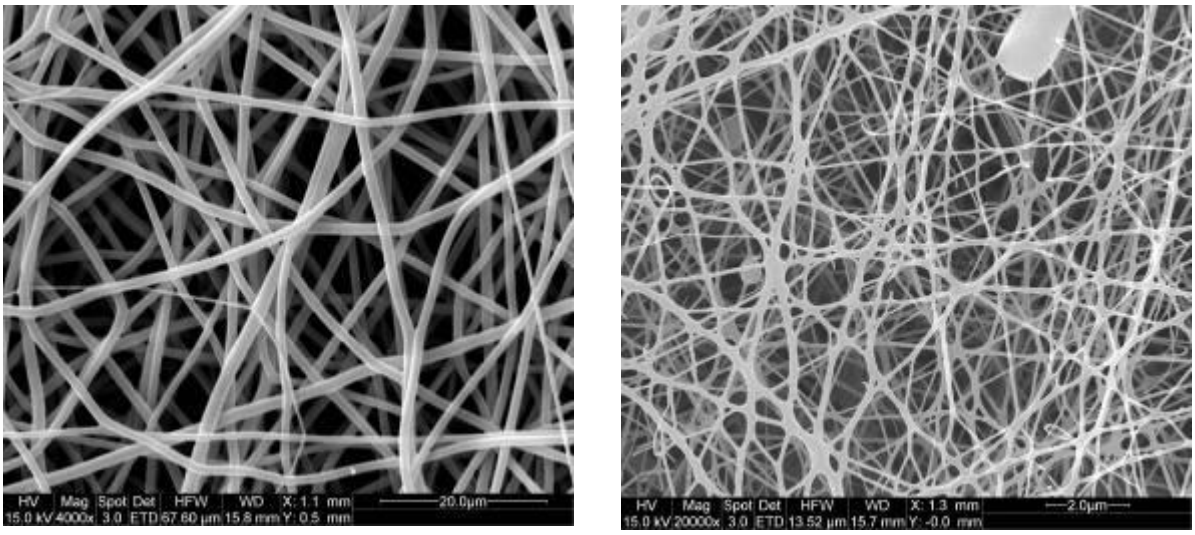


Figure 4.1: SEM images of PCL electrospun nanofiber without proteins encapsulation (left) and PCL electrospun nanofiber with proteins encapsulation (right)

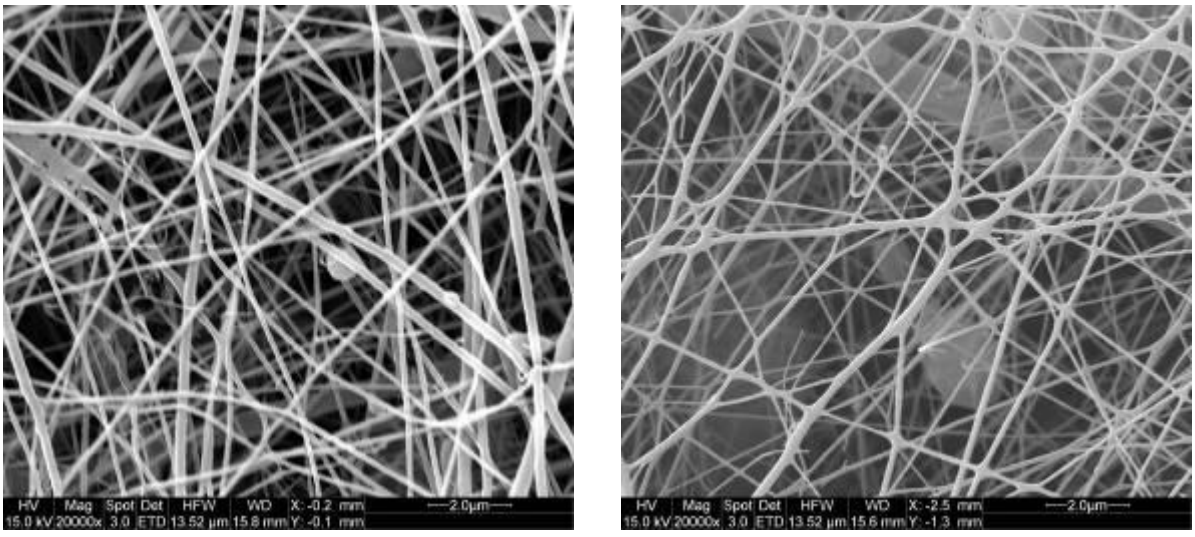


Figure 4.2: SEM images of PLLA electrospun nanofiber without proteins encapsulation (left) and PLLA electrospun nanofiber with proteins encapsulation (right)

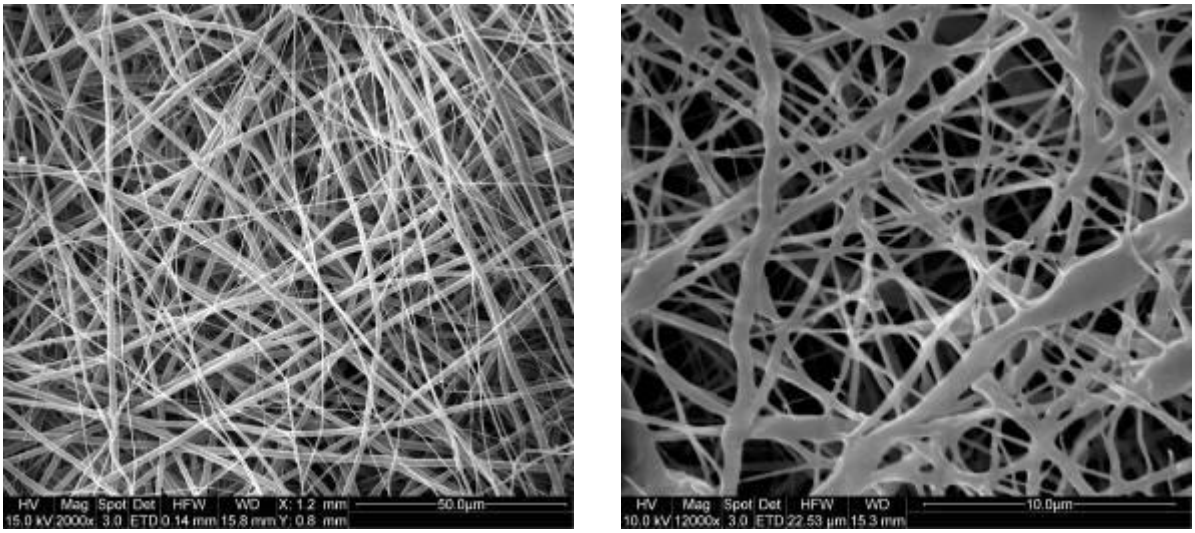


Figure 4.3: SEM images of PLLACL electrospun nanofiber without proteins encapsulation (left) and PLLACL electrospun nanofiber with proteins encapsulation (right)

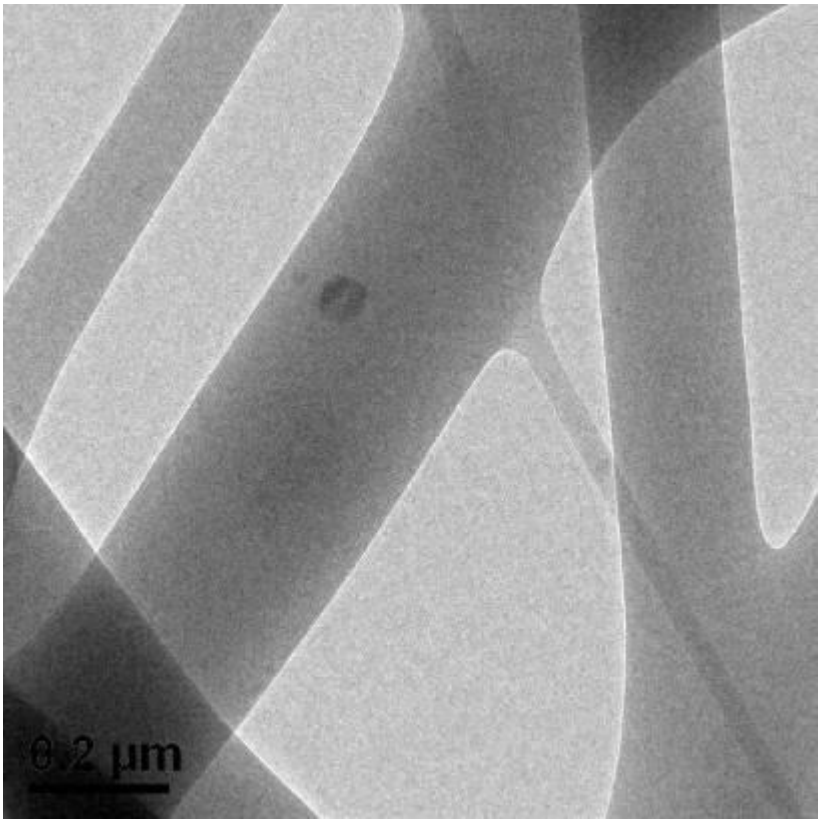


Figure 4.4: TEM image of PLLACL electrospun nanofiber with proteins encapsulation

Figure 4.1 shows SEM images of PCL electrospun nanofibers with and without protein encapsulation. The morphology of PCL electrospun nanofibers without proteins encapsulation is finer than PCL electrospun nanofiber encapsulated with proteins as small size beads are visible in the SEM image and the fibers are broader compared to the fiber prepared as-such. Figure 4.2 shows SEM images of PLLA electrospun nanofibers with and without proteins encapsulation. Non-uniform fiber deposition is seen in the SEM image for PLLA electrospun nanofiber without proteins encapsulation as well as formation of small size beads. Large size droplets were seen in PLLA electrospun nanofibers encapsulated with proteins as well as discontinuous fibers. Figure 4.3 shows SEM images of PLLACL electrospun nanofibers with and without proteins encapsulation. In both SEM images, the fibers are finely spun and broader fibers are seen in PLLACL electrospun nanofibers encapsulated with proteins. It is found that the average diameter of PCL, PLLA and PLLACL nanofibers are 298 nm, 376 nm and 428 nm respectively and the diameter analysis was done by using Image J.

Figure 4.4 on the other hand shows TEM image of PLLACL electrospun nanofibers with proteins encapsulation. A foreign particle is present in the fiber core indicating the crystalline form of the proteins mixture after it is spun. The images of PCL and PLLA electrospun nanofibers with proteins encapsulation were not available as the fiber mats were burnt under high magnification with high voltage in TEM attributed to the fiber mats preparation instead of a single fiber. Supposed that the carbon coating is applied to the metal mesh and nanofibers are electrospun on the mesh.

The morphology of the electrospun nanofibers loaded with the proteins mixture showed the fibers were not deposited in uniform at the ground collector. It is suspected that the presence of distilled water in the core solution containing proteins mixture dissolved in distilled water has affected the fibers morphology. Characterization on the electrospun nanofibers morphology were conducted by using SEM and TEM. The diameter of PCL, PLLA and PLLACL electrospun fibers were determined using analyzer. Since the fibers are in the diameter range of 200 to 1000 nm, 15 kV

acceleration voltage was used. Since the ultrafine nanofibers are above 200 nm diameter, the measured value is accurate and morphology study using FE-SEM is not necessary.

The presence of distilled water in core solution could affect the formation of electrospun nanofibers meshes because the volatility of solvents is one of the most important influence factors in the solidification of electrospun nanofibers (Su *et al.*, 2009). Since the drug/protein concentration used is 5% (w/w), the amount of water content in the core solution is relatively high. The higher the water content, the less uniform the nanofibers in the mats would be. This is because water has relatively low volatility and may not be able to completely evaporate during electrospinning.

The flow rates of the core solution (BSA, lysozyme and IgG/distilled water, 0.05 to 0.3 mL/h) are very slow compared to the shell solution (0.5 to 1.6 mL/h). During electrospinning process, the core solution is suspected to be in spherical shape particles or droplets and significantly elongated. Parts of these droplets are suspected to be broken into smaller particles or droplets under the electric force and result in the formation of the composite nanofibers with proteins incorporated. Based on the images, the influence of water content on the fibers morphology is insignificant. Though different flow rates are used, no proportional relationship between the average diameters of the fibers and the flow rates of the shell and core solutions were seen in the fibers. Therefore, the effect of shell/core solutions flow rate was very small.

The difference in the images brightness and contrast was due to the non-uniform thickness of the coating which interrupts the diameter measurement accuracy. The images however does not show any significant difference in the fiber diameters even though non-uniform fiber diameters are obtained in the three electrospun nanofibers. The diameter was measured based on the chosen non-coglutinated part of the fibers based on the images in Figure 4.1. However, none of the average diameters were below the 200 to 1000 nm fiber diameter range. It is proven that the electrospun fibers are in nanoscale. Figure 4.1 shows that the average diameter of the coaxial electrospun

nanofibers from PCL, PLLA and PLLACL incorporated with BSA, lysozyme and IgG protein mixtures.

It is observed that the core component was not clearly visible in the TEM image. This could be attributed to the flow instability of the core solution and the bending instability during electrospinning process. During coaxial electrospinning, the electrostatic repulsions between the surface charges of PCL, PLLA and PLLACL polymer solutions rapidly elongated the shell and high shear stress is produced at the interfaces the immiscible inner/outer dopes and further extending the inner proteins mixture solution along the outer PCL, PLLA and PLLACL shell. Subsequent rapid solvent evaporation preserved in the core-shell structure of the nanofibers (Jiang *et al.*, 2005). During electrospinning process particularly in bending instability, the droplets would be significantly elongated and will broke into smaller droplets under the electric forces (Su *et al.*, 2009).

4.3 Mechanical Property of Electrospun Nanofibers

Coaxial electrospun nanofibers created from PCL, PLLA and PLLACL encapsulated with BSA, lysozyme and IgG proteins mixture was expected to deliver the bioactive agents in controlled manner. Tensile properties of electrospun nanofibers is done by preparing the specimen in rectangular shape. This method is applied in terms of mechanical testing of nanofibrous membranes. Mechanical properties of the nanofibers are done to study the tensile strength of the electrospun nanofibers with and without drug loading. All of the mats were stretched till break.

Table 4.1: Tensile strength of PCL, PLLA and PLLACL electrospun nanofibers without proteins encapsulation

Sample	Maximum load (N)	Tensile stress at maximum load (N)	Tensile strain at maximum load (N)
PCL 1	1.08	1.04	8.41
PCL 2	1.75	1.96	14.15
PCL 3	2.61	2.09	19.88
PCL 4	3.05	2.18	20.72
PCL 5	3.36	2.27	22.09
Sample	Maximum load (N)	Tensile stress at maximum load (N)	Tensile strain at maximum load (N)
PLLA 1	1.08	1.59	41.7
PLLA 2	1.75	3.33	70.4
PLLA 3	2.61	3.49	78.86
PLLA 4	3.05	3.74	80.54
PLLA 5	3.36	4.02	83.45
Sample	Maximum load (N)	Tensile stress at maximum load (N)	Tensile strain at maximum load (N)
PLLACL 1	1.08	0.49	33.36
PLLACL 2	1.75	0.59	41.53
PLLACL 3	2.61	0.69	42.11
PLLACL 4	3.05	0.7	42.49
PLLACL 5	3.36	0.71	42.61

Table 4.2: Tensile strength of PCL, PLLA and PLLACL electrospun nanofibers with proteins encapsulation

Sample	Maximum load (N)	Tensile stress at maximum load (N)	Tensile strain at maximum load (N)
PCL 1	1.08	0.78	6.31
PCL 2	1.75	1.49	10.75
PCL 3	2.61	1.61	15.31
PCL 4	3.05	1.7	16.16
PCL 5	3.36	1.79	17.45
Sample	Maximum load (N)	Tensile stress at maximum load (N)	Tensile strain at maximum load (N)
PLLA 1	1.08	1.19	31.28
PLLA 2	1.75	2.53	53.5
PLLA 3	2.61	2.69	60.72
PLLA 4	3.05	2.92	62.82
PLLA 5	3.36	3.14	65.09
Sample	Maximum load (N)	Tensile stress at maximum load (N)	Tensile strain at maximum load (N)
PLLACL 1	1.08	0.37	25.35
PLLACL 2	1.75	0.45	31.56
PLLACL 3	2.61	0.53	32.42
PLLACL 4	3.05	0.55	33.14
PLLACL 5	3.36	0.59	34.22

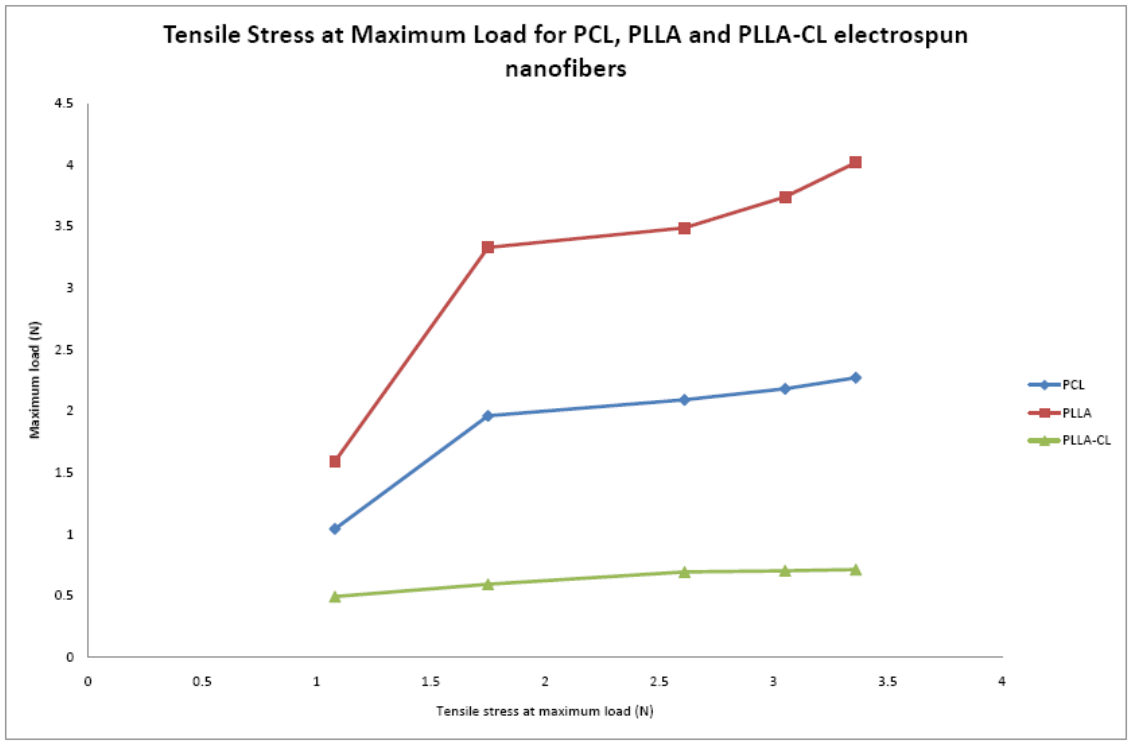


Figure 4.5: Tensile stress graph of PCL, PLLA and PLLACL electrospun nanofibers without proteins encapsulation

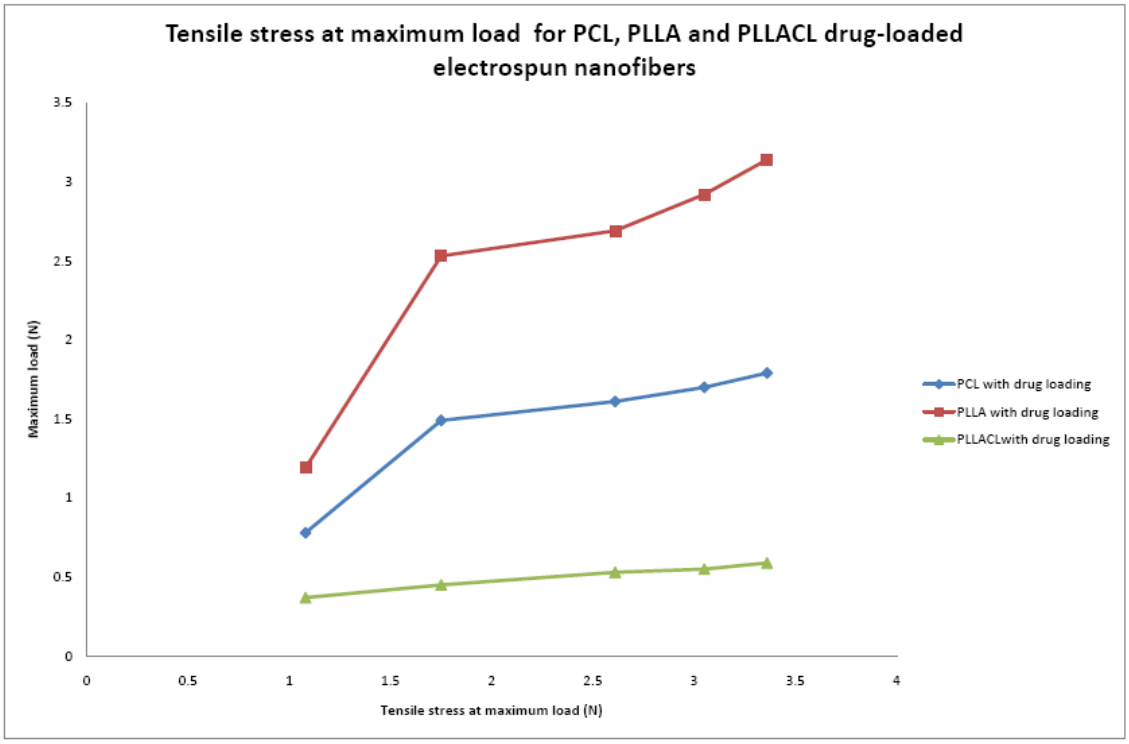


Figure 4.6: Tensile stress graph of PCL, PLLA and PLLACL electrospun nanofibers with proteins encapsulation

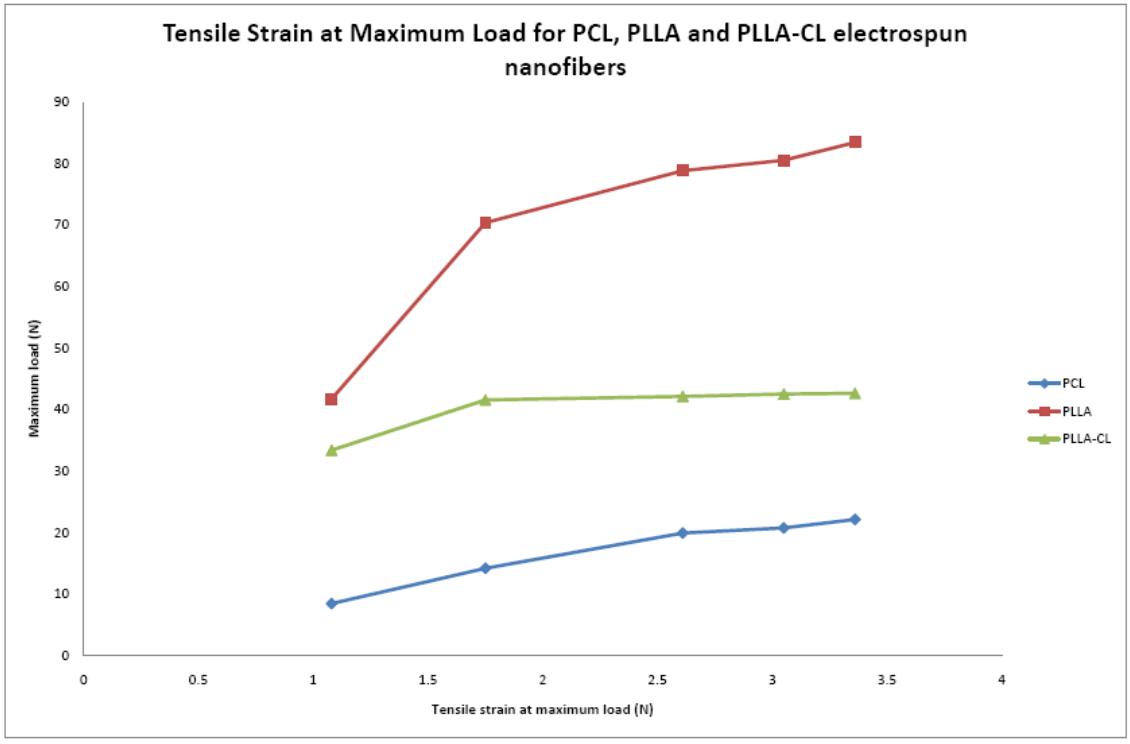


Figure 4.7: Tensile strain graph of PCL, PLLA and PLLACL electrospun nanofibers without proteins encapsulation

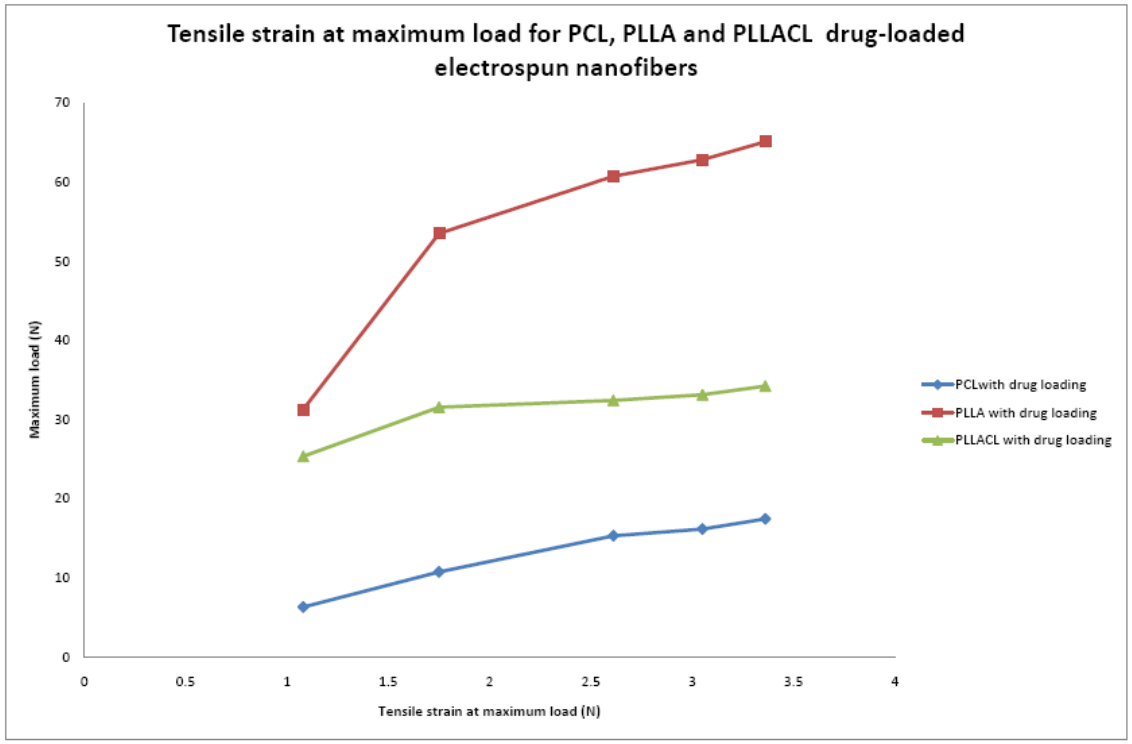


Figure 4.8: Tensile strain graph of PCL, PLLA and PLLACL electrospun nanofibers with proteins encapsulation

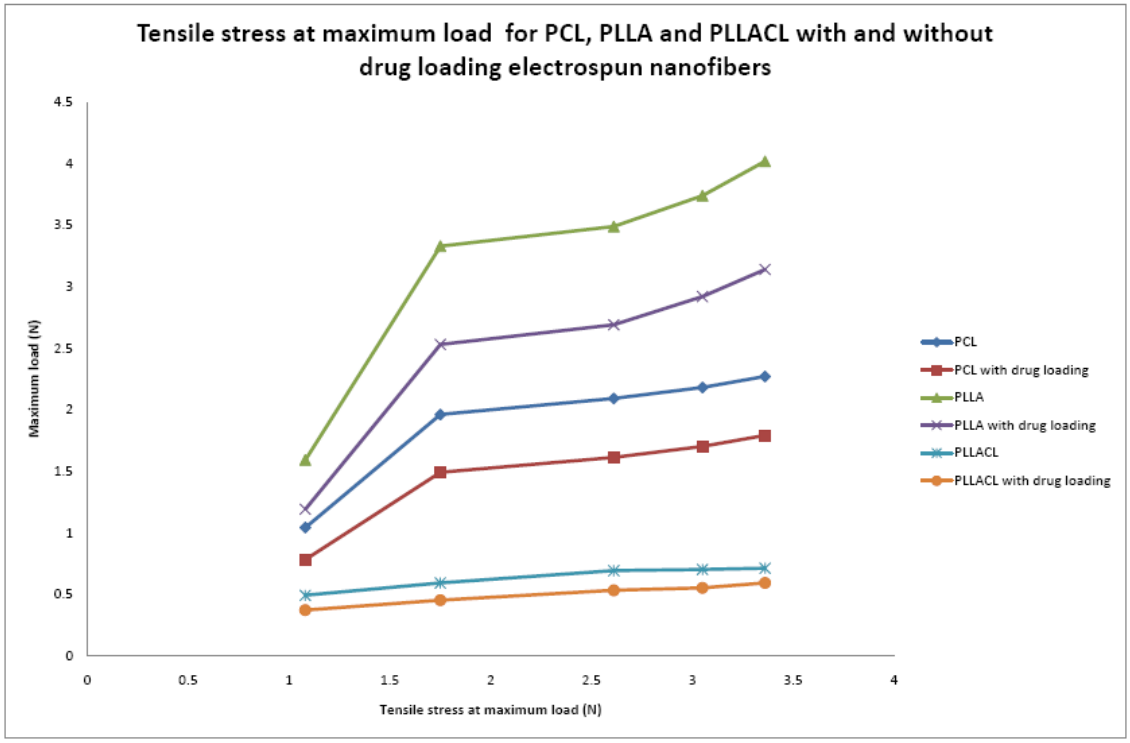


Figure 4.9: Tensile stress graph of PCL, PLLA and PLLACL electrospun nanofibers with and without proteins encapsulation

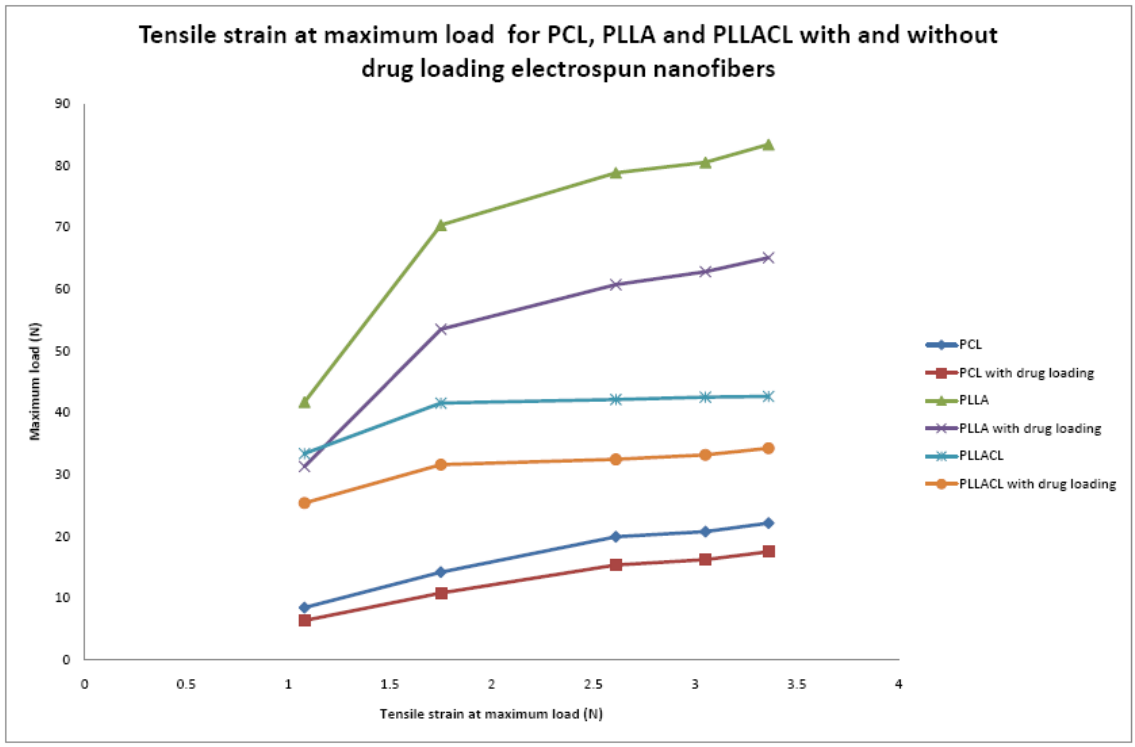


Figure 4.10: Tensile strain graph of PCL, PLLA and PLLACL electrospun nanofibers with and without proteins encapsulation

Figure 4.5 shows tensile stress of PCL, PLLA and PLLACL electrospun nanofibers without proteins encapsulation, Figure 4.6 shows tensile stress of PCL, PLLA and PLLACL electrospun nanofibers with proteins encapsulation. Figure 4.7 shows tensile strain of PCL, PLLA and PLLACL electrospun nanofibers without proteins encapsulation. Figure 4.8 shows tensile strain graph of PCL, PLLA and PLLACL electrospun nanofibers with proteins encapsulation. Figure 4.9 shows tensile stress graph of PCL, PLLA and PLLACL electrospun nanofibers with and without proteins encapsulation and Figure 4.10 shows tensile strain graph of PCL, PLLA and PLLACL electrospun nanofibers with and without proteins encapsulation.

Based on the figures, it can be said that polymers electrospun without drug loading have better strength in terms of tensile stress and tensile strain compared to polymers that are electrospun with proteins encapsulation. Tensile stress of polymers both with and without drug loading indicate that PLLA has better strength and elasticity as compared to PLLACL and PCL while tensile strain of polymers with and without drug loading indicate that PLLA has better strength as compared to PCL and PLLACL. Tensile strength is defined as the maximum stress that a material can withstand while being stretched or pulled before the specimen cross-section starts to significantly contract (Ramakrishna *et al.*, 2006). The figures showed that all the polymers were able to withstand the maximum loading of 1.08 N, 1.75 N, 2.61 N, 3.05 N and break upon reaching the maximum loading of 3.36 N.

During electrospinning, the processing conditions may affect the crystalline structure of the electrospun fibers as the diameters of the fibers with and without drug loading are identical. The change is diameter size from micro scale to nano scale might affect the crystalline stricture of the electrospun fibers as well (Ramakrishna *et al.*, 2006). The average strength of PCL, PLLA and PLLACL electrospun nanofibers without drug loading is 0.3 MPa, 3.23 MPa and 0.64 MPa respectively which is higher than that of coaxial electrospinning which is 0.23 MPa, 2.45 MPa and 0.49 MPa for PCL, PLLA and PLLACL with proteins mixture as drug loading electrospun nanofibers. This could be attributed to the core-shell structure of PCL, PLLA and PLLACL

nanofibers for the proteins incorporated within the polymers contributed less to the mechanical performance. It should be noted that protein solutions with low concentration couldn't be used to fabricate nanofibers by electrospinning. Nevertheless, all the drug-loaded coaxial electrospinning mats were identical to the mats electrospun as-such. The mechanical properties of the nanofibrous mats could not be directly translated into the mechanical properties of nanofibers (Su *et al.*, 2009).

4.4 *In Vitro* Multiple Proteins Release Study

Multiple proteins release profile from PCL, PLLA and PLLACL electrospun nanofibers are shown in Figure 4.11, Figure 4.12 and Figure 4.13. Experiments were performed in triplicate. The release kinetics of coaxial electrospinning can be illustrated by two stages: an initial fast release before the inlections (stage I) followed by a constant release (stage II) (Su *et al.*, 2009). In stage I, controlled release was seen in PCL, PLLA and PLLACL electrospun nanofibers. The drug release behaviour was related with the distribution of drug in the fiber mats. In electrospinning process, the ions were easily attracted at the surface of nanofibers. Proteins mixture molecules were easily distributed on the surface of nanofibers. The meshes produced from coaxial electrospinning showed a relatively stable behaviour of the proteins mixture. The method of coaxial electrospinning made the proteins mixture encapsulated in the inner part of the electrospun nanofibers during electrospinning process.

Table 4.3: Spectrophotometer values for PCL, PLLA and PLLACL electrospun nanofibers with proteins encapsulation in % Abs using UV-Vis Spectrophotometer

Time / Polymer	0 h	4 h	8 h	12 h	16 h	20 h	24 h
PCL	0.000	0.009	0.022	0.061	0.149	0.162	0.202
PLLA	0.000	0.022	0.053	0.064	0.085	0.127	0.190
PLLACL	0.000	0.015	0.054	0.082	0.146	0.221	0.291

Table 4.4: Spectrophotometer values for PCL, PLLA and PLLACL electrospun nanofibers with proteins encapsulation in % Conc using UV-Vis Spectrophotometer

Time / Polymer	0 h	4 h	8 h	12 h	16 h	20 h	24 h
PCL	0.000	0.008	0.028	0.061	0.155	0.171	0.214
PLLA	0.000	0.023	0.052	0.065	0.082	0.155	0.218
PLLACL	0.000	0.014	0.052	0.082	0.143	0.213	0.284

Table 4.5: Cumulative release value (in %), % Abs based of multiple proteins encapsulated in PCL, PLLA and PLLACL electrospun nanofibers after 24 hours incubation

Time / Polymer	0 h	4 h	8 h	12 h	16 h	20 h	24 h
PCL	0.0	0.9	2.3	6.2	15.2	16.5	20.6
PLLA	0.0	2.2	5.4	6.5	8.6	12.9	19.4
PLLACL	0.0	1.5	5.5	8.4	14.9	22.6	29.7

Table 4.6: Cumulative release value (in %), % Conc based of multiple proteins encapsulated in PCL, PLLA and PLLACL electrospun nanofibers after 24 hours incubation

Time / Polymer	0 h	4 h	8 h	12 h	16 h	20 h	24 h
PCL	0.0	0.8	2.8	6.2	15.8	17.4	21.8
PLLA	0.0	2.4	5.3	6.6	8.4	15.8	22.2
PLLACL	0.0	1.4	5.3	8.3	14.5	21.7	28.9

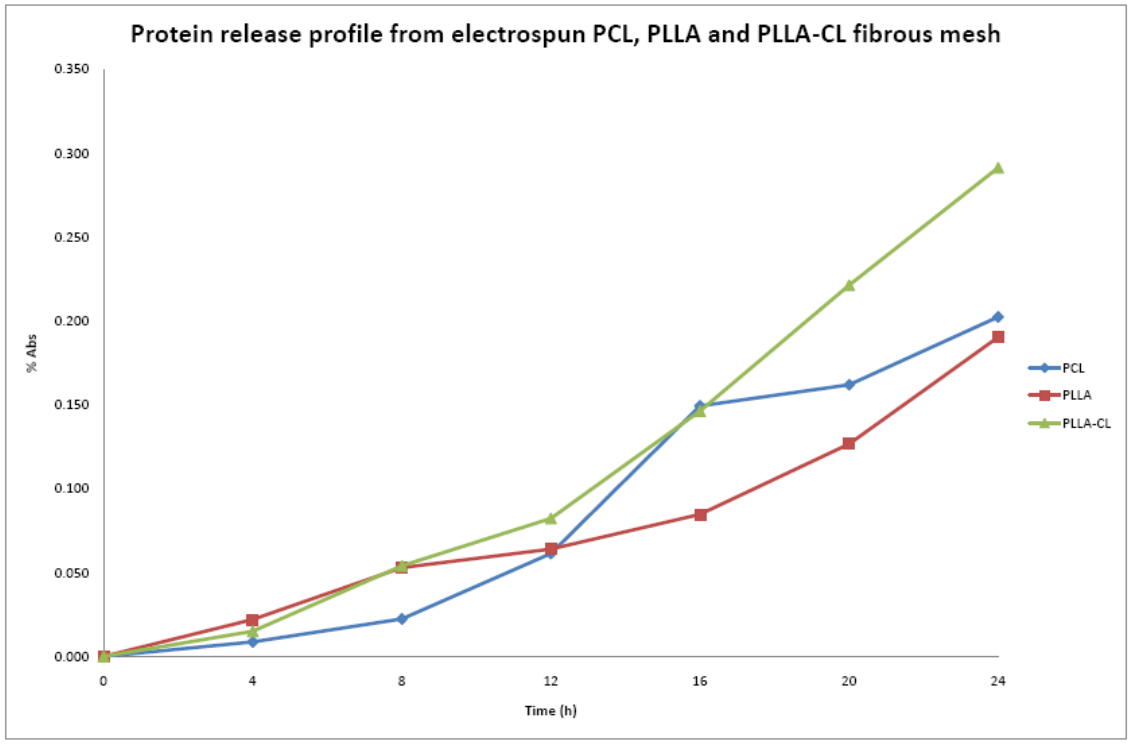


Figure 4.11: % Abs graph of PCL, PLLA and PLLACL electrospun nanofibers encapsulated with proteins after 24 hours incubation

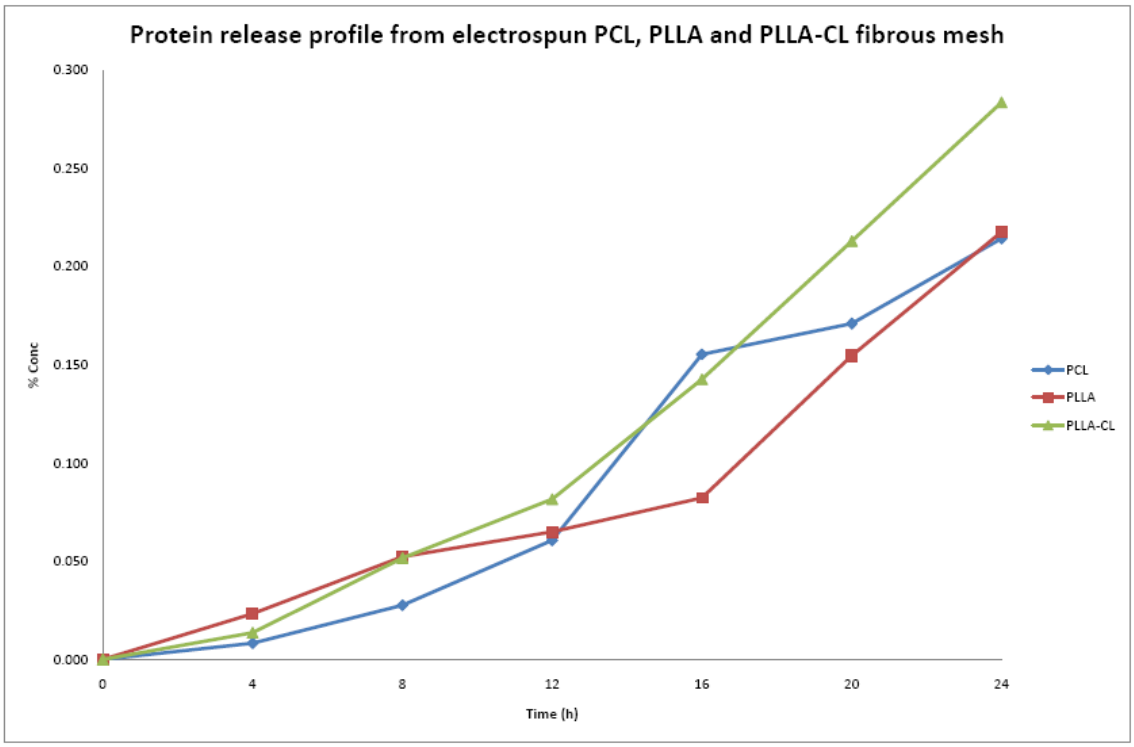


Figure 4.12: % Conc graph of PCL, PLLA and PLLACL electrospun nanofibers encapsulated with proteins after 24 hours incubation

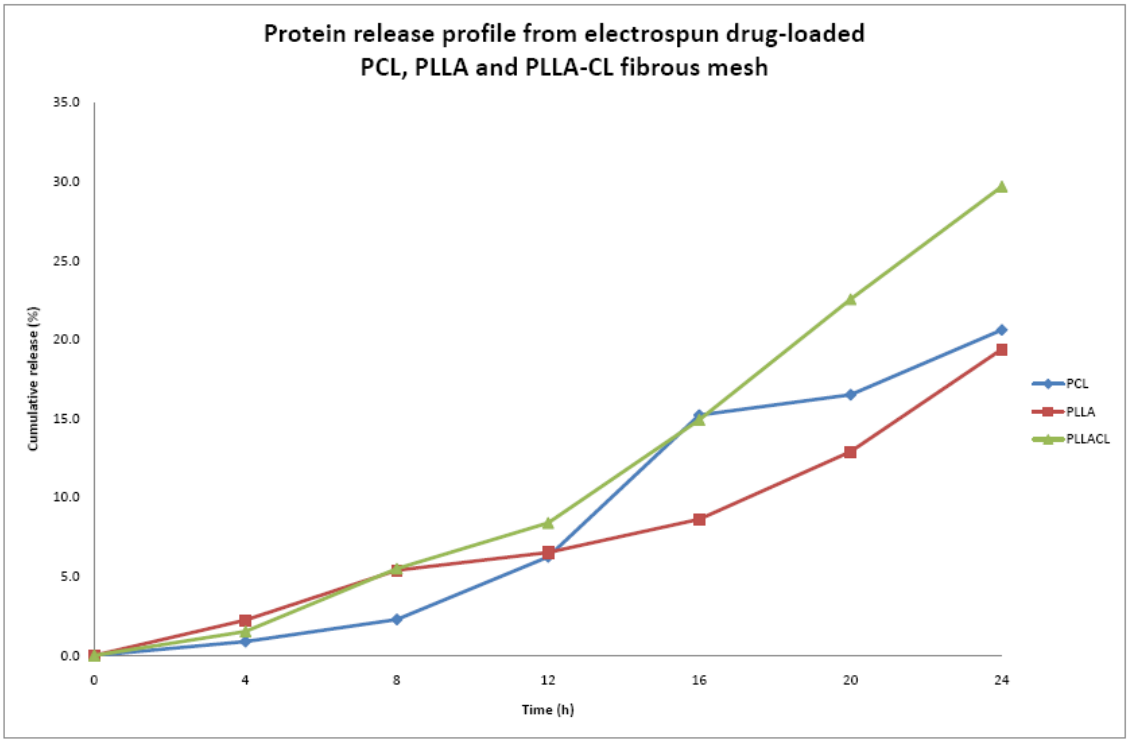


Figure 4.13: Cumulative release value (in %) of multiple proteins encapsulated in PCL, PLLA and PLLACL electrospun nanofibers after 24 hours incubation

Figure 4.11 shows the % Abs absorbance of PCL, PLLA and PLLACL electrospun nanofibers encapsulated with proteins after 24 hours incubation. Figure 4.12 shows the the % Conc absorbance of PCL, PLLA and PLLACL electrospun nanofibers encapsulated with proteins after 24 hours incubation and Figure 4.13 shows the cumulative release value (in %) of multiple proteins encapsulated in PCL, PLLA and PLLACL electrospun nanofibers after 24 hours incubation.

In the first four hours, 2.2% release of proteins mixture was seen in PLLA and is the highest as compared to PLLACL and PCL which 1.5% and 0.9% of proteins mixture released respectively. The release rate is defined as controlled release as the percentage increment was not significant. After 12 hours, PLLACL has the highest proteins mixture release rate of 8.4% compared to 6.5% and 6.2% for PLLA and PCL respectively. The release rate in each of the electrospun fibers is fluctuated depending on the extent of release. After 24 hours, 29.7% of proteins mixture was released in PLLACL while

20.6% and 19.4% proteins mixtures were released by PCL and PLLA electrospun nanofibers.

There are many factors contributing to the proteins mixture release profile. As the fibers are spun using coaxial electrospinning configuration, the proteins mixture are loaded into electrospun fibers forming a core-shell structure are able to release proteins in a sustained manner. As 10% (w/w), 16% (w/v) and 10% (w/w) polymer concentrations were used for the PCL, PLLA and PLLACL electrospun nanofibers respectively, the encapsulation of 5% (w/w) proteins mixture solution into the electrospun nanofibers does not seemed to affect the ionic and the hydrophobic-hydrophilicity interactions between the polymers and the drug as no burst release was indicated in the release profile. If so, the increase in the ionic strength would have decrease the release rate after the incubation period but no such property was found in the release profile.

PLLACL has better release rate compared to PCL and PLLA after 24 hours of incubation because PLLACL is a block copolymer emerge from the mixing of PCL and PLLA homopolymers. PLLACL has better compatibility and miscibility compared to PCL and PLLA homopolymers as it has properties that resemble the properties of the two homopolymers. As PCL and PLLA and semi-crystalline polymers, rather hydrophobic with high molecular weights, the characteristic implemented in PLLACL made the block copolymer as a potential medium to be used as carriers in the drug delivery system.

Theoritically, the proteins mixture release rate should be at a slow rate as the proteins mixture are encapsulated within the electrospun nanofibers. During the release process, the drug-loaded PCL, PLLA and PLLACL electrospun nanofibers were immersed in PBS solution and the inner proteins mixture should diffuse in PBS in the second stage. It is suspected that during 3 months storage of the drug-loaded electrospun nanofibers, the moisture is absorbed and the degradation of the polymers is initiated as the fibers are kept in parafilm-sealed petri dishes eventhough all the samples are dried

for an overnight and stored in a vacuumed dessicator. The entrapped moisture in the parafilm-sealed petri dishes has affected the fibers performance in vitro. The incorporation of proteins also affect the storage stability of the electrospun nanofibers as the degree of crystallization is affected by the strength of water polymer bonds.

As BSA, lysozyme and IgG are macromolecular drugs, a small portion of proteins is released by polymer degradation and another small portion is released by the diffusion mechanism. As the proteins are polypeptides, they usually have a limited solubility in the polymer that prevents their diffusion. Therefore, the release rate of the electrospun fibers as showed in Figure indicated that the proteins are released by polymer degradation. The initial release after 4 hours of incubation is attributed to thee release of proteins that is absorbed onto the fiber surface or present close to the surface in the pores by diffusion. The degradation of the polymers is suspected to occur very slowly during the 3 months drug carrier storage.

The release rate could be modulated by varying the feed rate of the core and shell solutions as higher feed rate will result in a more rapid proteins release (Jiang *et al.*, 2005). It is also suspected that the release medium appeared on the nanofibers surface caused by the dissolution of the core content within the vicinity of the fiber surface generating the initial slight burst release.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Drug delivery systems using PCL, PLLA and PLLACL electrospun nanofibers for controlled release of multiple proteins were successfully developed using coaxial electrospinning technique. Upon designing the drug delivery systems, a set of experimental designs were taken into consideration for the purpose of achieving a sustained release of multiple proteins using PCL, PLLA and PLLACL electrospun nanofibers as drug carriers. The state of drug molecule in the electrospinning was achieved by using coaxial electrospinning and the drug was formulated through reservoir type structure. *In vitro* release study was investigated in this study and the the drug delivery systems were stored for three months to investigate the drug configurations in terms of bioavailability and bioactivity. Therapeutic proteins namely BSA, lysozyme and IgG were used as the core of the coaxial fibers and biodegradable polymers were used as the shell structure. In this study, the type of polymers was chosen as the evaluating parameter by using PCL and PLLA homopolymers and PLLACL block copolymer as the building material of the electrospun nanofibers.

The characterization of the electrospun PCL, PLLA and PLLACL polymers electrospun fibers was performed on the electrospun fibers morphology and mechanical

strength. Using SEM and TEM to analyze to fibers surface structure and fiber diameters, all the fabricated fiber diameters are 298 nm, 376 nm and 428 nm respectively for PCL, PLLA and PLLACL electrospun nanofibers which are in the submicron scale. The mechanical strength was analyzed by using tensile strength showed that the three fibers were able to withstand the maximum of 3.36 N.

The multiple proteins release profiles were analyzed by immersing the prepared electrospun nanofibers encapsulated with multiple proteins with 0.05 M PBS solution at pH 7.4. The samples, prepared in triplicate are incubated for 24 hours inside the incubator. At selected time intervals which is every 4 hours, the absorbance was measured for each of the prepared electrospun fibers by using UV-Vis spectrophotometer. A graph of cumulative release in percentage was plotted against release time in hours. After 24 hours, a controlled release was displayed by the three drug delivery systems with PLLACL has the highest release rate of 29.7% followed by 20.6% and 19.4% from PCL and PLLA electrospun nanofibers respectively. The result indicates that the objectives of this study are achieved.

5.2 Recommendation

It is recommended that longer incubation time is implied to study the release profile of the multiple proteins until constant values are achieved for the three drug delivery systems. Most of the literatures reviewed studied the release profile of the bioagents incorporated within the electrospun nanofibers up to 14 days for clinical drugs and 96 days for therapeutic proteins.

For the purpose of commercialization, the toxicity analysis of the polymeric drug delivery systems should be conducted to investigate the safety and health effects of the electrospun nanofibers upon consumption. Apart from that, the drug configuration analysis in terms of bioavailability and bioactivity of the incorporated bioagents should be performed by manipulating the storage period to determine the appropriate shell life

of the electrospun nanofibers for biomedical application especially in therapeutic proteins delivery as proteins denaturation is one of the main concern for such application.

Biological components analysis should also be conducted to analyze the bioactivity of the encapsulated biomaterials inside the electrospun nanofibers. For drug delivery of therapeutic proteins application, the concentration of each proteins should be tested by using High Performance Liquid Chromatography (HLPC) at the specific time intervals throughout the incubation period. This is to study the release profile of each proteins encapsulated within the electrospun nanofibers as the release of each proteins will not be uniform and the interactions between the proteins will somehow affect the release rate.

In vivo release study should also be performed to widen the drug delivery application to tissue engineering especially for clinical drugs. This will enhance the biomedical application of the electrospun nanofibers. Optimization of the parameter evaluation and processing parameters should also be conducted so that a highly effective drug delivery system can be designed and can be proposed for commercialization in drug delivery market.

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APPENDICES

Preparing 10 wt% polymer A solution.

ρ solvent A= 1.483 g/cm^3
ρ solvent B= 0.791 g/cm^3
ρ polymer A= 1.14 g/cm^3

Mass solvent A=6.9315 g
Mass solvent B=2.3125 g
Mass polymer A= 0.75 g

Volume solvent A=$(6.9315\ g)/(1.483\ g/cm^3)$=4.674 ml
Volume solvent B=$(2.3125\ g)/(0.791\ g/cm^3)$=2.9235 ml
*1 cm^3=1 ml

Dissolve 0.75 g polymer A into mixture of 4.674 ml solvent A and 2.3125 ml solvent B. Stir the solution vigorously overnight.

Preparing 10 wt% drug loading polymer solution.

wt%= $(weight\ of\ solute)\ /\ (weight\ of\ solution)$ x 100

Weight of solution=6.9315 g + 2.3125 g=9.244 g
Weight of solute (drug A)=0.1744 g

Dissolve 0.1744 g drug A in polymer solution and stir the solution vigorously overnight.

Preparing 10 wt% polymer A solution.

ρ solvent A= 1.483 g/cm^3
ρ solvent B= 0.791 g/cm^3
ρ polymer A= 1.14 g/cm^3

Mass solvent A=6.9315 g

Mass solvent B=2.3125 g
Mass polymer A= 0.75 g

Volume solvent A=$(6.9315\ g)/(1.483\ g/cm^3)$=4.674 ml
Volume solvent B=$(2.3125\ g)/(0.791\ g/cm^3)$=2.9235 ml
*1 cm^3=1 ml

Dissolve 0.75 g polymer A into mixture of 4.674 ml solvent A and 2.3125 ml solvent B. Stir the solution vigorously overnight.

Preparing 10 wt% drug loading polymer solution.

wt%= $(weight\ of\ solute)\ /\ (weight\ of\ solution)$ x 100

Weight of solution=6.9315 g + 2.3125 g=9.244 g
Weight of solute (drug A)=0.1744 g

Dissolve 0.1744 g drug A in polymer solution and stir the solution vigorously for 30 minutes before electrospinning.

Prepared 5 wt% 20 ml stock solution of drug B.

ρ distilled water=1 g/cm^3

Weight of solute=1 g

1 g of drug B is dissolved in 20 ml of distilled water.

Prepared 5 wt% 20 ml stock solution of drug C.

Weight of solute=1 g

1 g of drug C is dissolved in 20 ml of distilled water.

*Drug B and drug C stock solutions are stored in 4°C refrigerator.

Prepared 10 wt% polymer C solution without surfactant addition.

1. 1 g of polymer C is weighed and dissolved in 6.7843 ml solvent C.
2. The solution is stirred vigorously for an overnight.

Prepared 10 wt% polymer C solution with surfactant addition.

1. 0.8 g of polymer C is weighed and dissolved in 10 ml solvent C.
2. 0.08 ml of surfactant A is added to the polymer solution.
3. The solution is stirred vigorously for an overnight.

*Without surfactant, the solution is more viscous and clear.

Prepared the polymer solution by dissolving 1 g polymer C in 2.8602 ml solvent C and 4.749 ml solvent D mixture (using 70:30 ratio).

Polymer C is dissolved in 70% solvent C and 30% solvent D.

70/100 x 9 g solvent=6.3 g solvent C
30/100 x 9 g solvent=2.7 g solvent D

ρ solvent C=1.3266 g/cm^3
ρ solvent D=0.944 g/cm^3

Volume solvent C=$6.3\ g/1.3266\ g/cm^3$=2.8602 ml
Volume solvent D=$2.7\ g/0.944\ g/cm^3$=4.749 ml

Prepared 2 sets of polymer solution.

Set 1
Dissolved 0.5 g polymer C in 3.10 ml solvent C and 1.40 ml solvent D.

Set 2
Dissolved 0.5 g polymer C in 3.10 ml solvent C and 1.40 ml solvent D.

Added 0.05 g drug A into the polymer solution 30 minutes before electrospinning.

Prepared 2 sets of polymer solution with polymer solution concentration of 16 w/v%.

Set 1
Dissolved 0.8 g polymer B in 5 ml solvent C.

Set 2
Dissolved 0.8 g polymer B in 5 ml solvent E.

Prepared the polymer solution with polymer solution by dissolving I dissolved 0.8 g polymer B in 5 ml solvent E.

Added 0.08 g drug A into the polymer solution 1 hour before electrospinning.

Prepared 5 wt% drug C solution and 10 wt% drug B solution.

5 wt% drug C solution
Dissolved 0.25 g drug C in 5 ml distilled water.

10 wt% drug B solution
Dissolved 1 g drug B in 10 ml distilled water.

Prepared 5 wt% drug mixture solution.

5 wt% drug mixture solution
5 ml of 5 wt% drug C and 5 ml of 5 wt% drug B were mixed in a bottle and stirred for 2 hours. The drug mixture solution were stored for an overnight in the 4°C refrigerator.

Prepared 10 wt% polymer A solution and 16 w/v% polymer B solution.

Prepared 5 v/v% IgG stock solution by diluting 0.25 ml of IgG in 5 ml distilled water.

Preparing 10 wt% polymer A solution in solvent E

Dissolve 1 g polymer A in 10 g solvent E.

10 g solvent E=$10\ g\ /1.596\ g/\ cm^3$=6.266 ml solvent E.

Dissolve 10 g polymer A in 6.266 ml solvent E or dissolve 5 g polymer A in 3.133 ml solvent E.

Preparing 15 wt% polymer A solution in solvent E

Dissolve 1.5 g polymer A in 10 g solvent E (6.266 ml).

Preparing 18 w/v% polymer B solution in solvent E

Dissolve 1.8 g polymer A in 10 ml solvent E or dissolve 0.9 g polymer A in 5 ml solvent E.

Preparing 11 wt% polymer C solution in solvent E

Dissolve 0.9 g polymer C in 5 ml solvent E.

Preparing 15 wt% polymer C solution in solvent C and solvent D (using 70:30 ratio)

Dissolve 1.5 g polymer C in 7 g solvent C and 3 g solvent D.

Dissolve 1.5 g polymer C in 5.2766 ml solvent C and 3.178 ml solvent D or dissolve 0.75 g polymer C in 2.65 ml solvent C and 1.6 ml solvent D.