แผ่นเส้นใยพอลิแล็กทิกแอซิดที่มีประจุบวกและการตอบสนองทางชีวภาพ

นายวชิรศิลป์ แก้วประเสริฐศรี

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POSITIVELY CHARGED POLY(LACTIC ACID) FIBER MAT AND BIOLOGICAL RESPONSE

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
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Field of Study	Petrochemistry and Polymer Science
Thesis Advisor	Assistant Professor Varawut Tangpasuthadol, Ph.D.

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วชิรศิลป์ แก้วประเสริฐศรี : แผ่นเส้นใยพอลิแล็กทิกแอซิดที่มีประจุบวกและการตอบสนองทางชีวภาพ (POSITIVELY CHARGED POLY(LACTIC ACID) FIBER MAT AND BIOLOGICAL RESPONSE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.วราวุฒิ ตั้งพสุธาดล, 39 หน้า.

มีการนำพอลิแล็กติกแอซิดไปใช้ประโยชน์อย่างกว้างขวางและรวมทั้งทางชีวการแพทย์ ในงานนี้ได้เพิ่มความชอบน้ำของพอลิแล็กทิกแอซิดด้วยการติดประจุบวกผ่านหมู่ควอเทอร์นารีแอมโมเนียมที่ปลายสายโซ่พอลิเมอร์ทั้งสองด้าน ได้สังเคราะห์พอลิแล็กทิกแอซิดที่ปลายสายโซ่ทั้งสองด้านมีประจุบวก (PLAdi+) โดยการทำปฏิกิริยาสองขั้นตอน ขั้นตอนแรกได้สังเคราะห์พอลิแล็กทิกแอซิดที่มีหมู่คาร์บอกซิลที่ปลายสายโซ่ทั้งสองด้านโดยปฏิกิริยาพอลิคอนเดนเซชันระหว่างแอล-แล็กทิกแอซิดที่มีซักซินิกแอซิดร่วมอยู่ในปฏิกิริยา จากนั้นติดหมู่ไกลซิดิลไตรเมทิลแอมโมเนียมคลอไรด์ (glycidyltrimethyl ammonium chloride, GTMAC) ไปที่หมู่คาร์บอกซิลที่ปลายสายโซ่ทั้งสองด้านของพอลิแล็กทิกแอซิด จะได้ PLAdi+ น้ำหนักโมเลกุล 3,670 ดาลตัน นำ PLAdi+ ไปผสมกับพอลิแล็กทิกแอซิดทางการค้า (น้ำหนักโมเลกุล 60,000 ดาลตัน) ที่อัตราส่วนพอลิแล็กทิกแอซิดทางการค้าต่อ PLAdi+ เท่ากับ 100:0 100:10 100:30 100:50 และ100:70 นำพอลิเมอร์ผสมที่ได้ไปขึ้นรูปเป็นเส้นใยด้วยเทคนิคอิเล็กโทรสปินนิง ได้เส้นใยที่มีเส้นผ่าศูนย์กลางลดลงจาก 365±83 นาโนเมตรเป็น 109±29 นาโนเมตรเมื่ออัตราส่วนพอลิแล็กทิกแอซิดทางการค้าต่อ PLAdi+ เท่ากับ 100:70 นอกจากนี้การเพิ่มพอลิแล็กทิกแอซิดที่ปลายสายโซ่ทั้งสองด้านมีประจุบวกยังสามารถลดค่ามุมสัมผัสของน้ำจาก 135±1° เป็น 50±3° และ 144±5° เป็น 55±3° เมื่อวัดแบบสแตติก และไดนามิก ตามลำดับ นอกจากนี้เส้นใยยังถูกนำไปทดสอบฤทธิ์การต้านเชื้อแบคทีเรียและทดสอบการยึดเกาะและการเพิ่มจำนวนของเซลล์ไฟโบรบลาสต์ แอล 929 พบว่าเส้นใยที่เตรียมได้ไม่มีฤทธิ์การต้านเชื้อแบคทีเรีย แต่พบว่าเส้นใยที่เตรียมได้จากพอลิแล็กทิกแอซิดทางการค้าผสมกับ PLAdi+ และจากพอลิแล็กทิกแอซิดทางการค้าผสมกับ PLAdiCOOH ที่อัตราส่วน 100:70 เป็นเส้นใยที่มีความเหมาะสมสำหรับการเพาะเลี้ยงเซลล์ไฟโบรบลาสต์

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2558 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5672247523 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEYWORDS: POLY(LACTIC ACID) / ELECTROSPINNING / HYDROPHILICITY / CELL ADHESION AND POLIFERATION

WACHIRASIN KAEWPRASERTSRI: POSITIVELY CHARGED POLY(LACTIC ACID) FIBER MAT AND BIOLOGICAL RESPONSE. ADVISOR: ASST. PROF. VARAWUT TANGPASUTHADOL, Ph.D., 39 pp.

Poly(lactic acid) (PLA) has been widely used in several applications including biomedical applications. In this work the hydrophilicity of PLA was enhanced by attaching positively charged quaternary ammonium groups at both sides of polymer chain ends. The positively charged PLA or PLAdi+ was synthesized by two-step synthesis method. First, PLA having carboxylic groups on both chain ends (PLAdiCOOH) was prepared via polycondensation of L-lactic acid in the presence of succinic acid. Then the carboxyl groups of PLAdiCOOH were attached to glycidyl trimethylammonium chloride (GTMAC) to form PLAdi+ having M_n of 3,670 Da, It was then blended with a commercialized PLA (M_n of 60,000 Da) with mass ratio of PLA:PLAdi+ 100:0, 100:10, 100:30 100:50 and 100:70. The polymer blends were then used to produce non-woven fiber mats by electrospinning technique. The diameter of PLA/PLAdi+ fiber was found to be decreased from 365±83 nm to 109±29 nm with mass ratio of PLA:PLAdi+ 100:70. The addition of PLAdi+ also caused a reduction of air-water contact angle of the fiber mats from 135±1° (PLA) down to 50±3° and 144±5° (PLA) down to 55±3° measured by static and dynamic water contact angle, respectively. Moreover, the electrospun fiber mats were further evaluated for antibacterial testing and fibroblast L929 cell adhesion and proliferation. All electrospun fiber mats, however, did not possess antibacterial activity. But the fiber mats of PLA/PLAdi+ and PLA/PLAdiCOOH with mass ratio of PLA:PLAdi+ (100:70) were found to be an acceptable material for fibroblast cell culture.

Field of Study:	Petrochemistry and Polymer Science	Student's Signature	.....
		Advisor's Signature	.....
Academic Year:	2015		

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CHAPTER I

INTRODUCTION

1.1 Statement of problem

Poly(lactic acid) (PLA) has been widely used in several applications such as rigid packaging, flexible film, tissue engineering, staple fiber and biomedical materials [1-3], due to its biodegradable and biocompatible properties. In the area of biomedical engineering, electrospinning has been a versatile method to produce non-woven fibers from polymers, providing high surface area and high amount of pores which affect infiltration of air and water, resulting in promotion of cell proliferation. PLA is, however, hydrophobic, making it unsuitable to be used in certain applications that water accessibility is needed. Therefore, to increase its hydrophilicity, a number of modification methods of PLA were reported [4-7], such as graft copolymerization and blending with hydrophilic polymer [8-10]. Recently, to increase the hydrophilicity, PLA having two positively charged chain end (PLAdi+) has been developed [11, 12].

From the success of our previous works to increase the hydrophilicity by blending PLAdi+ with a commercialized PLA and then electrospinning fiber mats, this work aims to further systematic study by controlling fiber mats diameter. In addition, to show the potential application of the synthesized fiber mats, biological response will be also investigated.

This work has been conducted as a continuing research to combine low molecular weight PLAdi+ ($\overline{M}$n = 3,670 Da) with a commercialized PLA ($\overline{M}$n = 60,000 Da). Afterward, the polymer blend was used to produce fiber mats by applying the electrospinning technique. Finally, the effects of PLA/PLAdi+ ratios on hydrophobicity and fiber diameter were reported. Investigation on antibacterial and cell adhesion, proliferation on the modified fiber mats were also investigated.

1.2. Poly(lactic acid)

Poly(lactic acid) (PLA) has a structure of aliphatic polyester, which can be produced by two methods; condensation and ring-opening polymerizations (**Figure 1.1**). In ring opening polymerization method, PLA was prepared from lactide, a cyclic dimer of lactic acid. In condensation polymerization method, PLA was directly synthesized from lactic acid, which is derived from the fermentation of sugar from carbohydrate sources such as corn and tapioca [1]. The advantages of the

condensation polymerization include polymerization of inexpensive material and easy method [13]. Since PLA is bio-based, biodegradable and biocompatible, it has received much popularity in several applications as mentioned before. However, the major drawbacks of PLA due to its hydrophobicity limit its biomedical applications. So PLA has been modified mainly concerning two aspects; chemical and physical modification.

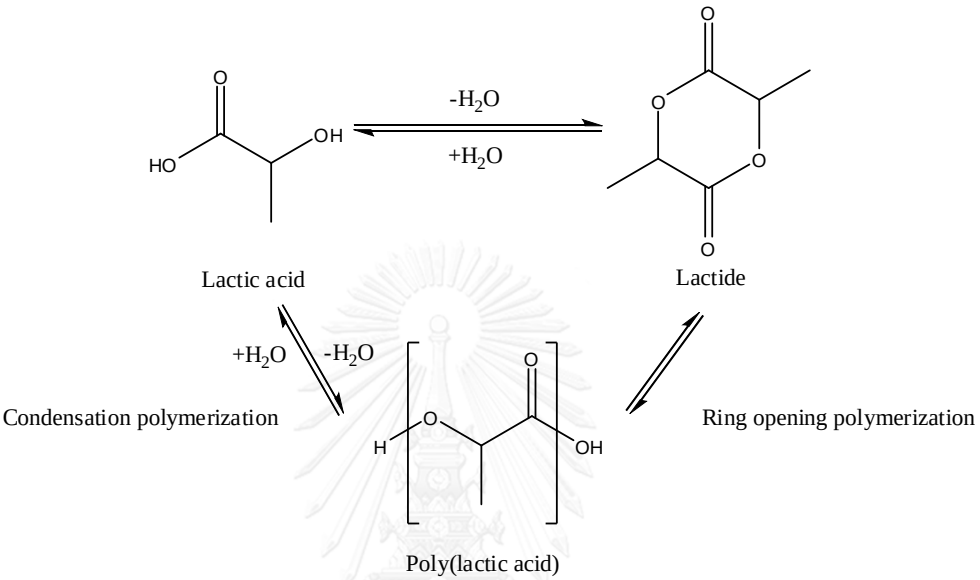


Figure 1.1 Synthetic route of poly(lactic acid)

1.3 Modifications of PLA

1.3.1 Chemical modifications

The hydrophilicity of PLA can be enhanced through graft copolymerization of PLA with variable hydrophilic groups such as hydroxyl group, carboxyl group, amino group and inorganic group as shown in **Table 1.1**

Table 1.1 Chemical modification of PLA by grafting with hydrophilic groups.

Authors		Reactant	Functional groups	Water contact angle (°)	
				PLA	Grafted PLA
In 2003, Ma *et al*. [4]	PLLA	Polyhydroxyethyl methacrylate (PHEMA)	-OH	82	51
	PLLA	Polymethacrylic acid (PMAA)	-COOH	82	51
In 2006, Deng *et al*. [5]	PLLA	RGD peptide	$-NH_2$	102.9	57
In 2007, Xu *et al*. [6]	PLLA	Hydroxyapatite	$Ca_5(PO_4)_3$ (OH)	130	78
In 2012 Guo, *et al*. [7]	PLA	Acrylic acid	-COOH	84	59

From previous studies, Khomein [11] and Chalermbongkot [12] synthesized PLA having two positively charged chain end (PLAdi+) by two-step reactions. In this method, PLA modified with carboxyl groups on both chain ends (PLAdiCOOH) was first synthesized. Then, PLAdi+ was obtained via ring opening reaction of GTMAC with the carboxylic end group of PLAdiCOOH. After fabricating as film [11] air water contact angle decrease from 57° for PLLA to 45° for the modified PLLA. and electrospun fiber mats [12] (air water contact angle decreased from 137° for PLLA to 123° for the modified PLLA mixed with PLLA), it was found that the hydrophilicity of PLAdi+ could be increased compared with PLLA before modification. Moreover, the degradation rate of PLA mixed with PLAdi+ was faster with increasing the PLAdi+ content in PLA solvent cast films [14].

1.3.2 Physical modifications

Polymer blending is an effective, simple, and versatile method to develop new materials without synthesizing new polymers. The physical properties of PLA can be

tuned by blending another polymer with PLA. Hydrophilic polymers such as HCl-doped poly(aniline-co-3-aminobenzoicacid) (3ABAPANI) as a positively charged polymer (PLA:3ABAPANI = 55:45) [8], gelatin (PLA:gelatin 1:1) [9] and polyethylene glycol (PEG) (PLA:PEG 50:50) [10] containing hydroxyl group were blended with PLA in order to reduce its hydrophobicity for biomedical applications.

1.4 Electrospinning

A basic processing of electrospinning is to use a high voltage electric field to produce fibers from polymeric fluid to leave on the collector as shown in **Figure 1.2**. When applying an electric filed, the polymeric fluid was elongated into a conical liquid as known as Taylor cone at the needle tip because the electrostatic interactions [15]. Once electric applied exceeds the surface tension of the Taylor cone, finally the polymeric fluid is jetted onto the collector plate to gather as fiber mats.

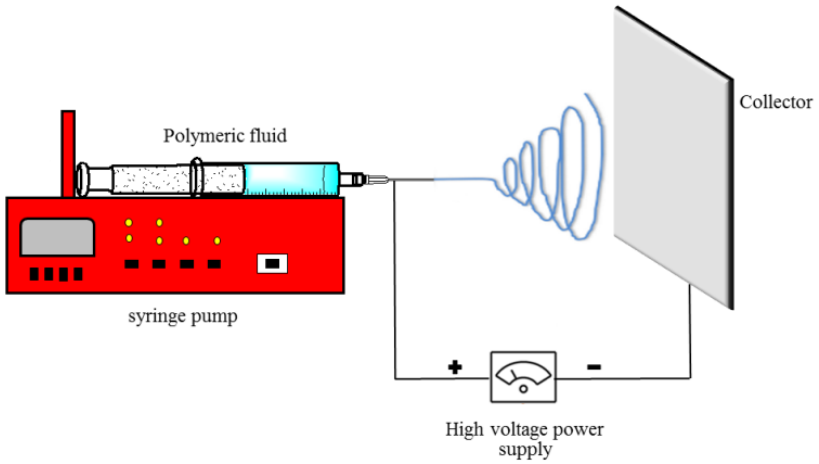


Figure 1.2 Diagram of electrospinning process.

The general parameters that affect the fiber morphology were showed in the **Table 1.2**

Table 1.2 The effects of electrospinning parameters on the morphological of fiber mats [16]

Parameter	Effect on fiber morphology
Increasing applied voltage	Fibers diameter were decreased
Increasing flow rate	Fibers diameter were increased (beaded morphologies occur if the flow rate is too high)
Increasing distance between needle tip and collector	Fibers diameter were decreased (beaded morphologies occur if the distance between the needle tip and collector is too short)
Increasing polymer concentration (viscosity)	Fibers diameter were increased (within optimal range)
Increasing solution conductivity	Fibers diameter were decreased (broad diameter distribution)
Increasing solvent volatility	Fibers exhibit micro texture (pores on their surfaces, which increase surface area)

Conductivity is one of the important parameters that affects the size and morphology of electrospun fiber mats. The conductivity can be increased by adding high polar solvent such as methanol [17], ethanol, dimethyl sulfoxide [18], dimethylformamide [19], cationic salt such as NaCl, NaH_2PO_4, KH_2PO_4 [20], LiCl, $NaCO_3$, $CaCl_2$ [21], and cationic surfactant such as dodecyltrimethylammonium bromide (DTAB) [22] and octadecyltrimethylammonium bromide (OTAB) [23] into the electrospinning solution. Therefore, the high conductive solution was jetted from the needle tip at a higher rate to obtain fibers with a smaller diameter. Besides cationic molecules used in the previous studies to reduce diameter of PLA fiber, in this work, in order to maintain PLA characteristics such as biodegradation and biocompatibility, we are interested in using PLAdi+ to reduce fiber diameter. Not only cationic molecule can reduce the diameter but also associated with the antibacterial activity of fiber [24-29].

1.5. Antibacterial activity

Positively charged polymers would bind to the cell membrane of bacteria with electrostatic attraction associated with the negatively charged due to phosphate groups on the cell membrane [30]. There were reports that attaching polymers with positively charged functional as polyamine group such as polyethylenimine (PEI) [24],

hexamethylene biguanide hydrochloride [25] and trimethylammonium group such as trimethylamioethyl methacrylate chloride [26], [2-(methacryloyloxy)ethyl] trimethylammonium chloride [27] and linear and branched N-alkyl-N-methyl PEIs, subsequent quaternization with alkyl bromide [28] showed an increase antibacterial activity of the films and tissue engineering. Moreover, positively charged polymer can be introduced by photochemical process [29].

1.6. Cell response

PLA electrospun fiber mats were usually applied in biomedical applications such as tissue engineering, tissue regeneration and dressing wound which involve cell such as blood cells [31], fibroblast L929 cells [9], human umbilical vein endothelial cells (HUVECs) [32], and MC3T3-E1 mouse calvaria-derived osteoprogenitor cell [33]. In this work, fibroblast L929, adipose from mouse, was selected to study cell response on the PLA fiber mats. Fibroblast L929 cell line is usually used for the testing of cell adhesion, proliferations and cytotoxic properties because its high activity and proliferation rate. However, it has many factors in relationships of cell response on the substrates such as the hydrophobicity and diameter of fiber mats which this work concern.

1.7 Objective

In this work, low molecular weight PLA ($\overline{M}$n = 3,670 Da) having two positively charged chain end (PLAdi+) was synthesized by chemical reaction between carboxyl end group of PLAdiCOOH and the epoxide ring of glycidyl trimethylammonium chloride (GTMAC). PLAdi+ blended with commercialized PLA was electrospun into non-woven fiber mats. The PLAdi+ content in the electrospinning solutions was varied to assess the effect on diameter and hydrophilicity of electrospun fibers. Moreover the electrospun fiber mats were studied for antibacterial testing and fibroblast L929 adhesion and proliferation.

CHAPTER II

EXPERIMENTAL

2.1 Material

L-Lactic acid solution (LLA, 88 wt%) was supplied from Carlo Erba Reagent, France. Tin (II) chloride dihydrate ($SnCl_2.2H_2O$), succinic acid (SA), triethylamine (Et_3N), para-toluene sulfonic acid (*p*TSA), and glycidyl trimethylammonium chloride (GTMAC) were purchased from Sigma-Aldrich, USA. Methanol (MeOH), ethanol (EtOH), sodium chloride (NaCl), and chloroform-d were supplied from Merck, Germany. Chloroform, tetrahydrofuran (HPLC grade), and dimethyl sulfoxide (DMSO) were purchased from RCI Labscan Limited, Thailand. Commercial PLLA, $\bar{M}$n 60,000 Da (Ingeo™ 4043D), was provided by NatureWorks LLC. Staphylococcus aureus TISTR 746 and Escherichia coli TISTR 117 were provided by Thailand Institute of Scientific and Technological Research (TISTR), Thailand. Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) were purchased from Difco (USA). All the above chemicals were analytical grade and used as received without further purification.

2.2 Instrumentation

2.2.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

The synthesized polymer samples dissolved in $CDCl_3$ were analyzed on 400 MHz NMR (Varian, model Mercury-400 nuclear magnetic resonance spectrometer, USA). Proton chemical shifts were reported in parts per million (ppm) relative to tetramethylsilane (TMS) signal as a reference.

2.2.2 Gel Permeation Chromatography (GPC)

Molecular weight of the polymers were obtained by Waters 600 controller chromatograph equipped with two HR (Waters) columns (HR2 and HR4) (MW resolving range = 100-500,000 Da) at internal column temperature 35 °C and a refractive index detector (Waters 2414). THF (HPLC grade) was used as a solvent for the polymers and as an eluent for GPC with a flow rate of 1.0 mL/min. Sample injection volume was 80 µL. The retention time was calculated for polymer molecular weight reported in Dalton relative to polystyrene standards (molecular weight of 7 polystyrene standards: 996 – 188,000 Da).

2.2.3 Ramé-Hart Contact Angle Goniometer

Hydrophilicity of the fiber mats was determined by static and dynamic air-water contact angle measurement using a Ramé-Hart Contact Angle Goniometer (USA), model 100-00, equipped with Gilmont syringe and 24-gauge blunt needle tip. A droplet of DI water was placed on the sample surface and reported as mean ± standard deviation from 10 different points by the syringe.

2.2.4 Scanning Electron Microscope (SEM)

Diameter and morphology of fiber mats and cell visualization were characterized by using a JEOL JSM-6610LV field emission scanning electron microscope operated at 15 kV. The fiber mats were coated with gold by ion-sputtering before the analysis. The fiber mats diameter were measured by using SemAfore version 5.21 program and reported as mean ± standard deviation from 50 different points of two SEM micrographs.

2.2.5 UV/Vis Absorbance Micro plate Reader

MTT assay was performed using Themo scientific multiscan FC micloplate reader at 540 nm. OD was investigated by using SkanIt for HP multiskan FC 3.1 program. And the optical density (OD) of bacteria suspensions was determined by MV, Bausch **ε** Lomb and Multi-Detection Microplate reader Model All, Bio-Tec Instruments, Inc.

2.3 Experimental

2.3.1 Synthesis of PLAdi+

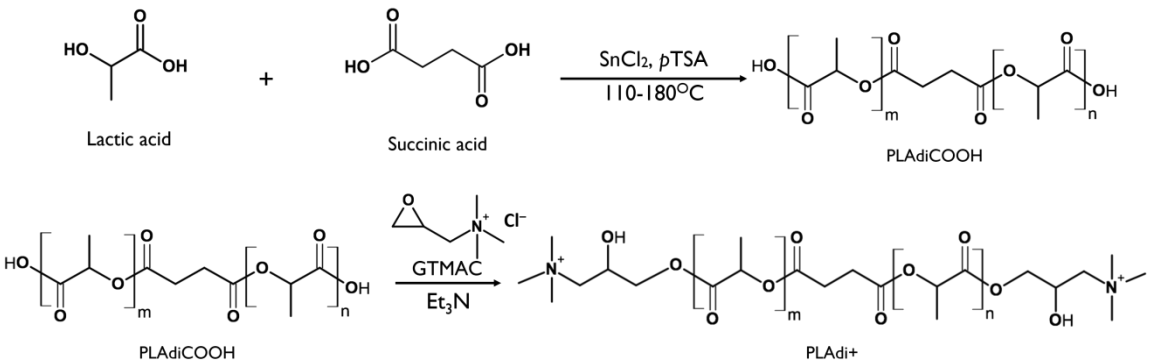


Figure 2.1 Synthesis of PLAdi+ from lactic acid.

PLA attached with positively charged chain end (PLAdi+) was synthesized from lactic acid. The first step was to prepare PLA with two carboxyl chain ends (PLAdiCOOH)

and the second step was to synthesize PLAdi+ as shown **Figure 2.1**. The first step, PLAdiCOOH was synthesized via condensation polymerization using $SnCl_2$ and *p*TSA as catalyst and co-catalyst, respectively. The synthesis method was carried out according to previous work [34]. L-Lactic acid solution (88%wt), succinic acid (4 mole% of L-Lactic acid), and one half-portion of *p*TSA (overall *p*TSA is 1 equiv mole of $SnCl_2.2H_2O$) were added into three-neck round bottom flask equipped with magnetic stirrer and distillated at 110°C for 2 hr to obtain lactide oligomers via dehydration. Then, to remove water by-product, the temperature was increased to 140° and 160°C for 70 and 120 min, respectively, under reduced pressure. Finally, polymerization occurred by adding $SnCl_2.2H_2O$ (0.4 wt % of L-Lactic acid) and the remaining half-portion *p*TSA was added to the prepared oligomer solution while the temperature was increased to 180°C for 4 hrs. The polymer was precipitated in cold ethanol. After vacuum dry, the final product was white powder. In the second step [12], PLAdiCOOH, GTMAC (3 equiv), triethylamine (1 equiv), and DMSO, as solvent, were added into a round bottom flask and stirred at 70°C for 24 hr. Then, the polymer solution was precipitated in 2M NaCl aqueous solution. The precipitated polymer was collected and washed with de-ionized water. White powder of PLAdi+ was obtained after lyophilization.

2.3.2 Preparation of electrospun fiber mats

Three types of electrospun fiber mats were produced; from pure commercialized PLA, a series of PLA/PLAdi+ mixtures, and PLA/PLAdiCOOH mixtures with mass ratios of 100:10, 100:30, 100:50 and 100:70. The polymer solutions were dissolved in $CHCl_3$/MeOH (3:1) mixture. Then polymer solutions (2 mL) were loaded into 5 mL syringe with 26G blunt needle tip and the syringe was fixed horizontally on syringe pump. The pump was controlled at a flow rate of 1 mL/hr with 20 kV. The gap between needle tip and collected plate was 20 cm. The electrospun fiber was jetted onto 10 cm × 10 cm aluminum sheet as a collector as shown in **Figure 2.2**.

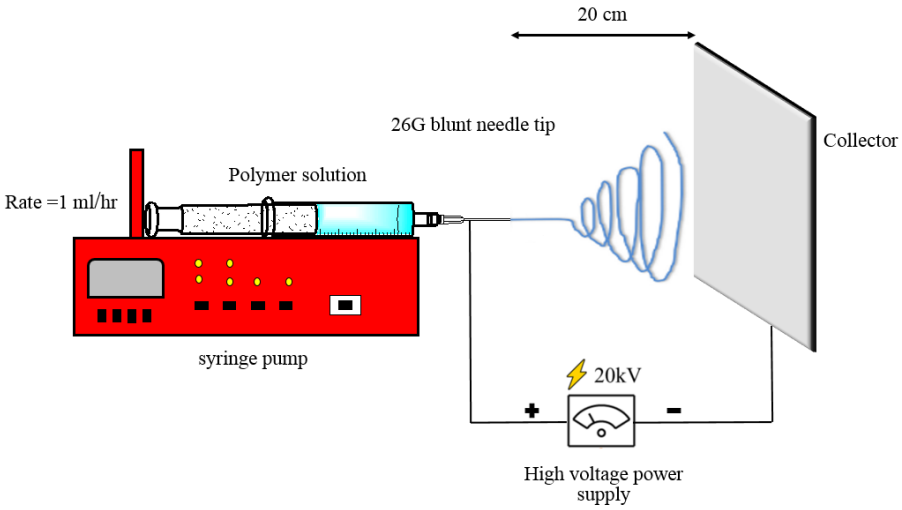


Figure 2.2 Schematic of electrospinng set-up and parameter

2.3.3 Biological test on the electrospun fiber mats.

2.3.3.1 Antibacterial testing.

S. aureus TISTR 746 (Gram positive) and *E. coli* TISTR 117 (Gram negative) were used to study antibacterial test by colony counting and clear zone. Before conducting any antibacterial test, the bacteria was first grown aerobically overnight on Mueller Hinton Agar (MHA) slant at 37 °C. Sterile deionized water (5 mL) was added in tube ager slant to obtain bacterial suspension. The bacterial suspension was prepared to obtain optical density (OD_{600}) of 0.1. The optical density was determined by UV-visible spectrophotometer at 600 nm.

Colony counting

A (OD_{600} = 0.1) bacteria suspension (15µL in deionized water), 2.5mL Mueller Hinton Broth (MHB) and electrospun fiber mats on round aluminium plate (diameter 1.5 cm) were put into the test tube. The test tube was incubated in shaking incubator at 37 °C, 110 rpm for 24 hrs. Then 100 µL of bacterial solutions was diluted to 10^4, 10^6 and 10^8 times. The diluted bacteria suspensions were spread in triplicate onto MHA plate and incubate at 37 °C for 18 hrs. Finally, colony forming units were counted as measure of the assumed viable number of bacteria.

Clear zone

A (OD_{600} = 0.1) bacteria suspension (100 µL in deionized water) was spread in triplicate onto MHA plate (2×10^8 CFU/mL). The electrospun fiber mats on round

aluminium plate (diameter 1.0 cm) were attached on the MHA agar plate and incubate at 37 °C for 24 hrs. Finally, the clear zone was checked.

2.3.3.2 Fibroblast (L929) cell response on the electrospun fiber mats.

Fibroblast (L929) cell line from mouse was used to study cell adhesion and proliferation on the fiber mats by MTT assay. RPMI 1640 containing 5% fetal bovine serum (FBS), penicillin (10,000 U/L) and streptomycin (100 mg/ml) was used as cell culture media. Cell incubation was carried out under 5% CO_2 at 37°C for up to 5 days. The media was replaced every 2 days.

All polymer fiber mats (1 cm diameter) were transferred to 48-well tissue culture polystyrene (TCPS) plate and sterilized with UV light for 30 min. Then, the cells, approximate 2×10^4, were seed on the substrates and incubated under 5% CO_2 at 37°C for 1 hr. The media (500 µL) was added into the sample and incubated under 5% CO_2 at 37 °C. MTT assay was used to investigate cell adhesion and proliferation. After 1st, 3rd and 5th day, the media solutions was removed, and 0.5 mg/ml MTT/normal saline solution was added to the well plate and incubated under 5% CO_2 at 37 °C for 2 hr. Then the solution was removed and 200 µL DMSO was added to dissolve the purple crystals of formazan. The measurement was done by microplate reader at the wavelength of 540 nm. The cell proliferation ratio was determined for 1st, 3rd, and 5th day and evaluated using Eq. 1 where OD_{sample} indicated the optical density of the samples and OD_{TCPS} indicated the optical density on TCPS surface as a control.

$$\text{Cell proliferation ratio (\%)} = \left[\frac{OD_{sample\ 1,3,5\ days}}{OD_{TCPS\ 1,3,5\ day}}\right] \times 100 \qquad \text{Eq. (1)}$$

CHAPTER III

RESULT AND DISCUSSIONS

This chapter was divided into three parts. The first part is the synthesis of PLA having two positively charged chain ends (PLAdi+), which was produced from two-step reactions. The first step was to synthesize PLAdiCOOH, PLA with carboxyl groups on both chain ends, and the second step was to make PLAdi+. The second part is the preparation of electrospun fiber mats from pure PLA, a series of PLA/PLAdi+ mixtures, and PLA/PLAdiCOOH mixtures. The last part covers study on biological response of the fiber mats against bacteria and fibroblast (L929), in order to obtain initial results on the biological responses of the obtained PLA/PLAdi+ fiber mats.

3.1 Synthesis of PLAdi+

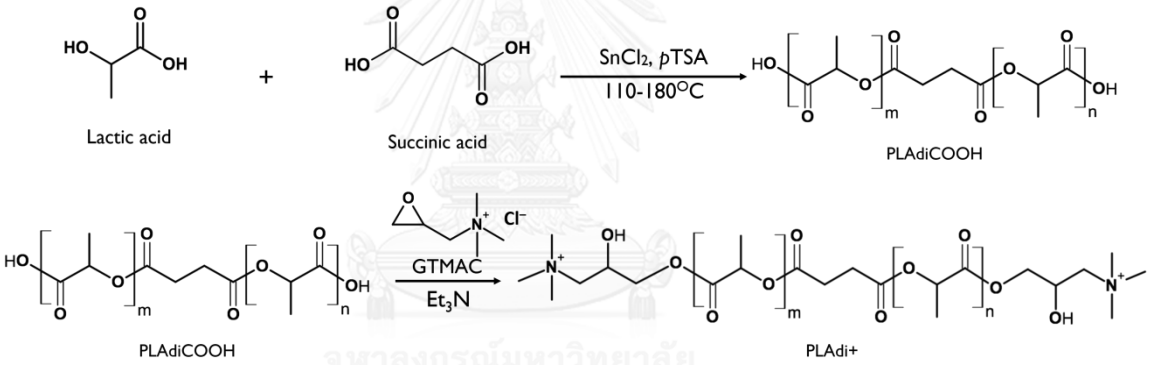


Figure 3.1 Synthesis of PLAdi+

3.1.1 Synthesis and Characterization of PLAdiCOOH

The telechelic dicarboxylic acid capped PLA (PLAdiCOOH) was synthesized via condensation polymerization of L-lactic acid monomer using $SnCl_2$ and *p*TSA as a catalyst and co-catalyst respectively. A small amount of succinic acid (4 mol%) was used to be the initiating point of esterification of L-Lactic acid to yield growing polymers having carboxyl group on both chain ends (**Figure 3.2**). In addition, succinic acid can also act as a coupling point between the two PLA chains to yield the same PLAdiCOOH structure. Percentage yield of PLAdiCOOH product was calculated (Eq. 2) based on weight of dried polymer product to theoretical weight. The PLAdiCOOH were synthesized in a good yield of 82%.

$$\% \text{ yield} = \left[\frac{weight\ of\ dried\ PLAdiCOOH}{(mole\ of\ succinic\ acid \times 84.09\ g) + (mole\ of\ L\text{-}lactic\ acid \times 72.05)}\right] \times 100 \qquad \text{Eq. (2)}$$

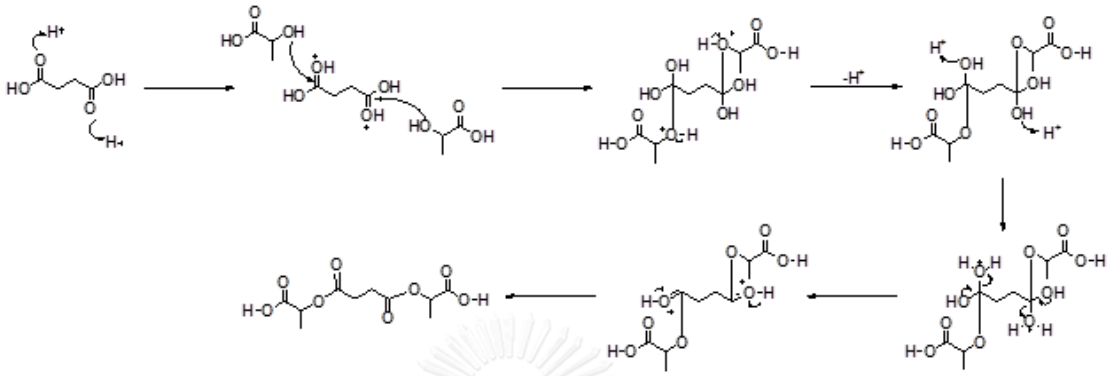


Figure 3.2 Mechanism of PLAdiCOOH synthesis

The molecular weight of obtained PLAdiCOOH was determined from ^{1}H NMR spectrum. Peak integrations of methylene protons (-CH_2-) of succinic acid at 2.8 ppm, and methine protons (-CH-) of lactide unit at 5.2 ppm were used to calculate its molecular weight (**Figure 3.3** and Eq. 3). The molecular weight of PLAdiCOOH determined by ^{1}H NMR was 3,670 Da, about 15% lower than that from GPC analysis (4,305 Da, PDI=1.23) (**Figure 3.4**). Moreover upon closer inspection of the NMR spectrum in **Figure 3.3**, weak signals belonging to the lactide unit located at the 'hydroxyl' chain end (1.5 ppm for methyl and 4.4 ppm for methine groups) were also observed. These signals suggest the presence of normal PLA chains that have hydroxyl in one end and carboxyl group in the other. The remaining normal PLA chains could be calculated from Eq. 4 which was 17%. This normal PLA occurred possibly due to low amount of succinic acid, thus providing less opportunity to react with lactic acid.

$$\bar{M}_n = \left[\left(\frac{a}{d+(c/4)}\right) \times 72\right] + 262 \qquad \text{Eq. (3)}$$

while 72 is the molar mass of lactide unit and 262 is the summation of molar mass of lactide unit at both COOH chain ends (178 g/mole) plus the middle succinic unit (84 g/mole)

$$\% \text{ Normal PLA chain} = \left[\frac{d}{d+(c/4)}\right] \times 100 \qquad \text{Eq. (4)}$$

while d is methane proton of normal PLA chain and d plus c/4 is total number of normal PLA and PLAdiCOOH chains.

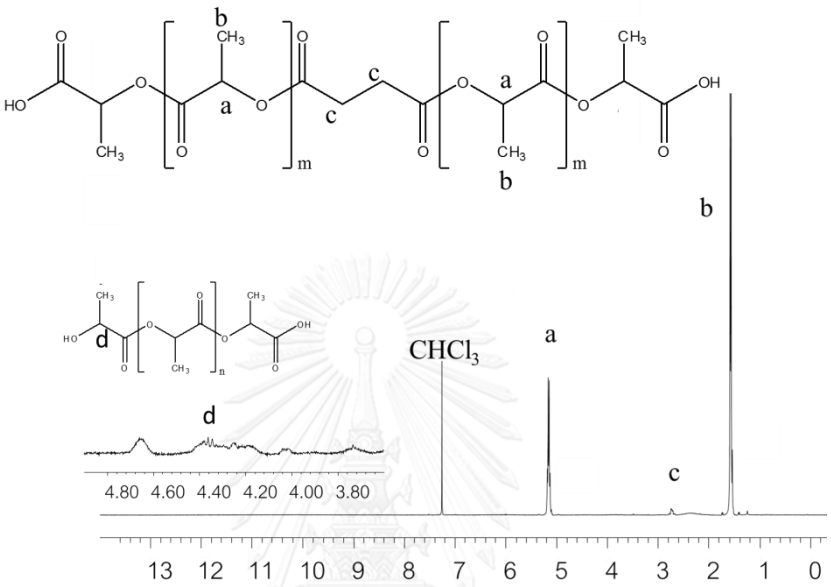
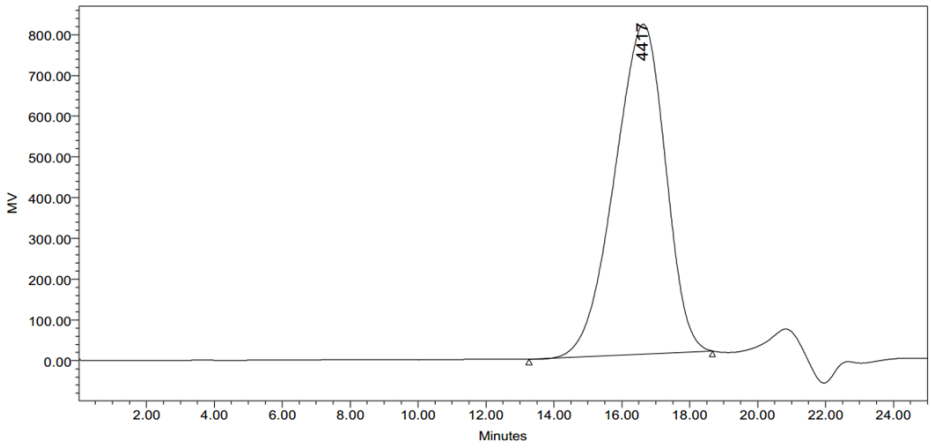


Figure 3.3 ^{1}H NMR spectra of PLAdiCOOH in $CDCl_3$. a is methine proton. b is methyl proton of lactide repeating unit, and c is methylene proton of succinic acid.



Mn	Mw	MP	Mz	MZ+1	PDI
4305	5317	4417	6596	8143	1.235114

Figure 3.4 Chromatogram from GPC analysis of synthesized PLAdiCOOH dissolved in THF.

3.1.2. Synthesis and Characterization of PLAdi+

PLA having positively charged ammonium groups at both chain ends (PLAdi+) was synthesized via ring opening reaction of GTMAC with the carboxylic end groups of PLAdiCOOH. This reaction was carried out based on previous studies [12, 34], reporting that carboxylate ion (-COO-) was more reactive than hydroxyl (OH) in the ring opening reaction of GTMAC. The addition of triethylamine (Et_3N) helps converting the carboxylic acid to a stronger nucleophile, the carboxylate ion (**Figure 3.5**).

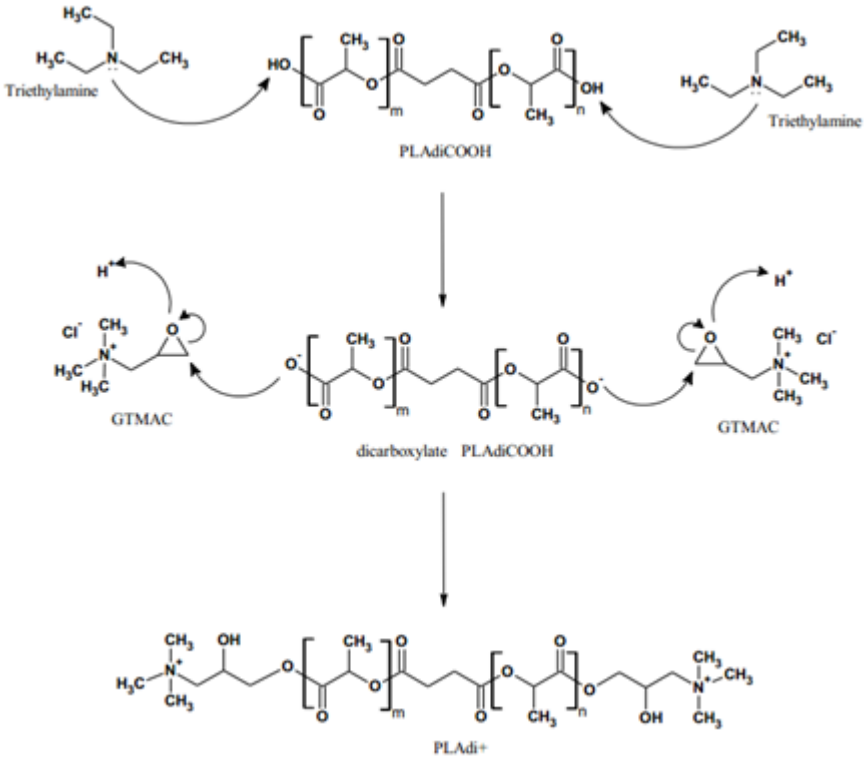


Figure 3.5 Mechanism of base-catalyzed substitution reaction in PLAdi+ synthesis

After lyophilization, the PLAdi+ product was obtained. The percentage yield of PLAdi+ was 76%. The success of attachment of GTMAC onto PLAdiCOOH was confirmed by the appearance of methyl proton (-CH_3-) of quaternary ammonium group at 3.4 ppm. The degree of substitution (%DS) was also evaluated from ^{1}H-NMR spectroscopy. The DS as high as 95% was obtained possibly because the molecular weight of PLAdiCOOH was not so high that it could hinder the ring-opening reaction (**Figure 3.5**).

From the ^{1}H NMR spectrum of PLAdi+ (**Figure 3.6**), peak integrations of methine protons (-CH-) of lactic unit at 5.2 ppm and methylene protons (-CH_2-) of succinic acid

at 2.8 ppm (**Table 3.1**) were used to calculate its molecular using Eq. 5. The molecular weight of PLAdi+ determined by ^{1}H NMR was 3,660 Da.

$$\bar{M}_n = \left[\left(\frac{a}{d+(c/4)}\right) \times 72\right] + 565 \qquad \text{Eq. (5)}$$

where 72 is the molar mass of lactic unit and 565 is the summation of molar mass of lactic unit at both polymer end (144 g/mole) plus the middle succinic unit (84 g/mole) and plus the both GTMAC chain ends attached to the PLAdiCOOH and Cl- (337 g/mole)

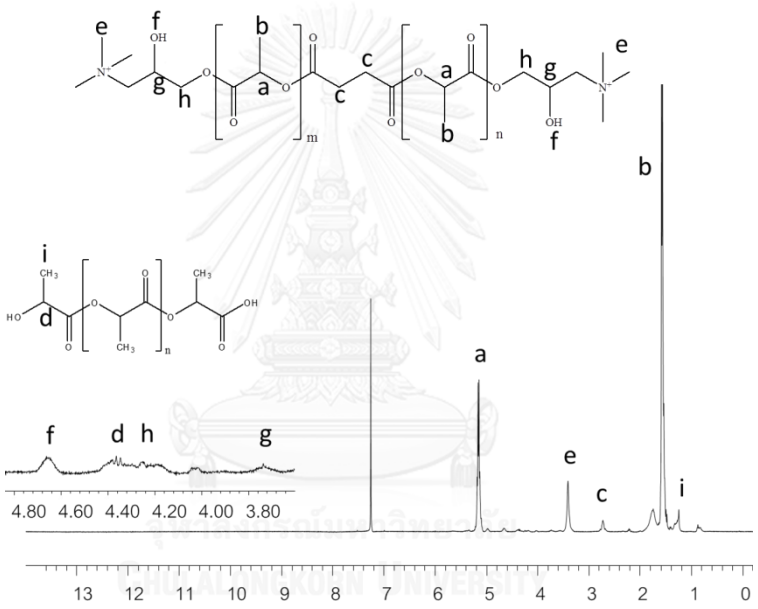


Figure 3.6 ^{1}H NMR spectra of PLAdi+ in $CDCl_3$

Table 3.1 Complete assignment of ^{1}H NMR chemical shift of PLAdi+ in $CDCl_3$.

Unit	chemical shift (ppm)	Unit	chemical shift (ppm)
a	5.2	f	4.7
b	1.5	g	3.8
c	2.8	h	4.3
d	4.4	i	1.2
e	3.4		

Furthermore, ^{1}H NMR was used to calculate the degree of substitution (%DS) of GTMAC on PLAdiCOOH chain end as shown in Eq. 6. The degree of substitution of GTMAC on PLAdiCOOH determined by ^{1}H NMR was 95%.

$$\%DS = \frac{1}{2}\left[\frac{e/9}{d+(c/4)}\right] \qquad \text{Eq. (6)}$$

where e/9 is total number quaternary salt unit per one chain, d is total number of PLA chain, and c/4 is total number of PLAdiCOOH chain.

3.2 Characterization of electrospun fiber mats

3.2.1 Morphology and diameter of electrospun fiber mats

The electrospining technique has been widely used to produce the non-woven micro and nano fibers. In this work, all fibers were produced with identical processing parameter. The electrospun fiber mats were produced into three types; from pure commercialized PLA, from a series of PLAdi+ mixed with commercialized PLA with mass ratios for PLA:PLAdi+ equaled 100:10 (+10) , 100:30 (+30), 100:50 (+50), and 100:70 (+70). And PLAdiCOOH mixed with PLA with mass ratios for PLA:PLAdiCOOH equaled 100:10 (CO10) , 100:30 (CO30), 100:50 (CO50), and 100:70 (CO70). The polymer solutions were dissolved in $CHCl_3$/MeOH (3:1) mixture. MeOH was also added in order to improve the conductivity of polymer solution [17, 35] and easily to be evaporated from the electrospun fiber mats. The electrospun fiber mats was very thin and white in color (**Figure 3.7**).

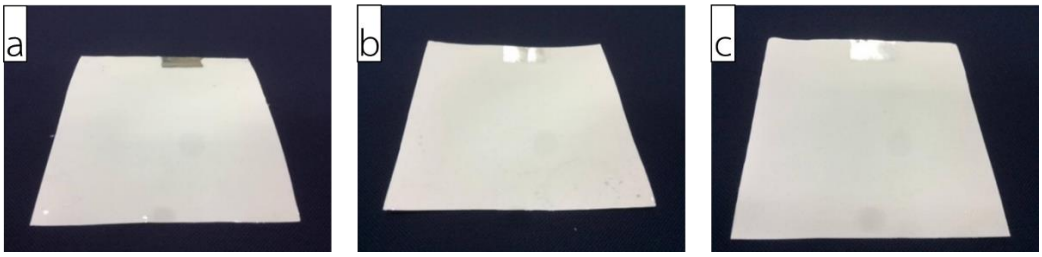


Figure 3.7 Electrospun fiber mats of PLA (a), +70 (b) and CO70 (c).

The amount of positive charge from PLAdi+ in the fiber mats was determined using Eq. 7 and 8. It was found that the amount of positive charges increased from 0.47×10^{-5} mol/g to 2.14×10^{-4} mol/g when PLAdi+ was increased from 100:10 to 100:70 (**Table 3.2**).

$$Amount\ of\ positive\ charge\ (mole) = \left[\frac{\frac{Weight\ of\ sample \times \%DS}{100}}{M_w\ Of\ PLAdi+}\right] \times 2 \qquad \text{Eq. (7)}$$

$$Amount\ of\ positive\ charge\ \text{per}\ gram\ \left(\frac{mole}{gram}\right) = \frac{Amount\ of\ positive\ charge}{Weight\ of\ PLA + Weight\ Of\ PLAdi+} \quad \text{Eq. (8)}$$

Table 3.2 Amount of positive charge in the electrospinning solutions.

Samples (PLA/PLAdi+)	Amount of positive charge/ total weight (mol/g)
100:10 (+10)	0.47×10^{-5}
100:30 (+30)	1.20×10^{-4}
100:50 (+50)	1.73×10^{-4}
100:70 (+70)	2.14×10^{-4}

From SEM images, the fiber surface was smooth and bead-free (**Figure 3.8**). When increasing the PLAdi+ content in the polymer mixture, the fiber diameter decreased from 365±83 to 109±29 nm (**Figure 3.9**). This change can be explained by two phenomena. The first is the plasticizing effect of low molecular weight PLAdi+ that infiltrated into the commercialized PLA. The second is the increased positive charged portion in the PLA/PLAdi+ solution helps pushing the polymer solution out of the needle. Therefore, the solution is jetted from the needle tip at a higher rate and fibers

with smaller diameter [12, 20]. Meanwhile, mixing PLAdiCOOH into PLA solution resulted in diameter reduction from 365±83 to 229±44 nm which was arising from only the plasticizing effect **Figure 3.9**. It was however found that increasing the PLAdiCOOH content further from 50 to 70 did not help reduce the fiber diameter. From these results, it can be concluded that the positive charge in the form of PLAdi+ doped into high molecular weight PLA solution can effectively reduce the fiber diameter by combining between the increasing of positively charged portion and plasticizing effect.

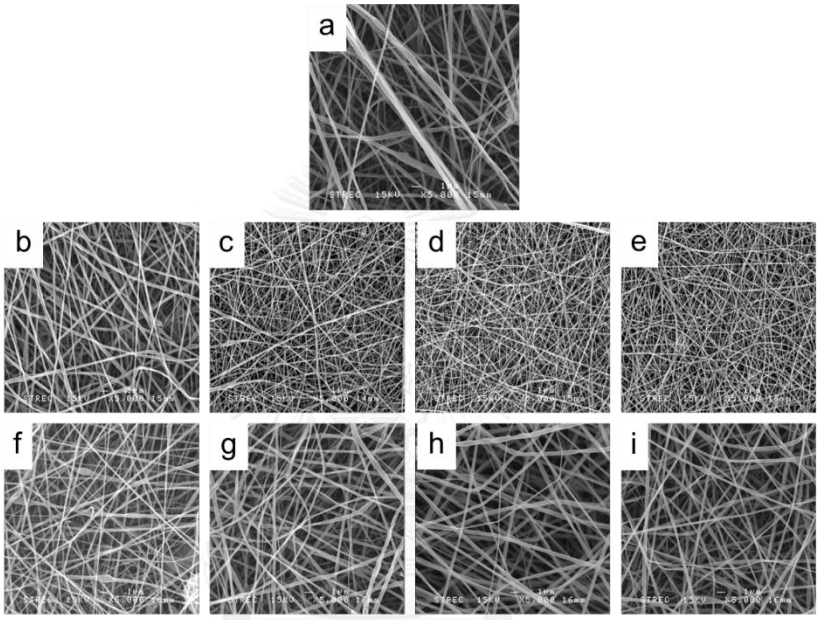


Figure 3.8 SEM images of (a) PLA, (b) +10, (c) +30, (d) +50, (e) +70, (f) CO10, (g) CO30, (h) CO50, and (i) CO70 electrospun fiber mats (5000× magnification, scale bar = 1 μm)

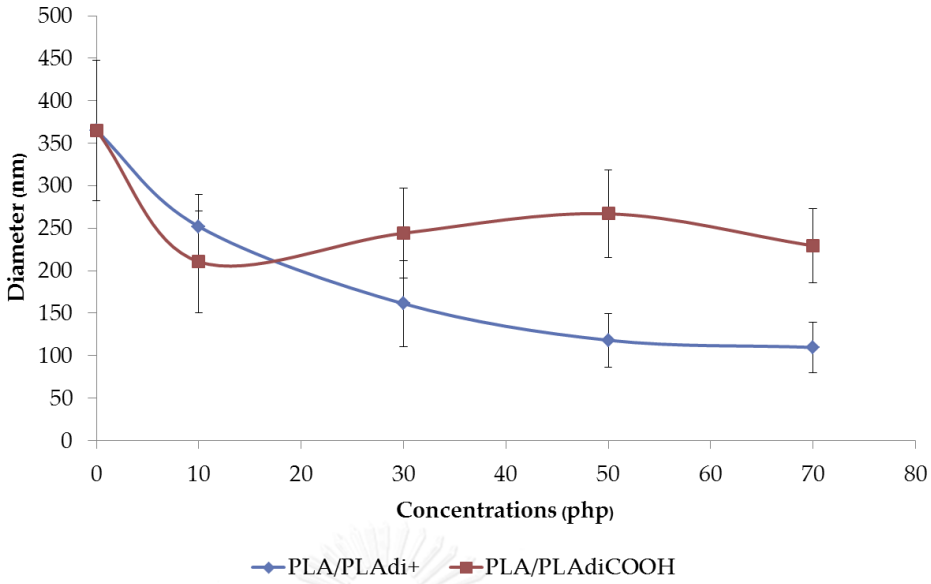


Figure 3.9 Average diameters of PLA, PLA/PLAdi+ and PLA/PLAdiCOOH electrospun fiber mats

3.2.2 Hydrophobicity of the electrospun fiber mats

The hydrophobicity of fiber mats was determined by air-water contact angle using two methods, static and dynamic water contact angle.

Static water contact angle, the results shown in **Table 3.3** were the air-water contact angles measured at 4 consecutive time points (0, 30, 60 and 90 sec). The addition of PLAdi+ caused a reduction of air-water contact angle from 135° (PLA) down to 122° (+70 fiber mats). Upon continuous observation for 90 sec, the +70 fiber mat slowly absorbed water droplet, causing a dramatic decrease of contact angle value to 50°. Meanwhile the presence of polar carboxylic group in PLAdiCOOH only slightly reduced the contact angle (from 135° to 128°), and no water absorption was observed. The contact angles of the other fiber mats were shown at only 2 consecutive time points (0 and 30 sec) because after 30 sec their contact angles remained unchanged.

Table 3.3 Air-water contact angle (°) of PLA, PLA/PLAdi+, and PLA/PLAdiCOOH electrospun fiber mats. The angle measurement was done at 0, 30, 60, and 90 see after water was dropped onto the samples.

Samples	Contact angle (°)			
	0 sec	30 sec	60 sec	90 sec
PLA	135±1	134±1	-	-
+10	130±3	129±4	-	-
+30	128±2	128±2	-	-
+50	122±1	122±1	-	-
+70	122±1	74±5	59±8	50±3
CO10	130±1	129±2	-	-
CO30	128±2	128±2	-	-
CO50	128±1	127±2	-	-
CO70	128±1	127±2	-	-

Dynamic water contact angle was measured to evaluate relative hydrophobicity of the prepared fiber mats. As shown in **Table 3.4**, the advancing water contact angle was decreased from 144±5° (unmodified PLA) to 55±3° (+70 fiber mats) while the presence of carboxylic group in PLAdiCOOH only slightly reduced the water contact angle (from 144±5° to 128±5°) indicating more hydrophilic fiber mats after modifying PLA chain end with positively charged quaternary ammonium groups. Receding water contact angles of all substrates were unmeasurable. They were totally wet implying the hydrophilicity of these fiber mats after being wet with water droplet. This may be explained as a consequence of hydrophilic carbonyl group and quaternary ammonium groups being present at the fiber/air interface. In addition, high porosity on the electrospun fibrous surfaces provided high roughness resulting in extremely high contact angle hysteresis (advancing-receding) and water droplet pinning effect.

Table 3.4 Advancing and receding water contact angle (°) of PLA, PLA/PLAdi+, and PLA/PLAdiCOOH electrospun fiber mats.

Samples	Contact angle (°)	
	Advancing	Receding
PLA	144±5	N/A
+10	140±4	N/A
+30	130±8	N/A
+50	119±7	N/A
+70	55±3	N/A
CO10	140±5	N/A
CO30	137±9	N/A
CO50	133±6	N/A
CO70	128±5	N/A

N/A: not measurable

3.3 Biological test on the electrospun fiber mats

3.3.1 Antibacterial testing

The antibacterial activity of electrospun fiber mats were reported as colony counting, and clear zone method.

From the result of colony counting method, the total number of replication competent (viable) cell as mean colony forming unit per volume (CFU/mL) against *S. aureus* bacteria is shown in **Figure 3.10**. Considering the data of PLA/PLAdi+ fiber mats, the statistical comparison of antibacterial activity against *S. aureus* with PLA and PLA/PLAdiCOOH were not significantly different as identified by using Anova ($p < 0.05$). This indicates that the amount of positive charge in fiber mats is insufficient to antibacterial activity.

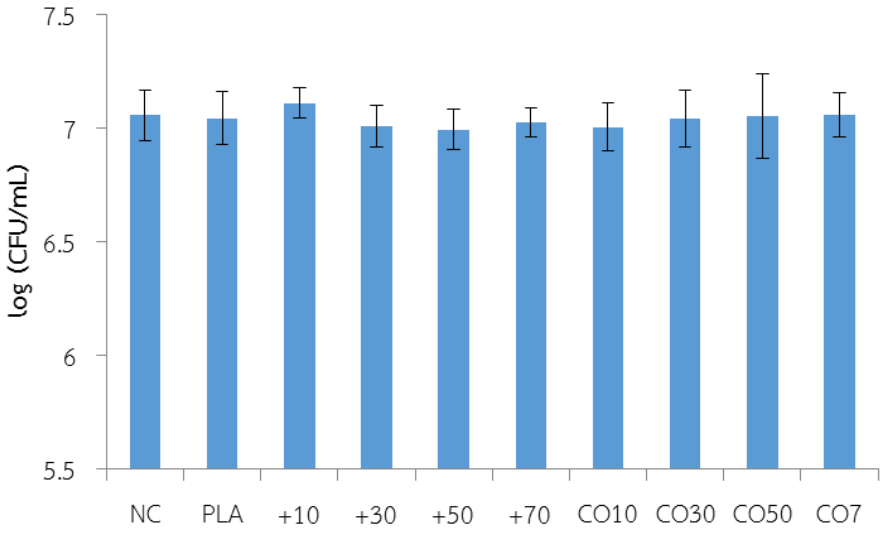


Figure 3.10 Viable cell counts of *S .aureus* bacteria suspension incubated with electrospun fiber mats for 24 hr.

The bacteriostatic activities of PLA, +70 and CO70 fiber mats were also examined by clear zone tested. *S. aureus* (Gram positive) and *E. coli* (Gram negative) were chosen in this study. **Figure 3.11** shows the inhibition zones of the fiber mats and negative controls as round aluminium plate against *S. aureus* and *E. coli* after incubated at 37°C for 24 hrs. All fiber mats showed no activity against the tested bacteria. This result is consistent with colony counting method.

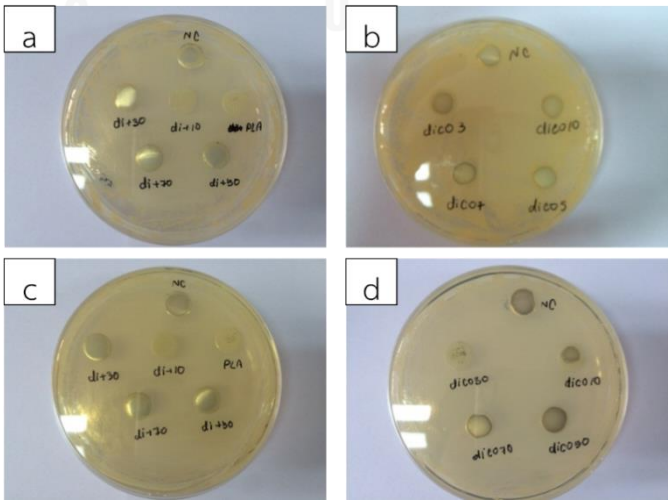


Figure 3.11 Clear zone of inhibition of electrospun fiber mats against both Gram positive bacterial (*S.aureus*) (a, b) and Gram negative bacterial (*E.coli*) (c, d).

3.3.2 Fibroblast (L929) cell response on the electrospun fiber mats.

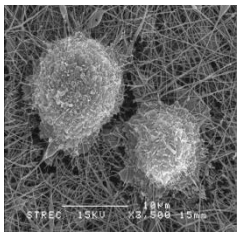
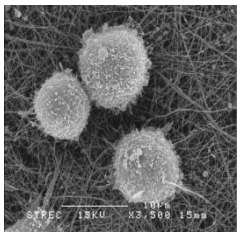
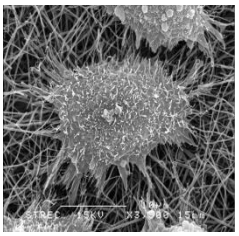
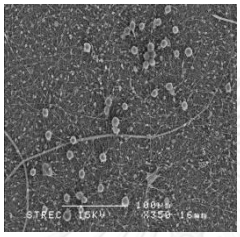
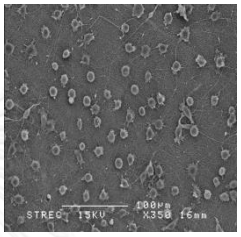
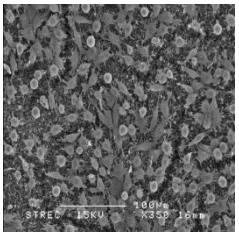
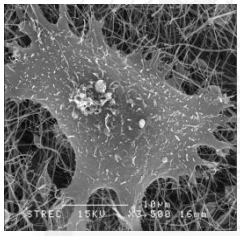
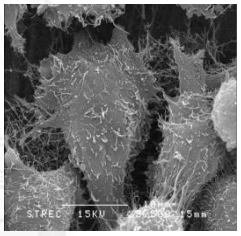
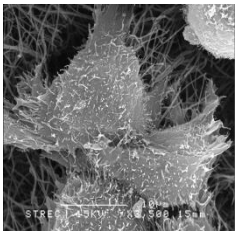
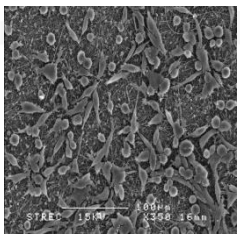
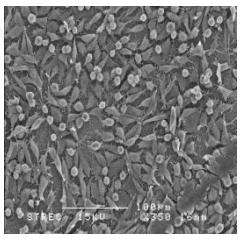
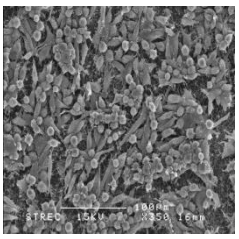
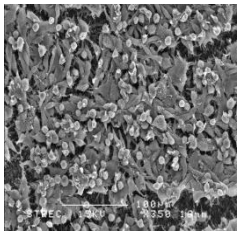
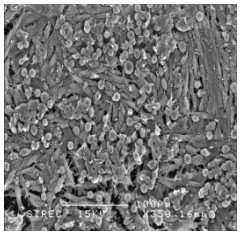
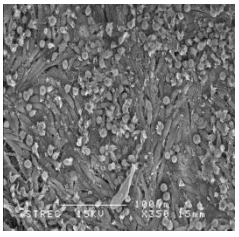
Before undergoing the fibroblast (L929) cell response test, all fiber mats were sterilized by UV irradiation. During the sterilization period, the polymers degraded to some extent **(Table 3.5 and Figure A-1-A-12).** From these results, sterile period of 30 min was chosen because it's enough to aseptically inoculate for cell adhesion and proliferation studies.

Table 3.5 Molecular weight of PLA, +70, and CO70 electrospun fiber mats determined by GPC before and after UV sterilization for 18 hr, 60 min and 30 min

Sample	Before	After		
		30 min	60 min	18 hr
PLA	48.9 kDa	44.5 kDa	38.9 kDa	18.2 kDa
+70	58.6 kDa	57.8 kDa	38.8 kDa	20.0 kDa
CO70	56.6 kDa	56.1 kDa	36.9 kDa	27.4 kDa
	4.3 kDa	4.2 kDa	3.3kDa	1.3 kDa

Examination of morphology and adhesion of fibroblast (L929) cell on PLA, +70 and CO70 electrospun fiber mats was carried out by SEM. The SEM images show the interesting effect of CO70 electrospun fiber mats on cell growth. The fibroblast cells have a spread morphology (18.79±1.09 μm) since 1st day (**Table 3.6**), while the fibroblast cells on PLA and +70 spread at a lesser extent with a diameter of 11.41±1.26 and 9.11±1.01 μm, respectively. However, on the PLA and +70 electrospun fiber mats the fibroblast (L929) cells eventually spread to the same extent as the one on CO70 on the 3rd day. By the 5th day, cells completely covered the surface of +70 and CO70 fiber mats.

Table 3.6 SEM images of fibroblast (L929) cell line on the electrospun fiber mats on the 1st, 3rd, and 5th day. (350× and 3500x magnification, scale bar = 1μm).

Date	magnification	PLA	+70	CO70
1	3500x			
	350x			
3	3500x			
	350x			
5	350x			

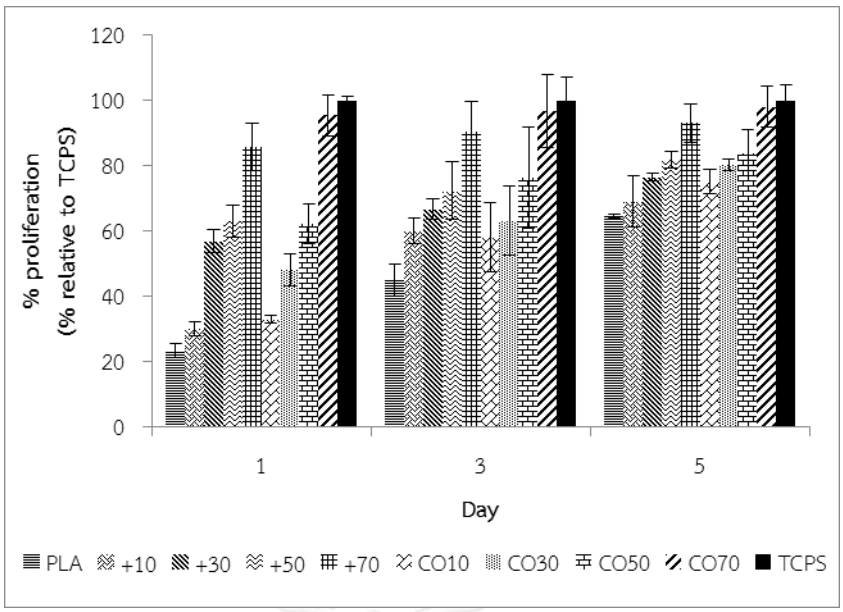


Figure 3.12 Degree of proliferation (relative to TCPS) of L929 on the 1st, 3rd, and 5th days. Approximately 2×104 per well were seeded on the electrospun fiber mats as determined by MTT assay. Error bars represent absorbance variability measured at 540 nm where n=3, Anova ($p < 0.05$)

The degree of proliferation was calculated based on absorbance as illustrated in Eq. 1. **Figure 3.12** shows percentage of cell proliferation of fibroblast (L929) cell on electrospun fiber mat in the 1st, 3rd and 5th day. Statistically comparing between the 1st and 3rd day, the percentage of cell proliferate for each fiber mats was not significantly different as identified using Anova ($p < 0.05$). However, on the 5th day, the percentage of cell proliferate on the fiber mats with PLAdi+ and PLAdiCOOH over 30 php were significantly higher than PLA ($p < 0.05$) indicating more hydrophilicity of PLA/PLAdi+ and PLA/PLAdiCOOH. Although the contact angle of +70 was lower than CO70, the percentage of cell proliferate on the +70 and CO70 were not significantly different as identified using Anova ($p < 0.05$). It might be because of the larger diameter of CO70 (229±44 nm) compared with +70 (109±29) resulting in the lower fiber density of CO70 as shown by SEM images (**Figure 3.8**), as well as more cell attachment. This finding was also consistent with the previous studies, they found that the greater diameter of PDLLA fibers (2.14 μm) than PLLA fiber (0.25 μm), provided higher MC3T3-E1 cell attachment [33]. Moreover, another work also supported that the greater diameter of poly(D,L-lactide-co-glycolide) (diameter range of 25 to 100 μm in 2×5 cm rectangular shapes) provided lower fiber density resulting in higher porosity which could promote adhesion of BALB/c C7 mouse fibroblast cells [36]. Not only surface polarity, but also surface topography affects cell adhesion and proliferation. However,

the attachment and proliferation on the +70, CO70, and tissue culture polystyrene (TCPS) plate were not significantly different as identified using Anova ($p < 0.05$). From these results, it can be conclude that the electrospun fiber mats of PLA mixed with PLAdi+ and PLAdiCOOH were found to be an agreeable material for cell culture because the degree of cell proliferation on +70 and CO70 comparison with TCPS was not different as identified by Anova ($p < 0.05$). However, fibroblast (L929) cell on the surface of other fiber mats were viable and proliferated, confirming biocompatibility of the PLA/PLAdi+ electrospun material.

CHAPTER IV
CONCLUSION

4.1 Summary

Low molecular weight PLAs with dicarboxylic chain end (PLAdiCOOH) and PLAs with positively charged chain end (PLAdi+) were successfully synthesized from lactic acid monomer in the presence of succinic acid using $SnCl_2$ and *p*TSA as a catalyst and co-catalyst respectively. PLAdi+ was mixed with commercialized PLA and electrospun into fiber with diameter as low as 109 nm. The size and hydrophobicity of PLA/PLAdi+ was decreased because of the increasing positive charge in the polymer blend. The electrospun fiber mats of PLA/PLAdi+ and PLA/PLAdiCOOH were found to be an agreeable material for cell culture since they showed a comparable fibroblast L929 proliferation as tissue-cultured polystyrene (TCPS), Moreover, the fibroblast cell was also able to proliferate on the surface of all PLA/PLAdi+ fiber mats prepared in this work, suggesting that they can be considered as biocompatible fiber mats. The PLA/PLAdi+ fiber mats did not, however, possess an antibacterial activity possibly because the amount of positive charges in the fiber mats was insufficient. It is anticipated that the developed PLA/PLAdi+ fiber mats should be applicable as biomedical material such as dressing wound.

4.2 Future direction

To increase positively charged by branched PLA structure would be quite interesting. Thus, the tricarbally acid and tricarboxylic acid, is appropriate molecules that would increase the number of PLA chain end to 3 and 4 carboxylic end groups which can be future derivatized to 3 and 4 quaternary ammonium containing molecule. Moreover, to study in cell response without the effect of fiber density, fiber mat with controllable density would be prepared in similar topography.

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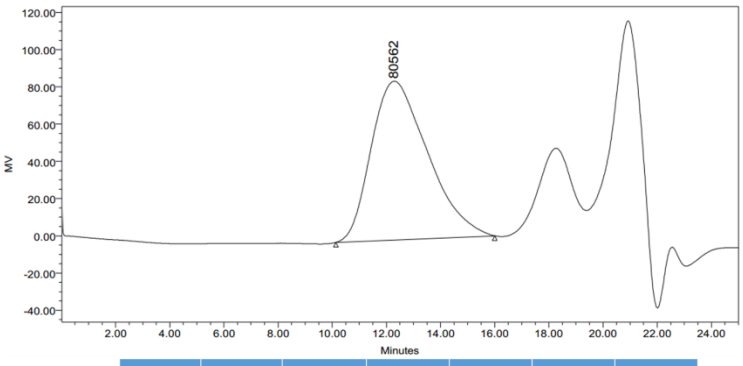
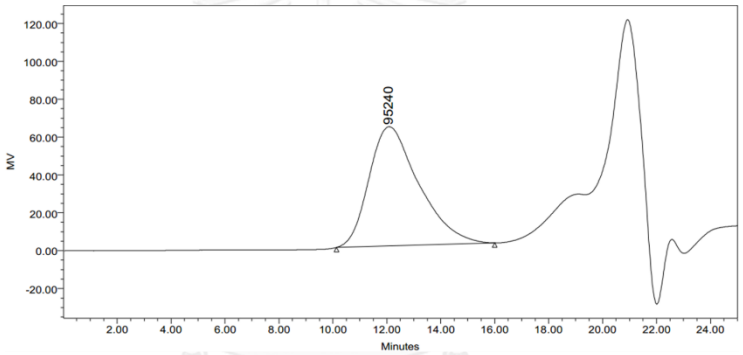
APPENDIX



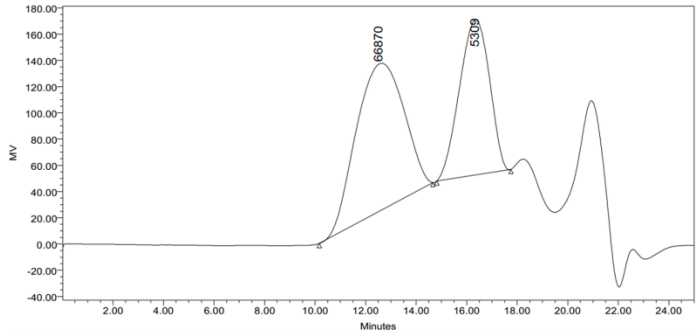
Broad	Mn	Mw	MP	Mz	MZ+1	PDI
1	48868	84516	80562	133908	192102	1.729474

Figure A-1 Chromatogram from GPC analysis of PLA electrospun fiber mats dissolved in THF.



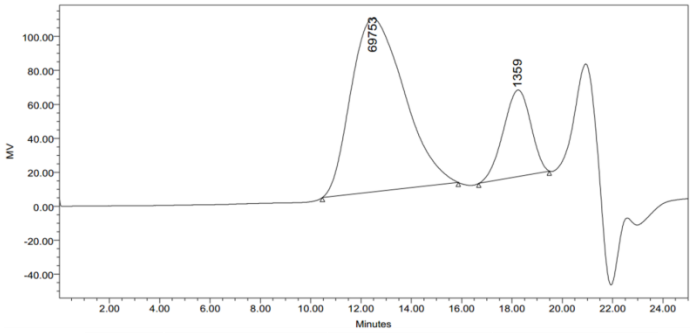
Mn	Mw	MP	Mz	MZ+1	PDI
58556	96509	95240	145270	201773	1.648146

Figure A-2 Chromatogram from GPC analysis of PLA/PLAdi+ (100:70) electrospun fiber mats dissolved in THF.



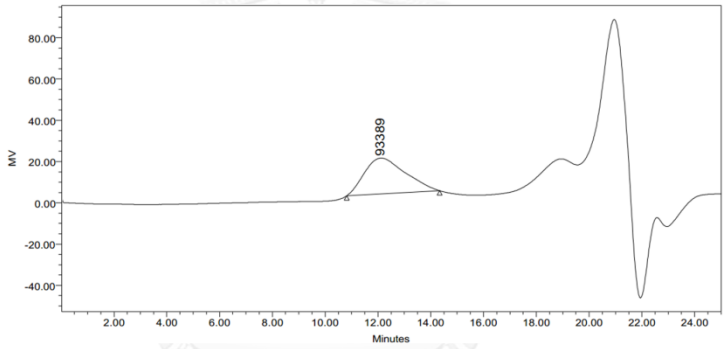
Broad	Mn	Mw	MP	Mz	MZ+1	PDI
1	56612	81670	66870	118299	161439	1.442624
2	4298	5301	5309	6464	7153	1.115938

Figure A-3 Chromatogram from GPC analysis of PLA/PLAdiCOOH (100:70) electrospun fiber mats dissolved in THF.



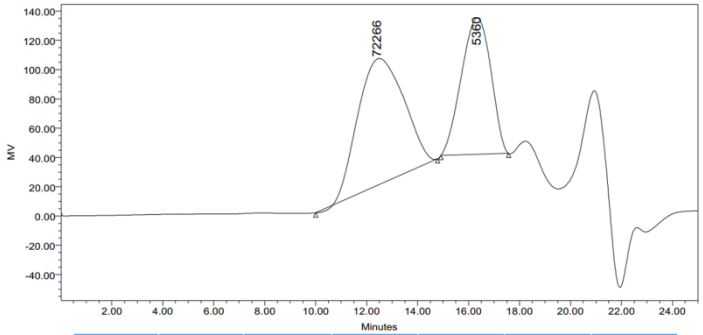
Broad	Mn	Mw	MP	Mz	MZ+1	PDI
1	44504	73205	69753	111817	154327	1.644914
2	1315	1527	1359	1764	2012	1.161446

Figure A-4 Chromatogram from GPC analysis of PLA electrospun fiber mats dissolved in THF after sterilized 30 min



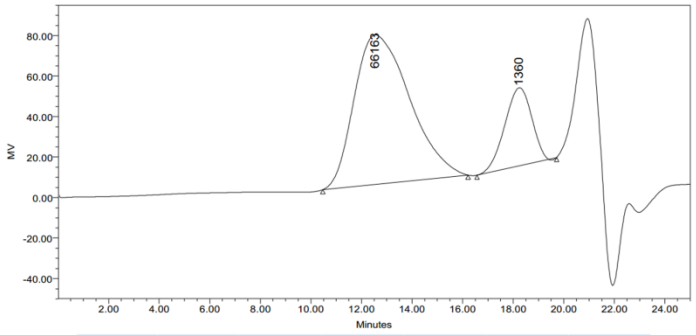
Mn	Mw	MP	Mz	MZ+1	PDI
57872	80981	93389	117201	142677	1.340478

Figure A-5 Chromatogram from GPC analysis of PLA/PLAdi+ (100:70) electrospun fiber mats dissolved in THF after sterilized 30 min



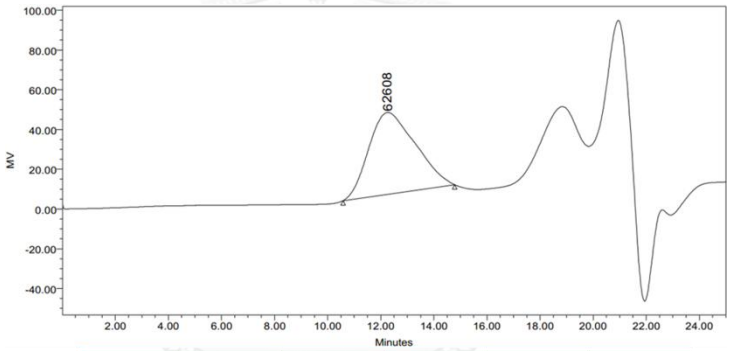
Broad	Mn	Mw	MP	Mz	MZ+1	PDI
1	56178	78648	72266	108422	140942	1.399977
2	4262	4824	5360	6438	7070	1.106793

Figure A-6 Chromatogram from GPC analysis of PLA/PLAdiCOOH (100:70) electrospun fiber mats dissolved in THF after sterilized 30 min



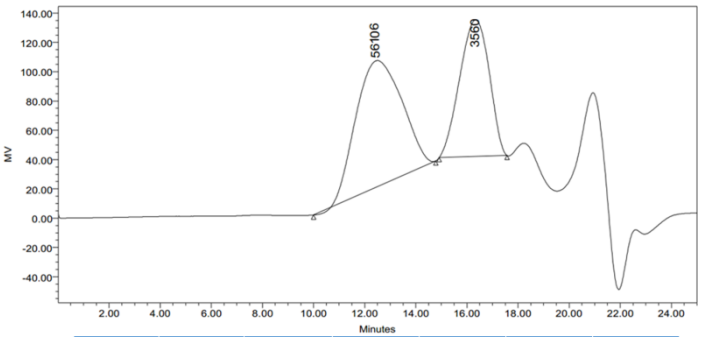
Broad	Mn	Mw	MP	Mz	MZ+1	PDI
1	38896	65369	66163	101112	141409	1.680623
2	1328	1530	1360	1758	2001	1.152321

Figure A-7 Chromatogram from GPC analysis of PLA electrospun fiber mats dissolved in THF after sterilized 60 min



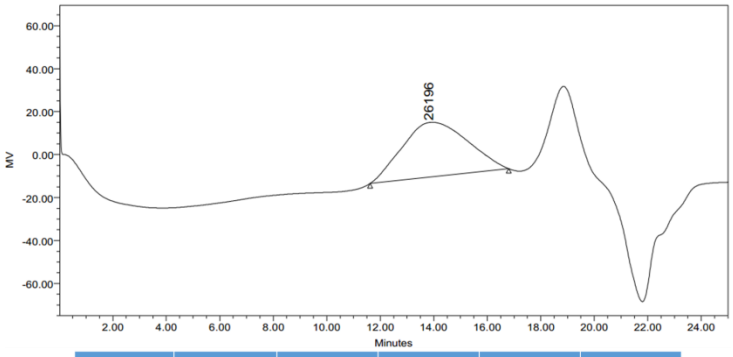
Mn	Mw	MP	Mz	MZ+1	PDI
38092	63341	62608	115036	148805	1.434636

Figure A-8 Chromatogram from GPC analysis of PLA/PLAdi+ (100:70) electrospun fiber mats dissolved in THF after sterilized 60 min



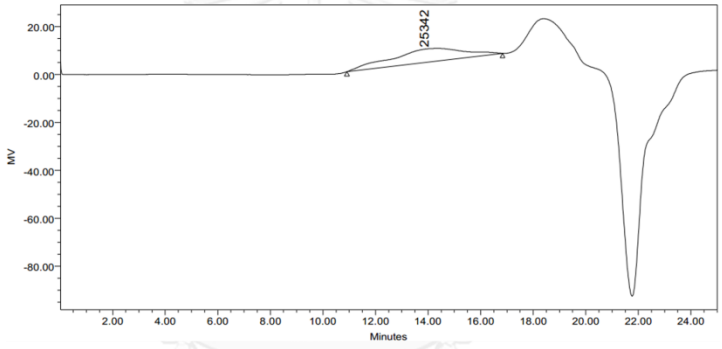
Broad	Mn	Mw	MP	Mz	MZ+1	PDI
1	36944	62489	56106	103790	120942	1.365927
2	3297	3760	3560	4438	5670	1.217832

Figure A-9 Chromatogram from GPC analysis of PLA/PLAdiCOOH (100:70) electrospun fiber mats dissolved in THF after sterilized 60 min



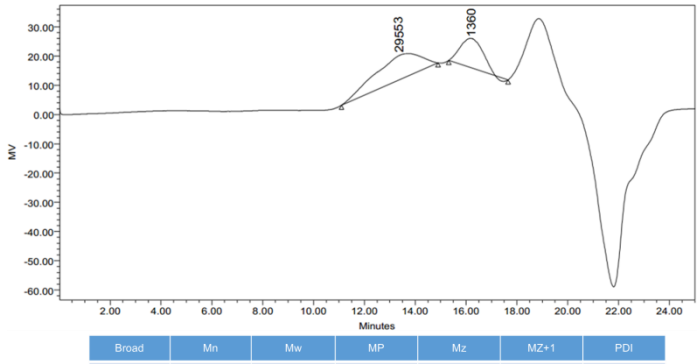
Mn	Mw	MP	Mz	MZ+1	PDI
18167	28920	26196	43852	59754	1.591864

Figure A-10 Chromatogram from GPC analysis of PLA electrospun fiber mats dissolved in THF after sterilized 18 hr



Mn	Mw	MP	Mz	MZ+1	PDI
20027	41640	25342	83163	126044	2.079247

Figure A-11 Chromatogram from GPC analysis of PLA/PLAdi+ (100:70) electrospun fiber mats dissolved in THF after sterilized 18 hr



Broad	Mn	Mw	MP	Mz	MZ+1	PDI
1	27460	31740	29553	72935	96931	1.381187
2	1346	1540	1360	1758	2001	1.059522

Figure A-12 Chromatogram from GPC analysis of PLA/PLAdiCOOH (100:70) electrospun fiber mats dissolved in THF after sterilized 18 hr

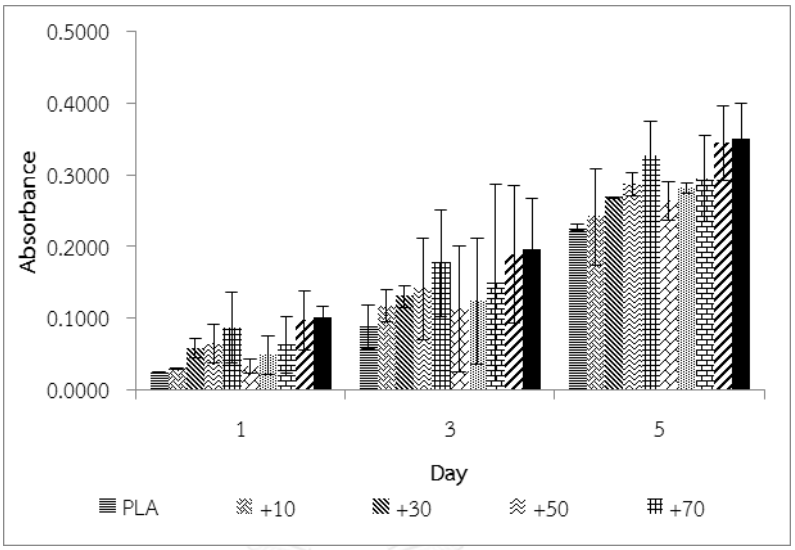


Figure A-13 Absorbance for cell proliferation of L929 at 540 nm on the 1st, 3rd, and 5th days. Approximately 2×104 per well were seeded on the electrospun fiber mats as determined by MTT assay. Error bars represent absorbance variability measured at 540 nm where n=3

VITA

Mr. Wachirasin Kaewprasertsri was born on April 4th, 1991 in Chonburi province, Thailand. He received the Bachelor Degree of Science, majoring in Applied Chemistry from Rangsit University in 2013. In the same year, he was admitted to Master Degree of Science, program in Petrochemistry and Polymer Science, at Chulalongkorn University and graduated in the academic year of 2015.

Proceeding

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