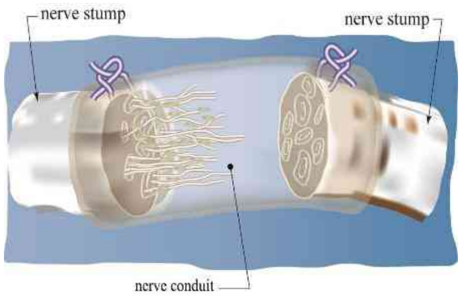
TOPIC
Thai Organic & Printed Electronics Innovation Center
NECTEC
a member of NSTDA
RGJ PhD.
โครงการปริญญาเอกกาญจนาภิเษก

Surface Modification for Improve Biocompatibility of Absorbable Nerve Guide Fabricated by Electrospinning and 3D printing

Introduction

"Tissue engineering"

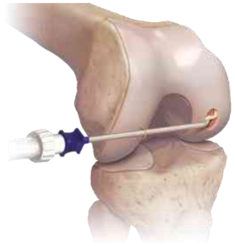
It is scientific filed mainly focused on the development of tissue and organ to **replace or support the function of defective or injured.**



Absorbable Nerve guide

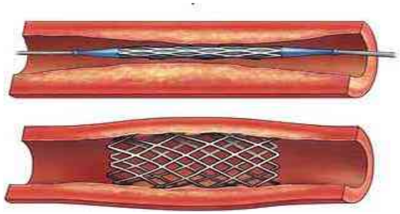
Absorbable Suture

Bone fixation



Cartilage

Wound dressing



Blood vessel

Biodegradable Polymers

Three-dimensional porous structures as scaffolds for tissue engineering and as controlled/sustained release drug delivery system.

Important Properties

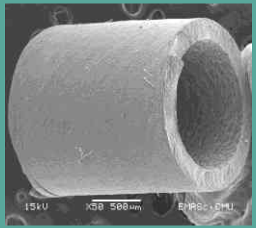
- **Acceptable shelf life**
- **Suitable degradation time**
- **Non-toxic degradation products**
- **Appropriate permeability**

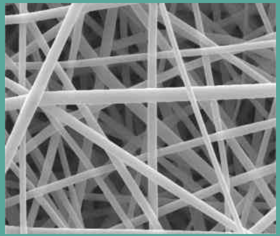
Medical Device Value Chain

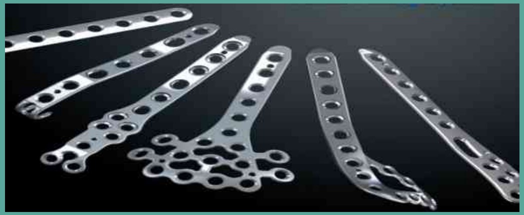
UPSTREAM

Production of medical grade resorbable polymers for use as medical device

MIDSTREAM





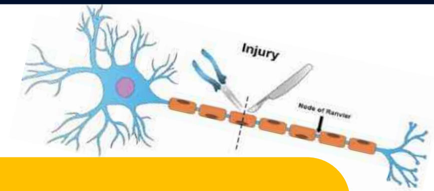


Fabrication, *in vitro* hydrolytic degradation, cytotoxicity and biocompatibility testing

DOWNSTREAM

***In vivo* and clinical testing**

What is Peripheral Nerve Damage?



Peripheral Nerve Damage can be caused by transportation and construction accidents, natural disaster and war damage.

Loss of motor and sensory function at the nerve targets.

Standard Nerve Repairs

Small gap

Large gap

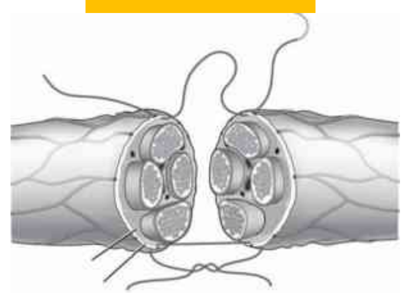


Figure 1. Nerve suturing

Figure 2. Nerve grafting

W. Bowers, E Carlson, S. Wenner. Hand clinic, **1989,** 5,445-453.
W.A. Lackington, A. Ryan, F. Brien. ACS Biomater. Sci. Eng., **2017,** 3, 1221−1235.

Nerve Graft

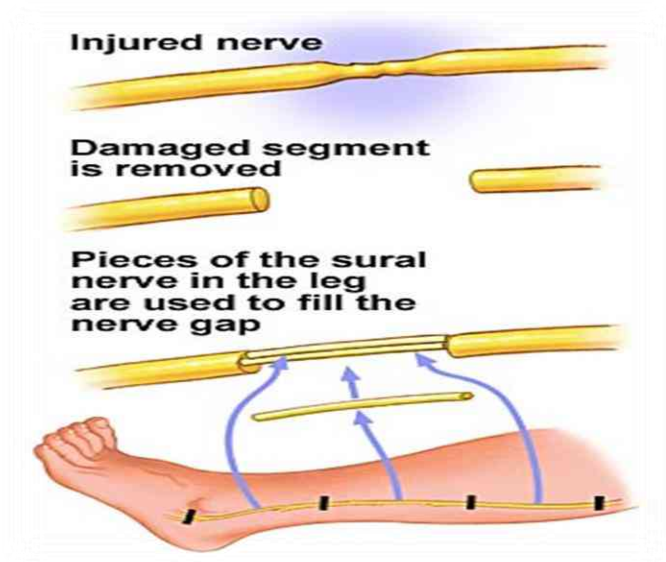


Figure 3. Sural nerve graft harvesting

Problems

- Loss of function at the donor site
- Need for multiple surgeries
- Mismatch between nerve and graft

Nerve Guide

- Simple and effective nerve repair
- Replaces the need for nerve grafting
- Natural materials or synthetic polymers

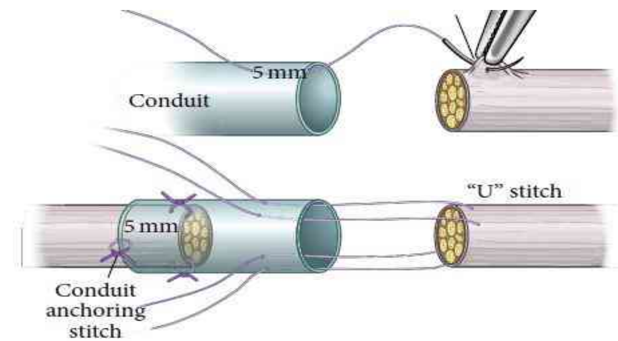


Figure 4. Nerve guides

Problems

Unsatisfactory mechanical or biological properties in large gaps

To solve these problems…?

http://www.mayoclinic.org/peripheral-nerve-injuries cite 2 Feb 2017

The US Food and Drug Administration (FDA) -approved nerve conduits

Table 1 : FDA-approved nerve conduit materials for clinical use

Product Name	Tube Materials	Price	Degradation time (months)
Neuro Tube®	poly(glycolic acid) (PGA)	€340 /12,988	3 months
Neurolac®	poly(DL-lactide-co-caprolactone)	€700-1800 / 26,740-68,760	16 months
NeuroMatrix ™ and Neuroflex ™ NeuraGen	Type-I collagen	€600 / 22,920 €1200 / 45,840	4-8 months 36-48 months
SaluTunnelTM	Poly(vinyacetate) (PVA)	Not stated	Non-degradable

Figure 5. Commercial nerve guide conduits

F. Tavangarian, Y. Li. Ceramics Internaltional, **2012,** 48, 6075-6090.

LITERATURE REVIEWS

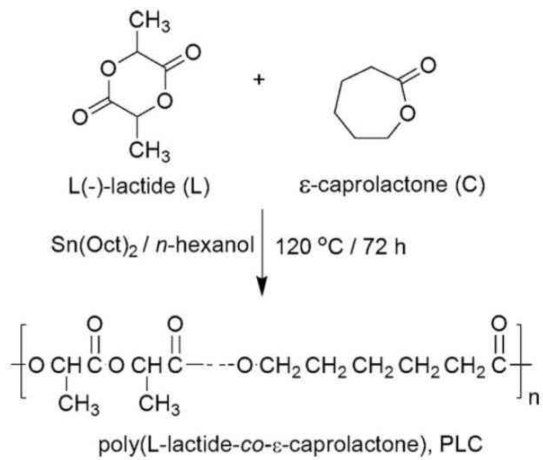
Effects of Copolymer Microstructure on the Properties of Electrospun Poly(L-lactide-co-e-caprolactone) Absorbable Nerve Guide Tubes

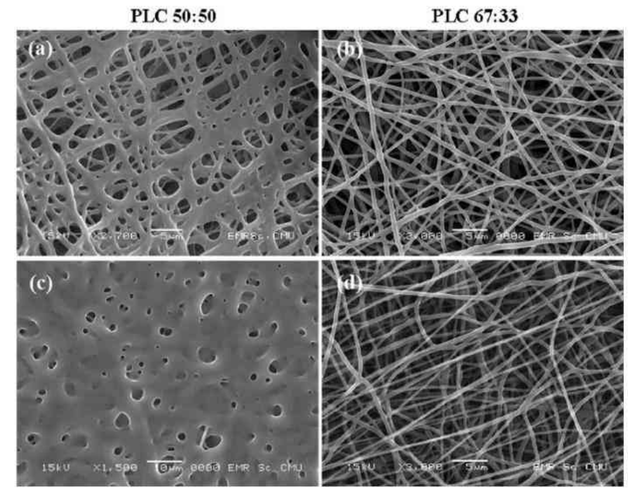
Thapsukhon, B.; Thadavirul, N.; Supaphol, P.; Meepowpan, P.; Molloy, R.; Punyodom, W. J. Appl. Polym. Sci 2013, 130, 4357–4366.

Material : PLC

Fabrication :

Electrospinning





re 8. SEM images of the PLC 50 : 50 and PLC 67 : 33 membranes: (a–b) after initial preparation and (c–d) after storage for 14 weeks.

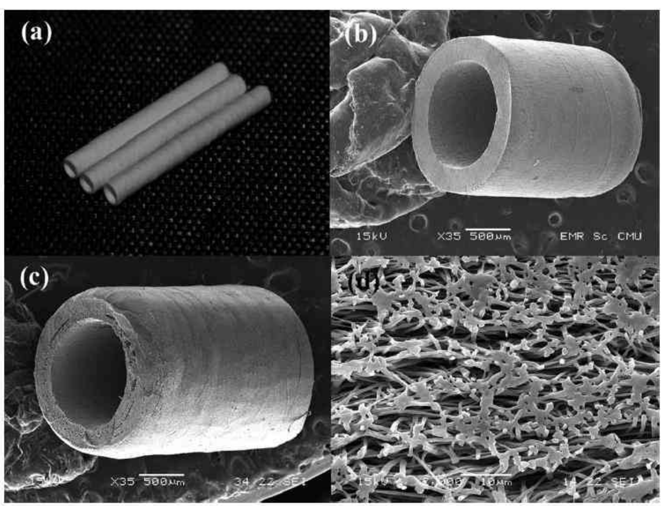
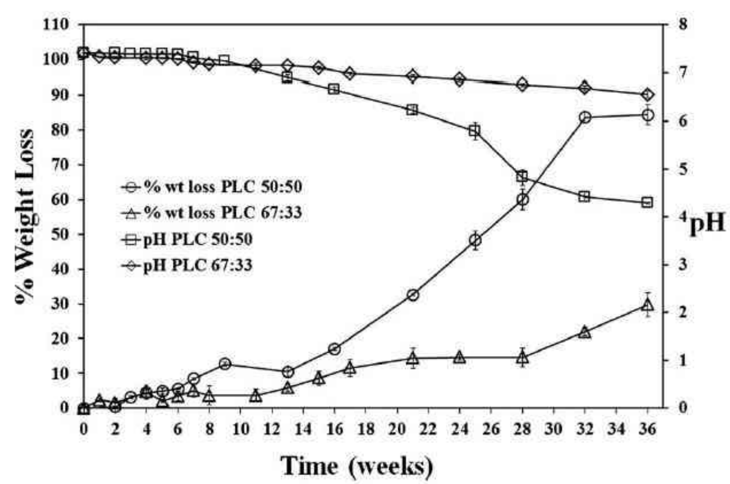


Figure 9. (a) Photographs and (b–d) SEM images of PLC copolymer tubes: (b) PLC 50 : 50 tube, (c) PLC 67 : 33 tube, and (d) cross-section of PLC 67 : 33 tube.



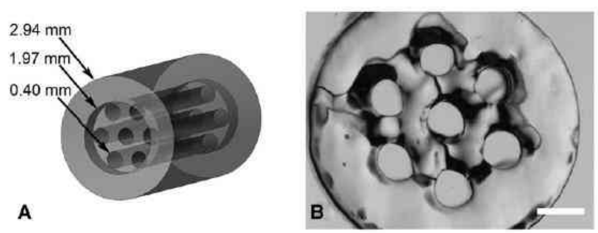
LITERATURE REVIEWS

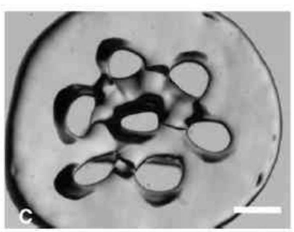
Fabrication of Off-the-Shelf Multilumen Poly(Ethylene Glycol) Nerve Guidance Conduits Using Stereolithography

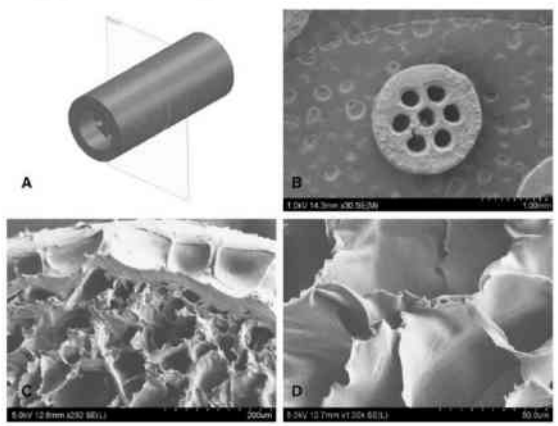
Arcaute, K.; Mann, B. K.; Wicker, R. B. Tissue Eng. Part C Methods 2011, 17 (1), 27–38.

Material : Poly(Ethylene Glycol)

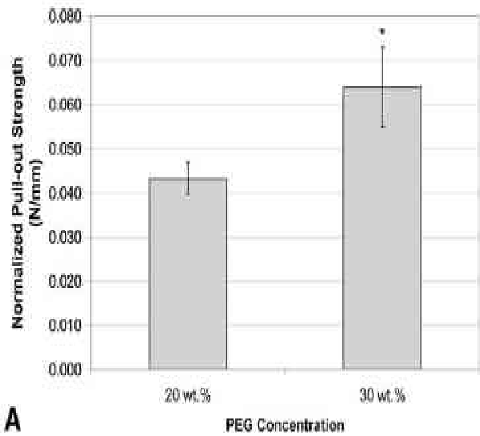
Fabrication : 3D Systems Model 250/50 SL machine (3D Systems)



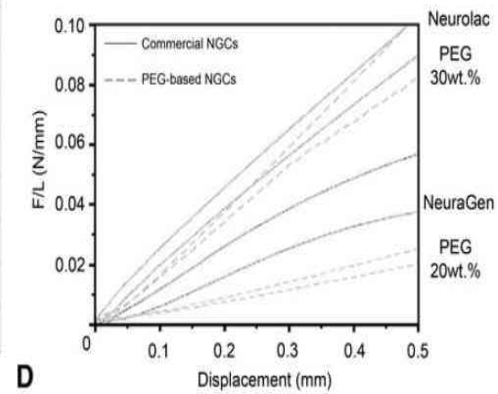




Pullout strength



Compressive resistance



SL shows promise for creating complex 3D tissue-engineered scaffolds in a rapid, efficient manner. Advantage offered by SL include the ability to easily alter the scaffold design by simply altering the CAD, allowing flexibility in the system, relatively simple scalability of the manufacturing process, and access to individual layers during fabrication enabling control over 3D material placement and structure creation.

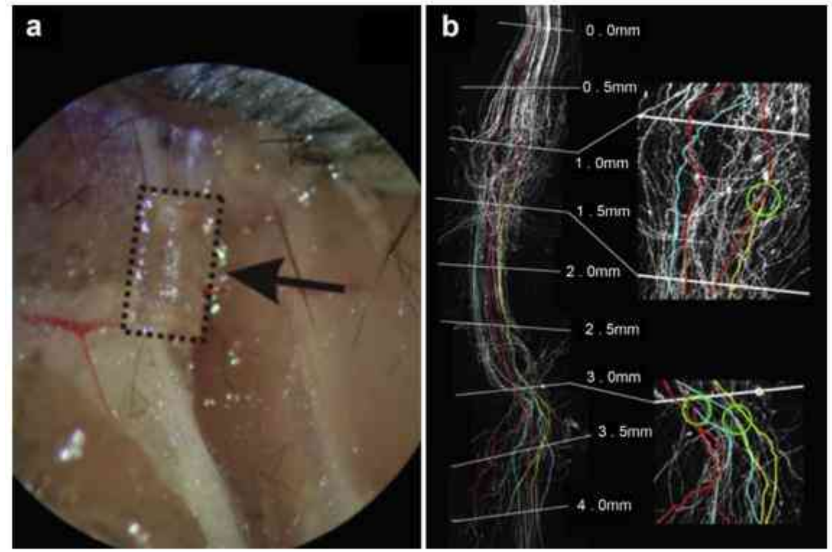
LITERATURE REVIEWS

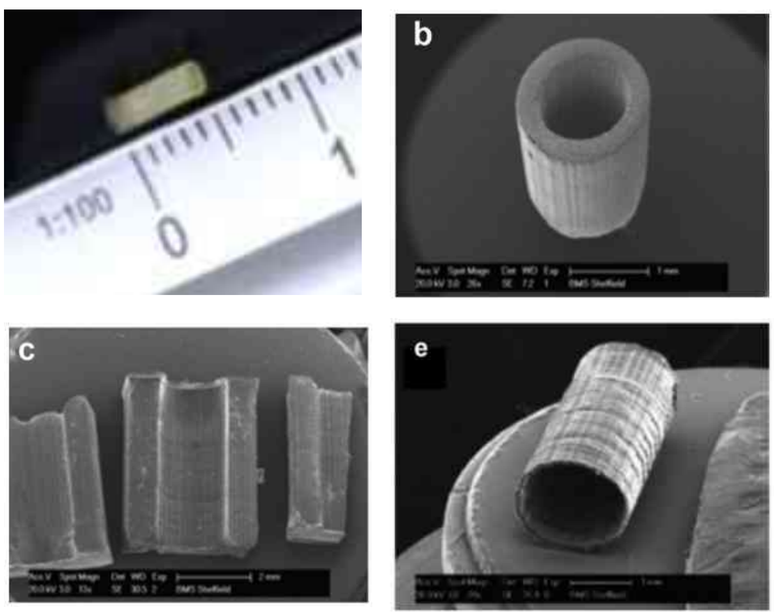
Nerve guides manufactured from photocurable polymers to aid peripheral nerve repair

Pateman, C. J.; Harding, A. J.; Glen, A.; Taylor, C. S.; Christmas, C. R.; Robinson, P. P.; Rimmer, S.; Boissonade, F. M.; Claeyssens, F.; Haycock, J. W. *Biomaterials* **2015**, *49*, 77–89.

Material : Poly(ethylene glycol) resin

Fabrication : Laser-based microstereolithography; μSL





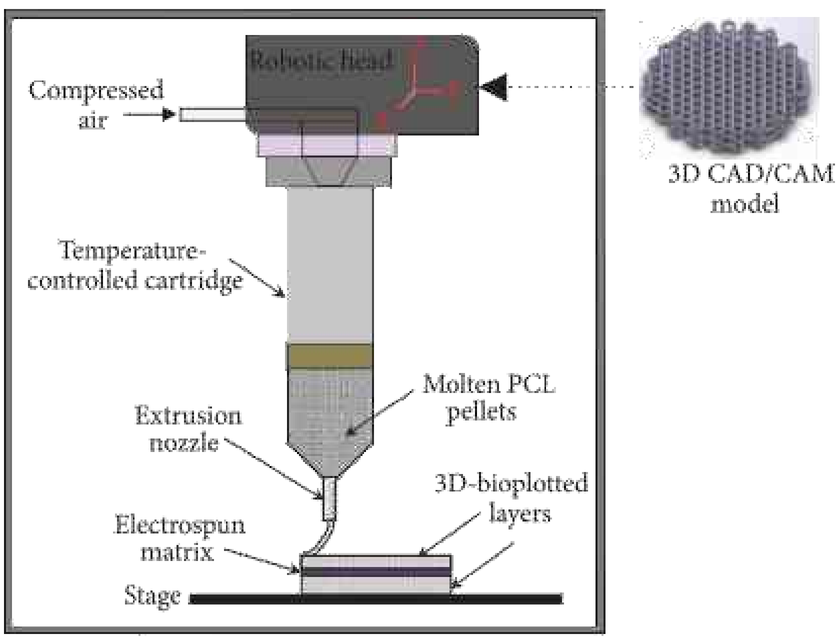
They observed that the photocurable form of PEG used herein was permissive for neuronal growth and experimental differentiation in vitro. Devices constructed from the bulk material had acceptable handling properties and performed comparatively with an autograft control in a thy-1-YFP-H mouse 3 mm gap injury model after 21 days, with the number of unique axons at the distal end in each repair group being similar.

LITERATURE REVIEWS

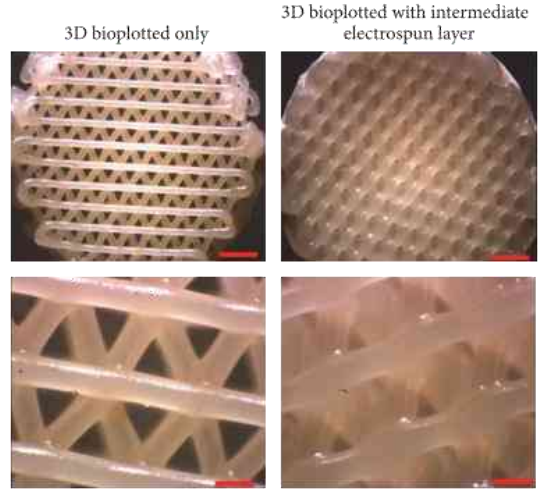
Fabrication and Evaluation of Electrospun, 3D-Bioplotted, and Combination of Electrospun/3D-Bioplotted Scaffolds for Tissue Engineering Applications, Mellor, L. F.; Huebner, P.; Cai, S.; Mohiti-asli, M. *Biomed Res. Int.* **2017**, *2017*.

Material : PCL

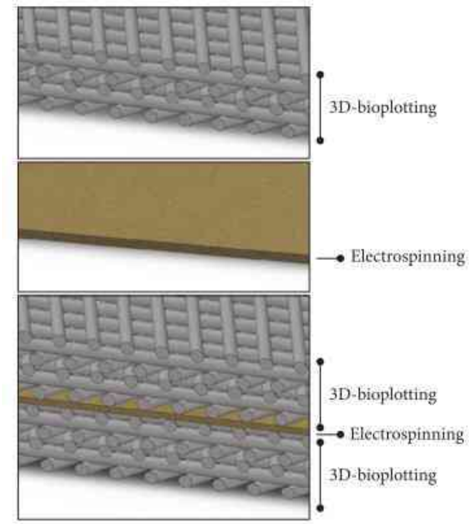
Fabrication : 3D Bioplotter® and electrospinning

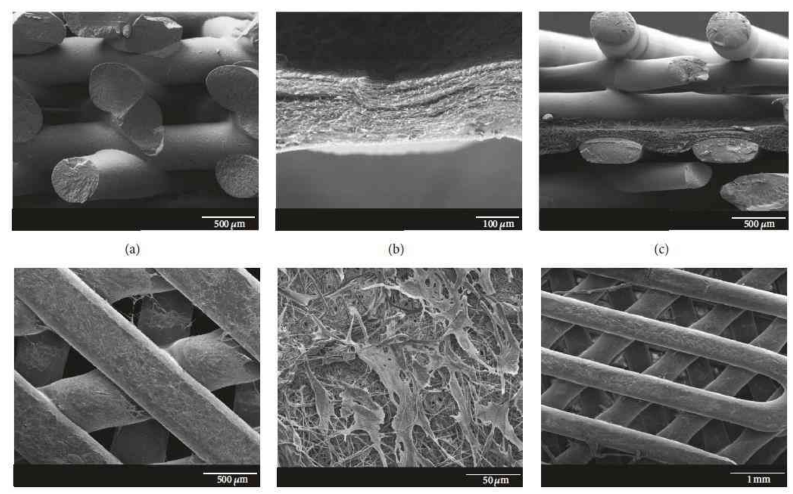


(a)



(b)

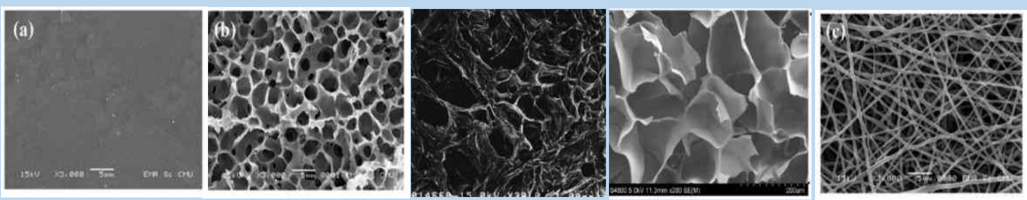




Fabrication techniques

Original techniques

Solution casting | Phase immersion precipitation | Freeze drying | Molding | Electrospinning



Non-porous | **Open pore** | **Interconnecting pore**

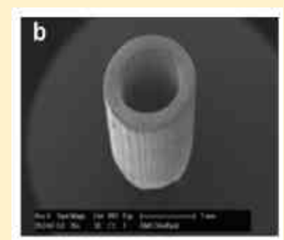
These techniques

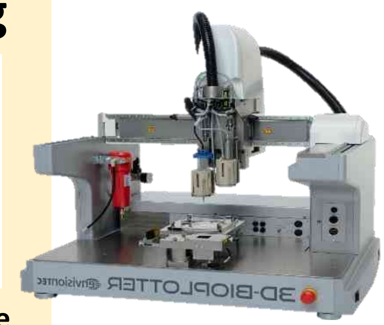
- They do not permit enough control of scaffold architecture, pore network.
- All of these technique often have lack of pore interconnectivity resulting poor cell infiltration and migration within the scaffold.

Novel techniques

lack of resolution to solve these problems.
Big challenge

3D printing





- **Size and shape are specified**
- **Less processing time**

Development of polymer scaffold that are **compatible with the suitable cell migration and infiltration.**

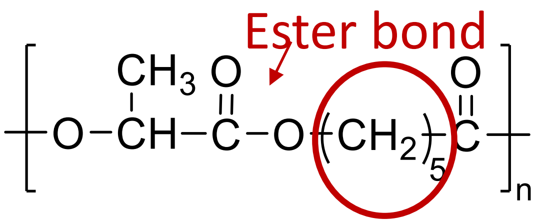
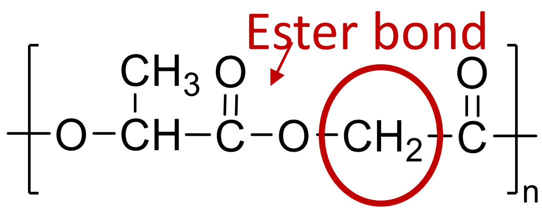
Yu Hu, Yao Wu, Zhi yuan Gou, Jie Tao, Jiu meng Zhang, Qian qi Liu, Tian yi Kang. Sci. Rep. **2016**. 6;32184.

B. Thapsukhon, D. Daranarong, P. Meepowpan, N. Suree, R. Molloy, K. Inthanonc, W. Wongkham, W. Punyodom. Journal of Biomaterials Science, Polymer Edition, **2014**, 25, 1028–1044.

C.J. Pateman, A.J. Harding, A. Glen, C.S. Taylor, C.R. Christmas, P.P. Robinson , S. Rimmer, F.M. Boissonade,F. Claeyssens, J.W. Haycock. Biomaterials**, 2015**, 49, 77-89.

Molecular Design

Copolymers

	Poly(L-lactide-*co*-ε-caprolactone), PLC	Poly(L-lactide-*co*-glycolide), PLGA
Molecular structure		
	✓ Flexibility ✓ Biocompatibility ✓ Controllable degradation time ✓ Nontoxic biodegradation products	✓ Bioabsorbability ✓ Biocompatibility ✓ Controllable degradation time ✓ Nontoxic biodegradation products

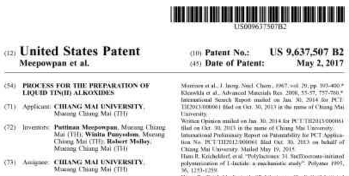
★ ***To compare the molecular structure of biodegradable copolyesters***

B.Schlosshauer, L. Dressmann, H. Schaller. Neurosurgery, **2006,** 59,740-748.

Summary of the Project : Bioplastic Innovation

Monomers | **Pure Grade Polymers** | **Medical Grade Polymers** | **Tech. Transfer**

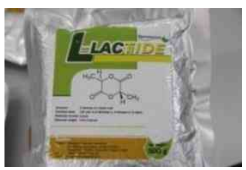
New Catalyst



- **US Patent No.** 9,637,507 B2 (May 2017)
- **JP Patent No.** 6246225 (Nov 2017)
- **Thai Patent Application** No. 1201006169
- EP, CN, SG **Patent Application**

2548-2549
CMU Novel Catalyst

IP 1: Process for the preparation of Liquid Tin(II) alkoxides



2550-2555
Monomers

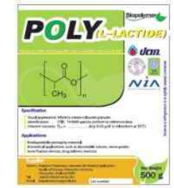
IP 2: Process for the production of lactide using liquid tin(II) alkoxides

Products : Lactide (L, D, DL), Glycolide

2555-2557
Pure Grade Polymers

IP3: Process for the production of polyesters via cyclic ester using liquid tin(II) alkoxides

Products : Pure grade PLA, PDLA, PCL, PGA, PLC, PLG



2558-2560
Medical Grade Polymers

- **Bioplastic Production Laboratory for Biomedical Applications**
- **ISO 13485** (Medical devices-Quality management systems – Requirements for regulatory purposes)
- **ASTM F1925-09**
- **มอก. 2677-2558**
- **Business Model (STeP)**

IP4: Process for the production of medical grade polyesters using liquid tin(II) alkoxides

Products : Medical grade polyesters (e.g. PLA, PLC, PLG)

2561-2564
Incubation & Technology Transfer

- ผลิตและจำหน่ายผลิตภัณฑ์
- พัฒนาผลิตภัณฑ์
- กลุ่มลูกค้า
- การอนุญาตให้ใช้สิทธิ์ (Licensing)

ขณะนี้ห้องปฏิบัติการดำเนินการผลิตและจำหน่ายผลิตภัณฑ์ โดยการสนับสนุนของ บริษัท ปตท. จำกัด (มหาชน)

Property Requirements in ASTM F1925-17

Table 1. Chemical Property Requirements for Virgin Semi-Crystalline Poly(lactide) Homopolymers and Poly(lactide)-based Copolymer Resins

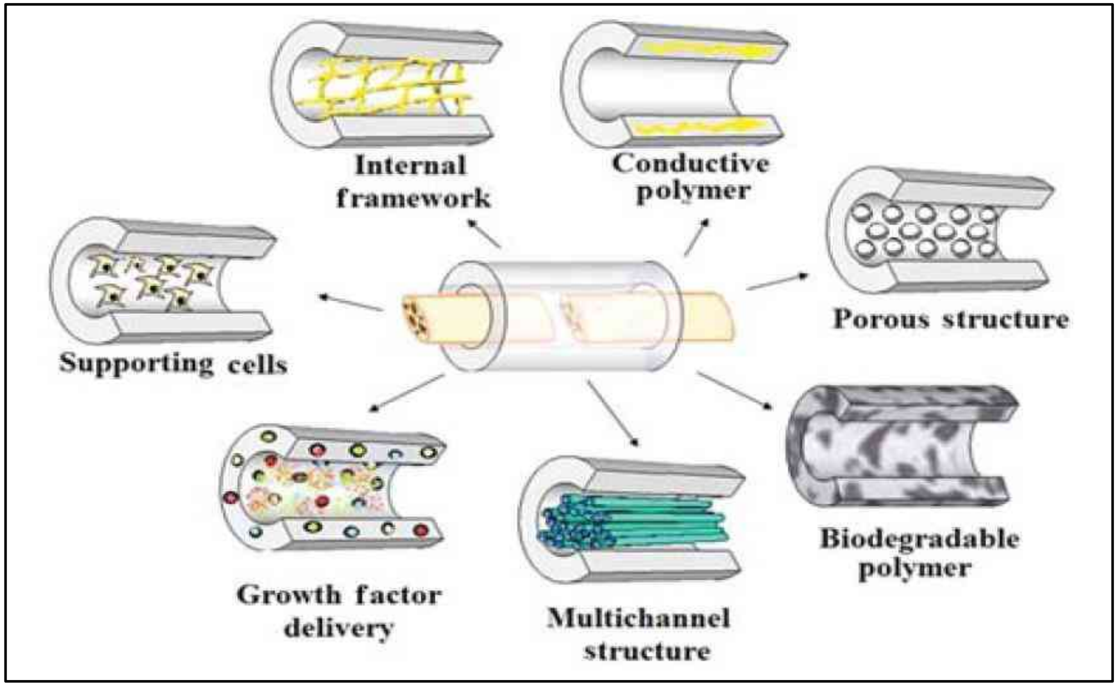
Specification	ASTM F1925-09	Laboratory Copolymer
Total Residual Monomer, (%)	< 2.0 % [A]	max. 1.08 % L-lactide max. 0.08 % ε-caprolactone 1.16 % ±0.01 Total
Total Solvent Residual(s), (in ppm)	< 1000 ppm	1 ppm
Residual Catalyst (in ppm as Sn)	≤ 150 ppm	61 ppm
Heavy Metals (ppm as Pb)	< 10 ppm (minus Sn)	1 ppm
Residual Water	≤ 0.5 % [B]	0.02 %
Copolymer Ratio	± 3 % of target (by mole)	< 3 % (71:29 molar ratio)
Specific Rotation	113° to 120°	118°±0.18

A Up to 3 % if deemed acceptable by the purchaser.
B Utilizing a moisture determination method agreed upon by the supplier and purchaser

ASTM F1925 – 09. Standard Specification for Semi-Crystalline Poly(lactide) Polymer and Copolymer Resins for Surgical Implants.

Objectives

OBJECTIVES



- To synthesize medical grade biodegadable copolyesters according to ASTM F1925-17 (Standard Specification for Semi-Crystalline Poly(lactide) Polymer and Copolymer Resins for Surgical Implants)
- To design and modify scaffold prototyped with to improved biocompatibility for enhanced nerve regeneration
- To optimize 3D printing conditions and/or electrospinning conditions for fabricating scaffold prototyped into various forms such as sheets or tubes for use as temporary scaffolds in reconstructive nerve surgery
- To evaluate the scaffolds in terms of their *in vitro* hydrolytic degradation, cytotoxicity and cell study

EXPERIMENT & RESULTS

01 Synthesis of Poly(L-lactide-co-ε-caprolactone), PLC

Monomer Synthesis

L-Lactide
monomer

ε-caprolactone
monomer

Copolymer Synthesis

- **Composition (LL : CL)** : 70:30

Copolymer

Copolymer PLC

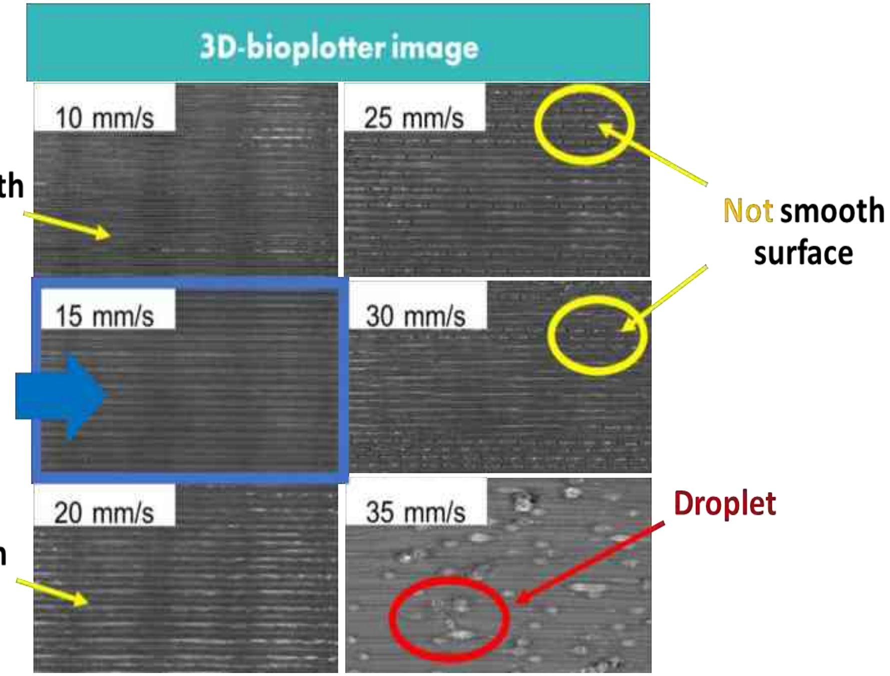
Characterization through
ASTM F1925-17
test methods

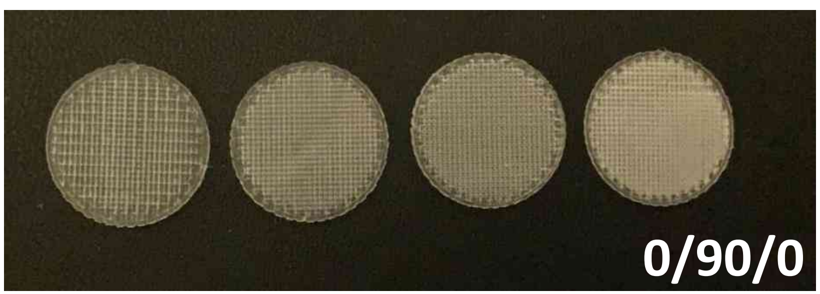
3D SHEET FABRICATION

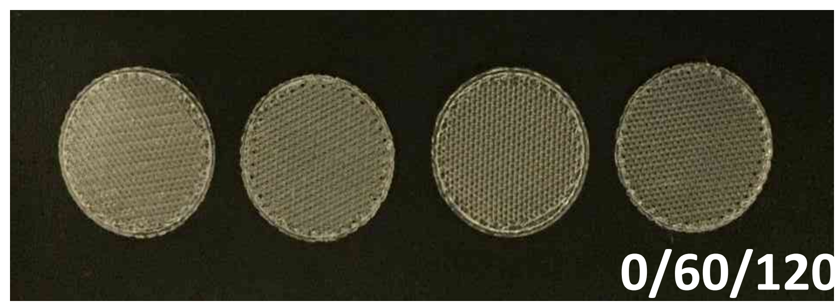
Optimal Fabrication Conditions

Parameter	Set value
Pressure	6.5 bar
Melting temp.	160 ^oC
Platform temp.	20 ^oC
Pattern	0/90/0 ^{o} 0/60/120 ^{o}
Printing speed	15 mm/s

Pressure (bar)	Speed (mm/s)
6.5	15.00

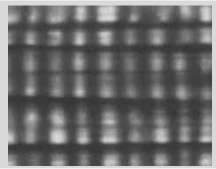
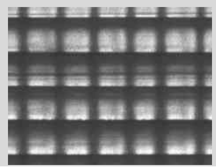
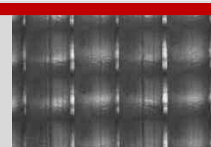
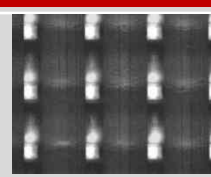
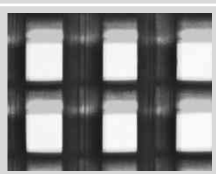






3D SHEET CHARACTERIZATION

Structural analysis

Distance Between Layer (mm)	PLC scaffolds	Light microscope image	Filament diameter (μm)	Pore size (μm)	Angle (°)
0.1			ND	ND	ND
0.2			ND	ND	ND
0.3			277.10 ± 7.82	37.92 ± 2.20	90
0.4			270.08 ± 2.95	14.94 ± 4.44	90
0.5			255.93 ± 2.28	242.10 ± 4.84	90

Optimal pore size = 10 to 20 μm

3D SHEET CHARACTERIZATION

Dilute Solution Viscometry

PLC

Weight (g)	Concentration (g/dL)	Ave-Corrective	η_r (t/t_0)	η_{inh} (dL/g)
$CHCl_3$	-	123.90	-	-
0.0254	0.1016	134.39	1.085	0.800
0.0253	0.1012	134.60	1.086	0.819
0.0250	0.1000	134.44	1.085	0.816
Intrinsic viscosity (dL/g)				0.810

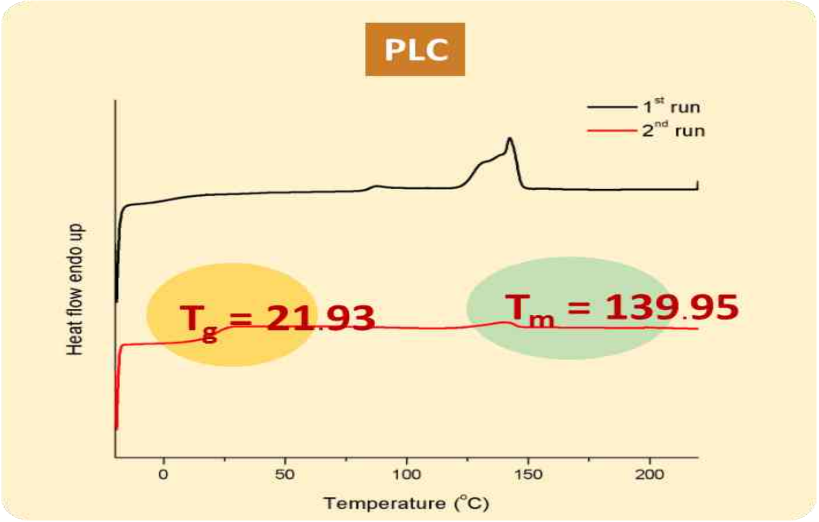
PLC Printed

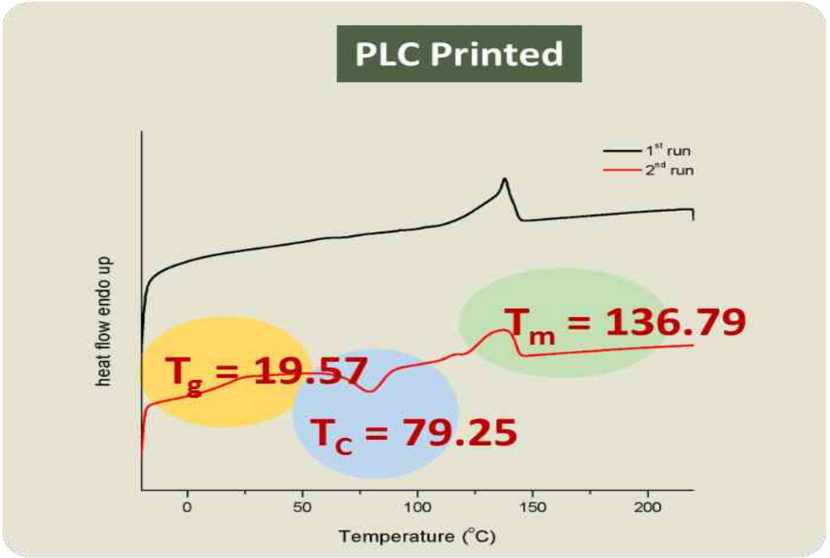
Weight (g)	Concentration (g/dL)	Ave-Corrective	η_r (t/t_0)	η_{inh} (dL/g)
$CHCl_3$	-	123.90	-	-
0.0254	0.1024	131.86	1.047	0.446
0.0253	0.1020	131.96	1.047	0.454
0.0250	0.1024	131.92	1.047	0.450
Intrinsic viscosity (dL/g)				0.450

Viscosity decreased 44%

3D SHEET CHARACTERIZATION

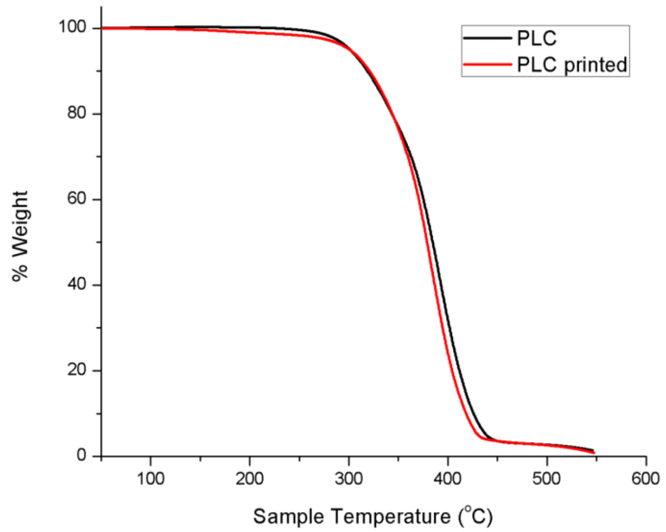
Differential scanning calorimetry , DSC





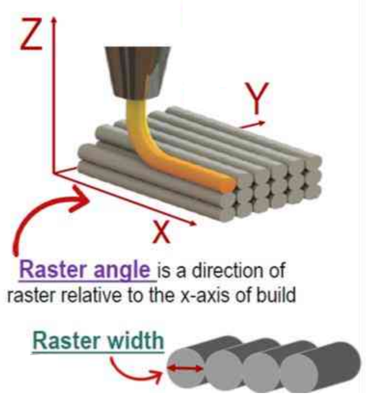
Decomposition Temperature

Sample	Start	Peak	End
PLC	250	390	450
PLC printed	152	385	450

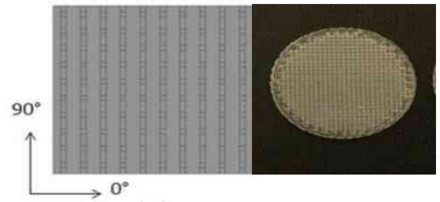


3D SHEET CHARACTERIZATION

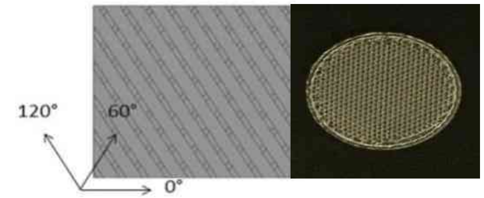
Mechanical properties



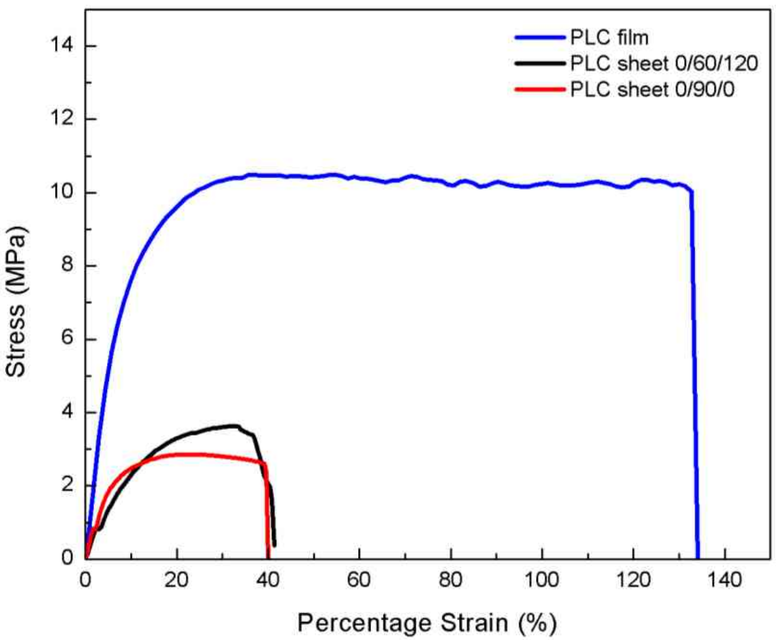
Printed



0/90/0° (Pattern 1)



0/60/120° (Pattern 2)



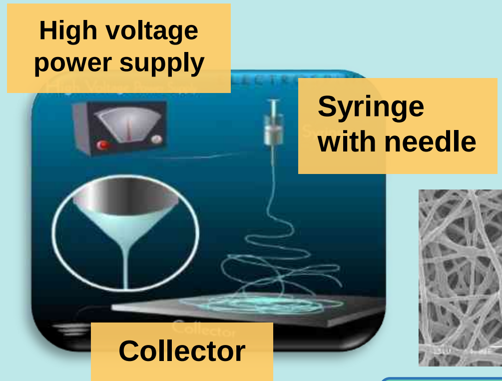
Tensile properties for suitable nerve conduits	Tensile Stress (MPa)	Percentage strain (%)	Young's Modulus (MPa)
	6.5-8.5	6-16	8-16

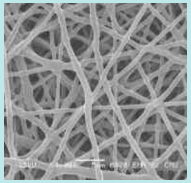
Samples	Tensile Stress (MPa)	Percentage Strain (%)	Young's Modulus (MPa)
PLC film	7.69 ± 1.10	181.40 ± 11.9	142.07 ± 3.34
PLC sheet 0/90/0 °	3.03 ± 0.14	18.38 ± 5.98	64.52 ± 2.27
PLC sheet 0/60/120 °	3.40 ± 0.18	25.26 ± 6.95	54.96 ± 10.95

Fabrication

Pressure (bar)	Speed (mm/s)	Distance Between strain (mm)
6.5	15.00	0.3

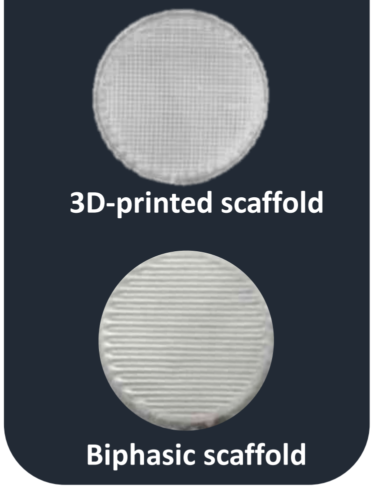
The 3D Printing technique





22% w/v PLC

The electrospinning technique

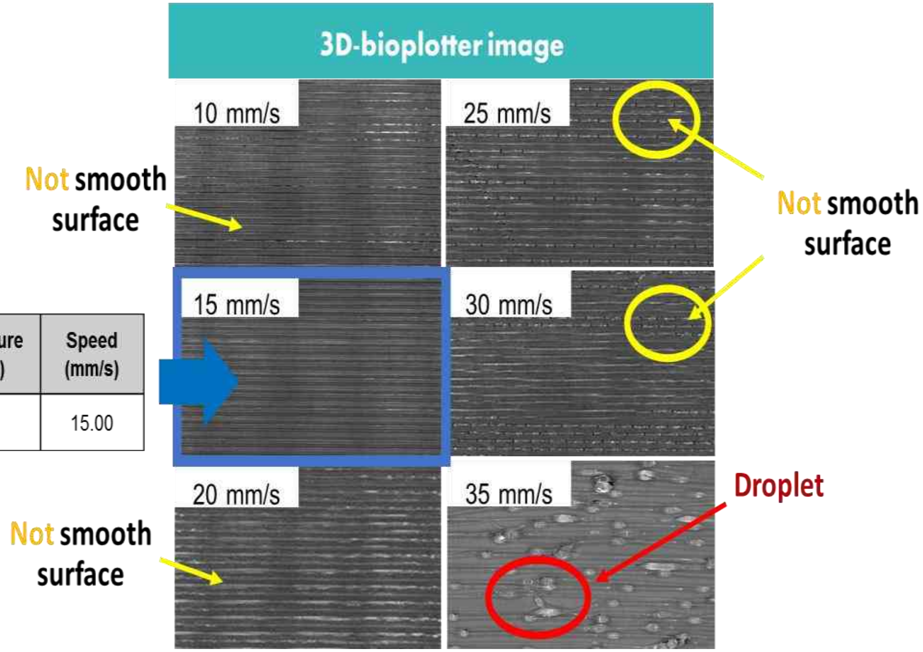


Vary Parameters

-Pressure: 3.0,3.5,4.0,4.5,5.0,5.5,6.0,6.5,7.0,7.5 bar

-Printing speed: 10.0,15.0,20.0,25.0,30.0,35.0 mm/s

Pressure (bar)	Speed (mm/s)
6.5	15.00



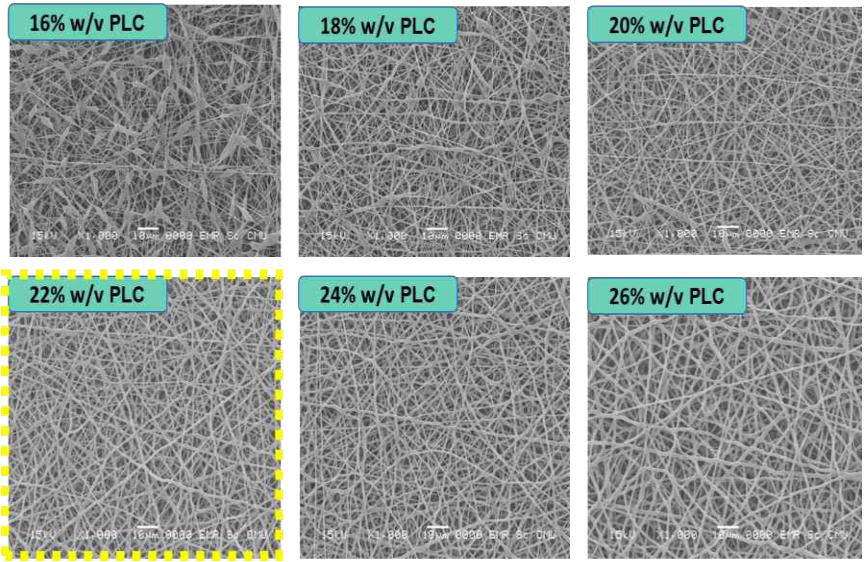


Figure 6. SEM images illustrating morphologies of electrospun PLC membranes from various polymer solution concentration (16, 18, 20, 22, 24 and 26% w/v) (Scale bars, 10 µm; ×1000 magnification.).

MORPHOLOGY RESULT

Cross-sectional views

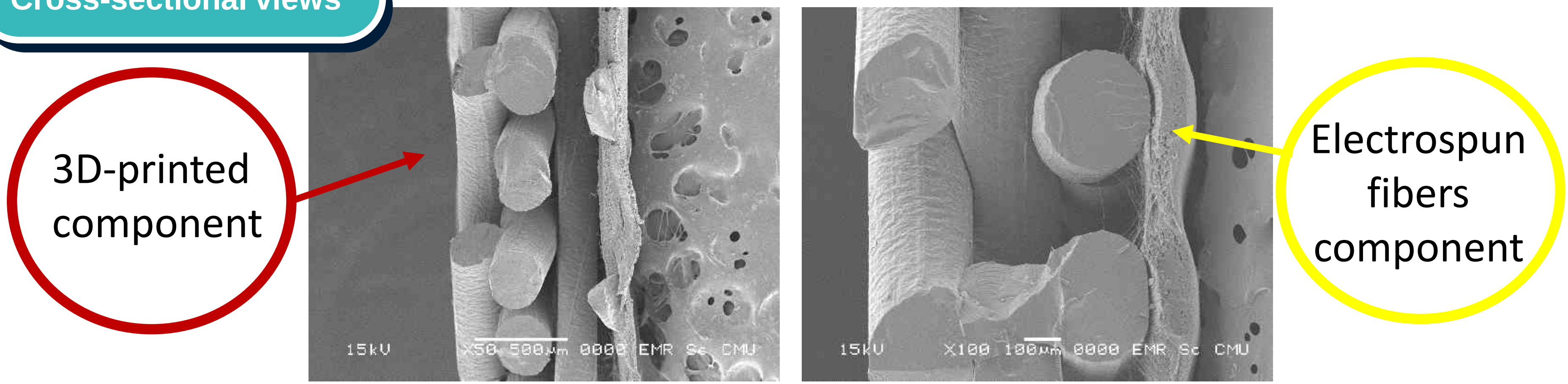


Figure 7. SEM images illustrating morphologies of the biphasic scaffold. Cross-sectional views of the electrospun fibers (right hand side) onto the 3D-printed component (left hand side).

Top views of electrospun fibers

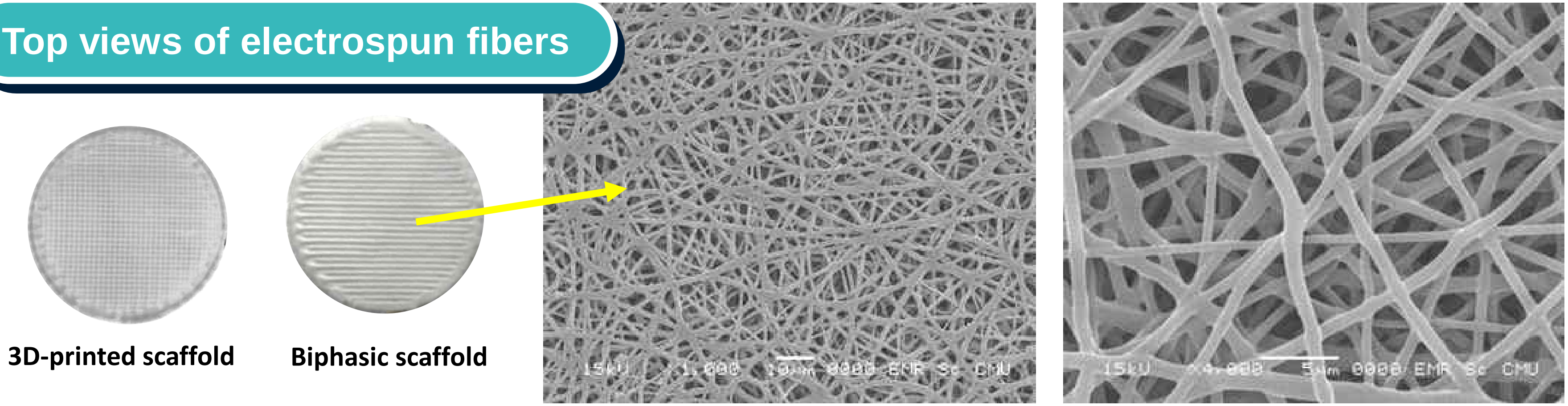


Figure 8. Fabrication of the biphasic scaffold. Top views of the biphasic scaffold by SEM showing Scale bars, 10µm; ×1000 magnification (right hand side) and Scale bars, 10µm; ×4000 magnification (left hand side)

CONCLUSIONS

Synthesized PLC

01

Ring-opening bulk polymerization,
Initiator : Sn(O*n*-$C_4H_9)_2$, LL:CL = 72:28 mole%.
T_g : 21.9 °C , T_m : 140.0 °C,
Intrinsic : Viscosity was 0.810 dl/g.

Fabricated 3D-sheets

02

Fabrication temperature is 160 °C, platform temperature is 20 °C, , pressure is 6.5 bar, printing speed is 15 mm/s, distance between the stand is 0.3 mm.

Pore size

03

Fiber Diameter = 177.10 ± 7.82 μm
Pore size = 17.92 ± 2.20 μm,
which is the appropriate pore size for use as nerve conduits.

Effect of Printing Process

04

After printing, the viscosity of the polymer decreased same as mechanical properties and the thermal properties due to the degradation of polymers during printing.

Successful to fabricate

05

The biodegradable scaffolds based PLA could be fabricate via combining of 3D printing and electrospinning techniques.

06

- These electrospun fiber is clearly embedded within the 3D printed construct and also these scaffolds were highly interconnected throughout the entire structure.

ACKNOWLEDGMENTS

TOPIC

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NECTEC

a member of NSTDA

RGJ PhD.

โครงการปริญญาเอกกาญจนาภิเษก

Bioplastic Production Laboratory for Medical Application (CMU)

THANK YOUR KIND ATTENTION