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BIODEGRADABLE ELECTROSPUN FIBERS FOR DRUG DELIVERY APPLICATION

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A thesis submitted in fulfillment of the

Requirements for the award of the degree of

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Faculty of Chemical & Natural Resources Engineering

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DECEMBER 2010

I declare that this thesis entitle “Biodegradable Electrospun Fibers for Drug Delivery Application” is the result of my own research except as cited in the 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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DATE : 3 DECEMBER 2010

I dedicate this entire work to my family especially to my beloved parents (Mr Jumaat Abd Moen and Mrs Norashikin Jantan), whose patient, support, and companionship have facilitated my study, and made my life enjoyable, to my grateful brother (Mohd Ridhwan). And not forget to all my friends for their enduring faith and unconditional love in good times and bad.

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ABSTRACT

The combined use of two techniques namely electrospray and spinning is made use in a highly versatile technique called electrospinning (electro+spinning). Controlled drug delivery systems have gained much attention in the last few decades. This is due to the many advantages compared with the conventional dosage forms such as improving therapeutic efficacy and reducing toxicity by delivering them at a controlled rate to the specific site. The objective of this research is to study the drug release behavior from the electrospun scaffolds and its antimicrobial effects on microbacteria culture. Non-steroidal anti-inflammatory drug (NSAID) was embedded in two types of polymer; polylactide acid (PLLA) and polycaprolactone(PCL) with the parameters of drug concentration; 5% of indomethacin concentration and no indomethacin concentration (control), and types of microbacteria used; *E.coli* sp., *B.cereus* sp., and *M.luteus* sp. All the microbacterial cultures were incubated for an overnight at 37°C. Results showed throughout the study, the absorbance for polymer with no drug concentration is higher than polymer with 5% of drug concentration as there is no drugs diffuse from the polymer to inhibit the microbial growth. The growth of microbacterial can be visualized directly from the plate to assess the viability of indomethacin release. In this study, it is clear that these PLLA and PCL-with indomethacin based electrospun scaffolds hold a future potential in drug delivery system.

ABSTRAK

Gabungan dari dua teknik iaitu elektrospray dan putaran dibuat digunakan dalam teknik yang sangat serbaguna disebut electrospinning (elektro + spinning). Sistem penghantaran dadah telah mendapatkan banyak perhatian dalam beberapa dekad terakhir ini. Hal ini disebabkan terdapat banyak kelebihan berbanding dengan bentuk dos konvensional iaitu seperti meningkatkan kejayaan terapi, mengurangkan toksisitasi dengan menyalurkan pada kadar terkawal di tempat yang spesifik. Tujuan kajian ini adalah untuk mempelajari perilaku sistem penghantaran dadah dan kesan antimikrob pada microbacteria. Dadah anti-inflamasi non-steroid (NSAID) telah tertanam dalam dua jenis polimer; asid polylaktid (PLLA) dan polikaprolakton (PCL) dengan parameter kepekatan dadah; 5% kepekatan indometasin dan tiada kepekatan indometasin (kawalan), dan jenis microbacteria yang digunakan; *E.coli* sp, *B.cereus* sp, dan *M.luteus* sp. Semua kultur mikrobakteria diinkubasi pada suhu 37 °C. Keputusan kajian menunjukkan, absorbansi untuk polimer yang tiada kepekatan dadah adalah lebih tinggi daripada polimer dengan 5% kepekatan dadah kerana tidak ada dadah meresap keluar dari polimer untuk menghalang pertumbuhan mikrob. Pertumbuhan mikrobakteria dapat dilihat secara terus dari piring petri untuk menunjukkan kadar penghantaran indometasin. Dalam kajian ini, telah jelas dibuktikan bahawa PLLA dan PCL-dengan indometasin adalah sangat berpotensi di masa depan dalam sistem penghantaran dadah.

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LIST OF ABBREVIATIONS / SYMBOLS

DDS	Drug delivery system
ECM	Extracellular matrix
PLLA	Poly-lactide acid
PCL	Polycaprolactone
NUS	National University of Singapore
LED	Light Emitting Diode

CHAPTER 1

INTRODUCTION

1.1 Research background

Electrospinning process seems to be the only method which can be further developed for mass production of one-by-one continuous nanofibers from various polymers. Although the term ‘electrospinning’’, derived from ‘‘electrostatic spinning’’, was used relatively recently (in around 1994), its fundamental idea dates back more than 60 years earlier. A polymer solution, such as cellulose acetate, was introduced into the electric field. The polymer filaments were formed, from the solution, between two electrodes bearing electrical charges of opposite polarity. One of the electrodes was placed into the solution and the other onto a collector. Once ejected out of a metal spinneret with a small hole, the charged solution jets evaporated to become fibers which were collected on the collector. The potential difference depended on the properties of

the spinning solution, such as polymer molecular weight and viscosity. When the distance between the spinneret and the collecting device was short, spun fibers tended to stick to the collecting device as well as to each other, due to incomplete solvent evaporation (Zheng et. al., 2003).

Yan et. al. (2009) defines electrospinning as a method which combined two techniques namely electrospray and sinning. The conventional spinning processes including wet-, dry- and wet/dry-spinning techniques are capable of producing fibers with diameter in the micrometer rang. Unlike the conventional spinning, electrospinning is capable of producing fibers with the diameter ranged from tens nanometer to several micrometer (Huang et, al, 2004). Additionally, the non-woven fibrous mats made of electrospun polymer nanofibers offer a unique capability to control the fibers diameters by adjusting applied voltages, collecting distances, solutions concentrations and solutions flow rates (Mo et. al., 2004). Unlike nanorods, nanotubes, and nanowires that are produced mostly by synthetic, bottom-up methods, electrospun nanofibers are produced through a top-down nano-manufacturing process, which results in continuous and low-cost nanofibers that are also relatively easy to align, assemble and process into applications (Badami et. al., 2006).

The important advantages of electrospinning technique are the production of very thin fibers to the order of few nanometers with large surface areas, ease of fictionalization for various purposes, superior mechanical properties and ease of process as suggested by many experts in this field. The possibility of large scale productions combined with the simplicity of the process makes this technique very attractive for many different applications (Agarwal et. al., 2008). Biomedical field is one of the important application areas among others utilizing the technique of electrospinning like filtration and protective material, electrical and optical applications, sensors, nanofiber reinforced composites etc.

Drug delivery systems have numerous advantages compared with conventional dosage forms, such as improve therapeutic effect, reduce toxicity and improve patient compliance, by delivering drugs at a controlled rate over a period of time to the site of action (Yan et. al., 2009). With respect to the local delivery of bioactive ingredient (such as growth factors), electrospun ultrafine fibers have been investigated as tissue engineering scaffolds and biofunctional scaffolds for other biomedical applications (Liang et. al., 2007). Plenty of polymeric materials, either non-degradable or biodegradable materials, can be used as delivery matrices. In the delivery system made from non-degradable materials, for example, drugs mainly released and driven by a concentration gradient. However, drugs release from biodegradable matrices by both diffusion and scaffolds degradation. For polymeric materials used in tissue engineering applications, biodegradable materials are generally more popular due to the reason that they eliminate gradually for the need of new tissue regeneration and replace the scaffolds.

Non-steroidal anti-inflammatory drugs (NSAID) indomethacin is used for controlling pain and inflammation in rheumatic diseases. Administration of acidic NSAIDs to arthritic patients relieves pain and inflammatory swelling (Giammona et. al., 1991). Beside the advantages of NSAIDs, they have few disadvantages. Their main disadvantage is a relatively short plasma half-life. This results in short activity duration, and a pronounced ulcerogenic potency (Adams et. al., 1969).

1.2 Problem statement

Despite its long history and some preliminary work in tissue engineering nearly 30 years ago, electrospinning has not gained widespread interest as a potential polymer processing technique for applications in tissue engineering and drug delivery until the last 5-10 years. This renewed interest can be attributed to electrospinning’s relative ease of use, adaptability, and the ability to fabricate

fibers with diameters on the nanometer size scale. Furthermore, the electrospinning process affords the opportunity to engineer scaffolds with micro to nanoscale topography and high porosity similar to the natural extracellular matrix (ECM). The inherently high surface to volume ratio of electrospun scaffolds can enhance cell attachment, drug loading, and mass transfer properties.

Various materials can be electrospun including: biodegradable, non-degradable, and natural materials. Electrospun fibers can be oriented or arranged randomly, giving control over both the bulk mechanical properties and the biological response to the scaffold. Drugs ranging from antibiotics and anticancer agents to proteins, DNA, and RNA can be incorporated into electrospun scaffolds. Suspensions containing living cells have even been electrospun successfully. The applications of electrospinning in tissue engineering and drug delivery are nearly limitless.

Therefore, drug therapy is designed to construct a new barrier system (using electrospun fibrous scaffolds based on biodegradable polymers), which also possesses local drug delivery capability. It was designed to demonstrate the incorporation and controlled-release of drug from electrospun fibrous scaffolds, by monitoring its ability to inhibit bacterial growth (Kim et al., 2004).

1.3 Research Objevtive

The objective of this research is to study the drug release behavior from the electrospun scaffolds and its antimicrobial effects on microbacteria culture.

1.4 Scope of study

There are some important tasks to be carried out in order to achieve the objective of this study. These important scopes have been identified for this research in achieving the objective, which are:

- to understand the basic principle of electrospinning in producing nanofibers,
- to study the drug release behavior from the PCL and PLLA electrospun scaffolds
- to observe the antimicrobial effects on E. Coli, B. Cereus, and M. Luteus microbial culture.

1.5 Significant of study

Nanofibers (fibrous scaffolds) play a significant role in bioengineering as it serves a lot especially in biomedical applications. The electrospun nanofibrous scaffolds can also be used as carriers for drugs, where the drug release profile can be finely controlled by the modulation of the scaffold's morphology, porosity, and composition.

The main advantage of this system is that it offers site-specific delivery of any number of drugs from the scaffold into the body. It is essential to use the electrospun scaffolds in healthcare and medicine for treatments where the drug loading capability can further aid in the healing process (K. Kim, 2007). Besides, this research is very economical as it is a cost effective research. Electrospun fiber mats provide the advantage of increased drug release as compared to cast-films due to the increased surface area. (Agarwal et. al., 2008).

CHAPTER 2

LITERATURE REVIEW

2,1 Non-steroidal anti-inflammatory drugs

Nonsteroidal anti-inflammatory drugs, usually abbreviated to NSAIDs or NAIDs, but also referred to as nonsteroidal anti-inflammatory agents/analgesics (NSAIAs) or nonsteroidal anti-inflammatory medicines (NSAIMs), are drugs with analgesic and antipyretic (fever-reducing) effects and which have, in higher doses, anti-inflammatory effects. The term "nonsteroidal" is used to distinguish these drugs from steroids, which, among a broad range of other effects, have a similar eicosanoid-depressing, anti-inflammatory action. As analgesics, NSAIDs are unusual in that they are non-narcotic. The most prominent members of this group of drugs are aspirin, ibuprofen, and naproxen, all of which are available over the counter in many areas (Stuart, 2010).

2.2 Indomethacin

Indomethacin is a non-steroidal anti-inflammatory drugs and antipyretic agent. Its activity does not depend upon pituitary-adrenal stimulation and it is fully active in adrenalectomized animals. As shown in **Figure 2.1** , chemically it is l-(ρ-chlorobenzoyl)-5-methoxy-2-methylindol-3-acetic acid, having the empirical formula of $C_{19}H_{16}NO_4Cl$ and a molecular weight of 357.8. It is relatively insoluble in water but soluble in the common organic solvents. Indomethacin has been used in the treatment of a group of patients in whom a clinical response might be anticipated from administration of a compound with anti-inflammatory, antipyretic, and possible analgesic properties.

Indomethacin is the drug of choice in acute gout, where relief is obtained more rapidly than with phenylbutazone. It is useful also in ankylosing spondylitis, in osteoarthritis, and in cases of rheumatoid arthritis with inflammatory features and swelling of joints. Although more time is needed before the true incidence of toxic effects can be evaluated, indomethacin appears to be a useful addition to the treatment of these rheumatic disorders (Hart & Boardman, 1963).

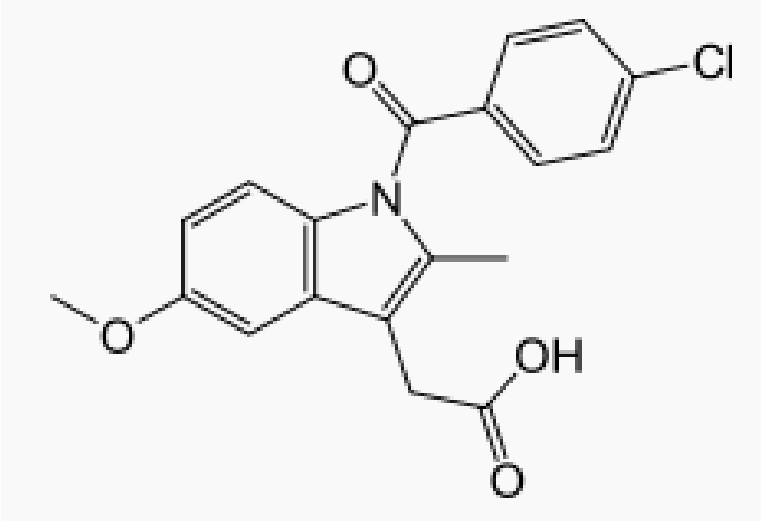

Figure 2.1 : Indomethacin chemical structure

2.3 Inflammatory Bowel Disease

In medicine, inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine. Ulcerative colitis (UC) and Crohn's disease represent the two main types of inflammatory bowel disease. Environmental factors (eg, geography, cigarette smoking, sanitation and hygiene), infectious microbes, ethnic origin, genetic susceptibility, and a deregulated immune system can result in mucosal inflammation. The main difference between Crohn's disease and UC is the location and nature of the inflammatory changes. Crohn's can affect any part of the gastrointestinal tract, from mouth to anus, although a majority of the cases start in the terminal ileum. Ulcerative colitis, in contrast, is restricted to the colon and the rectum. As a result of the inflammatory reaction, the intestinal wall is damaged leading to bloody diarrhea and abdominal pain. Usually the treatment is started by administering drugs with high anti-inflammatory effects (Daniel & Simon, 2007).

2.4 Electrospinning process

2.4.1 History of electrospinning

Early on, technical difficulties relating to a number of parameters prevented electrospinning from emerging as a feasible technique for spinning small-diameter polymer fibers. It was not until 1934, when Formhals patented a process and an apparatus that used electric charges to spin synthetic fibers, that electrospinning truly surfaced as a valid technique for spinning small-diameter fiber (Formhals, 1934). The apparatus employed by Formhals utilized a movable thread-collecting device that collected fibers in a stretched state, allowing for the collection of aligned fibers. Using this apparatus, Formhals was able to

successfully spin cellulose acetate fibers using an acetone/alcohol solution as the solvent. While Formhals' invention did show significant improvement over earlier electrospinning methods, there still existed some disadvantages. Due to the close proximity of the collector to the charged polymer solution, the solvent could not completely evaporate before the fiber jet reached the collector, resulting in the formation of a loose web structure. Sill and von Recum (2008) reviews that another consequence of incomplete solvent evaporation was that the fibers tended to stick to the collector as well as to each other, making removal problematic. Thus, in a second patent Formhals detailed a new process in which a greater distance was used between the spinning and collecting sites thus alleviating many of the problems seen with his earlier apparatus (Formhals, 1939). In his second patent, Formhals also describes the use of multiple nozzles for the simultaneous spinning of a number of fibers from the same polymer solution as well as a means to direct the fiber jets toward the collector. In 1940, Formhals patented a new process in which a polymer solution was directly electrospun onto a moving base thread to generate composite fibers (Formhals, 1940).

Following the work of Formhals the focus turned to developing a better understanding of the electrospinning process; however, it would be nearly 30 years before Taylor would publish work regarding the jet forming process. In 1969, Taylor published his work examining how the polymer droplet at the end of a capillary behaves when an electric field is applied. In his studies he found that the pendant droplet develops into a cone (now called the Taylor cone) when the surface tension is balanced by electrostatic forces. He also found that the fiber jet is emitted from the apex of the cone, which is one reason why electrospinning can be used to generate fibers with diameters significantly smaller than the diameter of the capillary from which they are ejected. Taylor (1969) subsequently determined that an angle of 49.3 degrees with respect to the axis of the cone at the cone apex (or a cone angle of 98.6 degrees) is necessary in order to balance the surface tension with the electrostatic forces by examining a variety of viscous fluids. Shortly after Taylor's work on the jet forming process was published, interest shifted away from a fundamental understanding of the electrospinning process to a deeper understanding of the relationships between individual

processing parameters and the structural properties of electrospun fibers (Sill and von Recum, 2008).

Baumgarten (1971) began to investigate the affect of varying certain solution and processing parameters (solution viscosity, flow rate, and applied voltage) on the structural properties of electrospun fibers. In his studies, Baumgarten used a polyacrylonitrile/ dimethylformamide (PAN/DMF) solution, which was ejected from a metal capillary. Using a high-speed camera he was able to determine that a single fiber was being drawn from the electrically charged pendant drop. Using this system he also discovered that fiber diameter had a direct dependence on solution viscosity, with higher viscosities giving larger fiber diameters. Furthermore, Baumgarten found that fiber diameter does not monotonically decrease with increasing applied electric field. Rather the fiber diameter decreases initially with an increase in applied field reaching a minimum and then diameter increases when the applied field is increased further. By varying the solution and processing parameters he was able to electrospin fibers with diameters ranging between 500 and 1100 nm. Approximately a decade after Baumgarten's initial work, other work began to examine electrospinning of polymer melts, which afforded new means of manipulating the structural properties of the electrospun fibers (Sill and von Recum, 2008).

The former responds to requirement of an optimal microenvironment for regenerative medicine, and the latter to demands of targeted and intracellular delivery. Chakraborty (2009) views electrohydrodynamics as referring to the dynamics of electrically charged fluids, constitutes the basis for electrospinning and electrospraying. In electrospinning, when electrical forces overcome the forces of surface tension in the charged polymer liquid, a charged jet ejected from the tip of a capillary tube elongates and moves towards a grounded surface. The solvent in the jet is evaporated during the flight, leading to a mat of nanofibers deposited on the surface. The fibers are continuous and can range in diameter from several nanometers to micrometers. A limited ordering of the fibers, such as alignment, can be obtained by manipulation of the collector or the electrical field. Jaworek and Sobczyk (2008) provide a concise summary of the physics governing electrospraying. Bulk forces that are important to electrospraying include

electrodynamic forces (proportional to the electric fields induced by the charged nozzle and emitted droplets), gravity, inertia, and drag force (proportional to jet velocity and the viscosity of the gas surrounding the jet). Surface stresses deforming the jet and acting against surface tension include electrodynamic stress (proportional to the charge density on the surface of the jet, and on the local electric field), pressure differential across the jet–air interface, and stresses due to liquid dynamic viscosity and inertia (Chakraborty, 2009).

2.4.2 Fabrication technique

Agarwal (2008) reviews that the emergence of coaxial electrospinning has allowed the development of many new designs of functional nanotechnological materials. As shown in **Figure 2.2**, coaxial electrospinning is simple and rapid technique to produce micro/nanotubes, drug- or protein-embedded 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 from100 nm to 300 μm) of fibers it can produce. 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 offer better drug stability, more complete drug encapsulation, and tighter control of release kinetics as compared to monoaxial fibers.

Coaxial electrospinning circumvents technical limitations of monoaxial electrospinning by its core–shell design, allowing cytokines and growth factors to be dissolved in aqueous solution for encapsulation. The core–shell design also allows better control over the release kinetics of the drug of interest due to an increased number of variable parameters. Changes in the shell and core material properties via variation in molecular weight, polymer type and addition of porogen can fine-tune the release profile (Taylor, 1969). Core–shell electrospun

fibers are generally designed to concentrate the drug in the core of the fibers as opposed to randomly distributing the drug throughout the fiber matrix. A simplified summary of the various parameters and their effects on drug release can be found in **Table 2.1**

Table 2.1 : Effect of alteration of electrospinning parameters on the release rate of various drugs in different polymer systems

Parameter	**Drug of Interest**	**Polymer carrier**	**Effect**
Polymer MW	Rhodamine 610	PCL PMMA	Release Rate ↓
Polymer Concentration	Rhidamine 610 Pactitaxel Tetracycline hydrochloride	PCL PMMA PLGA PLLA	Release Rate ↓
Core-drug interaction	Rhodamine 610	PCL	Release Rate ↓
Polymer degradability	Rhodamine 610 Paclitaxel	PCL PMMA PLGA PMMA	Release Rate ↑
Drug concentration	Paclitaxel	PLGA	Release Rate ↑
Porogen concentration	BSA PDGF-bb	PCL	Release Rate ↑
Porogen MW	BSA	PCL	Release Rate ↓↑

Porosity	Paclitaxel	PLGA	Release Rate
Flow Rate Ratio	BSA	PCL	Release Rate ↓

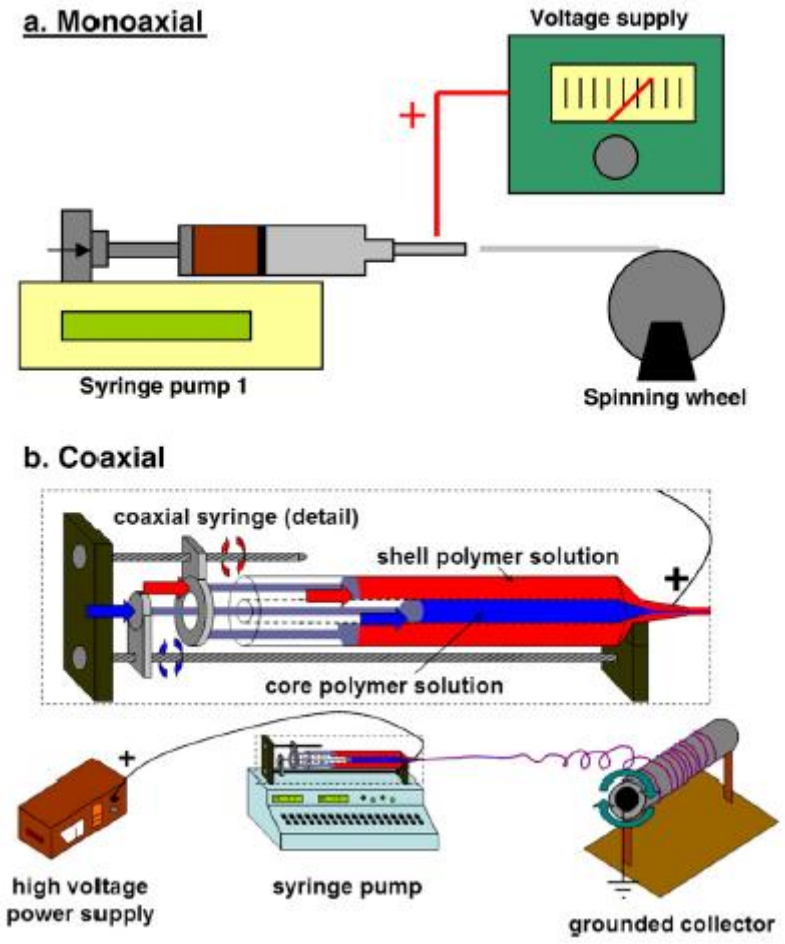

Figure 2.2 : Various electrospinning setup used to produce mono/coaxial fibers. a) Typical setup for monoaxial electrospinning involves the use of syringe pump to dispense a polymer solution through a needle gauge. A high voltage gradient is applied to the needle via a power supply and the formed fibers are accelerated towards the collecting ground (spinning wheel). b) Horizontal syringe-in-a-syringe design used for coaxial electrospinning.

2.4.3 Parameters in electrospinning

Despite electrospinning's relative ease of use, there are a number of processing parameters that can greatly affect fiber formation and structure. Grouped in order of relative impact to the electrospinning process, these parameters are applied voltage, polymer flow rate, and capillary-collector distance. Furthermore, all three parameters can influence the formation of bead defects.

2.4.3.1 Polymer types, molecular weight and concentration

Polymer type, molecular weight, and concentration are three crucial factors that determine the feasibility of electrospinning. Ramakrishna et al (2005) reviews that the types of polymer amenable to electrospinning can be classified by their hydrophilicity. Hydrophilic polymers (e.g. polysaccharides) or extracellular matrix proteins (e.g. collagen and hyaluronic acid) have been processed into electrospun fibers by dissolving the polymers in water, strong acids or a mixture of water and polar organic solvents. Changes in polymer concentration and molecular weight affect the viscosity and surface tension of the solution, and therefore greatly influence the electrospun product

2.4.3.2 Solvent properties

While the type of polymer determines the type of solvent used in the electrospinning process, different solvent properties play a crucial role in fiber formation. Three important characteristics of solvents to consider in the coaxial electrospinning process are surface energy, volatility, andmiscibility. The surface energy of the solvent influences the ability of the applied electrical potential to shear the polymer solution into electrospun fibers. The high surface tension also

causes more instability and resulted in a broad range of fiber diameters (Chakraborty et al., 2009).

Solvent volatility is critical in determining whether sufficient solvent evaporation can occur in the flight of the fiber between the needle and its designated ground. Insufficient solvent evaporation will lead to formation of ribbon-like fibers or fiber fusion. On the other hand, if solvent volatility is too high then it may lead to the drying of the jet even before jet whipping can thin the fiber. This in turn leads to the formation of large diameter fibers. Within optimum range of solvent volatility, fiber diameter has an inverse relationship with it. Drastic difference in solvent volatility introduces an extra level of difficulty for coaxial electrospinning. Solvent miscibility is another parameter to consider in order to ensure consistent core–shell electrospun fiber products

2.4.3.3 Applied voltage

The strength of the applied electric field controls formation of fibers from several microns in diameter to tens of nanometers. Suboptimal field strength could lead to bead defects in the spun fibers or even failure in jet formation. An insufficient electrical charge potential cannot overcome the surface tension of the polymer drop to form electrospun fibers (Ramakrishna et al., 2005). Coaxial electrospinning typically can be achieved at similar field strength as monoaxial electrospinning, depending on the solvent miscibility and surface tension of the core/shell solutions. Poor miscibility between core/shell solutions requires higher field strengths to overcome the solution surface tension; improving the solution miscibility (by adding secondary solvent to the shell solution) not only reduces the necessary applied voltage but also improves the fiber size uniformity (Sill & von Recum, 2008).

2.4.3.4 Flow rate

Polymer flow rate also has an impact on fiber size, and additionally can influence fiber porosity as well as fiber shape. At high flow rates significant amounts of bead defects were noticeable, due to the inability of fibers to dry completely before reaching the collector. Incomplete fiber drying also leads to the formation of ribbonlike (or flattened) fibers as compared to fibers with a circular cross section (Chakraborty et al., 2009).

2.4.4 Other parameters

Electrospinning distance, temperature and humidity are additional parameters that affect the size and morphology of the electrospun product. Electrospinning distance can influence fiber size and determine the final product morphology. Increasing electrospinning distance yields fibers of smaller size. Conversely, when there is inadequate electrospinning distance the electrospun fibers are more prone to fusion, as there can be residual organic solvent present during fiber deposition .

Temperature and humidity also affect the electrospun product morphology. Temperature elevation increases molecular mobility which in turn increases the solution conductivity while decreasing solution viscosity and surface tension. These conditions are favorable for decreasing the diameter of the electrospun fibers. Furthermore, this tend to decrease the crystallinity and increase the surface roughness of the electrospun product. Increase in relative humidity in the electrospinning chamber decreases the evaporation rate of polymer solvents and results in larger electrospun fiber diameters (Sill & von Recum, 2008)

2.4.5 Drug Delivery system

In medicine field, delivery of drug/pharmaceuticals to patient in the most physiologically acceptable manner has always been an important concern. Generally, the smaller the dimensions of the drug and the coating material required to encapsulate the drug, the better the drug to be absorbed by human being (Zheng et. al., 2003). Drug release and tissue engineering are closely related areas. Sometimes release of therapeutic factors can increase the efficiency of tissue engineering. Various nanostructured materials for applications in tissue engineering and in drug delivery have been reviewed by (Agarwal et. al., 2008). Modern therapeutics emphasizes pharmacokinetic and pharmacodynamic principle-driven administration of drugs. The scope of the term 'drug' has grown over the last few decades to include growth factors, bioactive proteins, and nucleic acids. This evolution continues to fuel new development of drug delivery systems (DDS) to realize the therapeutic potential of these delicate and macromolecular bioactive agents (Agarwal et. al., 2008). Drug delivery with polymer nanofibers is based on the principle that dissolution rate of a particulate drug increases with increasing surface area of both the drug and the corresponding carrier if needed.

Researchers are also increasingly interested in incorporating drugs into the fibers to enhance the functionality of these scaffolding materials. Drugs can be embedded in the fiber through dissolution or dispersion in the polymer solution. (Chakraborty et al., 2009). As the drug and carrier materials can be mixed together for electrospinning of nanofibers, the likely modes of the drug in the resulting nanostructed products are: (1) drug as particles attached to the surface of the carrier which is in the form of nanofibers, (2) both drug and carrier are nanofiber-form, hence the end product will be the two kinds of nanofibers interlaced together, (3) the blend of drug and carrier materials integrated into one kind of fibers containing both components, and (4) the carrier material is electrospun into a tubular form in which the drug particles are encapsulated. The modes (3) and (4) may be preferred. However, as the drug delivery in the form of

nanofibers is still in the early stage exploration, a real delivery mode after production and efficiency have yet to be determined in the future (Zheng et al., 2003).

Kenawy et al., (2007) investigated delivery of tetracycline hydrochloride based on the fibrous delivery matrices of poly (ethylene-co-vinylacetate), poly(lactic acid), and their blend. Electrospun fiber mats provide the advantage of increased drug release as compared to cast-films due to the increased surface area. Kanawung et al. (2007) have studied the release of model drugs like diclofenac sodium (DS) and tetracycline hydrochloride (TH) from electrospun PCL and poly- (vinyl alcohol) (PVA) fiber mats. The cumulative release of the model drugs increased monotonically with increasing immersion time and became practically constant at long immersion times. The release of antibiotic tetracycline hydrochloride and mefoxin is also studied using electrospun poly(lactic acid) PLA and poly(ethyleneco- vinylacetate) and PLA, respectively. Release studies of diclofenac sodium from electrospun poly(maleic anhydride-alt- 2-methoxyethyl vinylether) mats and poly(caprolactone-D,Llactide) are also known. Electrospun PLGA-based micro/nanofibers as implants for the sustained delivery of anticancer drug (paclitaxel) to treat C6 glioma (brin tumor) in vitro has been developed by Xie et al., (2006). The sustained release of drug with this system was achieved for more than 60 days. Mats of PVA nanofibers were developed as carriers of drugs for transdermal drug-delivery system by Supaphol et al., (2006). Four types of non-steroidal anti-inflammatory drug with varying water solubility, i.e. sodium salicylate, diclofenac sodium, naproxen, and indomethacin were tried as model drugs.

Weak electrolytes like poly(acrylic acid) (PAA) and poly(allylamine hydrochloride) (PAH) were also electrospun with methylene blue as model drug to evaluate the electrospun fibers for drugdelivery applications [100]. Sustained release of the model drug was achieved by using perfluorosilane networks on the fiber surfaces as capping layers. In general, the drug release profile is dependent on how good the drug is dispersed in the matrix polymer. In all the representative examples given above, the drugs are mixed (dissolved/dispersed in the polymer solution before electrospinning). There is always a probability of finding drug on

nanostructured surfaces besides being encapsulated inside thereby leading to burst release in the initial stages. The electrospun ultrafine fiber mats containing drugs may be used as drug release carriers or made into biomedical devices such as sutures and wound dressings (Agarwal et al., 2008).

Kim et al. (2004) incorporated hydrophilic antibiotic into poly(lactide-*co*-glycolide) to produce nanofibrous scaffolds. While polymer based electrospun nanofibers containing dispersions of poorly water-soluble pharmaceuticals as delivery systems. Biodegradable polymers are good candidates for applications in biomedical field due to their biocompatibility, their degradation and mechanical properties. Polycaprolactone (PCL) is a semi-crystalline polymer, rather hydrophobic, with a high molecular weight. It may be used in diffusion-controlled delivery systems. The main mode of degradation for caprolactone polymers is hydrolysis. This 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 pH and temperature of the environment. On the other hand, polyurethanes, as non-biodegradable polymer, have many medical applications. The applications include external devices such as wound dressing to many types of catheters and feeding tubes to long term implants such as pacemakers. Generally, polyurethanes are made of hard and soft domains (Giammona et al., 1991).

Electrospinning affords great flexibility in selecting materials for drug delivery applications. Either biodegradable or non-degradable materials can be used to control whether drug release occurs via diffusion alone or diffusion and scaffold degradation. Additionally, due to the flexibility in material selection a number of drugs can be delivered including: antibiotics, anticancer drugs, proteins, and DNA. Using the various electrospinning techniques a number of different drug loading methods can also be utilized: coatings, embedded drug, and encapsulated drug (coaxial and emulsion electrospinning). These techniques can be used to give finer control over drug release kinetics (Sill & von Recum, 2008). Other techniques for nanofiber generation, which are interesting yet not widely

used, are self-assembly and phase separation. Self assembly, such as that used to synthesize nanofibers from peptide amphiphiles, is attractive because of the mild condition of fabrication and the small size attainable. However, the technique is amenable only to a limited repertoire of polymers and difficult to process into a macroscopic structure. It is also challenging to obtain sustained release kinetics from these small fibers. The phase separation technique requires gelation of the polymer and extraction of solvent and suffers from a lack of control over fiber arrangement. The required solvent extraction step would also prematurely leach out any drugs entrapped in the fibers (Chakraborty et al., 2009).

When selecting a material to use in a drug delivery device a number of requirements must be met. As with materials used in tissue engineering applications, materials that undergo biodegradation are generally more popular due to the fact that they eliminate the need for explanation. However, biodegradable materials add an extra level of complexity to drug delivery devices as compared to non-degradable materials, which tend to release drug primarily by diffusion. Generally it is desirable to design a drug delivery device that gives controlled release of the desired agent; however, this may be difficult if the material begins degrading as the drug is being released. In a biodegradable system the drug may be released by diffusion as well as degradation of the material, which in some cases can lead to dose dumping resulting in local drug concentrations reaching toxic levels. Thus, special care must be taken to tailor both the release rate and the degradation rate if a degradable material is to be used. Chew et al. examined the release of b-nerve growth factor (NGF) stabilized in BSA from a copolymer consisting of ecaprolactone and ethyl ethylene phosphate (PCLEEP). Due to its relatively hydrophobic backbone, PCLEEP has a slow degradation rate demonstrating a mass loss of approximately 7% over a 3-month period. Using this system, Chew et al. observed a sustained release of NGF over a period of 3 months. Due to the relatively small amount of mass loss over this period they inferred that NGF release was occurring primarily via diffusion, demonstrating that a biodegradable system can be used to obtain a desirable release profile while still eliminating the need for a second surgery for implant removal.

Kenawy et al. (2007) examined the release of tetracycline hydrochloride from electrospun mats composed of PLA, poly(ethylene-co-vinyl acetate) (PEVA), and a 50:50 blend of the two. The electrospun mats were prepared by dissolving the polymer (14% w/v) and tetracycline hydrochloride (5 or 25 wt%) in a chloroform/methanol solution, thus producing polymer fibers containing embedded drug. The authors found that both polymer composition and drug loading affected the rate of drug release with PEVA demonstrating quicker release than either the 50:50 PEVA/PLA blend or PLA. Additionally, the authors compared the release profile obtained from electrospun mats to those obtained from corresponding cast films. Due to their larger surface areas, electrospun mats tended to give greater release of drug than did the corresponding films. Additionally, the electrospun PEVA and 50:50 PEVA/PLA mats gave a relatively smooth release over a period of 5 days, while eliminating the initial burst seen with the films (Sill & von Recum, 2008).

Recent work has examined the possibility of using electrospun matrices as constructs for giving controlled release of a number of drugs including antibiotics and anticancer drugs; as well as proteins and DNA for applications in tissue engineering. Following an invasive surgery in a region such as the abdominal wall, it is common to deliver antibiotics either systemically or locally in order to prevent infection at the surgery site. While infection is a potential complication with any surgery, one of the main complications encountered at the abdominal wall postsurgery is the formation of fibrotic bands linking separate surfaces in the peritoneal cavity, known as abdominal adhesion. Additionally, previous work has indicated that the formation of abdominal adhesions can be increased by certain bacteria. Thus, while the most promising technique for preventing abdominal adhesion postsurgery remains the use of a physical barrier to separate the injured site from the adjacent tissues, incorporation of antibiotics has also shown promise. A scaffold that is capable of providing barrier function along with controlled delivery of a substantial amount of antibiotic could potentially decrease the frequency and severity of abdominal adhesion formation postsurgery. Due to the ability to fabricate scaffolds containing pores on the nanometer size scale, which can either limit or eliminate cell migration; as well as scaffolds with inherently high surface area, which allows for high drug loadings and the ability to overcome

mass transfer limitations associated with other polymeric systems, electrospun matrices are able to meet both requirements of barrier function and drug delivery necessary to prevent abdominal adhesion.

Bolgen et al. examined the potential of PCL electrospun mats loaded with a commercial antibiotic (Biteral_) for preventing abdominal adhesion following surgery in a rat model. PCL (13% w/v) in chloroform/ DMF (30:70) was initially electrospun to form a nonwoven fibrous mat, which was then covered with a solution of Biteral_. Thus, drug loading was achieved by absorption of the Biteral_ solution into the electrospun mat and drug was only located at the surfaces of the electrospun fibers. In vitro release studies demonstrated that nearly 80% of the drug was released after the first 3 h, with complete release occurring after almost 18 h. The authors suggest that this rapid burst release of drug is advantageous as most infections occur within a few hours after surgery. Adhesion was then examined in vivo using a rat model with defects in the abdominal wall in the peritoneum. A control containing no scaffold was examined along with electrospun PCL mats with or without antibiotic. The authors found that both the extent and tenacity of abdominal adhesions was reduced using the antibiotic containing
electrospun PCL mat as compared to the control and the unloaded PCL mat. Additionally, the antibiotic containing electrospun PCL mat seemed to improve and accelerate the healing process as compared to the control and the unloaded PCL mat. However, the authors noted that the electrospun mats degraded much slower than the rate of the healing process, indicating that the fiber diameter must be reduced and lower molecular weight polymer must be used in order to tailor the degradation rate to the healing rate in vivo. Thus, antibiotic loaded electrospun scaffolds have potential applications in reducing abdominal adhesion postsurgery.

Kim et al. (2004) examined the controlled release of a hydrophilic antibiotic (Mefoxin_) from electrospun PLGA and PLGA/ PEG-b-PLA/PLA (80:15:5 by wt%) mats, but used a different method of drug loading than that found in Bolgen et al. Rather than absorbing the antibiotic onto the scaffold after electrospinning, Kim et al. (2004) dissolved both the polymer and the antibiotic in DMF prior to electrospinning, giving antibiotic embedded within the electrospun

fibers. The authors observed that the drug loading affected the morphology of the electrospun fibers, with solutions containing no drug giving a bead-and-string morphology and solutions containing 5 wt% drugs giving a completely fibrous structure. Additionally, the average fiber diameter decreased from 360 _ 220 nm (without drug) to 260 _ 90 nm (with 5 wt% of drug). The authors attribute this change in morphology and fiber diameter to the increased conductivity of the solutions containing drug. The authors also verified that the structure of the drug was unchanged by the electrospinning process using both UVevis and 1H NMR analysis, demonstrating that the antibiotic retained its bioactivity. An in vitro drug release study was conducted and demonstrated that the PLGA/PEG-b-PLA/PLA blend gave a more sustained release than did the PLGA mat. The authors suggest that due to the high ionic strength of the drug and the minimal physical interactions between the polymer and the drug in the PLGA system, the drug is primarily located at the surface of the fibers. However, incorporating the amphiphilic PEG-b-PLA block copolymer allows some of the hydrophilic antibiotic to be embedded in the polymer nanofibers. Additionally, electrospun PLGA and PLGA/PEGb- PLA/PLA mats containing 5 wt% antibiotic both demonstrated greater than 90% inhibition of Staphylococcus aureus growth. Thus, the authors demonstrated that antibiotic loaded electrospun mats can be used to effectively reduce infection; however, careful attention must be paid to polymer composition as it can affect drug loading and consequently the drug release profile.

One of the largest areas of research within the drug delivery field is the targeted and controlled delivery of anticancer drugs. Electrospun mats have the ability to overcome drug loading limitations seen with other drug delivery devices, such as micelles and liposomes, currently used for targeting tumors. While the size scale and topography of electrospun nanofibers can help aid in cell attachment and proliferation due to their close approximation to the extracellular matrix, the ability to control the spatial and temporal delivery of bioactive agents can further augment the scaffolds ability to promote cell attachment, proliferation, and differentiation. Jiang et al. used a coaxial electrospinning setup to fabricate biodegradable core-shell fibers with PCL as the shell and BSA containing dextran as the core. Both BSA loading and its release rate could be controlled by varying

the feed rate of the inner solution, with higher feed rates giving higher BSA loading and accelerated release. Addition of PEG to the shell was used to further control the release rate, and was shown to increase release of BSA. By varying the inner solution feed rate as well as the PEG content of the shell the authors were able to vary the release period from 1 week to approximately 1 month. Liang et al. examined the incorporation of DNA into electrospun PLGA scaffolds. Plasmid DNA was condensed in a poor solvent mixture, and was then encapsulated in micelles composed of a triblock copolymer (PLAePEGePLA) giving encapsulated DNA nanoparticles. The micelles were then dissolved in a solution of DMF with PLGA and electrospun, resulting in the formation of PLGA fibers containing encapsulated DNA nanoparticles. The DNA was encapsulated in the PLAePEGePLA triblock copolymer in order to protect it from degradation during electrospinning with the PLGA copolymer (Sill and von Recum, 2008).

An in vitro release study demonstrated that approximately 20% of the encapsulated DNA was released after a period of 7 days. Additionally, the authors also examined the encapsulation of DNA in PLGA fibers without first forming protective micelles using the PLAePEGePLA triblock copolymer. MC3T3 cells demonstrated no transfection when the PLGA/DNA system (without the PLAePEGePLA triblock copolymer) was used. However, the PLGA/PLAePEGePLA/DNA system did demonstrate some limited transfection. The authors attribute the lack of transfection in the system without the PLAePEGe PLA triblock copolymer to degradation of the DNA during the electrospinning process. This paper demonstrates the importance of protecting biological agents that are incorporated into electrospun scaffolds. Special care must be taken to ensure that the agents will retain their bioactivity. In drug release applications, the summary of representative elctrospun sytems in shown in **Table 2.2**

Table 2.2 : Summary of some of the representative electrospun systems studied for drug-release applications.

Electrospun mat	Drug
Poly(caprolactone) PCL	Diclofenac sodium Tetracycline hydrochloride Resveratrol Gentamycin sulfate Biteral
Poly(lactic acid) PLA	Tetracycline hydrochloride Mefoxin
Poly(vinyl alcohol) PVA	Diclofenac sodium Tetracycline hydrochloride Sodium Salicylate Naproxen
Poly(lactide-glycolide) PLGA	Paclitaxel (anticancer) Tetracycline hydrochloride
Gelatin	Centella asiatica-herbal
Cellulose acetate	Vitamin A and E

CHAPTER 3

METHODOLOGY

3.1 Materials

Polylactid acid (PLLA) and polycaprolactone (PCL) monomer fiber (molecular weight, Mw = 16 w/v) with 5% of NSAID indomethacin were obtained from NUS Nanoscience & Nanotechnology Initiative respectively as shown in **Figure 3.1** and **Figure 3.2**. The NSAID indomethacin was purchased from NUS Nanoscience & Nanotechnology Initiative too, and used without any further treatment. Phosphate buffer and nutrient broth were obtained from the laboratory. Micropipette with 100μL size and its tip are the most essential apparatus in the research.

Microbacteria used for bacteria test were *Escherichia Coli* sp, *Micrococcus Luteus* sp. and *Bacillus Cereus* sp. Those microbacteria was purchased from American Type Culture Collection (Manassas, VA) in the form of a freeze-dried culture (**E.coli = ATCC 8739, M.Luteus = ATCC 0729, B.Subtilis = ATCC 0663**),

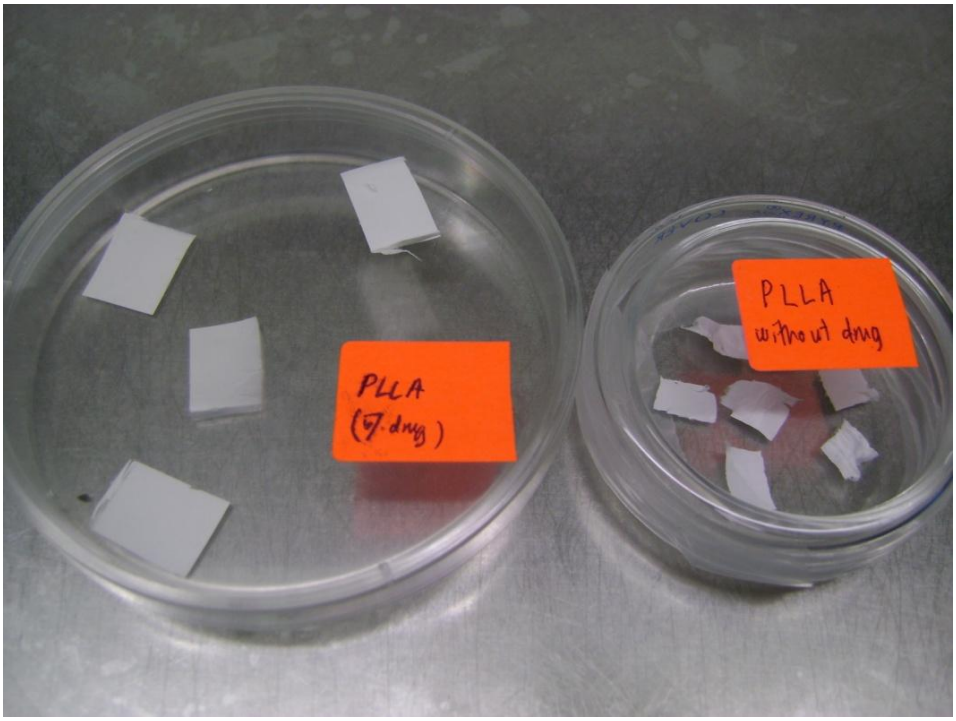

Figure 3.1 : PLLA polymer with 5% drug and PLLA with no drug concentration (control)

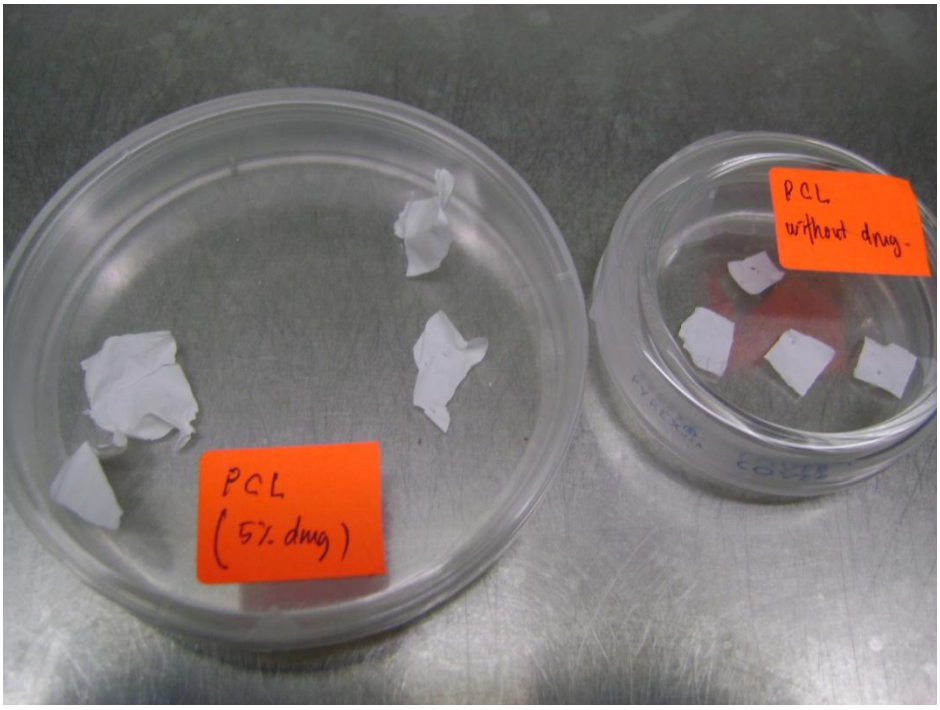

Figure 3.2 : PCL polymer with 5% drug and PCL with no drug concentration (control)

3.2. Preliminary Procedure

3.2.1 Work preparation

Nutrient Agar acts as the medium for the microbacteria to grow in petri plate. In **Figure 3.3**, the agar plate is prepared for the antimicrobial effect. Meanwhile, Nutrient Broth is prepared as shown in **Figure 3.4** as media for the use of cultivation of microbacteria. Nutrient broth is prepared as the microbacterial culture for the dynamic system of antimicrobial effect. Both Nutrient Agar and Nutrient Broth were autoclaved to make sure its sterility before used. In this research, all apparatus have to be in sterile condition before usage. Thus, micropipette tips, test tubes, bottles and petri plates have been autoclaved at 121°C for 20 minutes as shown in **Figure 3.5** Meanwhile, phosphate buffer is prepared as dilution blanks for use in microbiological testing rather than unbuffered water in order to standardize this potential variable due to the wide variation in the pH of purified water from multiple sources.

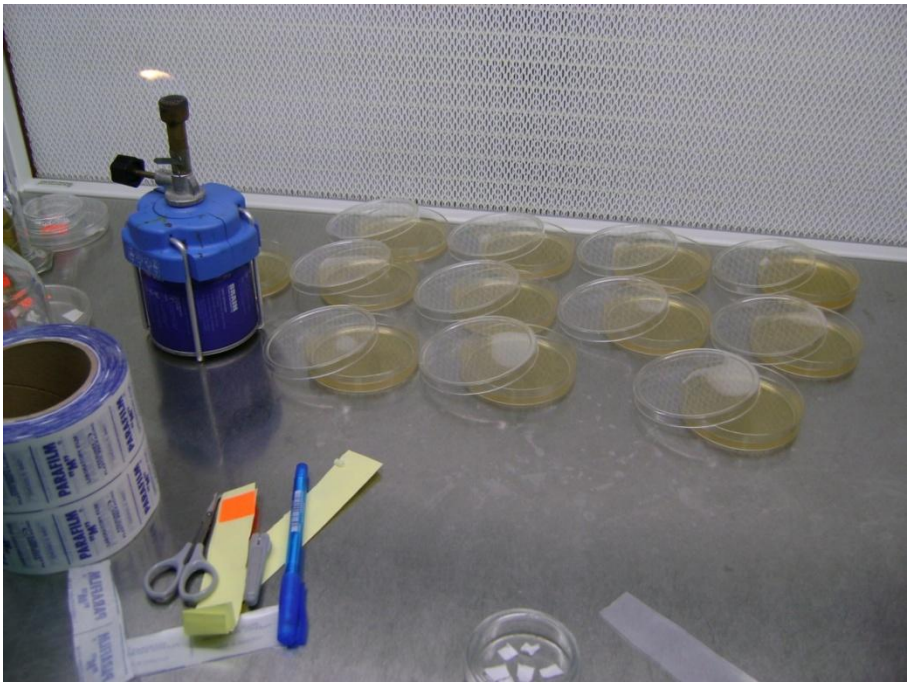

Figure 3.3 : Preparation of Agar Plate

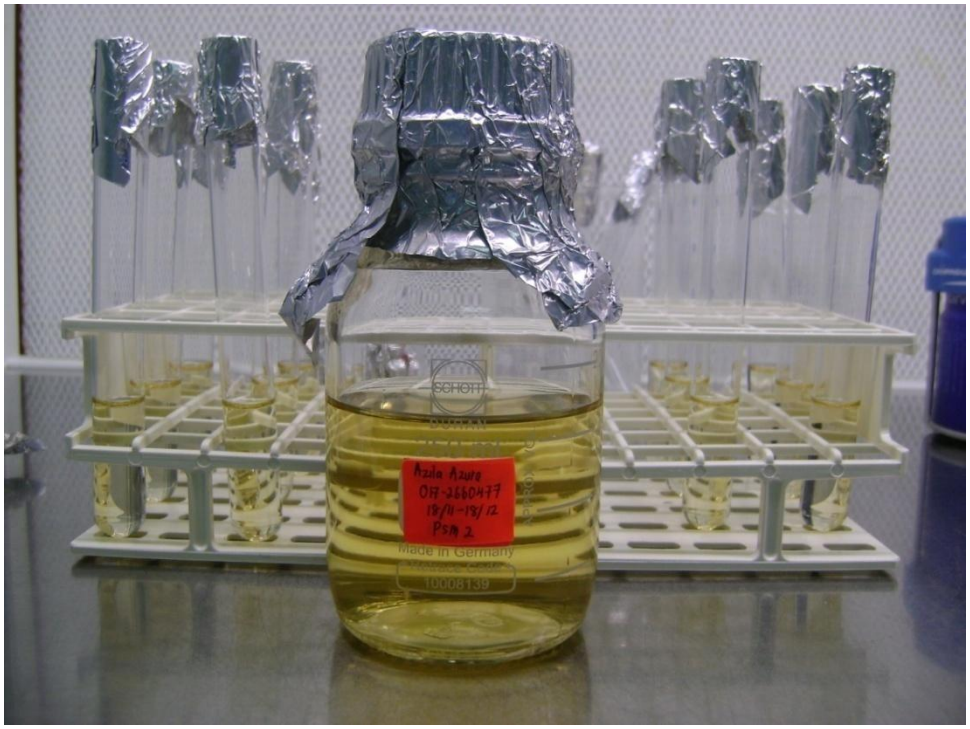

Figure 3.4 : Preparation of bacteria culture (Nutrient Broth)

Figure 3.5 : Apparatus need to be sterilized before used

3.2.2 Bacteria Streaking

In **Figure 3.6,** bacteria streaking were done to get a single colony for the microbacteria culture for the drug release study and antimicrobial effect. The bacteria colony is needed to make sure a uniform distribution of microbacteria culture in each test tubes and agar plates for testing.

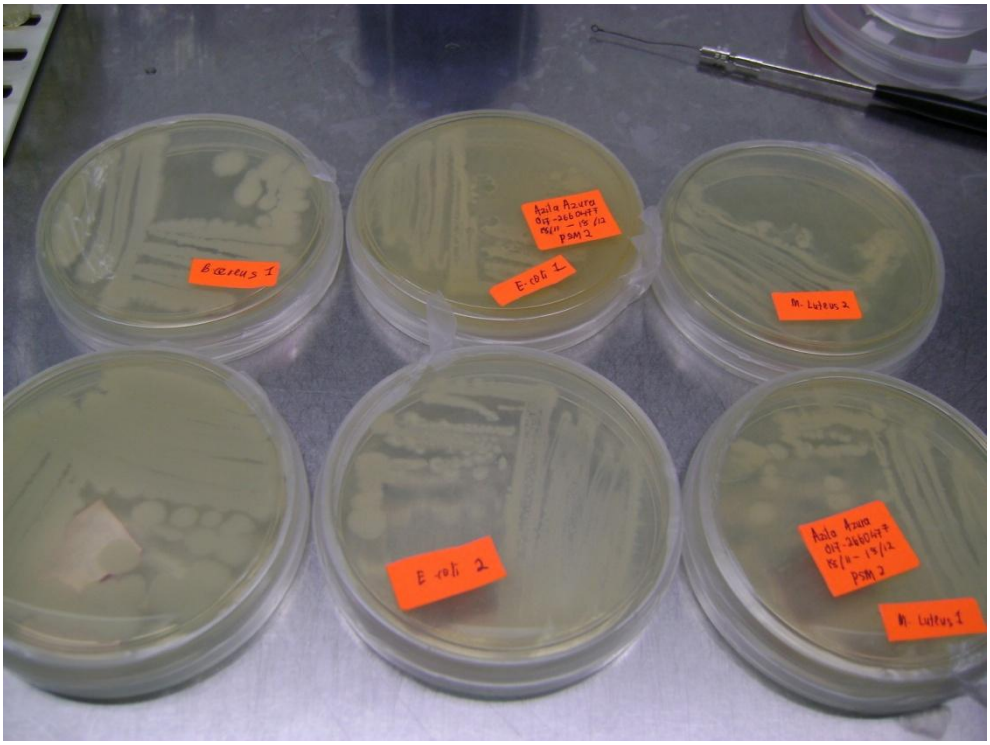

Figure 3.6 : Bacteria culture (by streaking method)

One single colony of the microbacteria was reconstituted in Nutrient Broth for growth and incubated at 37°C.

3.3 Drug release study

100µl bacteria solution was aliquoted into 5 ml volumes each multiwell tissue culture cell plate containing phosphate buffer solution, and standardized to a wavelength at 294 nm using Uv-Vis. To each aliquot, drug loaded scaffolds containing indomethacin were added as shown in **Figure 3.7**. All tested solutions were incubated at 37 °C with constant shaking at 130 rpm, and the absorbance at 294 nm was monitored after 24 hours of incubation.

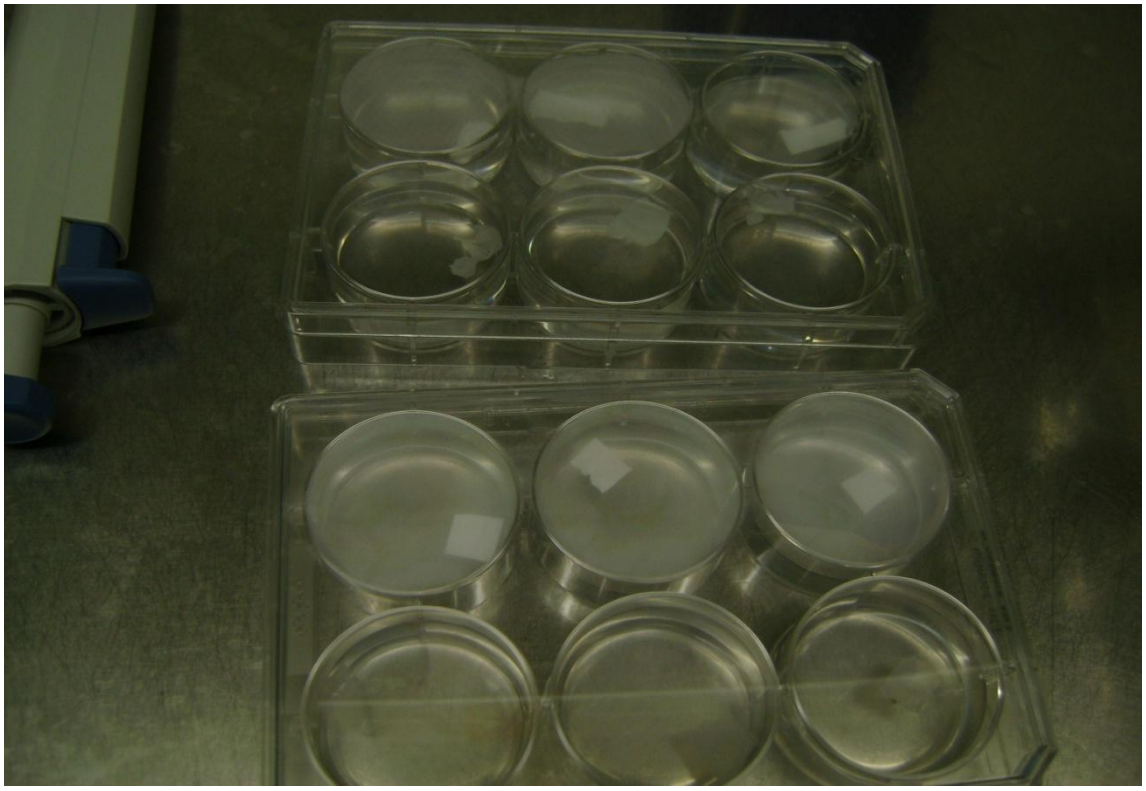

Figure 3.7 : Polymers were immersed in the multiwell cell plate

3.4 Bacteria test of the viability of released indomethacin

100µl bacteria solution was aliquoted into 5 ml volumes each test tubes containing nutrient broth, and standardized to a wavelength at 294 nm using Uv-Vis. To each aliquot, drug loaded scaffolds containing indomethacin were added as shown in **Figure 3.8** and **Figure 3.9**. All tested solutions were incubated at 37 °C with constant shaking at 130 rpm, and the absorbance at 294 nm was monitored after 24 hours of incubation.

In addition, a second viability test for the released drug was also adopted, which involved the use of bacterial colony formation. After the polymer was placed on the agar as shown in **Figure 3.10**, the plate was incubated at 37°C for 4 hours, allowing the incorporated drugs to diffuse from the scaffolds unto the agar. Then, an equal amount of microbial is pipette on the agar plate. A 100µl aliquot of S. aureus was spread directly onto the plate. In this test, scaffolds sections (1 x 1 cm^2) of varying polymers PLLA and PCL; with drug and without drug were incubated at 37 °C for 24 hours on agar plates, allowing the incorporated drugs to diffuse from the scaffold into the agar. Bacterial growth was visualized directly on the plate.

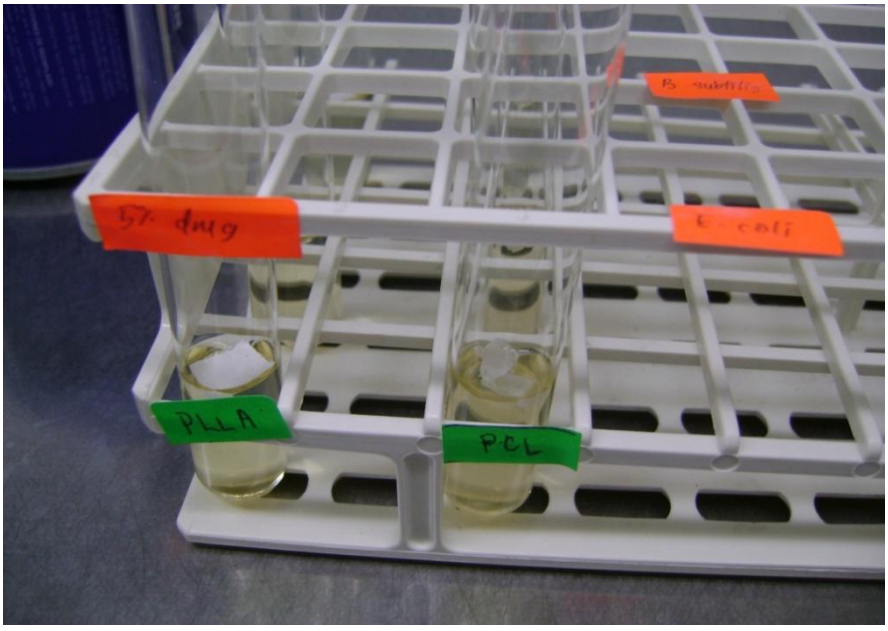

Figure 3.8 : Immersing of polymers with 5% drug concentration

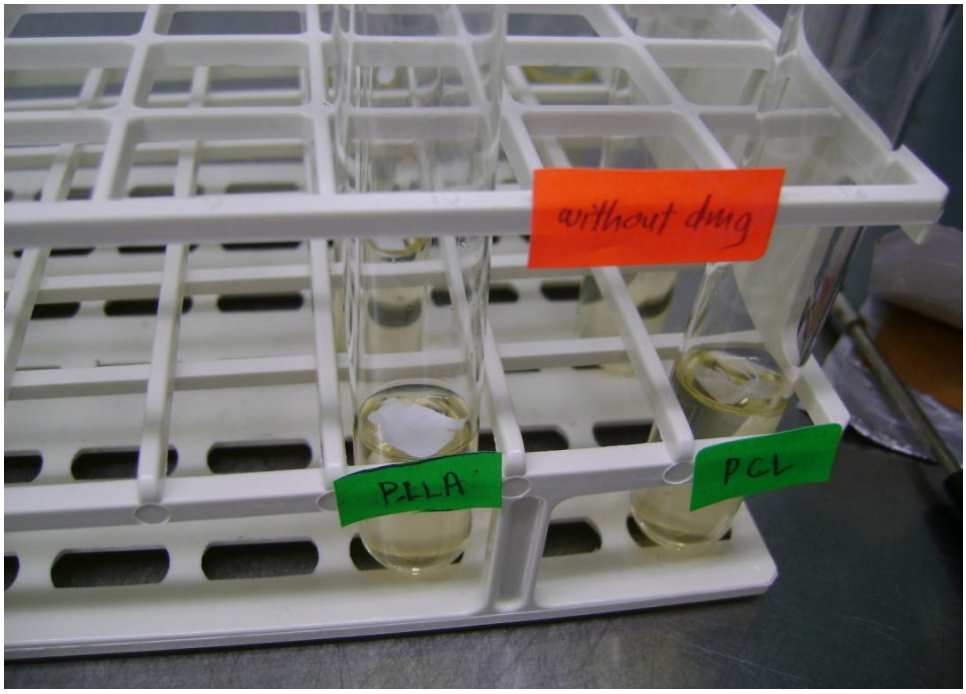

Figure 3.9 : Immersing of polymers with no drug concentration (control)

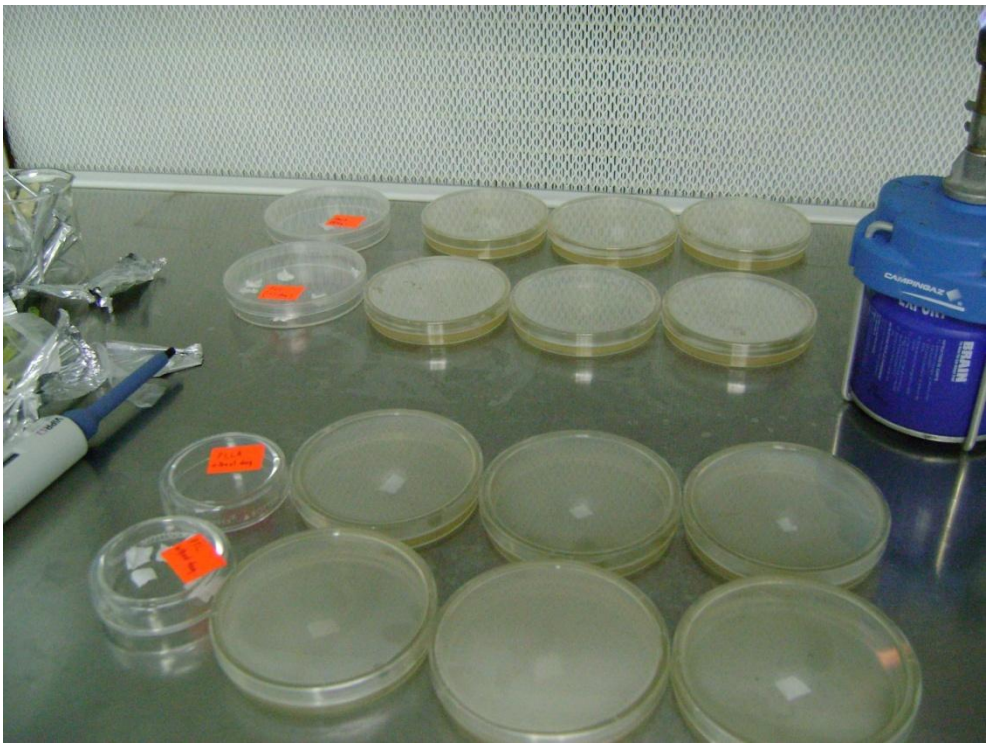

Figure 3.10 : Antimicrobial test

3.5 Analytical Technique

Uv-Vis Spectrophotometry is used to determine the absorption or transmission of Uv-ViS light (180 to 820 nm) by a sample. It can also be used to measure concentrations of absorbing materials based on developed calibration curves of the material. A sample is placed in the UV/VIS beam and a graph of the transmittance or absorbance versus the wavelength is obtained. Alternatively, samples are prepared in known concentrations and their absorbance is read by the UV/VIS Spectrophotometer. Results are then graphed to make a calibration curve from which the unknown concentration can be determined by its absorbance.

Uv-Vis spectroscopy is the measurement of the wavelength and intensity of absorption of near-ultraviolet and visible light by a sample. Ultraviolet and visible light are energetic enough to promote outer electrons to higher energy levels. Uv-Vis spectroscopy is usually applied to molecules and inorganic ions or complexes in solution. The Uv-Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law. The light source is usually a hydrogen or deuterium lamp for Uv measurements and a tungsten lamp for visible measurements. The wavelengths of these continuous light sources are selected with a wavelength separator such as a prism or grating monochromator. Spectra are obtained by scanning the wavelength separator and quantitative measurements can be made from a spectrum or at a single wavelength.

Single-Beam spectrophotometers are often sufficient for making quantitative absorption measurements in the UV-Vis spectral region. In **Figure 3.11**, single-beam spectrophotometers can utilize a fixed wavelength light source or a continuous source. The simplest instruments use a single-wavelength light source, such as a light-emitting diode (LED), a sample container, and a photodiode detector.

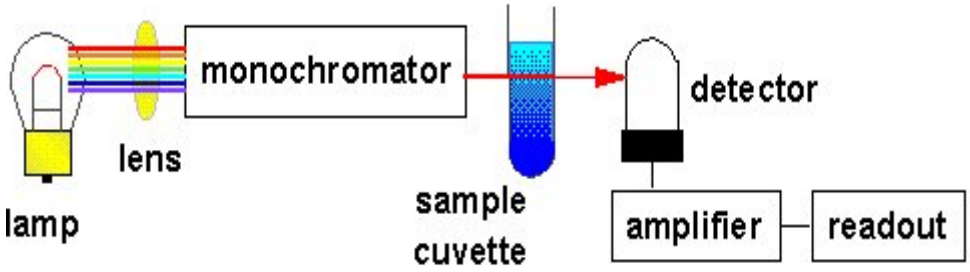

Figure 3.11 : *Schematic of a single beam uv-vis spectrophotometer*

3.6 Statistical analysis

The absorbance value of the bacteria solution was correlated directly with the concentration of bacteria in the solution. The percentage of bacterial inhibition was calculated by the absorbance difference between the bacteria solutions with drug (or medicated scaffold) and without the drug after 24 h using the following equation.

$$\text{Bacterial inhibition } \% = \text{abs (Ic \_ Is) / (Ic) x 100}$$

where Ic and Is are the absorbance of the control bacteria solution without drug or scaffold and of the bacteria solution with drug (or the medicated scaffold), respectively, at 294 nm after 24 h.

3.7 Summary of work

To accomplish the objective of the research, a well prepared plan has been done to make sure the research is completed within the time give. Thus, a summary of design method has been done to indicate the flow of work as shown in **Figure 3.12**

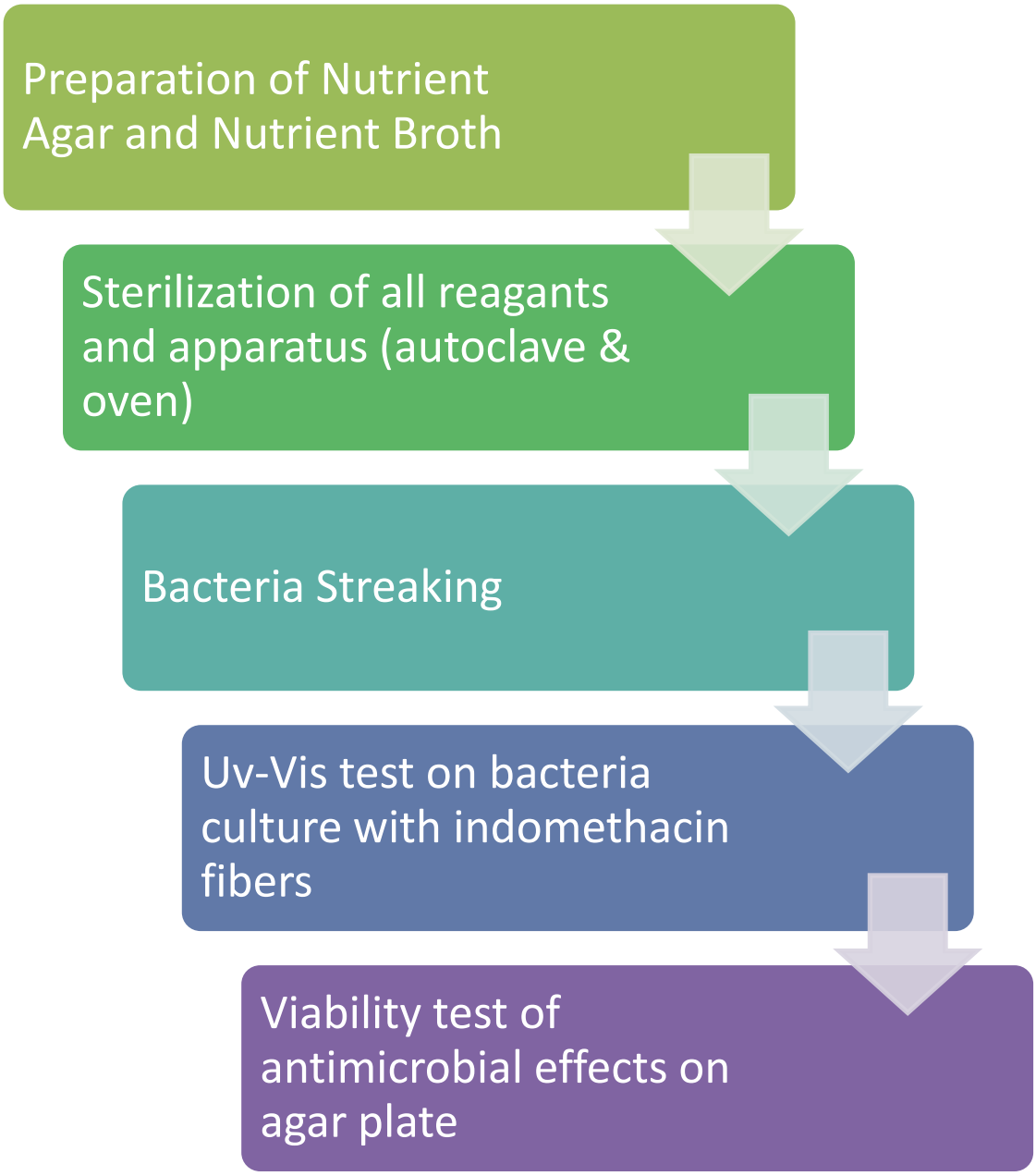

Figure 3.12 Summary of methodology and method design

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Results

The results obtained from the experiment were tabulated and plotted in graphical form. The calculations for percentage of bacterial inhibition and absorbance data using Uv-Vis are shown in Appendix A. The supported pictures were shown in Appendix B.

The parameters such as types of polymer; PLLA and PCL, polymer concentration; 5% drug concentration and no drug concentration(control), and lastly types of microbial culture used; *E.coli* sp., *B.cereus* sp., and *M.Luteus* sp. were analyzed. Firstly, the absorbance values using Uv-Vis were obtained for both parameters at 294nm wavelength. The bacteria cultures were incubated for 1 night at constant shaking, 130 rpm as shown in **Figure 4.1** and **Figure 4.2**. Then, the absorbance values were observed and tabulated after 24 hours as shown in **Table 4.1**, **Table 4.2**, **Table 4.3** and **Table 4.4**. As for the antimicrobial effects, **Figure 4.3** and **Figure 4.4** shows the polymer was placed in the agar plate containing microbial culture and incubated for an overnight.

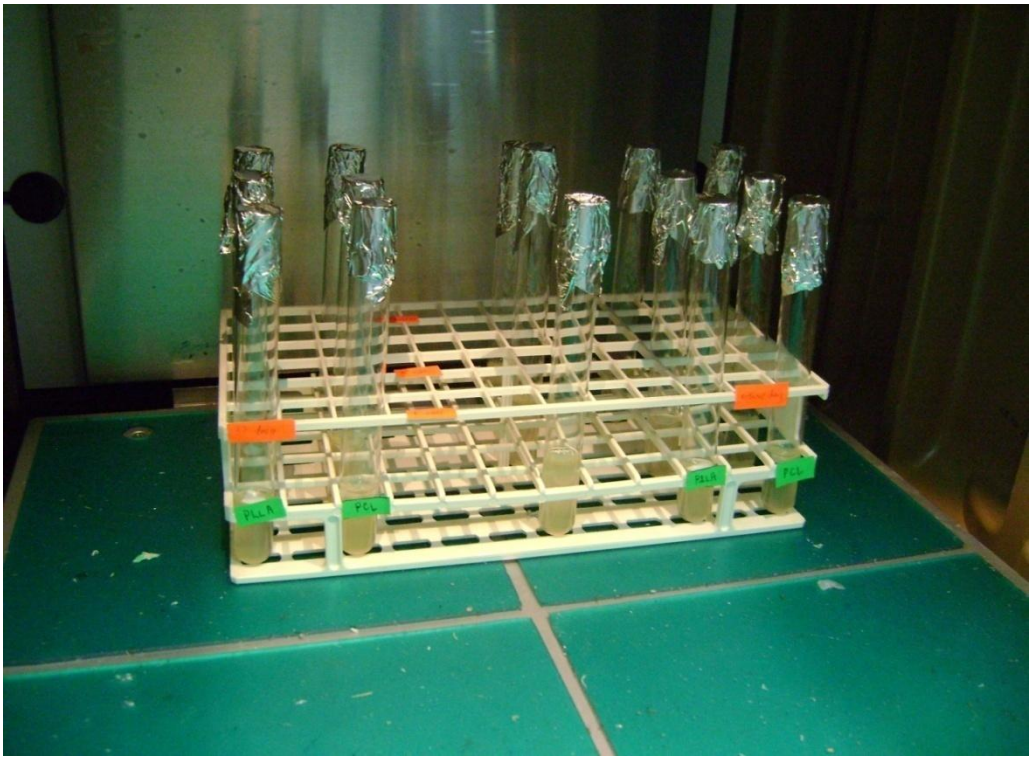

Figure 4.1 : Bacteria cultures(nutrient broth) incubated for an overnight

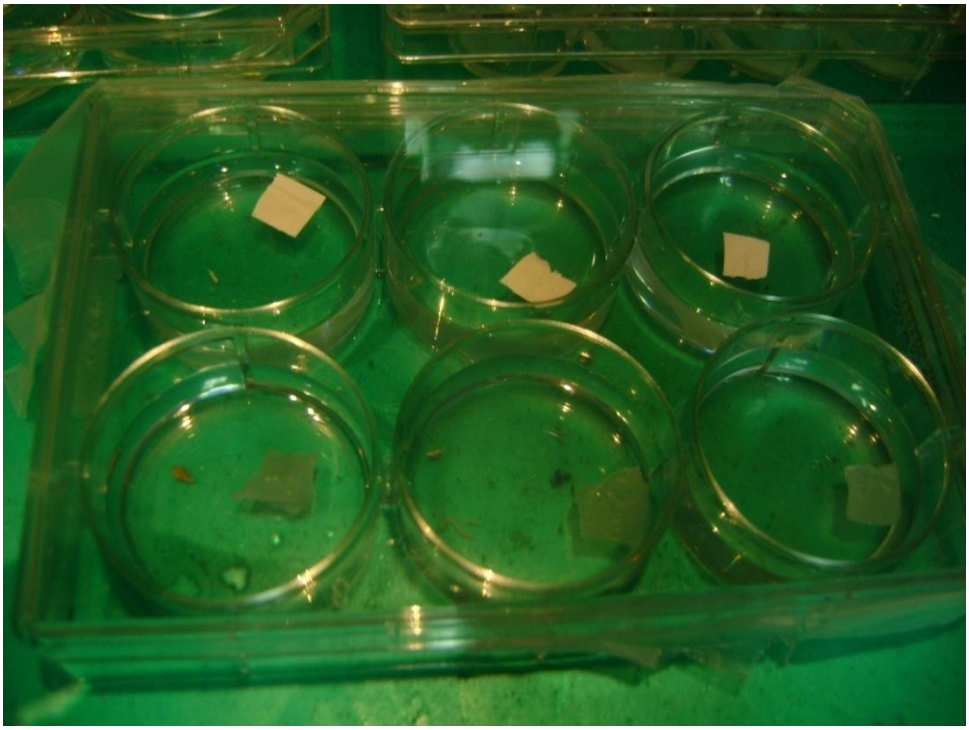

Figure 4.2 : Bacteria cultures(phosphate buffer) incubated for an overnight

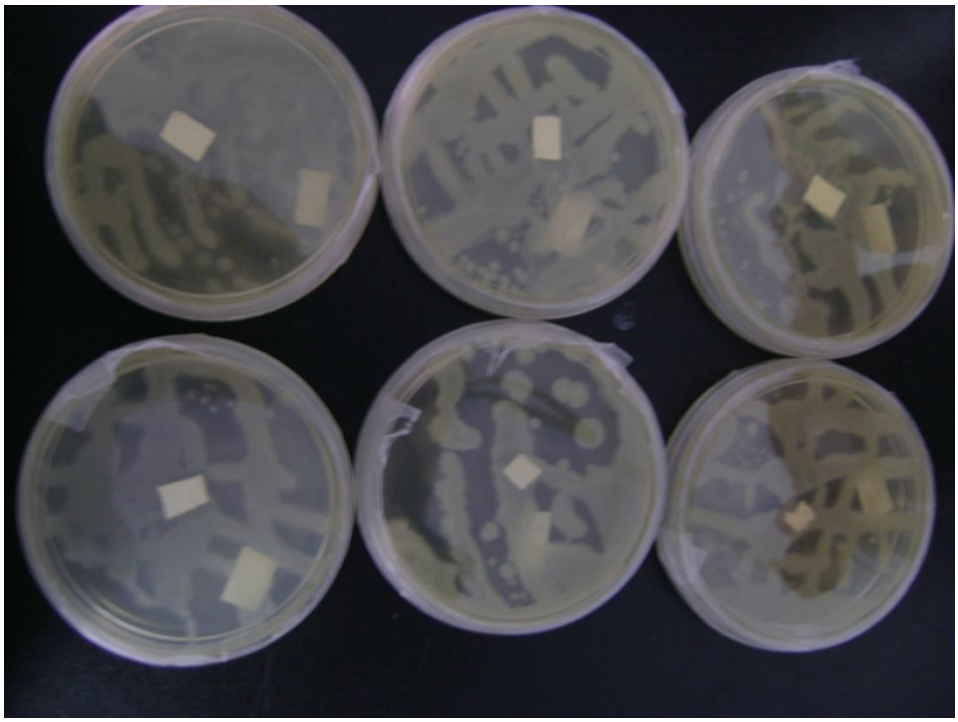

Figure 4.3 : PLLA polymer was incubated with the microbial culture for an overnight

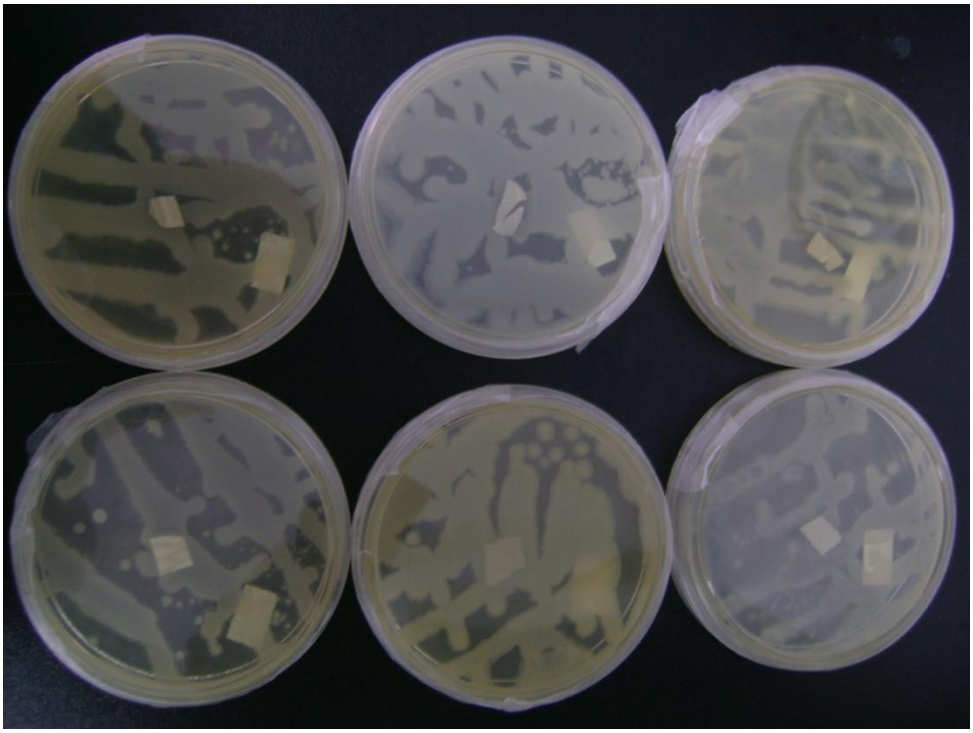

Figure 4.4 : PCL polymer was incubated with the antimicrobial culture for an overnight

Table 4.1 : Absorbance value for overall parameters in microbial culture(nutrient broth) before incubated

	5% drug		Without drug (control)	
	PLLA	PCL	PLLA	PCL
E. coli	0.023	0.026	0.619	0.704
B. cereus	0.018	0.031	0.706	0.671
M. Luteus	0.044	0.048	0.710	0.700

Table 4.2 : Absorbance value for overall parameters in microbial culture(nutrient broth) after incubated

	5% drug		Without drug (control)	
	PLLA	PCL	PLLA	PCL
E. coli	1.420	1.431	1.914	1.621
B. cereus	1.314	1.505	1.864	1.725
M. Luteus	1.665	1.728	1.955	1.818

Table 4.3 : Absorbance value for overall parameters in microbial culture(phosphate buffer) before incubated

	5% drug		Without drug (control)	
	PLLA	PCL	PLLA	PCL
E. coli	0.327	0.477	0.359	0.452
B. cereus	0.181	0.478	0.219	0.876
M. Luteus	0.355	0.454	0.386	0.110

Table 4.4 : Absorbance value for overall parameters in microbial culture(phosphate buffer) after incubated

	5% drug		Without drug (control)	
	PLLA	PCL	PLLA	PCL
E. coli	0.347	0.325	0.487	0.438
B. cereus	0.346	0.331	0.495	0.486
M. Luteus	0.311	0.295	0.350	0.350

4.2 Discussions

4.2.1 Overall absorbance after incubation for PLLA and PCL polymer

Based on the overall absorbance after incubation for PLLA and PCL polymer in nutrient broth as shown in **Figure 4.5 and Figure 4.6**, it shows that the absorbance of both polymers with no drug concentration (control) is higher compared with the polymer with 5% of indomethacin concentration. As the absorbance of polymer with 5% of drug concentration decreases after incubation, this shows that the drug diffused from the polymer into the microbial culture and thus inhibits the microbial growth. This will result in a lower absorbance value after incubation. In **Figure 4.7** and **Figure 4.8**, the absorbance for polymer with no drug concentration is higher as there is no drugs diffuse from the polymer to inhibit the microbial growth. This statement of justification was supported by Kenawy et al. (2007) who studies about the controlled release of ketoprofen(NSAID) from electrospun.

The samples were all incubated in an incubator shaker at 37 C because it is our body temperature. Thus the cultures need to be incubated at that degree so that we can analyzed the results precisely as the rate of release is likely to depend on the temperature (Kenawy et al., 2007).

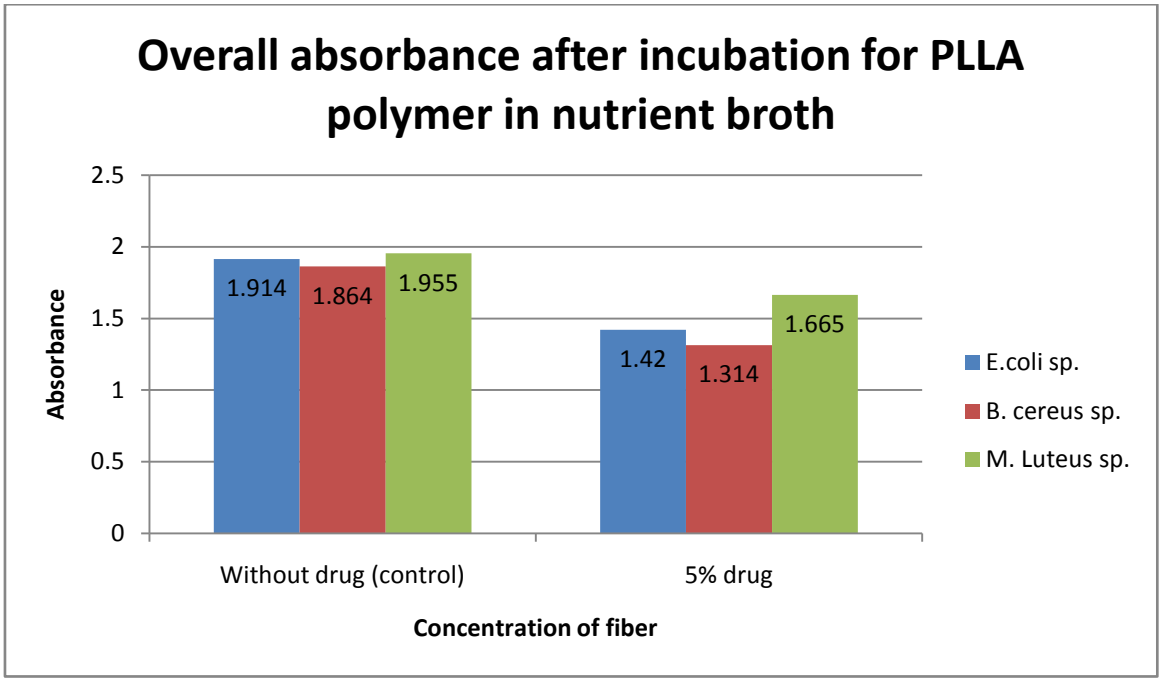

Figure 4.5 : Overall absorbance after incubation in nutrient broth for PLLA polymer

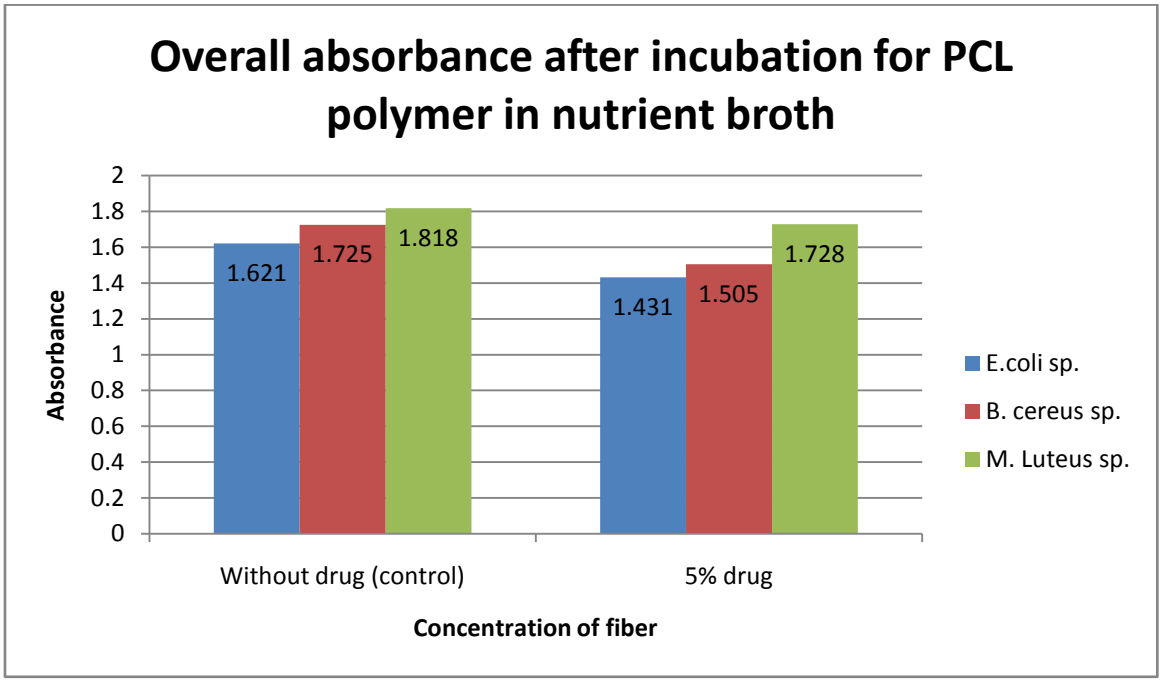

Figure 4.6 : Overall absorbance after incubation in nutrient broth for PCL polymer

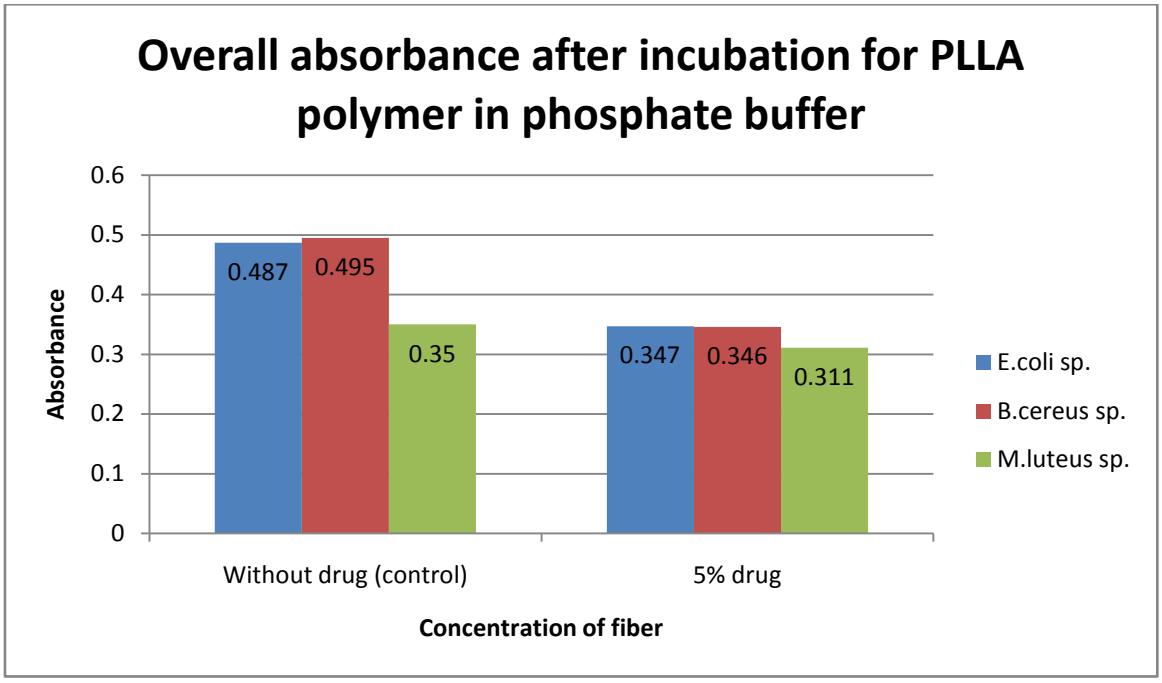

Figure 4.7 : Overall absorbance after incubation in phosphate buffer for PLLA polymer

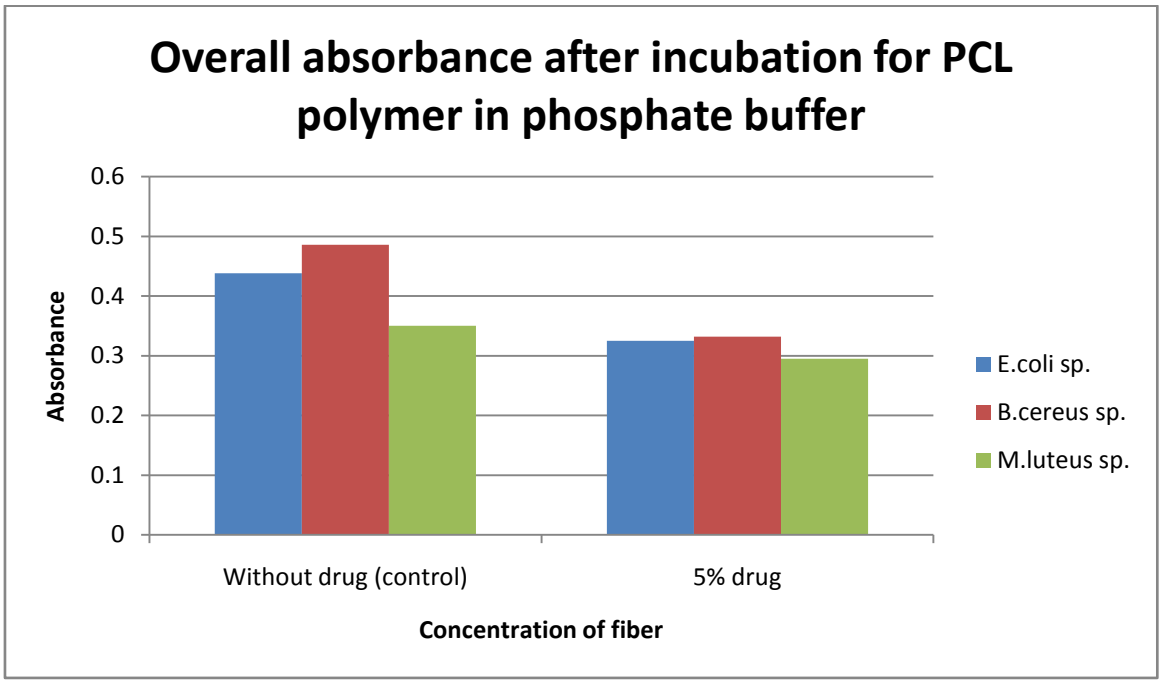

Figure 4.8 : Overall absorbance after incubation in phosphate buffer for PCL polymer

4.2.2 Drug release study

Indomethacin, a hydrophilic drug, was successfully incorporated into PLLA and PCL based electrospun nanofibrous scaffolds and could also be released from the scaffolds in aqueous conditions.Based on **Figure 4.9** and **Figure 4.10,** it shows the drug release behavior supports that the released drugs are effective in inhibiting microbacterial growth at specific concentrations tested. Phosphate buffer is used in the preparation of dilution blanks for use in microbiological testing rather than unbuffered water in order to standardize this potential variable due to the wide variation in the pH of purified water from multiple sources. A continued release of drugs is necessary to prevent the microbacteria further population (Kim et el., 2004).

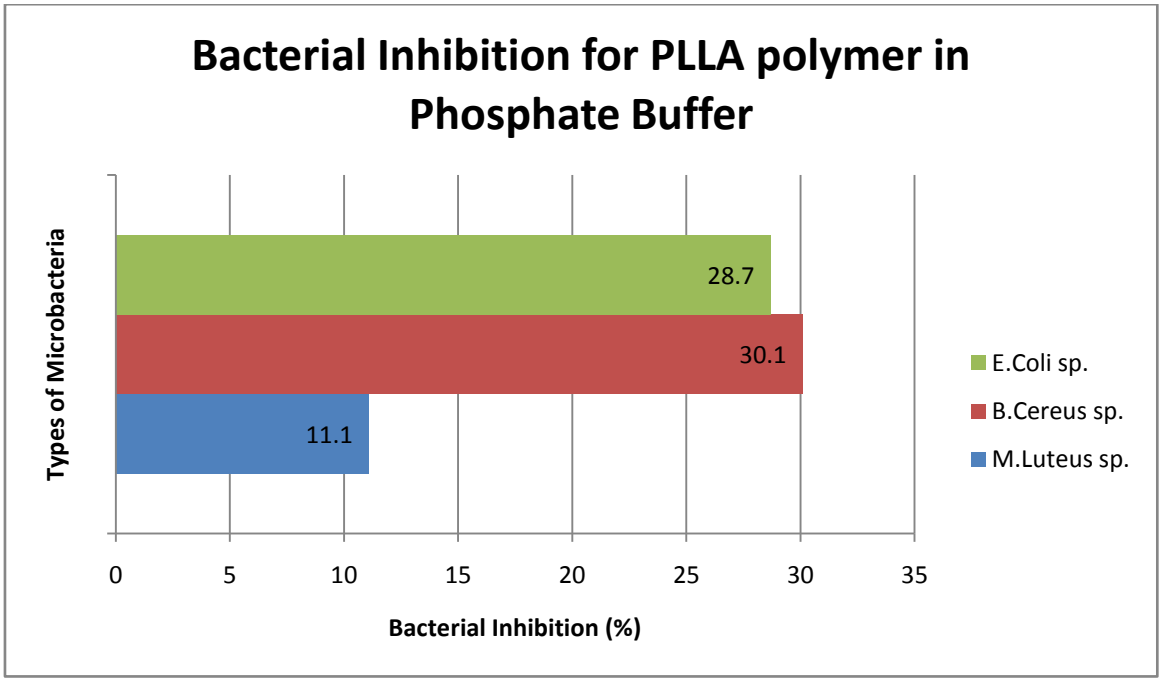

Figure 4.9 : Bacterial Inhibition for PLLA polymer in phosphate buffer

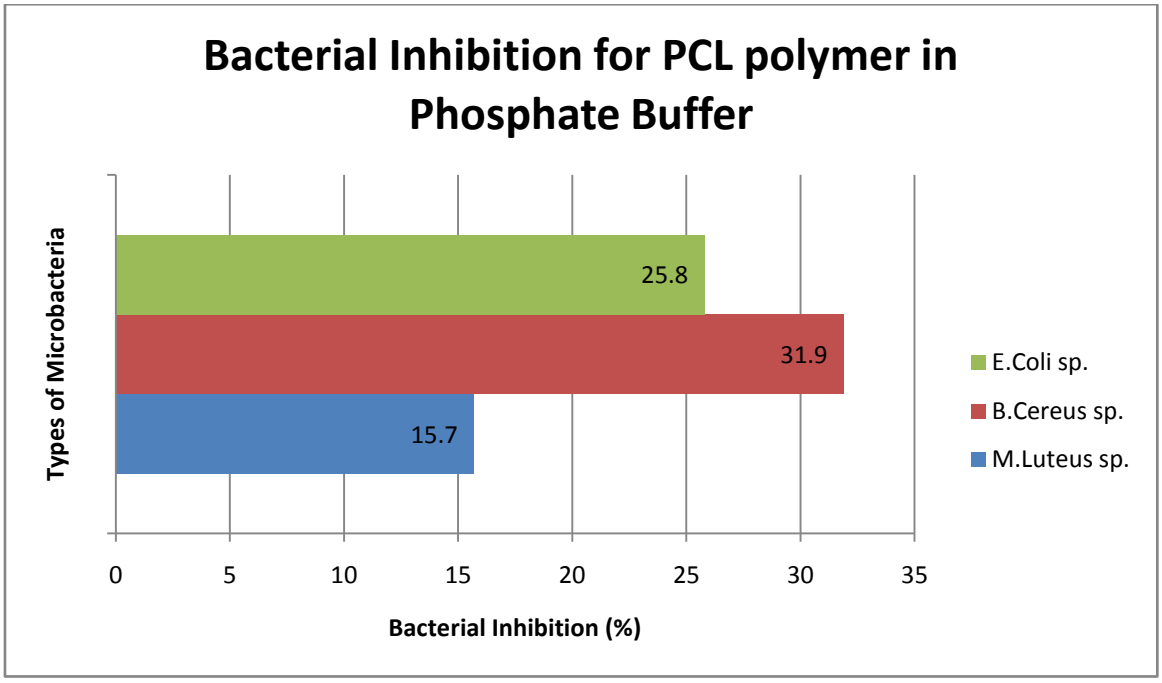

Figure 4.10 : Bacterial Inhibition for PCL polymer in phosphate buffer

4.2.3 Bacteria growth evaluation

The functionality of released indomethacin was investigated using microbacteria (*E.coli* sp., *B.cereus* sp., and *M.Luteus* sp.) inhibition experiments. Pure and released indomethacin at different concentrations was introduced into 5 ml of microbial cultures and the ability of each sample to inhibit microbial growth was monitored by the absorbance at 294nm in Uv-Vis spectrophotometry. In **Figure 4.11** and **Figure 4.12,** it is clearly seen that the released drugs are effective in inhibiting microbacterial growth at specific concentrations tested. These results confirm that indomethacin released from the scaffolds has retained it biological function, that process of electrospinning had no adverse effect on changing its structure.

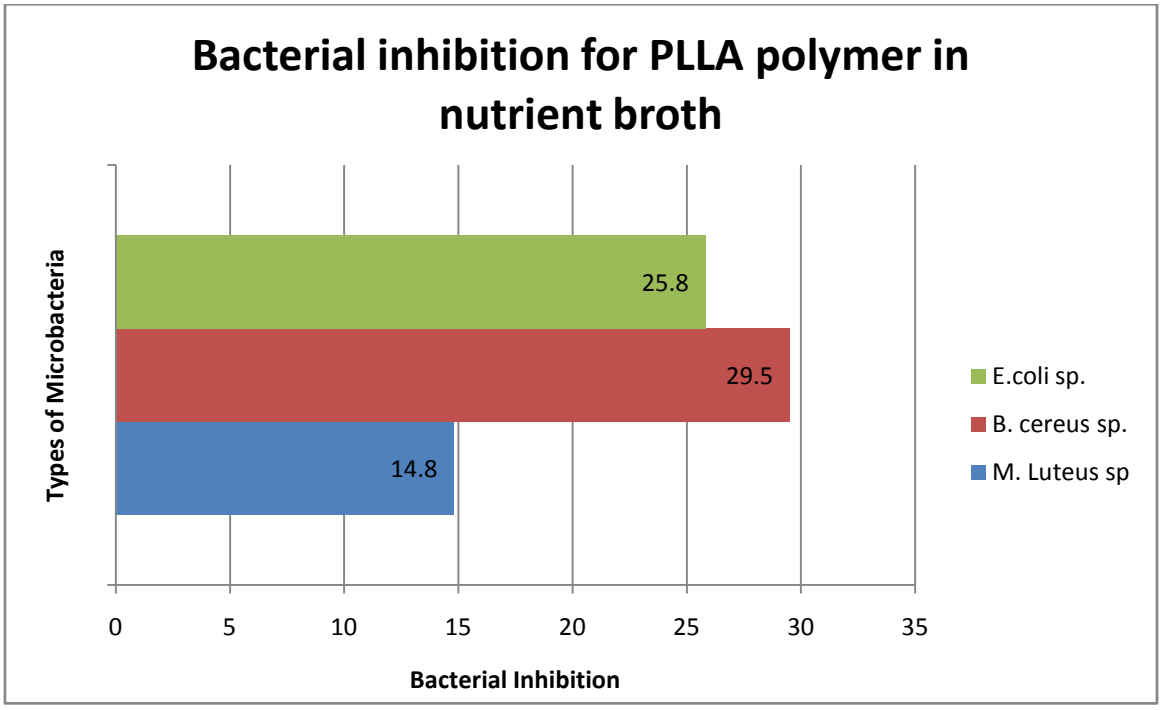

Figure 4.11 : Bacterial Inhibition for PLLA polymer in nutrient broth

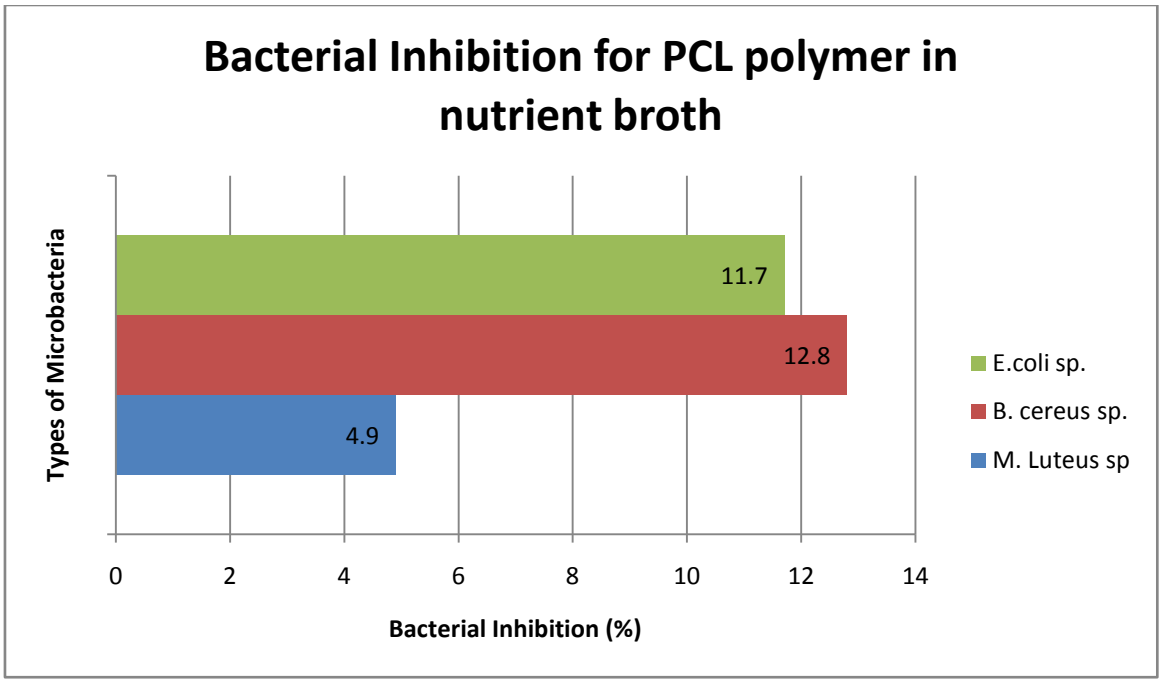

Figure 4.12 : Bacterial Inhibition for PCL polymer in nutrient broth

The effectiveness of electrospun scaffolds with and without indomethacin was tested to examine their ability to inhibit bacterial growth in a dynamic system (scaffolds mixed with microbacterial cultures). This result was supported by Kim et al (2004) who have successfully done the release of antibiotic drug from poly (lactide-co-glycolide) PLGA nanofibrous culture.

In addition to the liquid microbacterial culture test, the efficacy of the medicated scaffolds using a static system for bacterial growth on agar plates was also examined. The growth of microbacterial can be visualized directly from the plate to assess the viability of the medicated scaffolds. Agar region, where the scaffold was placed, shows the inhibition of microbacterial growth even though the inhibition area is very significant. In contrast, scaffolds containing no drug exhibit had no inhibitory effect on microbacteria.

E. coli sp. is a Gram-positive rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms), and can cause serious food poisoning in humans. The following characteristics are generally present in a Gram-positive bacterium:

a) cytoplasmic lipid membrane
b) thick peptidoglycan layer

    - teichoic acids and lipoids are present, forming lipoteichoic acids which serve to act as chelating agents, and also for certain types of adherence.

B. cereus sp. is a Gram-negative rod-shaped bacterium, and some of the strains are harmful to humans and can cause food borne illness. *M. Luteus* sp. is also a Gram-negative spherical bacterium that found in soil, dust, water and air. The bacterium colonizes the human mouth, mucosa, and upper respiratory tract. The following characteristics are displayed by Gram-negative bacteria:

a) cytoplasmic membrane

b) thin peptidoglican layer (which is much thinner than in Gram-positive bacteria)
c) Outer membrane containing lipopolysaccharide (LPS, which consists of lipid A, core polysaccharide, and O antigen) outside the peptidoglycan layer

As shown in **Figure 4.13,** the gram positive and gram negative bacteria are differentiated by their cell wall structure. Gram positive bacteria are those that are stained dark blue or violet by Gram Staining protocol. Besides, gram negative bacteria are bacteria that do not retain crystal violet dye in the Gram Staining protocol.

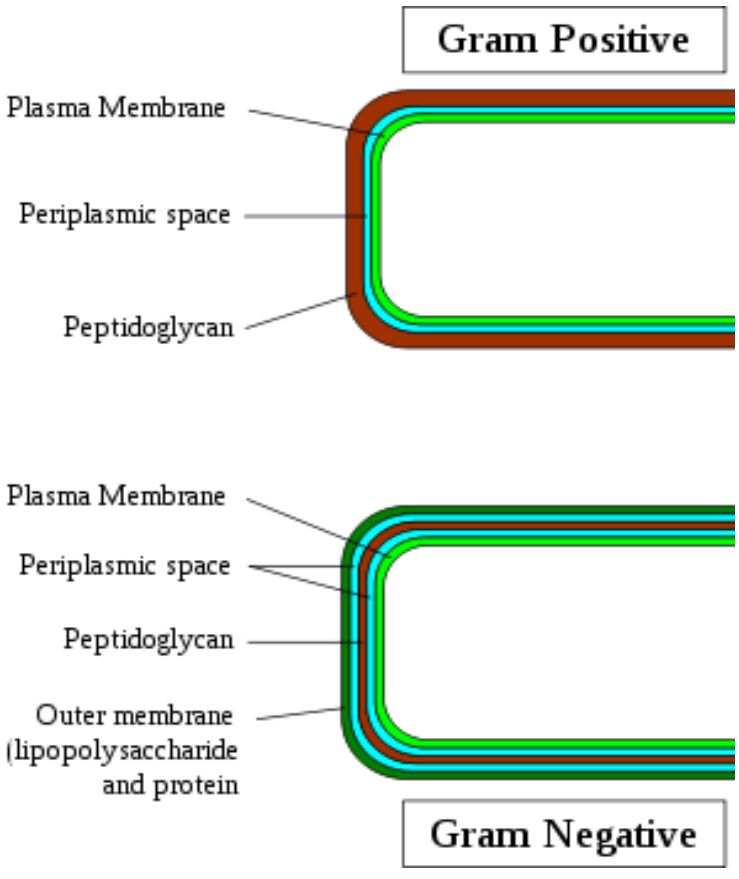

Figure 4.13 : Gram positive and gram negative bacteria cell wall structure

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Electrohydrodynamic techniques are promising tools for fabricating drug delivery system (DDS). Incorporation of drug into the delivery vehicle is usually a one-step process. High loading capacity, high encapsulation efficiency, simultaneous delivery of topographical and biochemical cues, ease of operation, and cost-effectiveness comprise other appealing features.

While electrospinning has emerged as a viable polymer processing technique for applications in drug delivery and tissue engineering, its true potential has yet to be realized. In the future it is important that research focuses on gaining a better fundamental understanding of the electrospinning process, but even more importantly on how this technique can be used as a tool in developing new systems for drug delivery and tissue engineering.

New system for the delivery of indomethacin as non-steroidal anti-inflammatory drug (NSAID) was developed. The drug was successfully incorporated and released from nanofibrous scaffolds without the loss of structural integrity or change in functionality.

Herein, a new electrospun fiber as a new approach for drug delivery system using electrospinning technique was developed. These fibers were biodegradable polymers such as polylactide acid (PLLA) and polycaprolactone(PCL). The release was monitored in phosphate buffer at the body temperature (37 ◦C) for the drug release behavior. The released indomethacin from electrospun scaffolds was found to be structurally intact as well as effective in its ability to inhibit *E.coli* sp., *B.cereus* sp., and *M.luteus* sp. bacteria growth in both a static (agar) and dynamic (liquid) environment.

In summary, the drug release behavior is affected by the parameter of types of polymer and drug concentration. For antimicrobial effect, the drug release is able to inhibit the microbacteria growth. As for the hypothesis, the higher drug concentration in the fiber, the higher microbacteria inhibition. It is clear that these PLLA and PCL-with indomethacin based electrospun scaffolds hold a future potential in drug delivery system.

5.2 Recommendation

Targeting is the ability to direct the drug-loaded system to the site of interest. Two major mechanisms can be distinguished for addressing the desired sites for drug release: (i) passive and (ii) active targeting. An example of passive targeting is the preferential accumulation of chemotherapeutic agents in solid tumors as a result of the enhanced vascular permeability of tumor tissues compared with healthy tissue. A strategy that could allow active targeting involves the surface functionalization of drug carriers with ligands that are

selectively recognized by receptors on the surface of the cells of interest. Since ligand–receptor interactions can be highly selective, this could allow a more precise targeting of the site of interest.

Nanoparticles provide massive advantages regarding drug targeting, delivery and release and, with their additional potential to combine diagnosis and therapy, emerge as one of the major tools in nanomedicine. The main goals are to improve their stability in the biological environment, to mediate the bio-distribution of active compounds, improve drug loading, targeting, transport, release, and interaction with biological barriers. The cytotoxicity of nanoparticles or their degradation products remains a major problem, and improvements in biocompatibility obviously are a main concern of future research.

For this specific research, I would like to recommend the use of colony counter as one of the analytical analysis for the inhibition area of microbacterial growth.

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APPENDIX A

CALCULATIONS

Bacterial inhibition % = abs (Ic _ Is) / (Ic) x 100

Table A 1 : Bacterial inhibition (%) in bacteria culture(broth) for PLLA polymer

Microbacteria	Absorbance		Bacterial inhibition (%)
	Without drug (control)	5% drug	
E.coli sp.	1.914	1.420	25.8
B. cereus sp.	1.864	1.314	29.5
M. Luteus sp.	1.955	1.665	14.8

Table A 2 : Bacterial inhibition (%) in bacteria culture(broth) for PCL polymer

Microbacteria	Absorbance		Bacterial inhibition (%)
	Without drug (control)	5% drug	
E.coli sp.	1.621	1.431	11.7
B. cereus sp.	1.725	1.505	12.8
M. Luteus sp.	1.818	1.728	4.9

Table A 3 : Bacterial inhibition (%) in bacteria culture(phosphate buffer) for PLLA polymer

Microbacteria	Absorbance		Bacterial inhibition (%)
	Without drug (control)	5% drug	
E.coli sp.	0.487	0.347	28.7
B. cereus sp.	0.495	0.346	30.1
M. Luteus sp.	0.350	0.311	11.1

Table A 4 : Bacterial inhibition (%) in bacteria culture(phosphate buffer) for PCL polymer

Microbacteria	Absorbance		Bacterial inhibition (%)
	Without drug (control)	5% drug	
E.coli sp.	0.438	0.325	25.8
B. cereus sp.	0.486	0.331	31.9
M. Luteus sp.	0.350	0.295	15.7

APPENDIX B

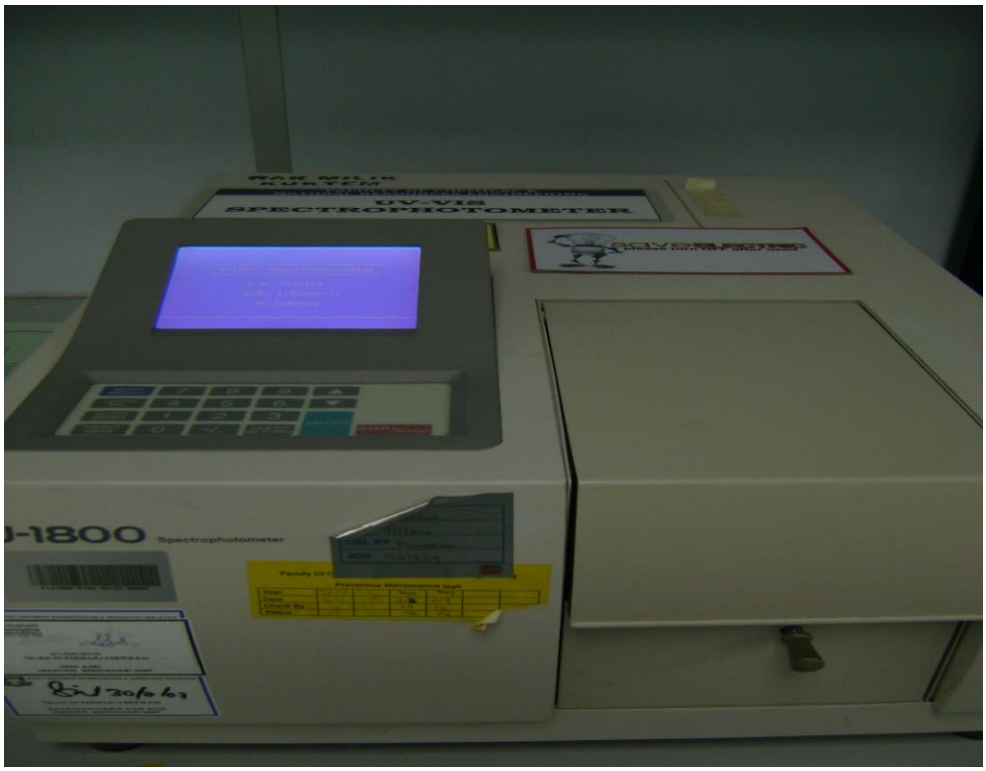
PICTURES

APPENDIX B 1 : Autoclave 50 L

APPENDIX B 2 : Incubator shaker

APPENDIX B 3 : Microbiological incubator

APPENDIX B 4 : Laminar flow : workplace



APPENDIX B 5 : Uv-Vis Spectrophotometer