ELECTROSPINNING DEVICE FOR BIOMEDICAL APPLICATIONS

BY

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ABSTRACT

In near future, Thailand will become an aging society. As a result, there will be an increase in the number of patients who suffer from elderly diseases and the accidents also may cause disease, injury and trauma can lead to damaged tissue. Therefore, the development of biomaterials would be one of the strategies to increase the treatment efficacy and capability to cope with the increased number of patients. Polymer fibers are one of the biomaterials used for medical applications. It has been reported that these fibers could enhance cell migration, wound healing, as well as stem cell differentiation. The common technique used to produce polymer fibers is electrospinning. It is the technique that applied electrostatic force to drive the flow of polymer solution towards the collector. The solvent evaporates whilst the solution is travelling through the air, and solid polymer fibers are deposited onto the collector as a result. It can be concluded from this study that the assembled device could produce electrospun PEO fibers in nanoscale. The variable that affects the fabrication of nanofibers include flow rate of syringe pump should be less than 0.5 ml/hr to avoid solution dripping from the needle tip during the electrospinning process. Moreover, the distance between the needle tip and collector should be 14 cm for 10 kV to allow sufficient evaporation for the polymer jet. The average diameter of electrospun fibers produced from the assembled device in this study are 136.79 nm from the static collector and 142.89 nm from the rotating collector. Therefore, the successful development of tissue engineering would be beneficial for patients around the world.

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The authors

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LIST OF ABBREVIATIONS/SYMBOLS

Abbreviations/Symbols	Term
A	Ampere
AC	Alternative current
cm	Centimeter
DC	Direct current
Ecm	Extracellular matrix
hr	Hour
kDa	Kilodalton
kV	Kilovolt
kΩ	Kiloohm
LCD	Liquid crystal displays
min	Minute
ml	Milliliter
mm	Millimeter
Mw	Molecular weight
m^2/g	Square meter per gram
MΩ	Megaohm
nm	Nanometer
PEO	Polyethylene oxide
R	Resistance
rpm	Revolutions per minute
SEM	Scanning electron microscopy
V	Volt
W	Watt
w/v	Weight per volume

CHAPTER 1
INTRODUCTION

1.1 Background and Significance of the Research

Nowadays, diseases, injuries, and accidents consistently occur in the global population which causes the damage, degeneration, or loss of one or more organs from the human body, such as nerves and bones. These incidents require a various type of therapeutic treatments to facilitate the tissue repair, replacement, or regeneration. Although the human body is having self-healing properties, their capabilities are dependent on the tissue types and also the severity of the injury [1]. In severe cases, tissue transplantation is typically used for treatments, including the autografting and allografting [2, 3]. Autografts are tissues transferred from other parts of the patient's body and possess the same properties as the native tissue. On the other hand, allografts are tissues from other donors. In some cases, xenografts are also used for transplantation, which is taken from animals [4]. However, autografts and allografts are commonly used by the doctors to treat the patients because of the quicker recovery time and high successful rate [5].

The advantages of autografting are the use of patients' own tissue that means the tissue is alive and consists of patients' own living cells and thus this is considered as the current "gold standard" [5]. Another advantages of using autografts is the low possibility of disease transmission and immune reaction [6]. Moreover, it is also different from allografting, which harvests the tissue from different donors that must be sterilized and preserved and thus allografts may degrade and lose some of the native tissue properties. However, the main advantage of allografting is usually the faster immediate postoperative recovery and lesser painful for the patients than autografting as it does not require tissue harvest surgery [7]. Likewise, there is also no donor site morbidity from allografting because allografts are obtained from reputable and reliable tissue banks with greater availability than autografts [4, 6]. Furthermore, the rehabilitation period for allografting could also be shorter due to the lesser operative injuries in the case that there is no complications from immune reactions [8]. Nonetheless, it should be noted that, since both autografting and allografting involve wound opening procedures, there is also a risk of pathological microbial infection on

the skin [9]. It can be seen that although these treatments have already been developed for a certain period of time, the disadvantages still exist in both techniques.

Recently, the field of tissue engineering are being widely studied to overcome the limitations of the current treatments. It is a branch of biomedical engineering that involves the use of biological science principles and a wide range of engineering discipline with the aims to regenerate the damaged tissues by combining cells, biomaterials or the scaffolds, and external stimulation [10, 11]. As biomaterials are one of the key components of tissue engineering, the development of biomaterials would be one of the strategies to increase the treatment efficacy and capability to cope with the increased number of patients [3]. Hence, the aim of this project is to design and manufacture an electrospinning device to fabricate polymer fibers, which are one of the biomaterials used for biomedical applications. It is expected that the findings from this study would be beneficial for further biomedical materials research that could help patients around the world.

1.2 Research Objectives

- To manufacture an electrospinning device that can produce polyethylene oxide fibers.
- To study the effect of electrospinning parameters on the fiber morphology.

1.3 Research Scope

There are three parts of electrospinning device to design and manufacture in this study, which consist of syringe pump, collector, and high voltage generator. The custom parts for syringe pump and collector are made by 3D printing, and the high voltage generator are adapted from the affordable components available commercially. There are two types of collector to study: static collector and rotating collector. The experiments will focus on the investigation of the effects of electrospinning parameters on the fiber morphology.

1.4 Research Outline

The following contents of this report is organized as follows:

Chapter 2 presents the relevant theory and literature review to this research.

Chapter 3 describes the design and implementation of electrospinning device.

Chapter 4 demonstrates the effect of concentration of the solution and the distance between the collector and needle tip on the nanofiber fabrication by the electrospinning method.

Chapter 5 discusses the obtained results and concludes the key points from this research, as well as suggesting the future work that can be further explored from this study.

CHAPTER 2
THEORY RELATED

2.1 Introduction to Tissue Engineering

Currently, the field of tissue engineering is widely studied with the aim to regenerate the damaged tissues by combining cells, biomaterials or scaffolds, and external stimulation [11]. In the history of tissue engineering, even though the term "tissue engineering" was officially coined in 1987 [12], the first historical reference that recorded about tissue engineering has existed since 1438–1490 by Fra Angelico. This person was an artist who painted the famous painting entitled, "The Healing of Justinian by Saints Cosmas and Damian" [13]. However, there was also an article in 500 - 400 BC, that may be the oldest written reference to "tissue engineering", which was Genesis II in which the text reads "*The Lord, breathed a deep sleep on the man and while he was asleep he took out one of his ribs and closed up its place with flesh. The Lord God then built up into a woman the rib that he had taken from the man*" [14].

In 1991, "*Functional Organ Replacement: The New Technology of Tissue Engineering*" was the first published article that used the term "tissue engineering", which is still being used nowadays [14].

In 1993, tissue engineering was defined by Langer and Vacanti as "*an interdisciplinary field which applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function*" [15].

Overview of tissue engineering strategies

The field of tissue engineering is important in regenerative medicine with the aim to provide biological substitutes that can regenerate, maintain, or improve the damaged tissues, which is related to many areas such as clinical medicine, materials science, genetics, biomedical engineering discipline and biological science [11, 16, 10]. Therefore, tissue engineering offers an alternative to the whole organ and tissue transplantation after damage caused by diseased, accident, or abnormally functioning [16].

Tissue engineering requires three main components that make up the tissue engineering triad, as shown in Figure 1:

(1) Scaffold in which cells are seeded.

(2) Cells or a source of cells such as stem cells from the donor tissue to support required tissue formation.

(3) Signaling molecules or growth factors [17].

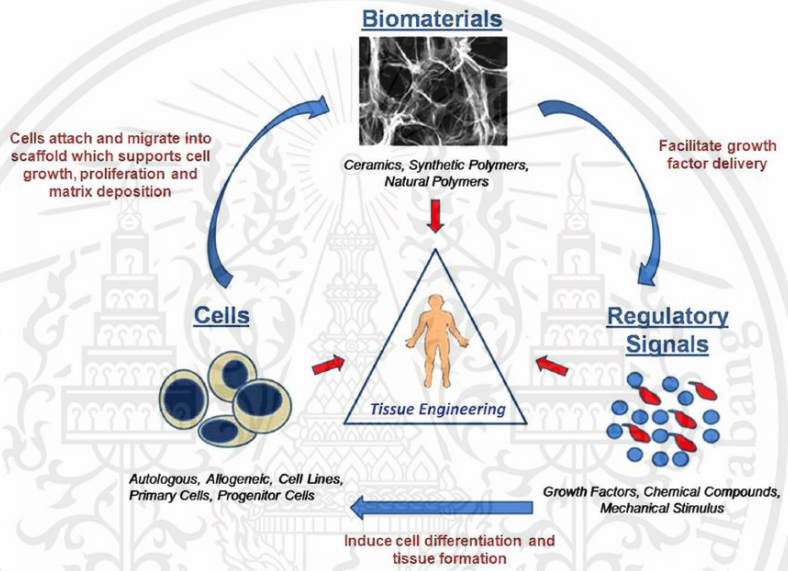


Figure 1. Three main components of Tissue engineering [17]

Scaffold

To achieve the aim of providing the biological substitutes, the use of a three-dimensional (3D) scaffolds is very important in the field of tissue engineering for enhancing cell adhesion, proliferation, and deposition of extracellular matrix (ECM) [11]. The scaffolds are produced from many biomaterials used in the field of tissue engineering to regenerate tissues, which is a form of 3D construct. A scaffold acts as a template to guide new tissue formation. Normally, it will use together with cells that are seeded into a scaffold and occasionally growth factors, as shown in Figure 1. The cell-seeded scaffold will be implanted into the injured site after being cultured *in vitro* to synthesize tissues, then the regeneration of tissues will be induced *in vivo* by the

interactions between the body and the tissue-engineered constructs to guide new tissue formation [2].

The use of scaffolds can be divided into two types: cell delivery scaffolds, which contain cells to be delivered to the injury site; and drug delivery scaffolds, which are delivering drug to the injury site [18].

Cells

Cell is the basic structural unit of all living substances. In the field of tissue engineering, cell is the main component to be used with scaffolds and occasionally growth factors to regenerate tissue or organ, as shown in Figure 1. There are two main categories of cells preferably used in tissue engineering, which are stem cells and progenitor cells. These cells can be found in almost every tissue, and they also have self-renewability and differentiation capability towards a various type of tissue-specific cells [18].

Signal

Signaling molecules or growth factors is also the third major component of tissue engineering that could regulate cellular activities, including cell adhesion, proliferation, migration, and differentiation, by adjusting the synthesis of protein, growth factors, and receptors [17]. Moreover, signals for tissue engineering could also be delivered in the form of external stimulation.

Examples of tissue engineering.

Normally, tissue engineering uses the cells from the native tissue to culture before seeding into the scaffold, including skin, cartilage, heart and bone tissue. For example, tissue engineering approach was used to repair the trachea by using a biodegradable scaffold tube, in which the amniotic fluid-derived mesenchymal stem cells were seeded, as shown in Figure 2 [19].

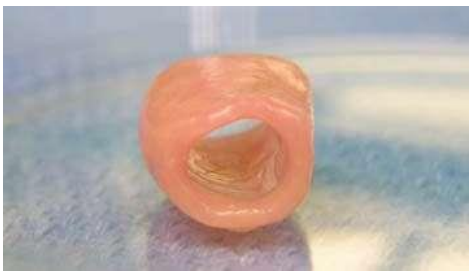


Figure 2. The biodegradable tissue engineered constructs loaded with mesenchymal stem cells [19].

2.1 Biomedical Materials for Tissue Engineering

Human tissues have a unique composition and highly organized structure which can help supporting the transport and mechanical load bearing to facilitate cellular and biological functions. Tissue injury, disease, trauma or aging can lead to the degeneration of one or more organs in the human body. Even though our tissues have their self-healing capability, but they have a certain limit that the natural healing process can be effective [20]. Therefore, it may need to use both natural biodegradable and biocompatible materials that can mimic the actual tissue and its structural organization in order to regenerate the functional tissue [21, 22]. Biomaterials play a significant role in the engineering of new functional tissues to replace the malfunctioning or lost tissues. They provide a temporary template and mechanical support to guide new tissue growth and organization, as well as providing the bioactive signals such as the cell-adhesion peptides and growth factors [23].

History of Biomaterial

Biological materials have existed alongside humans since the ancient times. According to records, the Romans and Egyptians used wood to make prostheses and plant fibers to sew their skin. However, biomaterial has the first definition only in 1982 as "*any substance, other than a drug, or a combination of substances, synthetic or natural in origin, which can be used for any period of time, as a whole or as a part of a system, which treats, augments or replaces any tissue, organ or function of the body*". The industrial revolution is the important development of biomaterials for tissue engineering because of the development of a synthetic biomaterial technology that enables the synthesis of polymers which are more suitable for medical devices than

metals. Nowadays, both natural and synthetic biomaterials are important elements used in tissue engineering strategies [11]. However, biomaterial has a new definition which was defined in 2018 as "*a material designed to take a form that can direct, through interactions with living systems, the course of any therapeutic or diagnostic procedure*", which is being applied until today [24].

Biomaterials can be categorized into three types [23]:

1) Natural materials such as collagen and alginate.
2) Acellular tissue matrices such as bladder and small-intestinal submucosa; and
3) Synthetic materials such as polyglycolic acid, polylactic acid, metallic components, ceramics, or composite materials.

Several types of scaffolds have been produced recently with various manufacturing system. However, the material selection for tissue engineering scaffold production is still being an issue. Currently, biological materials have also been used in tissue engineering alongside the natural or synthetic polymers, ceramics, metals, composite and hydrogels. Nonetheless, the important thing for determining the suitability of a scaffold is whether it meets the three requirements described below [11]:

1) Biocompatibility

Biocompatibility is the main properties required from the scaffold of tissue engineering. This property could direct cellular adhesion, proliferation and migration on the surface of the scaffold, either *in vitro* or *in vivo* [23]. Moreover, the description of biocompatibility is that the material has "*the ability to perform with an appropriate host response in a specific application*" without eliciting immune responses in the body that could lead to inflammatory reaction, the declined healing, or the tissue rejection [25]. However, the description of biocompatibility may differ in each application, though every biocompatible material should perform as intended without adverse effects.

2) Bioactivity

The bioactivity demonstrates the capability of a biomaterial to interact with the host tissue through their interfaces to promote tissue integration and triggering biological responses. Generally, the bioactivity of materials is higher when supplemented with chemical stimulating factors. Moreover, extracellular matrix proteins, including collagen, fibronectin, and laminin, can be used to supplement the material bioactivity to produce a biomimetic environment and regulate the cellular behavior [11].

3) Biodegradability.

The last necessary property of scaffold for tissue engineering is biodegradability. Because biomimetic scaffolds are not permanent implants, it is just a supporter to provide the sufficient time for the cells to produce the native extracellular matrix. Furthermore, they must also be non-toxic and easily disposed of from the body without destroying other tissues. On the other hand, it is also important to understand the *in vivo* degradation kinetics of biomaterials so as not to eliminate them too quickly or too slowly. These may be dependent on the interactions between the scaffold and cells, as well as the inflammatory responses from the host to the scaffold [11]. For example, nanofibrous scaffolds which are used for orthopedic tissue repair and regeneration. These biomimetic scaffolds reported to promote cellular activities through the interactions with the extracellular matrix proteins. [26]. Furthermore, biodegradable polymers are also of interest as the follow-up surgery is not required to remove any undesired leftover materials [27].

Apart from these aforementioned properties, the scaffolds are expected to possess good mechanical properties as well, such as the tensile and compressive strength. Biomaterials are also used as a part of the medical devices to recover the tissue functions, such as heart valves, artificial hips, or the dental implants [28]. However, it was suggested that most biomaterials are not as efficient for pediatric patients because of the continuous tissue growth that affects the environmental stability of the biomaterials [21].

Example for polymers used for tissue engineering.

- Polyphosphazene

Polyphosphazenes are biodegradable polymers that have various potential for tissue engineering applications. They are linear high molecular weight polymers with inorganic backbone, consisting of phosphorus and nitrogen linearly bonded by alternating single and double bonds [26]. The synthetic flexibility of polyphosphazene present the development of a variety of polymers with various physical, chemical and biological properties, which can be suitable for tissue regeneration [29]. Another feature is that these biodegradable polyphosphazenes undergo hydrolytic degradation. Due to the buffering capacity of phosphate and ammonia produced at the same time during the degradation of polyphosphazenes, the degradation by-products are non-toxic and have a neutral pH. Polyphosphazenes play an important role in drug delivery and tissue engineering applications. They have been used to deliver anti-inflammatory drugs, chemotherapy, growth factors, DNA, proteins and vaccines. Generally, several rapidly biodegradable polyphosphazenes are used for drug delivery applications and have more hydrophobic side group substituents in tissue engineering applications. They are usually used to produce three-dimensional, porous, biodegradable scaffolds that can provide temporary supports for the tissue growth. Polyphosphazenes have been widely investigated for use in orthopedic engineering, nerve guides, tendon and ligament tissue engineering and blood contact materials [30].

- Polylactide

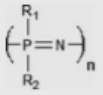
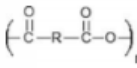
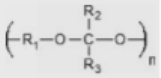
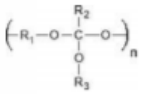
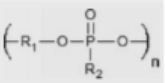
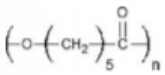
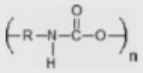
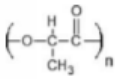
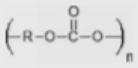
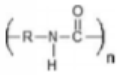
Poly(lactic acid) (PLA) is one of the most widely used synthetic polymers for the biomedical applications. It is a synthetic polyester that exhibits wide-ranging benefits in tissue engineering. As polylactic acid (PLA) has a chiral molecule, PLA is divided into four forms: poly(L-lactic acid) (PLLA); poly(D-lactic acid) (PDLA); poly(D,L-lactic acid) (PDLLA) that is a mixture of PLLA and PDLA; and meso-poly(lactic acid) [28]. It can be synthesized by ring-opening polymerization or polycondensation. The polymers of PLA family degrades through the hydrolysis into lactic acid, which is also a cell metabolic by-product. PLA nanofibers are also used to

produce scaffolds for regenerative medicine and drug delivery applications, and some of which are fabricated via electrospinning technique, as shown in Table I. The use of nanofibrous scaffolds provides desirable properties similar to native tissue, including high surface area, similar architecture to the native extracellular matrix, and tailorable mechanical properties. It was reported that electrospinning and thermally induced phase separation (TIPS) are among the most common techniques used to manufacture fibrous scaffolds [31]. The electrospun nanofibrous scaffolds have been widely studies for using as scaffolds for bone, cartilage, tendon, nerve and blood vessel regeneration [32].

- Polycaprolactone

Polycaprolactone (PCL) is a semi-crystalline polyester with high solubility in organic solvents. It also has biodegradable and biocompatible properties which could be used in various applications in tissue engineering. Polycaprolactone has also been used in drug delivery applications, but they have limited degradation, as shown in Table I. Although PCL has low tensile strength (~23 MPa), it has high elongation at breakage (4700%) that makes it to be a brilliant elastic biomaterial. Furthermore, PCL is also highly malleable that it could be processed into a wide range of shapes and sizes because of its low melting temperature and viscoelastic properties [26]. PCL has been shown to be one of the successful scaffolds for long-term bone implantation, and it is also bioresorbable. Since the majority of the inorganic bone components is made of hydroxyapatite, several recent studies have focused on the fabrication of composite PCL scaffolds loaded with hydroxyapatite nanoparticles [33]. PCL and PCL composites have also been used in the tissue engineering of other tissue types, including ligament, cartilage, skin, nerve, and vascular tissues [26].

TABLE I. Nanofibers for Biomedical Applications [27]

Polymer	Applications	Advantages	Disadvantages	λ, Degradation Rate Constant (s^{-1})	Structure
Polyphosphazenes	Tissue Engineering; Vaccine Adjuvant	Synthetic Flexibility; Controllable Mechanical Properties	Complex Synthesis	$4.5 \times 10^{-2} - 1.4 \times 10^{-7}$	
Polyanhydrides	Drug Delivery; Tissue Engineering	Significant Monomer Flexibility; Controllable Degradation Rates	Low-molecular Weights; Weak Mechanical Properties	$1.9 \times 10^{-3} - 9.4 \times 10^{-9}$	
Polyacetals	Drug Delivery	Mild pH Degradation Products; pH Sensitive Degradation	Low Molecular Weights; Complex Synthesis	6.4×10^{-5}	
Poly(ortho esters)	Drug Delivery	Controllable Degradation Rates; pH Sensitive Degradation	Weak Mechanical Properties; Complex Synthesis	4.8×10^{-5}	
Polyphosphoesters	Drug Delivery; Tissue Engineering	Biomolecule Compatibility; Highly Biocompatible Degradation Products	Complex Synthesis	1.4×10^{-6}	
Polycaprolactone	Tissue Engineering	Highly Processable; Many Commercial Vendors Available	Limited Degradation	3.5×10^{-8}	
Polyurethanes	Prostheses; Tissue Engineering	Mechanically Strong; Handle Physical Stresses Well	Limited Degradation; Require Copolymerization with Other Polymers	8.3×10^{-9}	
Polylactide	Tissue Engineering; Drug Delivery	Highly Processable; Many Commercial Vendors Available	Limited Degradation; Highly Acidic Degradation Products	6.6×10^{-9}	
Polycarbonates	Drug Delivery; Tissue Engineering; Fixators	Chemistry-Dependent Mechanical Properties; Surface Eroding	Limited Degradation; Require Copolymerization with Other Polymers	4.1×10^{-10}	
Polyamides	Drug Delivery	Conjugatable Side Group; Highly Biocompatible Degradation Products	Very Limited Degradation; Charge Induced Toxicity	2.6×10^{-13}	

2.2 Biomedical Materials Produced by Electrospinning

As mentioned previously, electrospinning is one of the methods to produce nanofibers by applying electrostatic force to drive the flow of polymer solution towards the collector. Nanofibers can be used in a wide range of biomedical applications such as dental applications, drug delivery, wound dressings, scaffolds for tissue engineering, and the manufacture of protective clothing [34]. The properties of the ideal polymers for biomedical applications should be biocompatible, biodegradable non-toxic, and mechanically strong [35]. Electrospun nanofibers can enhance cell adhesion, growth, migration, seeded [36]. The advantages of the electrospinning technique are the production of nano-sized fibers with almost defect-free and high surface-area-to-volume ratio. This makes the fibers strong and are also useful in applications that require a large surface area [37]. Furthermore, it also has high aspect ratio, ultrahigh porosity, good biocompatibility, good bioabsorbability and selective permeability that have high potential in the field of biomedical engineering, depending on the type of polymer used [38].

Example of electrospun nanofibers for biomedical applications

1) Wound Dressing/Healing

The wound dressing is one of the significant procedures in the biomedical field. Wound beds are moist, nutritious, and warm environment that provide an optimal condition for microbial growth [36]. Once the skin is injured, there is limited protection for the internal tissues from the external environment underneath the skin. In other words, there is a risk of microbial migration into the internal tissues, which causes infection, healing delay, and sometimes it can be fatal [36, 39]. Inflammation is an important part of wound healing because it is the early response from the tissue to injuries and infection, involving the elevation of pro-inflammatory cytokines and matrix metalloproteinases. The severe bacterial infection could prolong the inflammation and cause complications from the biofilms developed from the bacterial colonies [39]. It is understood that chronic diseases, burns, or even surgery can damage the skin and cause wound infection [40]. As a result, wound dressings are carried out to enhance the wound healing process by creating a sealed and moist environment, as shown in Figure 3 [41]. These techniques can be used to treat wounds and burns and

enhance skin regeneration, as well as reducing scar formation [36]. Normally wound dressing was originally produced from gauze and cotton, which have low cost and high absorbency. However, it just only isolates the wound from the contaminants and lets the wound healing process occur passively. Furthermore, it can also dehydrate and adhere to the wound which cause discomfort and pain, and potentially delay wound healing [40]. Therefore, the development of advanced functional wound dressing has been focused on in order to accelerate wound healing process and reducing the cost [39]. The example would be an antimicrobial dressing that provides moist environment, broad-spectrum antimicrobial behavior, and gas permeation [36].

Nowadays, there are many types of materials specifically used for wound dressings to treat different types of wound, as shown in Table III. Each type has different structures and functions for clinical uses, such as synthetic dressings, natural dressings, tissue engineered dressing, and medical dressings [40]. There are reports discussed the fabrication technique to produce nanofibers that could mimic the structure and architecture of the natural extracellular matrix. For this approach, the natural ECM is replaced by nanofibers to allow the host cells to grow by themselves and produce new ECM. Nanofiber mats were shown to initiate signaling pathways and induce fibroblast migration towards the dermal layer. For wound healing, it requires nanofibers with pore diameter between 500 nm and 1 mm and high surface area to protect the wounds from bacteria. The porous structure also facilitates the diffusion of drug molecules from the fibers. These nanofibers can be produced by electrospinning [36].

Figure 3. Schematic representation of a wound dressing that creates a sealed wound environment to prevent pathogens and promotes the wound healing process [41].

The structures of wound dressing materials have been developed to promote wound healing process, such as fibrous, sponges, films, hydrogels and hydrocolloids [42]. The electrospun nanofibrous membrane could provide three-dimensional support and mimic the structure of the natural ECM, which is supportive to cell growth, proliferation, and adhesion. Moreover, it also has great potential for wound dressing as the nanofiber mats have high porosity and small pore that can support bacterial isolation and allow gas exchange during the wound repairing state as well, as shown in Table II. Likewise, it has also been proved that the high surface to volume ratio of nanofibers is advantageous for drug delivery applications [40].

TABLE II. The characteristics of electrospun nanofibers that make them more suitable for wound dressings [40].

Ideal characteristic	**Advantages**
Fiber diameter (50~500 nm)	ECM-like structure and nanosized
High specific surface area	Promotes hemostasis of damaged tissues
High porosity (60%-90%)	Conducive to cell respiration and gas penetration
Cross-linked porosity	Can meet the need of cutting
Mechanical strength	Similar to human skin tissue

TABLE III. Examples polymer that produced by electrospinning for wound dressing

	Polymer	**Solvent**	**Wound types**	**Advantages**	**Disadvantages**
Synthetic	poly(lactic-co-glycolic acid) (PGA) [39, 43, 44, 45]	Dichloromethane		- biocompatibility - biodegradability - flexibility - minimal side effects - good mechanical properties - low immunogenicity - low toxicity	- there are some deficiencies limit.

	Polymer	Solvent	Wound types	Advantages	Disadvantages
	polycaprolactone (PCL) [39, 45]	Dichloromethane/ Dimethyl formamide	acute and chronic wounds	- biocompatibility - biodegradability - bioresorbable - non-toxic - can be easily processed into different shapes and forms	- poor antimicrobial properties.
	polyvinyl alcohol (PVA) [45, 46, 47]	Water		- can easily form hydrogels - biocompatibility - hydrophilic properties	- poor mechanical stability at swollen state.
	polyethylene glycol (PEG) [39, 45, 48]	Water/chloroform		- biocompatibility - non-toxic - non-immunogenic - flexible ether based polymer - good affinity	- no absorption capability
	polylactide (PLA) [42, 45, 49]	Chloroform		- biocompatibility - biodegradability - good mechanical properties	- no antimicrobial properties without suitable treatment
Natural	cellulose [45, 50, 51, 52]	Acetone	burns, chronic wounds, plastic/recon- structive surgeries	- biocompatibility - biodegradability - non-toxic - moisture retaining properties. - absorb exudates. - high porosity	- wound adherence - limited exudate absorption - residue deposition on a wound site.
	collagen [28, 45, 50]		bed sores, minor burns, foot ulcers,	- biocompatibility - mechanical strength - biodegradable	- increased infection rates

	Polymer	Solvent	Wound types	Advantages	Disadvantages
			large open cuts, chronic wounds, low to heavy exudation wounds, surgical wound	- metalloproteinases.	
	chitosan [39, 45, 50, 53, 54]	Mixture of dichloromethane and triflouro acetic acid (TFA)	acute wounds, pressure ulcers, hemorrhagic wounds	- biocompatibility - biodegradability - antimicrobial - nontoxic - high porosity	- low solubility

2) Drug Delivery Systems

The electrospinning technique is also widely applied in the field of drug delivery systems, as shown in Table IV. In general, the amount of drug delivered to the target site is much lower than the initial orally ingested drug dose due to the loss during the transportation inside the body, and this is unfavorable for patients because they are required to take extra dose to compensate the loss [41]. For this reason, electrospun nanofibers are used to enhance the drug delivery process to the target site by providing controllable and predictable drug release and dissolution inside the body once being implanted [55]. It could be expected that the use of electrospun nanofibers can help decreasing the excessive drug intake by the patients.

Materials used for drug delivery system should be biocompatible, biodegradable, and stimuli responsive [41]. Hence, electrospun nanofibers are excellent selection for drug delivery systems, because they can be produced from natural, synthetic, and composite materials in nano- and micrometer size and exhibit the aforementioned properties [56, 57], as shown in Figure 4. Moreover, the high surface-

to-volume ratio of the electrospun nanofibers could also improve the efficiency of drug delivery by accelerating the drug solubility. However, the structure of the nanofibers and surface morphology are the important factors for controlling the drug release and rate. Moreover, the drug stability upon the exposure to gastric acid and enzyme could be protected by loading into the electrospun polymers in order to maintain the bioactivity of the drug. On the opposite hand, nanofibrous scaffolds also serve as templates for the assembly of drug-loaded polymer systems [57].

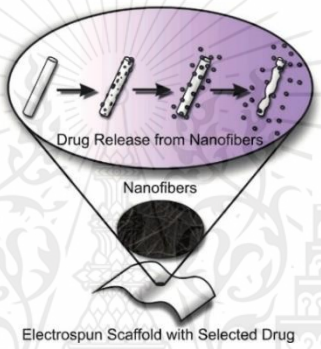


Figure 4. Release of drug components from an electrospun nanofiber [58].

TABLE IV. Examples polymer that produced by electrospinning for drug delivery systems.

	Polymer	**Solvent**	**Delivery system**	**Advantages**	**Disadvantages**
Synthetic	poly(lactic-co-glycolic acid) (PGA) [44, 45, 57, 58]	Dichloromethane	Mats	- biocompatibility - biodegradability - flexibility - minimal side effects - good mechanical properties - low immunogenicity - low toxicity	- there are some deficiencies limit.

	Polymer	Solvent	Delivery system	Advantages	Disadvantages
	polyvinyl alcohol (PVA) [45, 46, 47, 57, 58]	Water	Mats	- biocompatibility - hydrophilic properties	- poor mechanical stability at swollen state.
	polyethylene glycol (PEG) [45, 48, 57, 58]	Water/chloroform	Mats	- biocompatibility - non-toxic - non-immunogenic - flexible ether based polymer - good affinity	- no absorption capability
	poly(L- lactic acid) (PLLA) [57, 58, 59]	Water/chloroform / methyl chloride	Mats	- biocompatibility - biodegradability - degradation in living organisms	- high rigidity and hydrophobicity limit its use in some areas
Natural	Cellulose acetate [45, 50, 51, 52, 57, 58]	Acetone	Mats in hard gelatin capsules	- biocompatibility - biodegradability - non-toxic - moisture retaining properties. - absorb exudates. - high porosity	- limited exudate absorption
	Gelatin [31, 57, 58]	Glacial Acetic Acid and 2,2,2-Trifluoroethanol.	multilayered gelatin mesh	- biocompatibility - biodegradable - reduced side-effects - highly selective cell targeting. - more stable complexation between carrier and drug - higher drug encapsulation efficiency	- low antigenicity

	Polymer	Solvent	Delivery system	Advantages	Disadvantages
				- reduced immunogenicity	
	chitosan [45, 50, 53, 54, 57, 58]	Mixture of dichloromethane and triflouro acetic acid (TFA)	nanofilm	- biocompatibility - biodegradability - antimicrobial - nontoxic - high porosity	- low solubility

3) Tissue-engineered Scaffold

Electrospinning is a simple method to produce scaffolds with an interconnected pore structure and small diameter fibers. Electrospun fibrous scaffolds have been extensively use in tissue engineering field, including both 2D and 3D [60], as shown in Table V. Since the cells can attach to the fibers, the geometry and size of the fibers could affect cellular activities, such as cell proliferation and adhesion [36]. Normally, large-diameter fibers are not a good selection to mimic native structures because of the size difference, and this is why nanofibers are more preferable for tissue engineering [41]. Due to a wide range of available material properties for electrospun scaffolds, they have already been used in various types of tissue-specific applications, including vascular, neural, bone, cartilage, and tendon/ligament [61].

Vascular tissue engineering is the one type of tissue engineering that carries important task by regenerating or restoring the functions of blood vessels which are responsible for transporting blood from the heart towards the rest of the body. The electrospinning process is widely used for vascular tissue engineering to fabricate electrospun fibers at different lengths and diameters from both natural and synthetic polymers to be used as scaffolds for vascular transplantation [41].

Neural tissue engineering is the method for nerve regeneration in human body. Because of the lack of cellular cues that guide and support nerve regeneration, the regeneration of the brain after injury is usually slow or ineffective. Electrospinning has many advantages for the fabrication of neural scaffolds, using which the scaffold

properties and functions can be manipulated, such as the incorporation and delivery of bioactive molecules and the culture of different types of neural cells. Electrospun fibers have been highly successful to repair the injured brain by biological and physical cues [62].

Bone tissue engineering is the one type of tissue engineering that aims to regenerate the bone following the injuries. It is suggested that this approach would result in a shorter healing time compared to traditional procedures by using the technique that regulates bone cell migration, proliferation, and differentiation, as well as accelerating bone matrix formation. Electrospun nanofiber have been used to produce 3D macroporous nanofibrous (MNF) scaffold which yarns for bone tissue engineering [63].

Cartilage tissue engineering is the one type of tissue engineering that needs the biodegradable scaffold with suitable compressive strength and elasticity. Electrospinning can produce electrospun fibers from natural, synthetic, and composite polymers with the mentioned properties. For these reasons, electrospun fibers are also widely used in cartilage engineering [56].

Tendon and ligament tissue engineering also uses electrospun fibers for the regeneration and replacement of tendon and ligament tissues. These tissues have hierarchical structured morphology and non-linear mechanical properties and require fibrous texture to facilitate cell growth and extracellular collagen formation. Hence, electrospinning is one of the most promising methods to use in tendon and ligament tissue regeneration and the replacement because of the ability to produce the fibrous scaffolds as required [64].

TABLE V. Examples polymer that produced by electrospinning for tissue engineering scaffold.

	Polymer	Solvent	Tissue engineering types	Advantages	Disadvantages
Synthetic	Poly (glycolic acid) (PGA) [41, 65]	acetone, dichloromethane, chloroform, ethyl acetate, tetrahydrofuran	Blood vessels	- biocompatibility - biodegradability - tensile strength	
	polyethylene glycol (PEG) [41, 45, 48]	Water/chloroform	Blood vessels	- biocompatibility - non-toxic - non-immunogenic - flexible ether-based polymer. - good affinity	- no absorption capability
Natural	cellulose [41, 45, 51, 52]	Acetone	Bone tissue	- biocompatibility - biodegradability - non-toxic - moisture retaining properties. - absorb exudates. - high porosity	- limited exudate absorption - residue deposition on a wound site.
	collagen [28, 41, 45, 50]		Blood vessels	- biocompatibility - mechanical strength - biodegradable - metalloproteinases.	- increased infection rates
	Gelatin [41, 66]	Glacial Acetic Acid and 2,2,2-Trifluoroethanol.triflouro acetic acid (TFA)	Blood vessels	- biocompatibility - biodegradability	- low antigenicity

2.3 Principle of Electrospinning

Polymer fibers are one of the biomaterials used for several field applications. It has been reported that these fibers could enhance cell migration, wound healing, stem cell differentiation [67], solar cell fuel cell, polymer cell, air filtration, plant covering, as well as air and pollen filters [68]. There are various techniques of polymer fibers production. One of the most common technique used to produce polymer fibers is electrospinning. It is a simple technique that applied electrostatic force to drive the flow of polymer solution towards the conductive collector. The solvent evaporates whilst the solution is travelling through the air, and solid polymer fibers are deposited onto the collector as a result. The advantages of the electrospinning technique are the production of electrospun nanofibers with almost defect free and also has a high surface area to volume ratio. This makes it strong and is also very useful in applications that require a large surface area [37]. There are three major components of the electrospinning device include high voltage power supply, syringe pump, and a conductive collector, as shown in Figure 5. The process of the electrospinning started when a syringe pump is working to deliver the solution at a constant flow rate and the high voltage is applied to create an electric field between the needle tip and the conductive collector, then the polymer solution or polymer melt is drawn by electrostatic force and transforming into polymer at the conductive collector. Polymer fibers have a wide range of properties suitable for plenty of applications, including tissue engineering. Electrospinning can possibly produce nanofibers from almost any material, depending on the intended purpose because it works on a molecular level [69].

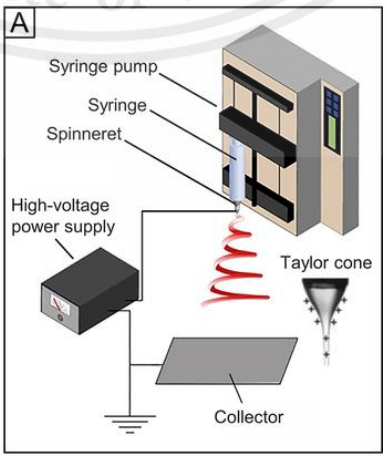


Figure 5. Component of electrospinning [67].

History of Electrospinning

The electrospinning had the first patent filed in 1900s by J. F. Cooley. Then, electrospun fibers were applied as wound dressing in 1974 and were studied as implantable vascular graft in 1978 [70]. In recent years, the electrospinning method has regained more interest maybe because of the attention in the field of nanotechnology. In fact, the name "electrospinning" of this method derived from "electrostatic spinning" [71].

Electrospinning process

The forming process using the electrospinning method is consists of three major components are high voltage generator, a syringe pump with a solution needle, and a conductive collector, as shown in Figure 5.

A) High voltage source

The high voltage source is the most important and quite complicated part in the electrospinning method to fabricate the fibers. The high voltage source that is used in the electrospinning process should be able to generate the high voltage 10 - 50 kV [72]. There are two devices which can be used as the high voltage source that are high voltage power supply and high voltage generator.

- AC to DC high voltage power supply

The high voltage power supply is the device that converts low AC voltage into high voltage by using high voltage transformer and then converts the output into DC using a rectifier.

- High voltage generator

The high voltage generator is a device that generates the step-up voltage from low to the high. There are many principles to construct of high voltage generator. For example, the principle of the Cockcroft-Walton uses voltage multiplier capacitors and diodes to convert AC or pulsing DC from low into high voltages [73]. The other principle is a switching circuit that uses the converter or the transformer, such as Flyback converter or transformer, to step up the low voltage to the high voltage [74].

- Heat sink/Fan

Heat sink is a device that usually comes with a build-in fan to absorb and disperse heat away from high temperature electrical and electronic components [75], as shown in Figure 6. Heat sink is made of high heat conducting materials, such as aluminum and copper, and has large surface area. The contact between cool air blown by the fan and the heat sink would induce heat convection from the source to the surrounding air [76].

Figure 6. Heat sink and fan [77, 78].

B) Syringe pump

A syringe pump is a device that is used to deliver fluid at an accurate and precise rate. Generally, the syringe pump consists of the pusher block, syringe holder, an internal stepper motor and an LCD touchscreen interface. There are 2 types of syringe pump which are medical syringe pump and research syringe pump. The medical syringe pump is a device used to deliver essential fluids, such as nutrients, blood, and drugs, to a patient. The research syringe pump is used in to deliver fluids in research laboratories that require high precision and low flow rate [79].

- Stepper motor

Stepper motor is an electric motor with rotating shaft as the main feature that provides steps which is the movement at a certain degree increment. This feature allows the precise control of shaft's angular position without using sensor by simply counting steps performed, as shown in Figure 7 [80].

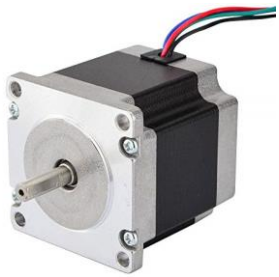


Figure 7. Stepper motor [81].

- Motor driver module

Motor driver is a device that connects between motors and control circuits, as shown in Figure 8. Since the motors require high current whereas the controller circuit uses low current signal to work, the function of this motor driver is to convert a low-current control signal to the higher current signal in order to drive the motor [82].

Figure 8. Motor driver module [83]

- Arduino Uno

Arduino Uno is a circuit board that contains all necessary components for microcontrolling applications, such as 14 digital input/output pins, 6 analog inputs, a USB connection, a power jack, and a reset button, as shown in Figure 9 [84].

Figure 9. Arduino Uno [85]

- Liquid crystal display (LCD)

Liquid crystal display or LCD is a device used to display information on the electronic devices, as shown in Figure 10 [86]. The principle is that the back of the screen is illuminated, also known as backlight, when an electric current is released to stimulate the crystal, it will make the crystal transparent, causing the light coming from the Backlight to appear on the screen. The other parts that are blocked by crystals have different colors depending on the color of the crystal [87].

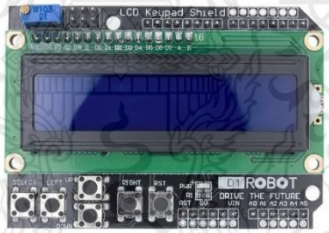


Figure 10. LCDs

C) Collector

The collector is grounded to create a stable potential in the process of electrospinning. The collector should be conductive to collect the nanofiber sheet. There are many types of collector used for the electrospinning technique, such as static collector or plane plate collector, rotating collector, grid type collector, and the edge type collector [88].

The tip of needle was connected to a high voltage power supply and a conductive collector was connected to ground of high voltage power supply to create an electric field between the needle tip and the conductive collector. A flow rate of solution was controlled using a syringe pump [89].

The formation of electrospun nanofibers would require the appropriate parameter settings, including the voltage, flow rate, the distance between needle and collector, as well as the polymer concentration [90].

Electrospinning uses a high voltage from 10 to 50 kV between a needle tip of a syringe filled with a polymer solution and a conductive collector [60]. Once the high voltage is applied to the needle tip, where polymer solution is pumped to, the surface tension of the solution is created by the electrostatic force. When the surface tension is overcome by the electrostatic force, the droplet is distorted into the cone shape called the Taylor cone. The charged polymer jet at the tip of the Taylor cone would then travel across the gap to the collector. For the static collector, randomly oriented fiber mats are formed, while the rotating collector could provide aligned nanofibers, as shown in Figure 11. This is because the rotating collector provided shearing and elongation forces that help adjusting the fiber direction and align the fiber layers [91].

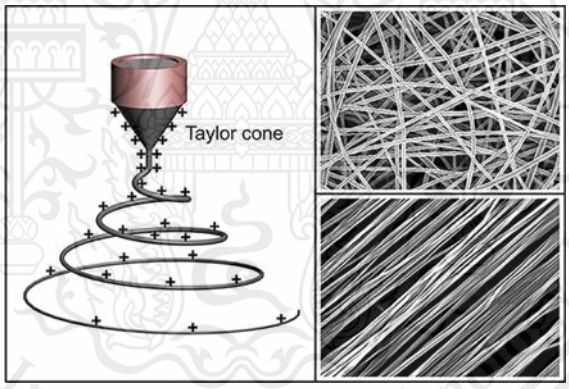


Figure 11. Taylor cone and forming nanofibers of static collector and rotating collector [67].

It was reported that the controlled the flow rate at 0.5 mL/hr, high voltage power supply at 12 kV, and distance between a conductive collector and a tip of solution needle is 18 cm. The polymer fibers with diameter about 0.1 mm could be continuously produced for 8 hours [92].

Although the electrospinning technique is practically a simple technique. However, the theoretical mechanism behind the spinning is quite complicated. The core of electrospinning method is to generate a continuous jet of a solution polymer to form nanofibers by immobilizing charges on the surface of a liquid droplet, and the spinning

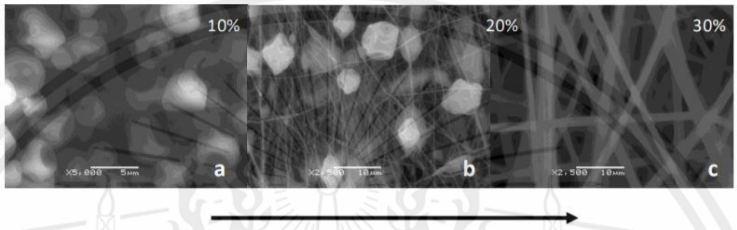
process is a result of whipping of a liquid jet. The electrostatic interaction between the surface charges on the jet and electric field causes the instability of the whipping of a liquid jet. The ability of the unstable fluid to survive the elongation and acceleration during the whipping process and form the nanofibers depends on the solution viscoelascity. Optimizing suitable parameters is important to produce electrospun fibers and is one of the most time consuming process for the research in the field as there a number of available parameters [67]. The electrospinning parameters can be separated into three major groups, which are the solution parameters, the process parameters, and the ambient parameters [68], as shown in Figure 12.

Solution parameters include solvent, solution temperature, additives, viscosity, concentration, solution surface tension, and molecular weight [68]. The concentration of polymer solution affects the controlled flow rate of solution and distance between a conductive collector and a tip of solution needle. The concentration of polymer solution will be an important factor that influences the morphology of the electrospun fibers, as shown in Figure 13. If the concentration of polymer solution is low, a polymer solution pumped out from the needle tip cannot form polymer fibers, and it will break into small droplets instead [93]. This event that occurs is called electrospraying [94]. On the other hand, the increased concentration of the polymer solution could increase the fiber diameter: however, in the case that the concentration of the solution is too high, the polymer solution flow would not be continuous due to the excessive viscosity [93].

Process parameters include flow rate, type of a collector, diameter of a needle, the distance between a tip of solution needle and conductive collector, and the applied high voltage which are also playing a crucial role in the production of fibers, as well as the reproducibility [68].

Ambient parameters include humidity and temperature which also have an impact on the morphology of the fibers [68].

Figure 12. Parameters of electrospinning [68].



The concentration of polymer solution

Figure 13. Scanning electron microscope of polymeric plus which the voltage is 15 kV and use different concentration of polymer solution by using dichloromethane-ethanol ratio 3:7 as a solvent [95].

The Advantage of Nanofibers Produce by Electrospinning.

There are many advantages of nanofibers produced by electrospinning, such as high porosity, high surface area (1 - 100 m^2/g), small diameters (10 nm - 10 mm), small fiber to fiber distance [68], low cost to produce, and the various types of material that can be produced [37].

The Disadvantage of Nanofibers Produce by Electrospinning.

Although electrospinning has many advantages, it also has several limitations. For example, the performance is dependent on the polymer used, and the limited performance and range of application of electrospun inorganic nanofibers due to their brittleness after calcination [96].

Other Applications of Electrospinning

Due to special characters of nanofibers such as production process, diameter, and fiber filament, these fibers can also be applied in a wide range of application beyond the biomedical field, including energy (solar cell, fuel cell, polymer cell), automotive (air and pollen filters, sound insulation, composite materials), agriculture (micro greenhouses, plant covering, agricultural protection), filtration (air filtration, oil filtration, fuel filtration, liquid filtration), technical textile (performance textile, windproof, water-resistant, highly breathable, antibacterial), and protective material (thermal protection, biological protection, chemical protection, magnetic protection), as shown in Figure 14 [68]. However, it should also be noted that nanofibers from electrospinning still require further improvement in regard to their functionality and the reproducibility of the electrospinning processes [97].

Figure 14. Applications of Electrospinning [68]

Techniques for Characterizing the Electrospun Nanofibers.

1) Scanning Electron Microscope (SEM)

A scanning electron microscope (SEM) is a type of electron microscope that uses a focused beam of electrons to produce images of the surface morphology of the sample. SEM has magnification ranging from 20X to approximately 100,000X which suitable to investigate the sample from the electrospinning technique that produces the fibers in nanoscale [98]. The maximum resolution obtained in an SEM depends on several factors such as the electron spot size and interaction volume of the electron beam with the sample. Some SEMs can obtain resolution below 1 nm [99].

Principle

SEM works by generating the electron beam at the top of the column and accelerating them towards the surface of the sample focused through the set of lenses, as shown in Figure 15. The pressure within the SEM chamber is at low vacuum state, and the level of vacuum depends on the type of microscope. The position of the beam is controlled by the scan coils, which scan the electron beam across the surface of the samples. Signals are produced from the electron-sample interactions and scattering. These signals are detected by an appropriate detector and processed into the image, as shown in Figure 15 [99].

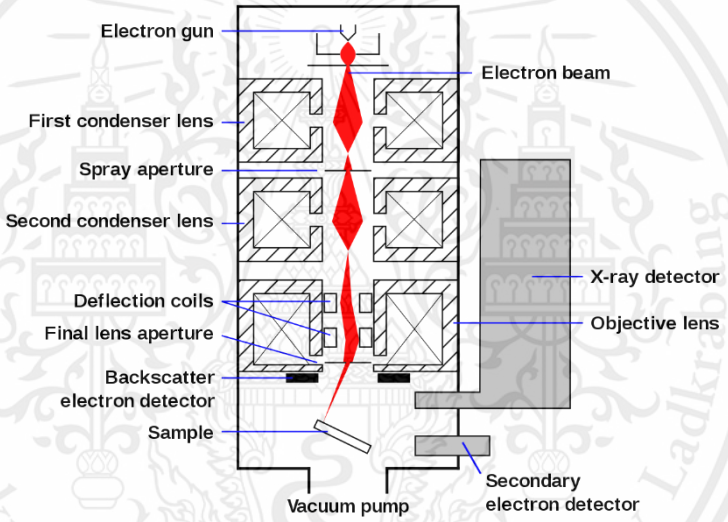


Figure 15. Schematic of a Scanning Electron Microscope [100].

2) ImageJ

ImageJ is an image processing program that is public domain Java. It can be used to analyze area, mean, min, max, angle and length from sample image using the available toolbar, as shown in Figure 16 [101].

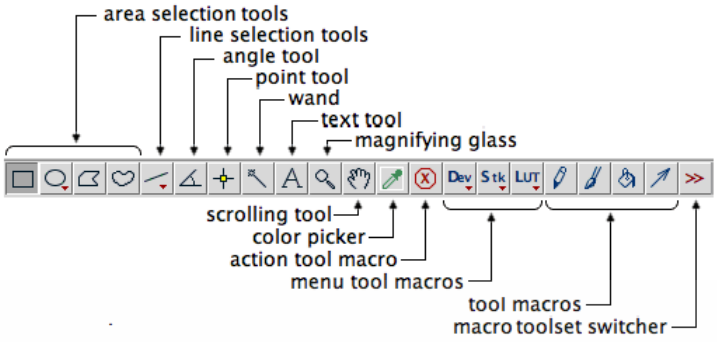


Figure 16. ImageJ toolbar [101].

CHAPTER 3
METHODOLOGY

There are 3 main parts of electrospinning device which consist of syringe pump, collector and high voltage generator. The purpose of this project is to design, manufacture, and assemble all three components of electrospinning device that can produce polyethylene oxide fibers. All experimental procedures are described in this chapter in details.

3.1 Prepare 7 % w/v polyethylene oxide solution.

Polymer solution is a crucial material for fiber fabrication by electrospinning, which is composed of polyethylene oxide (PEO) and water in this study. The reason why we use polyethylene oxide is that it is bio-inert and water soluble, which is safe and suitable for the device testing. It can be kept at room temperature. According to the preliminary tests, the PEO concentration used were 7% w/v. 5.95 grams of PEO powder (M_w = 200 – 300 kDa) were dissolved in deionized water and stirred until becoming homogeneous, as shown in Figure 17.

Figure 17. The preparation of 7 % w/v polyethylene oxide solution.

3.2 High voltage source

High voltage source used for generating the electrostatic field by applied electrostatic force to drive the flow of polymer solution towards the collector. It has been reported that the suitable voltage for electrospinning is ranging from 10 to 50 kV [72]. Furthermore, the increasing voltage would decrease junctions of fibers, which is

desirable for uniform fiber distribution [102]. Therefore, it is the first aim of the project to manufacture the cost-efficient generator that could achieve this level of voltage.

A. Voltage Measurement Circuit

Because the voltage used in electrospinning is beyond the limitation of the multimeter of 600 V, we have to assemble the voltage measurement circuit in order to scale the voltage down 1,000 times using the voltage divider theory. Figure 18 shows the calculation, which suggests that we could use 55 10-MΩ 1-W resistors and 1 550-kΩ 1-W resistor connected together in series, as shown in Figure 19.

$$V_{in} = 20000\ V,\ V_{out} = 20\ V,\ R2 = 550\ k\Omega$$

$$V_{out} = \frac{V_{in} \times V_{out}}{R_1 + R_2}$$

$$20 = \frac{20000 \times 550 \times 10^3}{R_1 + (550 \times 10^3)}$$

$$R_1 + (550 \times 10^3) = \frac{20000 \times 550 \times 10^3}{20}$$

$$R_1 = 549\ M\Omega$$

$$\text{Find Power ;}\quad P = \frac{V^2}{R} = \frac{(20\times 10^3)^2\ V}{549550\ k\Omega} = 0.73\ w$$

Figure 18. Calculation of voltage measurement circuit by using voltage divider formula.

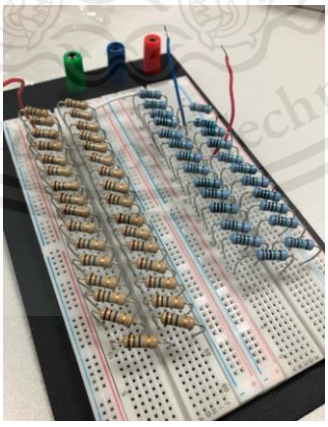


Figure 19. The assembled voltage measurement circuit.

In the experiment, we tested the voltage measurement circuit at various applied voltages for calculating the circuit tolerance, ranging from 30 to 120 V from the DC

power supply. The results exhibited a linear curve with the average tolerance of 1.18% in comparison with the calculation, as shown in Table VI and Figure 20

TABLE VI. Voltage Measured from the Measurement Circuit in Comparison with the Applied Voltage

R1exact = 561.52 MΩ	R2exact = 555 kΩ		
R1ideal = 549 MΩ	R2ideal = 550 kΩ		
Voltage from measurement circuit	**Voltage from Power Supply**	**Vout from calculation (V)**	**%error**
0	0	0	0
0.0296	29.93	0.03	1.351351351
0.0398	39.8	0.04	0.502512563
0.0492	49.8	0.05	1.62601626
0.0594	59.8	0.06	1.01010101
0.0692	69.8	0.07	1.156069364
0.079	79.7	0.08	1.265822785
0.0885	89.7	0.09	1.694915254
0.0986	99.6	0.10008	1.501014199
0.1084	109.6	0.11009	1.55904059
0.1185	119.6	0.1201	1.35021097
			1.183368577

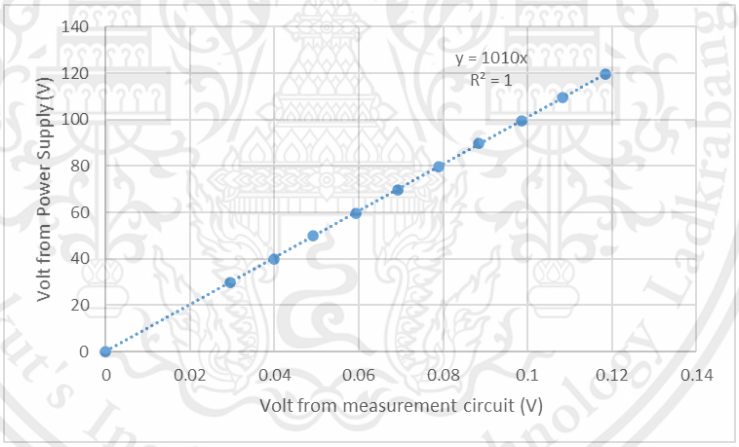


Figure 20. The relationship between the voltages from the power supply and the measurement circuit.

B. Mosquito Zapper Circuit

It is reported that the circuit of mosquito zapper provides high voltage in the range of 700 – 2,000 V [103]. Hence, it may be possible to adapt it as a high voltage source for electrospinning. We tested the voltage amplification rating of a mosquito zapper circuit, as shown in Figure 21, by using the in-house voltage measurement circuit and a DC generator with the applied voltage ranging from 1 to 8 V. The results are shown in Table VII and Figure 22 indicated that the mosquito zapper circuit could

amplify the voltage around 1,200 times. However, it was damaged after the applied voltage exceeded 5 V, which affects the result reliability afterwards.

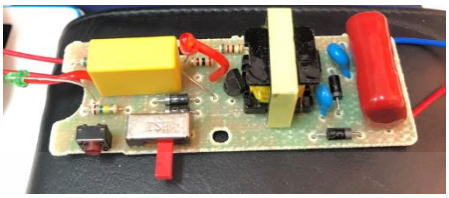


Figure 21. The mosquito zapper circuit.

TABLE VII. The Measured Voltage of the Mosquito Zapper Circuit.

Vin (v)	Vout (kv)
0	0
1	1.2
3	2.301
5	6.02
8	7.12

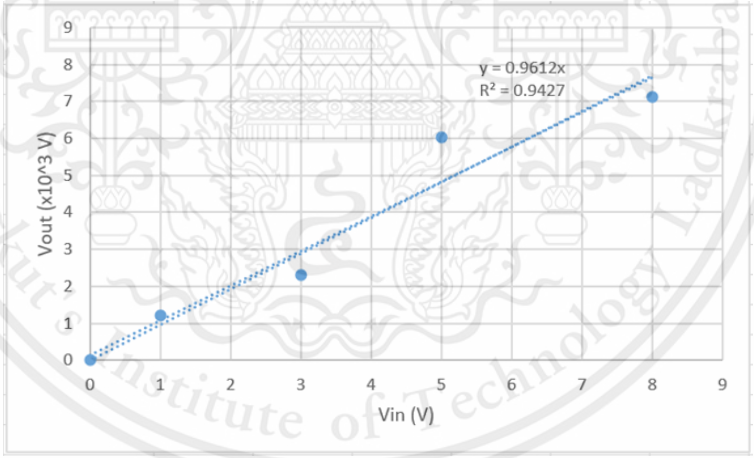


Figure 22. The output voltage of mosquito zapper circuit at various applied voltage.

3.2.1 High voltage generator

Subsequently, we have prepared another commercial 20-kV high voltage generator as shown in Figure 23 to be the high voltage source for our electrospinning device and the production of polyethylene oxide fibers.

Figure 23. 20 kV high voltage generator used for generating the electrostatic field.

3.2.2 High voltage power supply

Due to the instability of the high voltage generator, we also prepare the high voltage power supply as an alternative source for electrospinning device. It is noted that, even if we put heat sink and fan to reduce the heat, the generator only works stably for a few minutes. Therefore, we decided to use the high voltage power supply which has range of 2kV – 15kV for electrospinning experiment, as shown in Figure 24.

Figure 24 High voltage power supply

3.2.3 Measure the voltage of the high voltage source.

High voltage source can be measured by using high voltage probe that divides the actual voltage by 1000 times, as shown in Figure 25. This technique would overcome the maximum limit of 600 V of the digital multimeter, and thus the high voltage output from the generator and power supply could be quantified, as shown in Figure 26 and 27.

Figure 25. High voltage probe [104].

A. High voltage generator

- Input : DC 12 V Output : DC 9,600 V
- Input : DC 15 V Output : DC 11,400 V

Figure 26. Measure the voltage of high voltage generator.

B. High voltage power supply

- Input : AC 220 V Output : DC 10,600 V

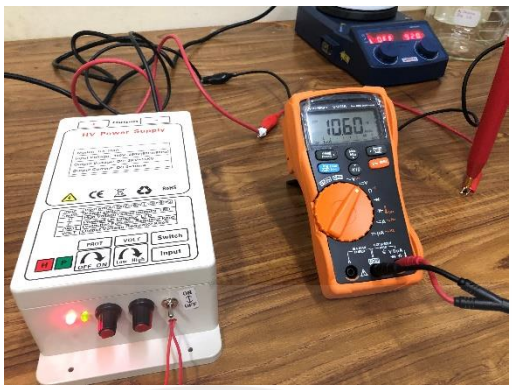


Figure 27. Measure the voltage of high voltage power supply.

3.3 Syringe pump

A syringe pump is used in this study to deliver the PEO solution at a controllable flow rate. The designed pump is driven via a stepping motor and controlled by the motor module. Lead screw with two guide rods are used to translate the angular motion from the motor into the linear motion, which could drive the syringe plunger, as shown in Figure 32.

In this project, we try to find the components that are inexpensive and easy to find in general market. We use the existing custom-made syringe pump left from the last project which provides flow rate in the range of 3 – 10 ml/min. We have already got the conductive plate as the static collector, syringe, and needle tip. The tip of needle that we used had to be cut into the blunt shape in order to allow the polymer jet flowing towards the collector in a straight line manner. To test the equipment, we have defined the input voltage to the generator = 10 V, input current = 1 A, distance between needle tip and collector = 10 cm, and flow rate = 3 ml/min, as shown in Figure 28. The preliminary results showed that syringe pump ejects the solution in the form of droplet spray, not nanofibers, and the deposited sample was still wet. These preliminary findings suggest that the flow rate used was too high and thus made the solvent evaporation more difficult. Hence, the following part of the experiment was to design the new syringe pump that could deliver polymer solution at much lower flow rate.

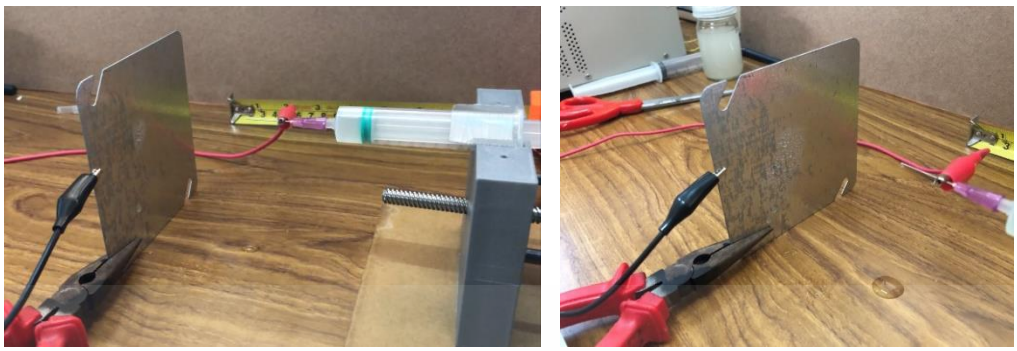


Figure 28. The result of experiment to check the equipment.

3.3.1 Design and manufacture the new syringe pump.

Several approaches were implemented in the design of new syringe pump to reduce the flow rate, including the changes in the size of lead screw and syringe, as well as the controlling software.

A. Hardware

- Lead screw was changed from 2 mm pitch, 8 mm lead to 1 mm pitch and 1 mm lead, as shown in Figure 29. This would cause the linear motion of 1 mm per revolution.

Figure 29. Size of lead screw

- There are a wide range of syringe size available in the market, as shown in Figure 30. In this study, the size was reduced from 20 ml to 10 ml in order to decrease the cross-section area and thus reduces the flow rate per unit of linear motion.

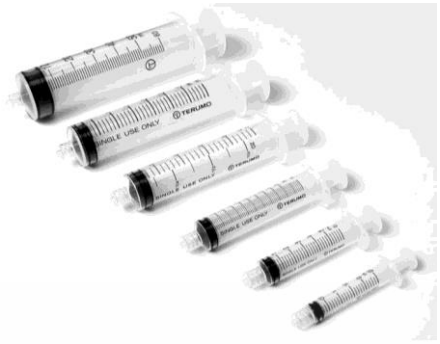


Figure 30. Size of syringe

- It is noted that the shape of the original syringe pump was also distorted during the operation with viscous polymer solution as the force generate was much higher than the water-based solution. Hence, the frame of the new syringe pump was modified to withstand the force during the operation. The new design is shown in Figure 31, 32, and 33.

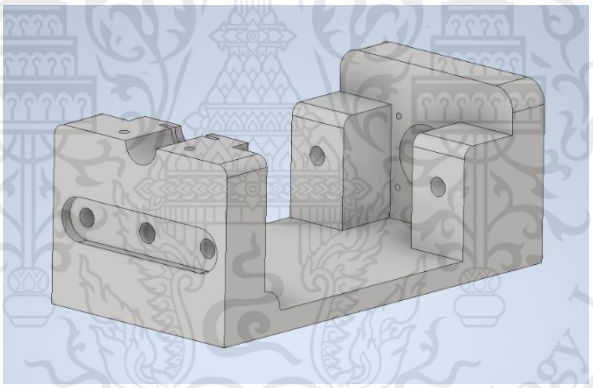


Figure 31. Syringe pump that is designed by Autodesk Inventor.

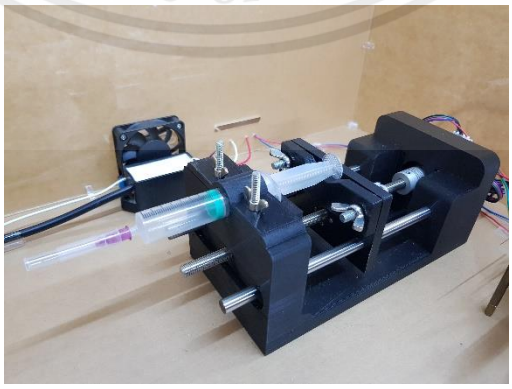


Figure 32. Syringe pump from 3D printer

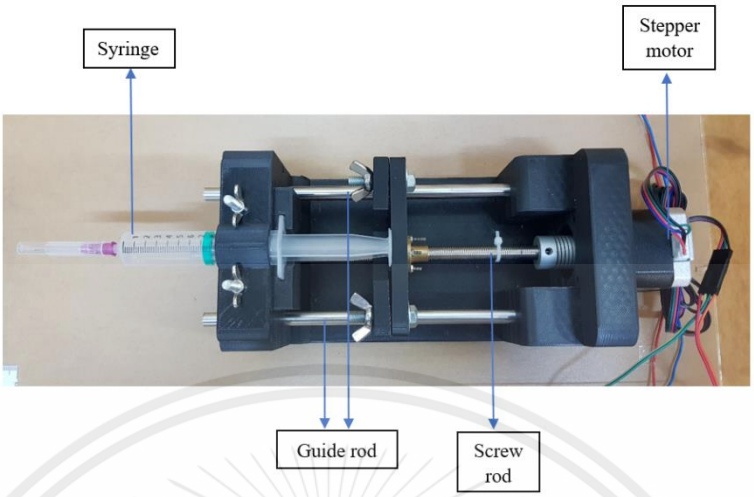


Figure 33. Components of the syringe pump.

B. Software

- Adjust the code of Arduino that controls the flow rate.

We have used the step counting method to control the movement of the motor and add the time delay between each step to reduce the flow rate of the syringe pump. The stepper motor moves 200 steps pre revolution, which is equivalent to the syringe plunger movement of 1 mm. Since the distance of 5 mm corresponds to 1 ml of 10-ml syringe. Therefore, it would require 1,000 steps to deliver 1 ml of the solution. Finally, we wrote the code to delay each step of stepper motor to define the flow rate of syringe pump, as shown in Figure 34. There are 4 flow rates for the new syringe pump which are 1 ml/hour, 1.5 ml/hour, 2.0 ml/hour, and 2.5 ml/hour, using the delay of 3.6 seconds, 2.4 seconds, 1.8 seconds, and 1.44 seconds between each step, respectively. The assemble controller is shown in Figure 35.

```
}

void loop() {
  // step one step:
  myStepper.step(-1);
  Serial.print("steps:" );
  Serial.println(stepCount);
  stepCount++;
  delay(4000);
}
```

Figure 34. The example code for define flow rate of syringe pump.

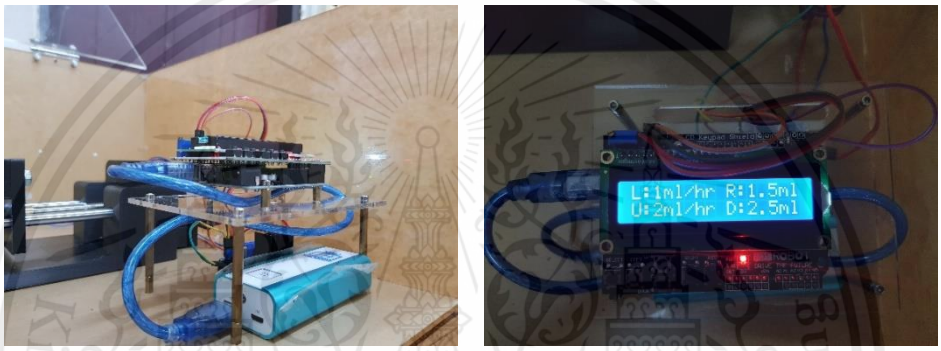
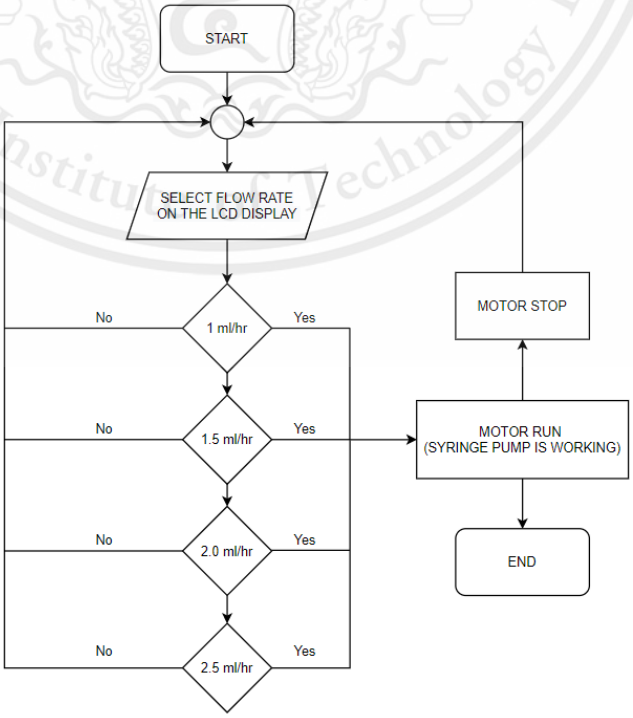


Figure 35. Controller of syringe pump

- Flowchart for controls the syringe pump.



The working process of our syringe pump is controlled by Arduino Uno, and the output information are shown on the LCD display. First, the input data is sent to the Arduino Uno by selecting the flow rate on the LCD display. Then, the motor will receive the corresponding output data from Arduino Uno. Finally, the motor will run, and syringe pump is working.

3.4 Collector

The collectors used in this study are of static and rotating types.

3.4.1 Static collector

The static collector is used for collecting random nanofiber sheet. We use the metallic plate for the static collector and cover by the aluminum foil to facilitate the sample removal and minimize the damage. The collector is composed of the conductive metal plate placed on the 3D printed stand, as shown in Figure 36. During the electrospinning process, the conductive part of the collector is connected to the ground of the high-voltage generator or power supply, as shown in Figure 37 and 38.

Figure 36. Base of the collector

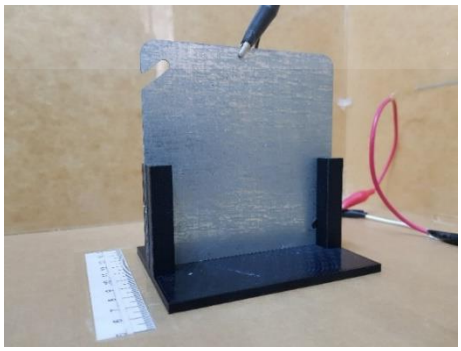


Figure 37. Metallic plate used for collecting the electrospun fibers.

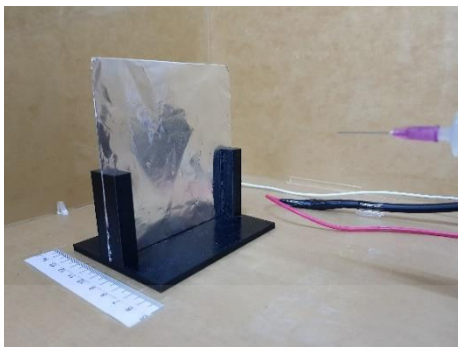


Figure 38. Metallic plate cover by aluminum foil used for collecting the electrospun fibers.

3.4.2 Rotating collector

For the rotating collector, the required properties would be conductive and having cylindrical shape. Therefore, aluminum can is chosen as it fits the required properties. Sandpaper was used to scrub the coated label out in order to expose the conductive surface of the can. The suitability of this can be used as rotating collector was tested by static electrospinning, as shown in Figure 39.

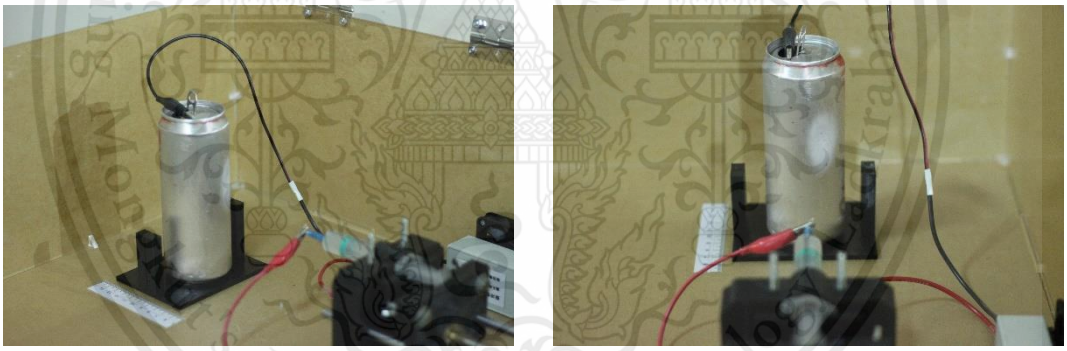


Figure 39. Testing the conductivity properties of the can.

To assemble the rotating collector, we design and fabricate a core and a base for the rotating collector by 3D printer, as shown in Figure 40. Aluminum foil was used to cover the rotating collector to facilitate sample removal. A diameter of the core for the rotating collector that we design is 56 mm and we use a DC motor to rotate the core of rotating collector that is rotating at the speed of 45 rpm. For the ground, we cut a piece of the can to be sheet that contacts the core of rotating collector to conduct electricity to crocodile clip, as shown in Figure 41, 42, and 43.

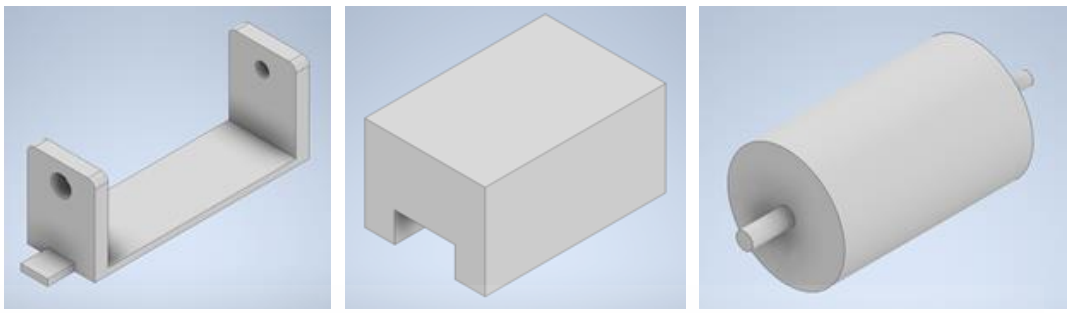


Figure 40. The base and core of rotating collector designed by Autodesk Inventor.

Figure 41. DC motor 45 RPM use for rotating the core of the rotating collector [105].

Figure 42. Rotating collector cover by aluminum foil used for collecting the electrospun fibers.

Figure 43. The average speed of the rotating collector is 52.5 rpm which is measured by the digital tachometer.

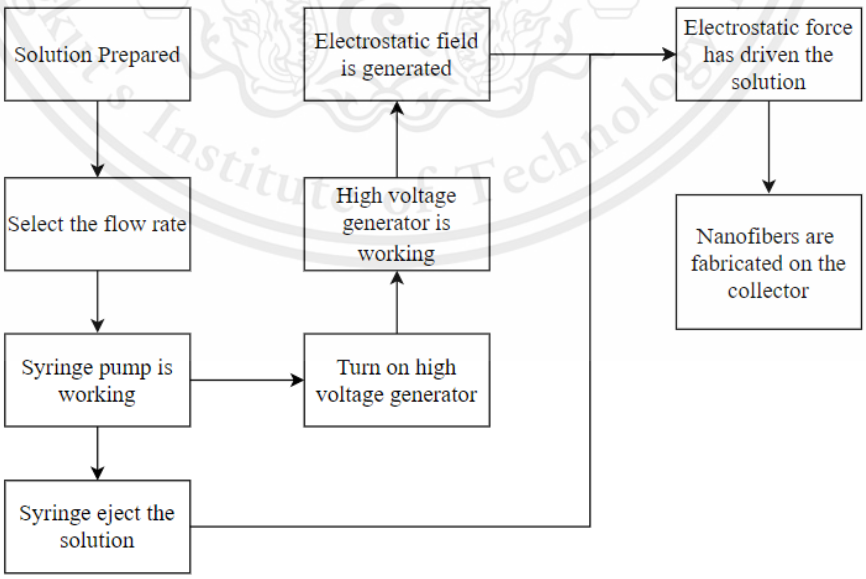
3.5 Manufacture the box to cover all device.

Since the electrospinning process involves the use of high voltage, the device should be fully enclosed in order to protect the nearby users from electrostatic effects, solution spillage, as well as the electrocution. Therefore, we make the box from acrylic sheet which is insulator to cover all device for safety and easy handling as shown in Figure 44.

Figure 44. The box covers all component of electrospinning device.

3.6 Nanofibers Fabrication

The flowchart is shown process of the electrospinning technique.



3.7 Investigation of the sample on the scanning electron microscope

Scanning electron microscopy (SEM) is a type of electron microscope with magnification ranging from 20X to approximately 100,000X that can produce image in nano scale. SEM is used for analyzing the fiber morphology. Since the samples are non-conductive, they have to be coated with gold prior to the imaging process.

3.8 Analysis of the sample by using ImageJ.

After obtaining the SEM images, the fibers were analyzed by image analysis software, which is called ImageJ. Using this software, the diameter of the electrospun fibers could be measured. The interface of ImageJ software is shown in Figure 45.

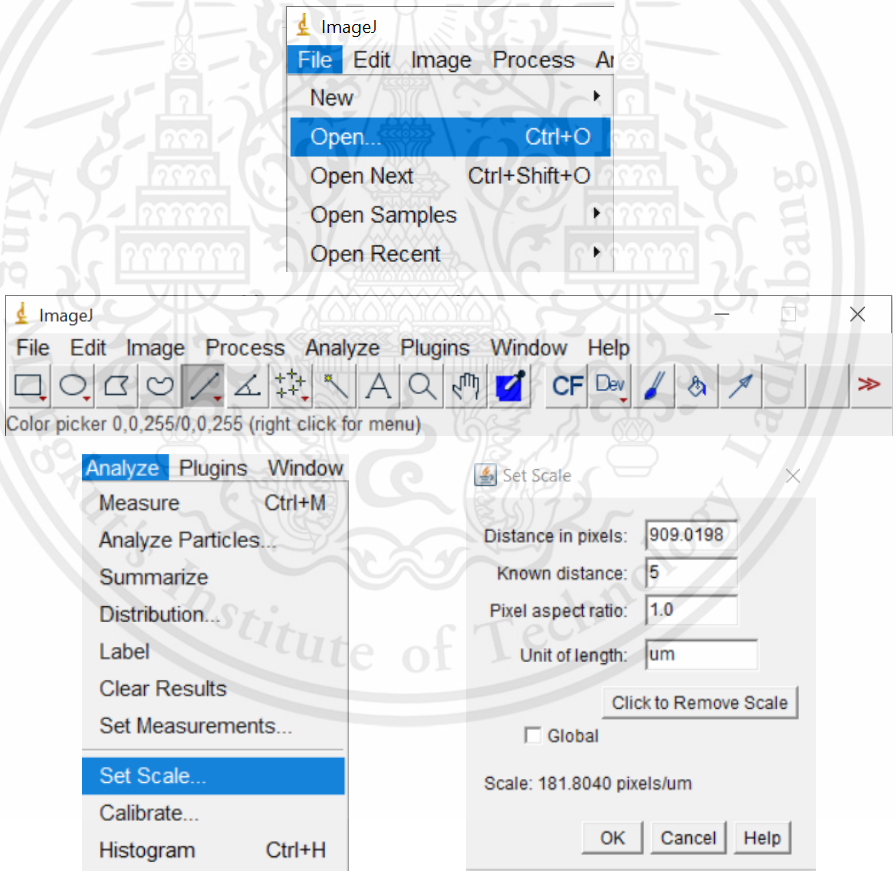


Figure 45. ImageJ program appearance.

Procedure

1. File > Open (to open the image want to measure)
2. Click on straight button > draw the line on the scale line.
3. Analyze > Set scale > fill the known distance of the image > fill the unit of length > OK. (to set the scale of the image)
4. Click on straight button > draw a cross section line.
5. Analyze > Measure (to measure the sample)
6. Ctrl + D (to keep the previous line)
7. Repeat no.4-6 (to measure other lines)

Note: Press Shift button to help scale uniformly.

CHAPTER 4

EXPERIMENTAL RESULT

As mentioned in the objective, this project aims to study the effect of electrospinning parameters on fibers morphology. Therefore, the experiment will focus on the effects of the distance between needle tip and collector, the voltage source, and also the collector type on the fiber morphology.

4.1 Electrospinning experiment with static collector.

4.1.1 Experiment to produce the nanofibers by focusing on the effects of the distance between needle tip and collector using high voltage generator.

Define controlled variables.

- 7 % w/v polyethylene oxide
- voltage = 10 kV
- current = 2 A
- flow rate = 1.5 ml/hr.
- time = 10 min

A. Distance at 6 cm

Figure 46. The result of static collector which distance at 6 cm.

The result from Figure 46 showed that at the first period of the experiment with 6 cm distance between the tip and collector, solution was ejected onto the collector in a spray droplet form and perhaps mixed with the nanofibers on the collector. The deposited sample on the collector looks like a cluster with droplet stain at the center.

B. Distance at 8 cm

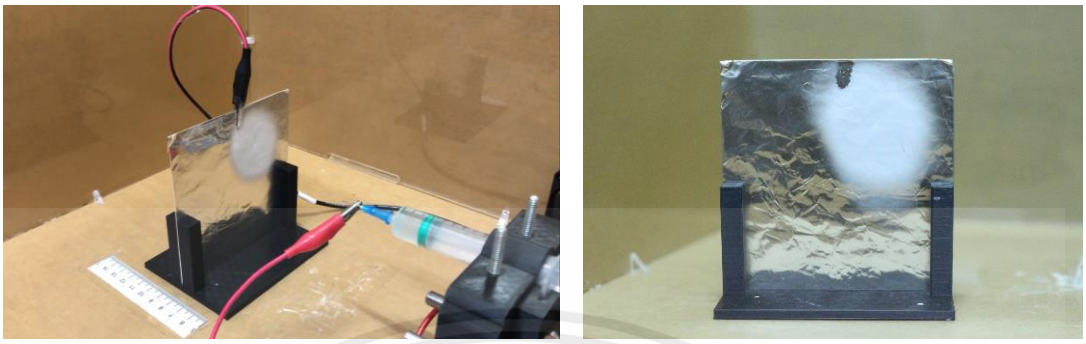


Figure 47. The result of static collector which distance at 8 cm.

The result from Figure 47 showed that at the first period of the experiment with 8 cm distance between the tip and collector, solution was ejected onto the collector in a spray droplet form before changing to the fiber form. Nanofibers fabricated on the collector look like cluster, although there was still some sign of droplet stain.

C. Distance at 10 cm

Figure 48. The result of static collector which distance at 10 cm.

The result from Figure 48 showed that the experiment with 10 cm distance between the tip and collector, solution was ejected onto the collector most likely in the fiber form without the droplet. It is also noted that the area of the deposited sample is larger than 6 and 8 cm distance. However, it also needs to be mentioned that the high voltage generator used in this part of the study only works stably for a short period of time, which is not ideal for electrospinning process. Hence, the following part of the experiment has switched to the more stable high voltage power supply.

4.1.2 Experiment to produce the nanofibers by focus on the effects of the distance between needle tip and collector using high voltage power supply.

Define controlled variables.

- 7 % w/v polyethylene oxide
- voltage = 10 kV
- flow rate = 1 ml/hr.
- time = 30 min

A. Distance at 8 cm

Figure 49. The result of static collector which distance at 8 cm using high voltage power supply.

The result from Figure 49 showed that the experiment with 8 cm distance between the tip and collector, solution was ejected onto the collector in a mixture between droplet and fiber form. It is noticed that the deposited sample cover only small area, showing huge droplet at the center surrounded by white stain.

B. Distance at 10 cm

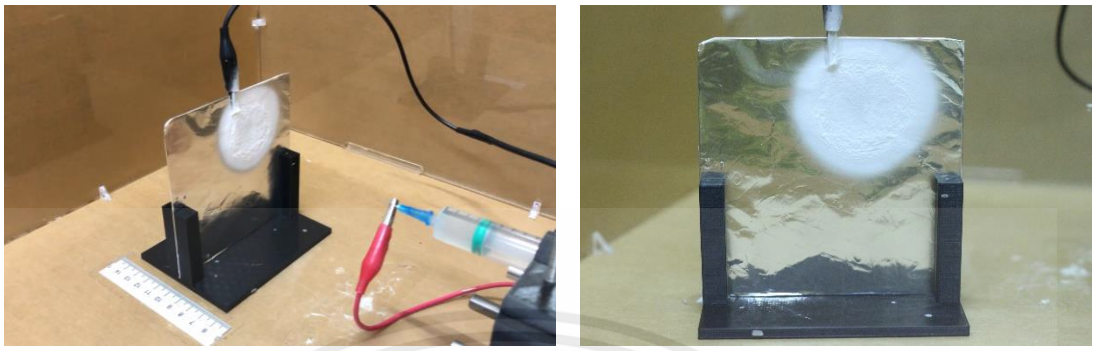


Figure 50. The result of static collector which distance at 10 cm using high voltage power supply.

The result from Figure 50 showed that the experiment with 10 cm distance between the tip and collector, solution was ejected onto the collector most likely in the fiber form without the droplet. However, its macroscopic morphology is not smooth and also exhibits spiky morphology.

C. Distance at 12 cm

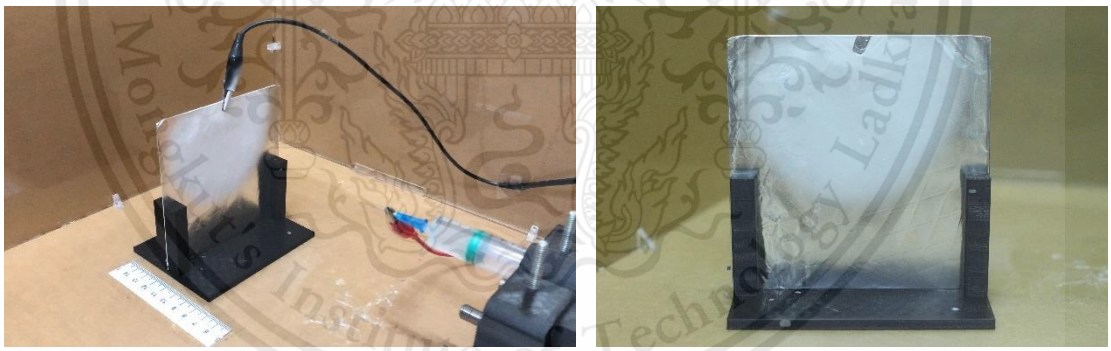


Figure 51. The result of static collector which distance at 12 cm using high voltage power supply.

The result from Figure 51 showed that the experiment with 12 cm distance between the tip and collector, solution was ejected onto the collector most likely in the fiber form without the droplet. Although its morphology is smoother and larger than 10 cm distance, the thickness is also visually thinner.

D. Distance 14 cm

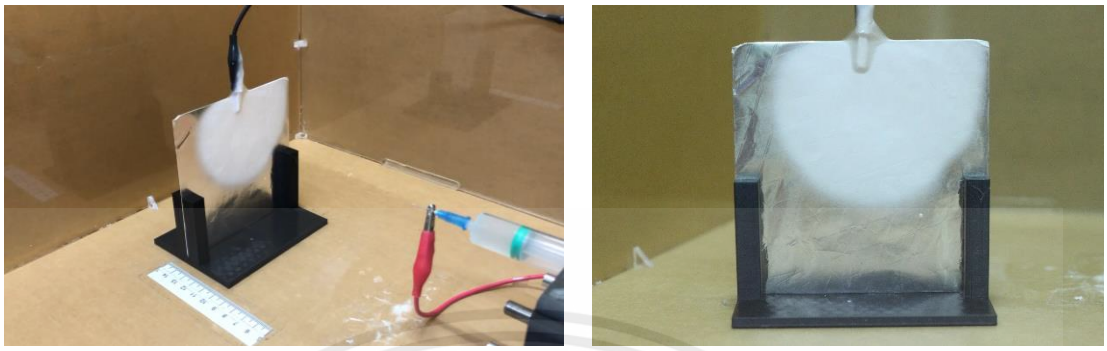


Figure 52. The result of static collector which distance at 14 cm using high voltage power supply.

The result from Figure 52 showed that the experiment with 14 cm distance between the tip and collector, solution was ejected onto the collector most likely in the fiber form without the droplet. The fibrous morphology is obviously noticeable near the clip. However, the morphology and deposited area are quite similar to 12 cm distance.

According to the aforementioned experiment, the result with distance at 12 and 14 cm is not clearly distinguishable to be selected for the experiment with rotating collector. Furthermore, it is also noticed that the solution was dripping from the needle tip during the long-term electrospinning process, which suggests that the flow rate may be too high. So, we try to decrease flow rate to 0.5 ml/hr and compare between 12 and 14 cm distances again.

A. Distance at 12 cm

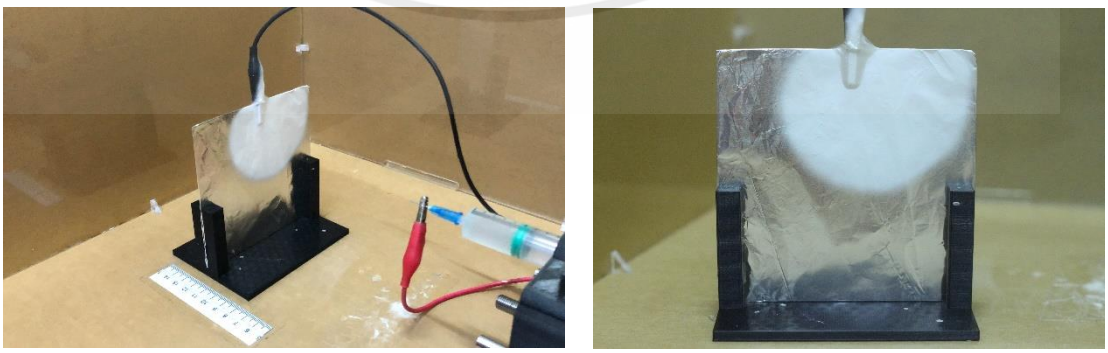


Figure 53. The result of static collector which distance at 12 cm and flow rate is 0.5 ml/hr. using high voltage power supply.

The result from Figure 53 showed that with slower flow rate, the deposited sample becomes thicker and smoother than 1 ml/hr flow rate at the same distance.

B. Distance at 14 cm

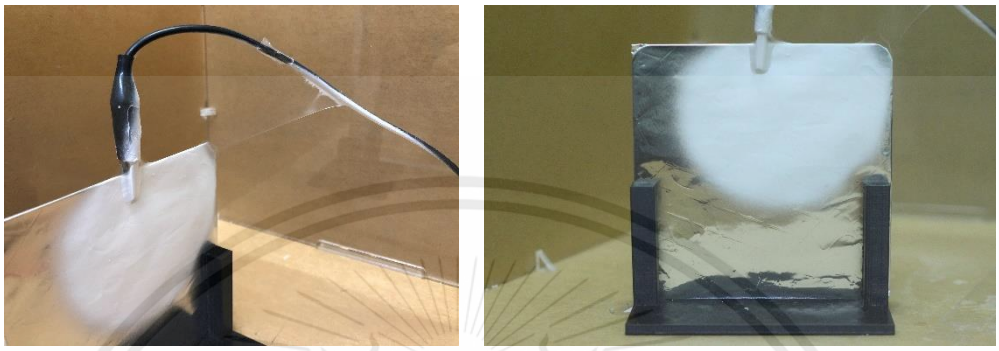


Figure 54. The result of static collector which distance at 14 cm and flow rate is 0.5 ml/hr. using high voltage power supply.

The result from Figure 54 showed that fibers are asymmetrically deposited on the collector towards the right side of the Figure. The sample looks smoother and thicker than the 12 cm distance. However, some fibers are deposited onto the cable crocodile clip.

It can be seen that the distance of 14 cm is the most suitable for this study. Since, we observed that some fibers are deposited asymmetrically and also onto the cable crocodile clip, we rearrange the position of the high voltage power supply and do the experiment again. The result from Figure 55 showed that the process is reproducible and the spinning process has become symmetric.

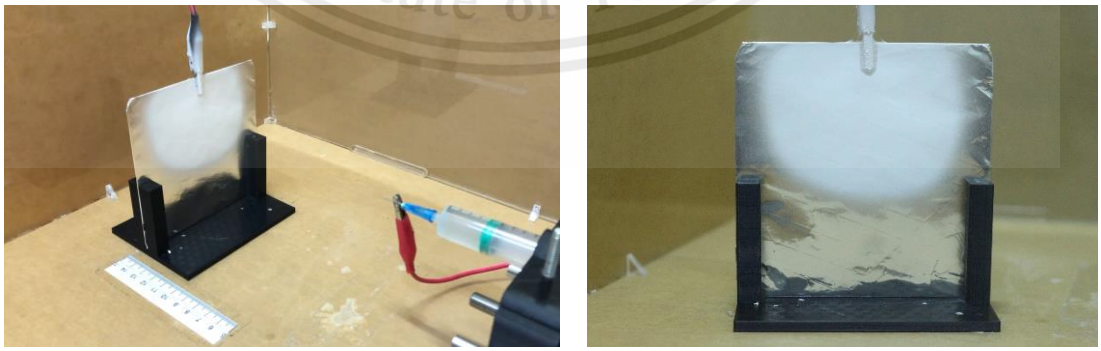


Figure 55. The result of static collector which distance at 14 cm and flow rate is 0.5 ml/hr. from rearranging the position high voltage power supply.

4.2 Electrospinning experiment with rotating collector.

The first part of this section was conducted before we obtained the high voltage power supply and thus, we used high voltage generator for the preliminary experiment.

4.2.1 Experiment to produce the nanofibers by focus on the effects of the distance between needle tip and collector using high voltage generator.

The distance of 10 cm was used for preliminary experiment just to test the device and equipment. The result from Figure 56 and 57 showed that nanofibers were deposited almost all over the surface of collector. It is smooth but not thick enough.

Define controlled variables.

- 7 % w/v polyethylene oxide
- voltage = 10 kV
- current = 2 A
- flow rate = 1.5 ml/hr.
- distance 10 cm
- time = 10 min
- speed of rotating collector 50 rpm.

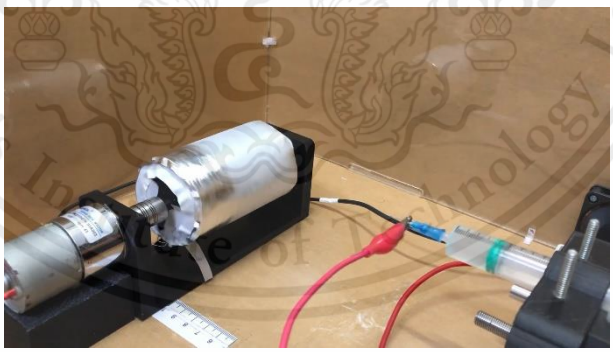


Figure 56. The electrospinning experiment with rotating collector using high voltage generator.

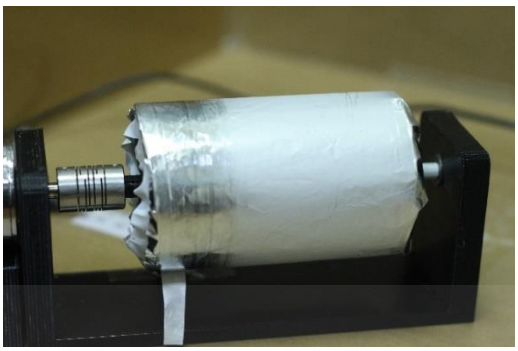


Figure 57. The result of experiment with rotating collector using high voltage generator.

4.2.2 Experiment to produce the nanofibers by focus on the effects of the distance between needle tip and collector using high voltage power supply.

After we obtained the high voltage power supply, we continue our experiment using the parameters from the static collector experiment, which are 14 cm distance and 0.5 ml/hr flow rate. The result from Figure 58, 59, and 60 showed that the fibers are also deposited all over the surface of rotating collector with smooth and thick morphology.

Define controlled variables.

- 7 % w/v polyethylene oxide
- voltage = 10 kV
- flow rate = 0.5 ml/hr.
- distance 14 cm
- time = 1 hour
- speed of rotating collector 50 rpm.

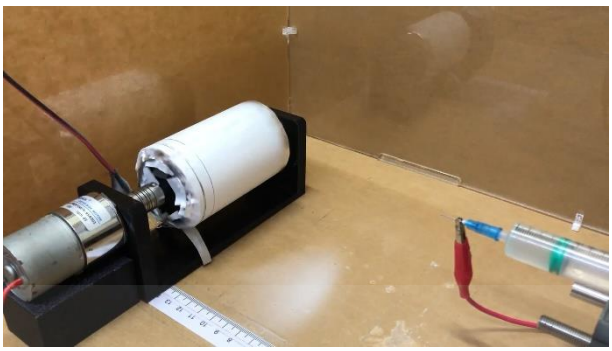


Figure 58. The electrospinning experiment with rotating collector using high voltage power supply.

Figure 59. The result of experiment with rotating collector using high voltage power supply.

Figure 60. The result of experiment with rotating collector after unfolding an aluminum foil.

4.3 Investigation of the sample on the scanning electron microscope

The SEM images taken from the samples deposited on the aluminum foil from static and rotating collectors are shown in Figure 61 and 62, respectively. It is found that the samples from both collectors exhibit random orientation.

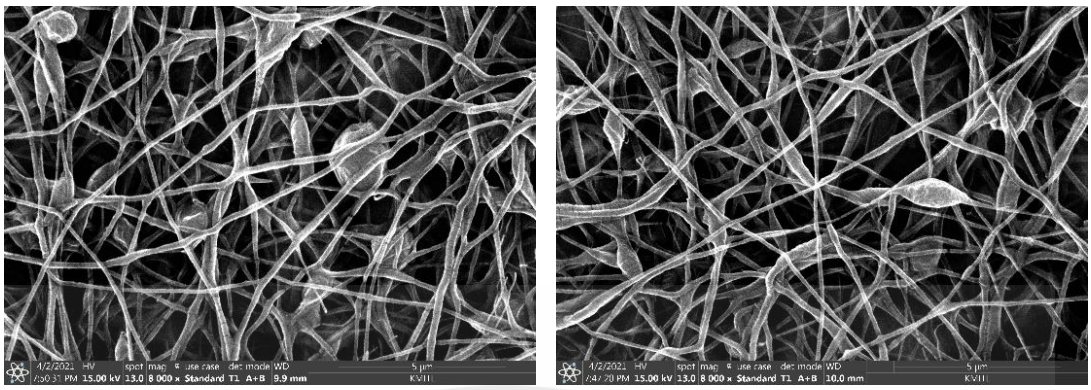


Figure 61. SEM image of fibers that are deposited on the static collector.

The result showed that the fibers are random direction and there are some spherical beads.

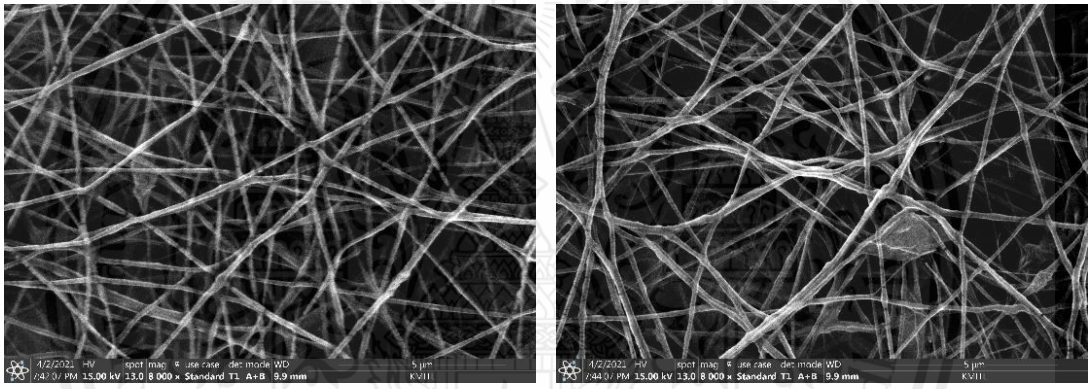


Figure 62. SEM image of fibers that are deposited on the rotating collector.

4.4 Data analysis of the fibers using ImageJ.

The fiber diameter data from static and rotating collectors measured by ImageJ software are shown in Table VIII and IX, respectively. The mean fiber diameter of static and rotating collectors are 136.79 and 142.89 nm, respectively, as shown in Table X. The histograms of the fiber diameter are shown in Figure 63 and 64.

TABLE VIII. Data analysis of the fibers from static collector.

No.	Length (nm)	No.	Length (nm)	No.	Length (nm)
1	143	35	128	69	151
2	183	36	159	70	157
3	155	37	151	71	145
4	168	38	121	72	136
5	121	39	174	73	156
6	121	40	178	74	185
7	173	41	140	75	165
8	176	42	100	76	203
9	162	43	104	77	164
10	176	44	102	78	133
11	91	45	117	79	140
12	104	46	128	80	119
13	125	47	123	81	132
14	102	48	167	82	122
15	125	49	134	83	148
16	132	50	147	84	109
17	91	51	125	85	103
18	140	52	111	86	132
19	179	53	114	87	115
20	137	54	176	88	144
21	120	55	136	89	157
22	164	56	145	90	142
23	187	57	127	91	144
24	92	58	176	92	112
25	123	59	106	93	140
26	98	60	139	94	129
27	157	61	125	95	135
28	110	62	201	96	110
29	103	63	209	97	141
30	99	64	116	98	125
31	166	65	98	99	174
32	116	66	124	100	125
33	126	67	106		
34	95	68	119		

TABLE IX. Data analysis of the fibers from rotating collector.

No.	Length (nm)	No.	Length (nm)	No.	Length (nm)
1	160	35	168	69	153
2	147	36	131	70	154
3	176	37	164	71	141
4	152	38	127	72	146
5	145	39	120	73	146
6	112	40	175	74	150
7	125	41	158	75	141
8	155	42	100	76	180
9	121	43	97	77	185
10	127	44	132	78	158
11	141	45	124	79	104
12	157	46	136	80	161
13	117	47	204	81	152
14	132	48	123	82	166
15	117	49	167	83	126
16	151	50	116	84	156
17	137	51	123	85	140
18	144	52	115	86	130
19	159	53	117	87	151
20	132	54	120	88	118
21	150	55	126	89	148
22	132	56	124	90	153
23	119	57	143	91	159
24	131	58	158	92	167
25	142	59	136	93	163
26	155	60	126	94	158
27	118	61	120	95	117
28	126	62	109	96	147
29	147	63	135	97	190
30	164	64	99	98	158
31	171	65	169	99	151
32	150	66	190	100	140
33	154	67	180		
34	141	68	141		

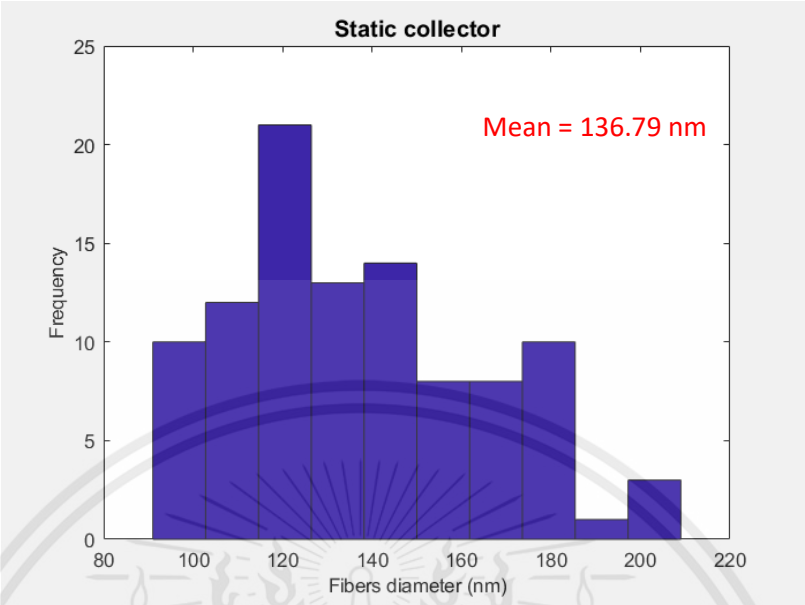


Figure 63. Histogram of fibers diameter from static collector.

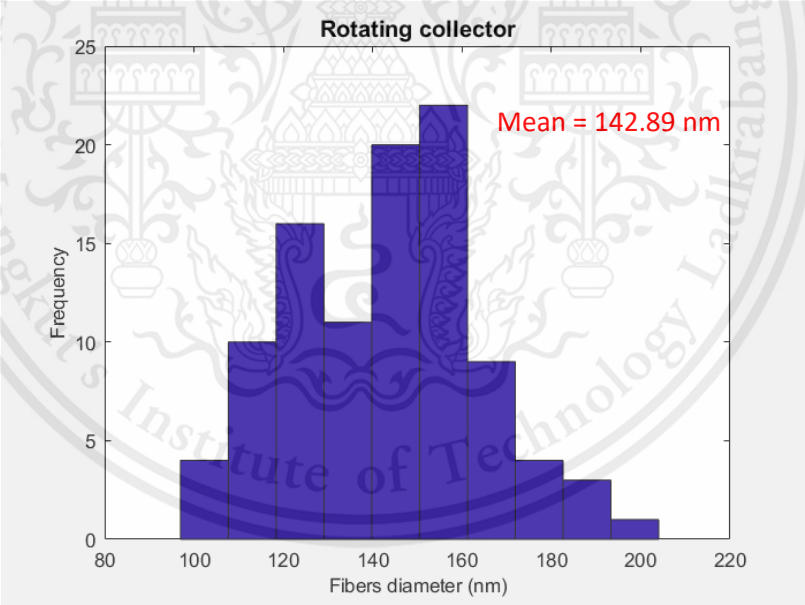


Figure 64. Histogram of fibers diameter from rotating collector.

TABLE X. The diameter range of fibers collected on the different collectors.

Collector	Diameter (nm)	Average Diameter (nm)
Static	91-209	136.79
Rotating	99-204	142.89

CHAPTER 5
CONCLUSION

5.1 Discussion

5.1.1 Electrospinning experiment with static collector using high voltage generator.

Based on the results, it was found that the distance between collector and needle tip has played an important role in the morphology of the deposited samples. The results showed that the area of the deposited sample increase with increasing distance. This is expected to be a result of the greater projected area from the needle tip as the distance increases. Moreover, droplets were seen from the deposited sample with shorter distance and the droplets disappear as the distance increases. This is likely to be the insufficient evaporation time at short distance. In other words, if the distance is short, the polymer jet arrives the collector faster, and thus it has short evaporation time. On the other hand, with greater distance, the polymer jet is allowed to evaporate longer, and thus the dried fibers are formed on the collector. Furthermore, the stability of the voltage also affects the continuity of the electrospinning. It was experienced in this study that the temperature of the high voltage generator increases shortly after it starts working, and the voltage subsequently drops once the temperature reached a certain level. Therefore, the electrospinning has to be stopped, and the sample with insufficient thickness could only be obtained.

5.1.2 Electrospinning experiment with static collector using high voltage power supply.

The findings from the experiment that used high voltage power supply with more stable voltage has shown similar results as the high voltage generator, but with significantly longer operating time. Moreover, spiky morphology was also observed in this part of the experiment, which is possibly the effect of the electrostatic force that attracts the loose part of the sample. On the other hand, the deposited area could be extended further with 12 and 14 cm distance, and it was also noticed from this part that the flow rate of 1.0 ml/hr. was too high as the solution was dripping from the needle tip during the long-term electrospinning. Therefore, the optimal condition for static collector was found to be 14 cm distance and 0.5 ml/hr. In addition, it should also be

noted that the arrangement of the high voltage source also influences the pattern of deposition. If there is high voltage source located near the electrospinning area, the electrostatic field would be disturbed which leads to the asymmetric fiber deposition.

5.1.3 Electrospinning experiment with rotating collector.

The results from the rotating collector part have shown that the parameters can be directly translated from the static collector experiment. The macroscopic morphology of the sample was quite similar to that of the static collector.

5.1.4 Analysis of the sample on ImageJ

From the SEM images, the result of static collector showed that fibers are aligned in random direction and there are some spherical beads. The reason why the spherical beads form on the surface of fibers may be because the solution has low extensional viscosity or the charge density on the tip of a needle or electrospinning jet is not enough. On the other hand, the sample from rotating collector is also in random direction, but clearer and there are a few spherical beads. However, the result of rotating should be aligned nanofibers because the rotating collector provided pulling force apart from high shear and elongation forces that help adjusting the fiber direction and align the fiber layer. It may be because the speed of the rotating collector is too low. From analysis of the sample on ImageJ, the diameter of the nanofibers of static collector is ranging from 91 - 209 nm, the average is 136.79 nm. The part of rotating collector is ranging from 99 - 204 nm, the average is 142.89 nm. It is observed that the diameter of the nanofibers of static collector is distributed but rotating collector is gathered in the range of about 140 - 160 nm. However, it would require further experiment to gain better understandings on the role of each parameter.

5.2 Conclusion

It can be concluded from this study that the assembled device could produce electrospun PEO fibers in nanoscale. This indicates that the design and manufacturing of all three parts are successful. The optimal flow rate from this study is 0.5 ml/hr to avoid solution dripping from the needle tip during the electrospinning process. Moreover, the distance between the needle tip and collector should be 14 cm for 10 kV

to allow sufficient evaporation for the polymer jet. On the other hand, the stability of the high voltage source is also crucial for long electrospinning time, which could produce thick fiber as a result. The observed issues from this study would be the presence of beads and the insufficient alignment of the fibers from rotating collector. This is expected to be mitigated in future by adjusting the polymer solution and the rotational speed of the collector, respectively. The average diameter of electrospun fibers produced from the assembled device in this study are 136.79 nm from the static collector and 142.89 nm from the rotating collector.

5.3 Suggestion

- More parametric studies could be conducted in future, such as the effects of polymer concentration, and the voltage.
- It would also be worth increasing the rotation speed of the rotating collector to investigate whether or not the aligned fibers can be obtained.

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