การสังเคราะห์และทดสอบคุณลักษณะของโฟมพอลิยูริเทนทางการแพทย์ชนิดสลายตัวได้ทางชีวภาพ: อิทธิพลของไอโซไซยาเนตและสัดส่วนเชิงโมลของพอลิออล

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SYNTHESIS AND CHARACTERIZATION OF BIODEGRADABLE MEDICAL POLYURETHANE FOAM: INFLUENCE OF ISOCYANATES AND POLYOLS MOLAR RATIO

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Thesis Title	SYNTHESIS AND CHARACTERIZATION OF BIODEGRADABLE MEDICAL POLYURETHANE FOAM: INFLUENCE OF ISOCYANATES AND POLYOLSMOLAR RATIO
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ภวัฐพงศ์ ทรัพย์ศุภวัฒน์ : การสังเคราะห์และทดสอบคุณลักษณะของโฟมพอลิยูริเทนทางการแพทย์ชนิดสลายตัวได้ทางชีวภาพ: อิทธิพลของไอโซไซยาเนตและสัดส่วนเชิงโมลของพอลิออล (SYNTHESIS AND CHARACTERIZATION OF BIODEGRADABLE MEDICAL POLYURETHANE FOAM: INFLUENCE OF ISOCYANATES AND POLYOLSMOLAR RATIO) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. ดร.ปิยะสาร ประเสริฐธรรม, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.นพวรรณ โม้ทอง , 83 หน้า.

งานวิจัยนี้เป็นการศึกษาการสังเคราะห์โฟมพอลิยูริเทนทางการแพทย์ที่สามารถย่อยสลายได้ทางชีวภาพและไม่เป็นพิษเพื่อนำไปประยุกต์ใช้เป็นวัสดุในทางการแพทย์ โดยใช้สารตั้งต้นที่ไม่เป็นพิษต่อร่างกาย โดยพอลิคาโปรแลคโตนไดออล (PCL) และพอลิโพรพิลีนไกลคอล (PPG) ถูกใช้เป็นส่วนอ่อนของพอลิยูริเทน ส่วนพอลิไอโซไซยาเนตที่ประกอบไปด้วย บิวเทนไดไอโซไซยาเนต (BDI) และ ไลซีนไตรไอโซไซยาเนต (LTI) นั้นจะใช้เป็นส่วนแข็ง ในงานวิจัยนี้จะใช้ chain extender ซึ่งสังเคราะห์จากกรดแลคติก (LA) และเอทิลีนไกลคอล (EG) โดยมีการศึกษาผลของสัดส่วนเชิงโมลระหว่าง PCL กับ PPG และประเภทของพอลิไอโซไซยาเนต คือ BDI กับ LTI โครงสร้างของผลิตภัณฑ์ที่ได้จะถูกยืนยันด้วย FTIR ซึ่งพบว่ามีโครงสร้างของพอลิยูริเทน รวมถึงตำแหน่งพีคของกลุ่มไอโซไซยาเนตก็หายไปด้วย และจากผลการทดลองในส่วนอื่นพบว่าเมื่อทำการเพิ่มปริมาณสัดส่วนของ PPG มากขึ้นจะส่งผลให้อุณหภูมิเปลี่ยนสถานะคล้ายแก้ว (T_g) และความสามารถทางกลลดลงยกเว้นกรณีของ PU-BDI-30 และ PU-BDI-50 แต่ความสามารถในการดูดซับน้ำจะสูงขึ้นตามสัดส่วนของ PPG ซึ่งส่งผลโดยตรงกับความสามารถในการย่อยสลายของพอลิยูริเทนเมื่อทำการย่อยด้วยสารละลายเกลือฟอสเฟตบัฟเฟอร์ (PBS) ที่อุณหภูมิ 60 องศาเซลเซียส อย่างไรก็ตามผลของปริมาณ PPG ที่มีต่อความสามารถในการย่อยสลายของพอลิยูริเทนเมื่อทำการย่อยด้วย (PBS) กับเอนไซม์ไลเปสยังไม่พบความแตกต่างที่เกิดขึ้น นอกจากนี้ยังพบว่า LTI จะทำให้ได้พอลิยูริเทนที่มีค่า T_g และสมบัติทางกลรวมถึงความสามารถในการย่อยสลายที่สูงกว่า BDI ด้วย ในส่วนของความเป็นพิษจากผลิตภัณฑ์ที่เกิดจากการย่อยสลายของพอลิยูริเทนจะประเมินด้วยวิธี MTT โดยใช้เซลล์ L929 เป็นตัวทดสอบ จากผลการทดสอบพบว่าผลิตภัณฑ์ที่เกิดจากการย่อยไม่มีความเป็นพิษ

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต .....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ปีการศึกษา 2557 ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5670325421 : MAJOR CHEMICAL ENGINEERING
KEYWORDS: ALIPHATIC ISOCYANATE / BIODEGRADABLE / POLYURETHANE FOAM / CHAIN EXTENDER

PHAWATPHONG SAPSUPHAWAT: SYNTHESIS AND CHARACTERIZATION OF BIODEGRADABLE MEDICAL POLYURETHANE FOAM: INFLUENCE OF ISOCYANATES AND POLYOLSMOLAR RATIO. ADVISOR: PROF. PIYASAN PRASERTHDAM, Dr.Ing., CO-ADVISOR: NOPPAWAN MOTONG, Ph.D., 83 pp.

In this research, biodegradable medical polyurethane foam was synthesized from biocompatible material that suitable for medical applications. Polycaprolactone diol (PCL) and polypropylene glycol (PPG) was used as soft segment, while butane diisocyanate (BDI) and lysine triisocyanate (LTI) was used as hard segment. Degradability of polyurethane was improved by the addition of chain extender synthesized from DL-lactic acid (LA) and ethylene glycol (EG). Polyurethane was prepared with various polyols molar ratios and type of polyisocyanate.The FTIR spectra showed the functional group of urethane linkage and the disappearance of free isocyanate group in polyurethane structure. The increasing of PPG content result in the decrease of glass transition temperature (T_g), compressive strength except for PU-BDI-30 and PU-BDI-50 while the water adsorption increase with the high PPG content directly affected on the degradation of polyurethane during hydrolytic degradation in phosphate buffer saline (PBS) solution at 60 ^oC. However, the degradation of polyurethane during enzymatic degradation in PBS with lipase was not showed the significant difference. LTI provided polyurethane with higher T_g, compressive strength and degradability than BDI. The toxicity from degradation product was evaluated using L929 cell with MTT assay, the results demonstrated that synthesized polyurethane foam did not have toxicity.

Department:	Chemical Engineering	Student's Signature .....
Field of Study:	Chemical Engineering	Advisor's Signature .....
Academic Year:	2014	Co-Advisor's Signature .....

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CHAPTER I
INTRODUCTION

From the increasing need of new materials with properties adapted to functional applications in medicine, construction, electrical, environment, etc. Polyurethane foam (PUF) has gained a considerable position as an important polymer suitable for biomaterials and biomedical device applications because they can combine biocompatibility with a good processability and mechanical resistance [1-4]. However the use of polyurethane in the field of medical materials remains largely unexplored.

Biodegradable polyurethane is generally prepared from polyol, isocyanate and chain extenders. Soft segment (SS) is derived from polyol while the hard segment (HS) is formed from isocyanate and chain extender. But a major limitation on the type of isocyanate is the toxicity of the degradation products. In this research lysine triisocyanate (LTI) and butane diisocyanate (BDI) have been carefully chosen to synthesize this PUF because they can biodegrade in-vitro and in-vivo to the nontoxic decomposition products [5, 6]. The polyurethane based on polycaprolactone diol (PCL) has been widely studied. Indeed, PCL is hydrophobic material, biocompatible, biodegradable and has a very mind inflammatory response with tissues. However, the rate of degradation of PCL is rather slow due to the crystallinity and hydrophobicity. So, it is demonstrated that combine PCL with a hydrophilic materials as a soft segment enhance the biodegradation of polyurethane [7, 8]. Polypropylene glycol (PPG) is well known as a comonomer for soft segment of polyurethane backbone. PPG present many attractive properties [8-9] such as hydrophilicity, nontoxic degradation products and widely used for polyurethane synthesis. In this research the effect of polyol molar ratio between hydrophilic material (PPG) and hydrophobic material (PCL) as the soft segment will be investigated

Tatai L. [9] investigated the effect of chain extender structure on the mechanical, thermal properties and in-vitro degradation of a series of polyurethane containing degradable chain extender. The result demonstrated that the degradable chain extender based on lactic acid (LA) and ethylene glycol (EG) did not have a considerable effect

on mechanical and thermal properties but have responsible for high mass loss. In this research, the biodegradable chain extender based on lactic acid and ethylene glycol will be prepared.

The purpose of this study is to develop biodegradable and biocompatible PUF based on aliphatic isocyanate (LTI, BDI), polyol (PPG, PCL) and LA-EG chain extender. PUF with well tolerated and degrade to toxically harmless product, suitable for wound dressing or wound support during the surgical healing period are expected, such as skin graft immobilization, wounds, burns and ulcers covering and closure of oroantral communications [10-17]. This research aim to synthesize, characterize and investigate the PUF qualities in term of mechanical behavior of these new biodegradable medical PUF because it is important for the assessment of their potential application for medical devices.

1.1 Objective and research scopes

1.1.1 Objectives

Biodegradable polyurethane foam (PUF) has gained a considerable position as an important polymer suitable for biomaterials and biomedical device applications because they can combine biocompatibility with a good processability and mechanical resistance. They are currently used as medical materials such as scaffold, bone adhesive in case of polyurethane film. However, in term of polyurethane foam, there are a few research and application. This research aim to synthesize and characterize biodegradable medical polyurethane foam based on biocompatible materials with various polyols molar ratio and types of aliphatic isocyanate.

1) To study the effect of polyols molar ratio and types of isocyanate on the properties of PUF: mechanical, thermal properties, degradability and toxicity.
2) To study the effect of hydrophilic material and degradable chain extender on the degradability of PUF.
3) To evaluate the in vitro degradation process of PUF in PBS and enzymatic system (to imitate a condition of human body system).

1.1.2 Research scopes

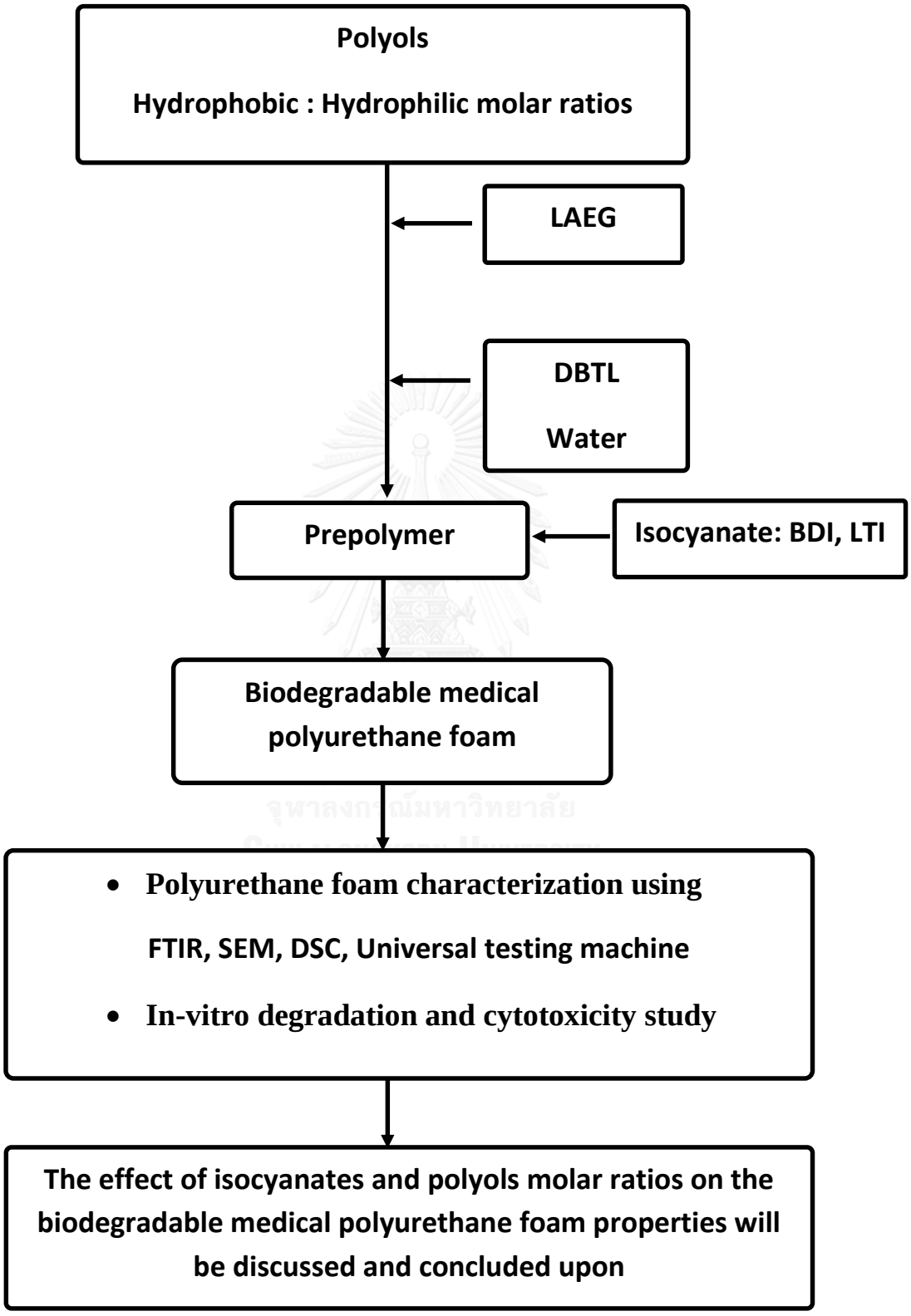
This research scopes are to synthesize polyurethane foam from soft segment: PCL and PPG, and hard segment: LTI and BDI increasing degradability by chain extender. The scope details are listed below.

1) To synthesize PUF using PCL and PPG as soft segment, BDI and LTI as hard segment and chain extender with various polyols molar ratio.
2) To compare the influence of polyols molar ratio and types of polyisocyanate on mechanical properties, thermal properties, degradability and cytotoxicity of biodegradable polyurethane.
3) The molecular weight of these products was investigated in from of viscosity average molecular weight (M_v) and related to weight average molecular weight (M_w) using viscometer and Mark - Houwink equation
4) The thermal properties, glass transition temperature (T_g), Melting temperature (T_m) were investigated by DSC.
5) The mechanical properties were determined by universal testing machine to provide acceptable strength.
6) The degradability of PUF was performed by hydrolytic degradation and enzymatic degradation test using phosphate buffer saline (PBS) solution. PBS with pH 7.4 and 60 ^oC was used for hydrolytic degradation test and PBS with lipase form porcine pancreas was used for enzymatic degradation.
7) The cytotoxicity of PUF was evaluated using MTT assay according to ISO 10993

1.2 Benefits

1) To synthesize and describe biodegradable polyurethane foam process with various polyols molar ratio and types of isocyanate understandingly.
2) To get various types of biodegradable polyurethane foam with adaptable parameter, for instance, density, compressive strength and degradability.
3) The properties of aliphatic isocyanate, polyol and chain extender with appropriate condition bring about biodegradable polyurethane foam suitable for biomedical material development.

1.3 Research Methodology



CHAPTER II
THEORY AND LITERATURE REVIEWS

2.1 Polyurethane foam

Polyurethane is a polymer consisting of a chain of organic unit joined by urethane linkage, based on the reaction of polyisocyanate with polyol containing hydrogen compounds. These groups will readily react to form polyurethane. Polyol act as a soft segment (SS) while polyisocyanate act as a hard segment (HS) as follows:

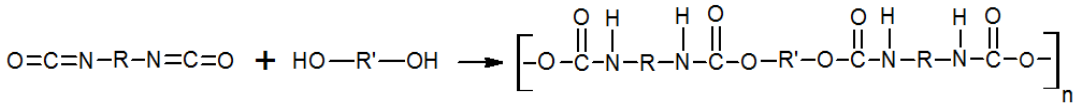


Figure 2.1 Polyurethane reaction

The hydrogen on the nitrogen atom of the urethane group can react with additional isocyanate to form an allophanate group.

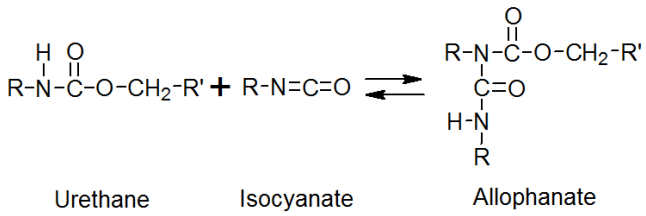


Figure 2.2 Allophanate reaction

The formation of allophanate is a high temperature and reversible reaction. If this group formed in flexible foam, the allophanate linkage would enhance crosslinking of the polymer. The catalyst using in foam formulations do not increase the rate of this reaction, and the allophanate can significantly formed by the high temperature reaction.

For the synthesis of polyurethane foam, polymer must be blown by the formation of bubble and a gas. A source of gas is the carbon dioxide produced from the reaction between isocyanate group and water

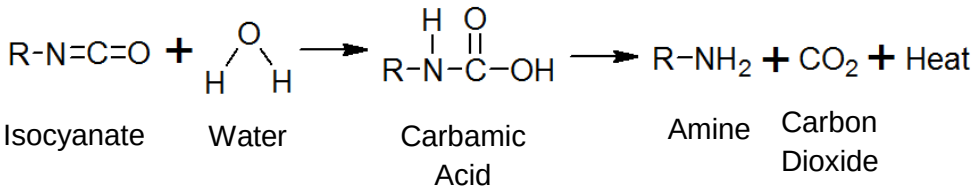


Figure 2.3 Gas producing reaction

The intermediate product of this reaction is carbamic acid, which inherently decompose to amine and carbon dioxide. Diffusion of carbon dioxide into bubbles previously nucleated in the medium causes the enlargement of the medium to make foam. Even more, reaction of the amine with additional isocyanate provides a disubstituted urea.

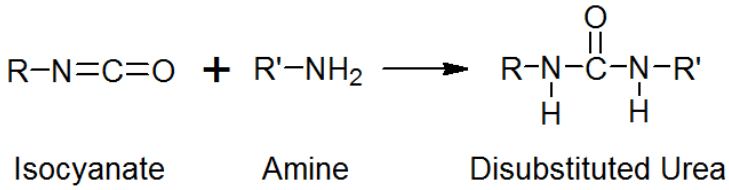


Figure 2.4 Disubstituted urea forming reaction

Again, if the reactants are polyfunctional, a cross-linked polymer will result. Another conceptual method of cross-linking the polymer is by reaction of hydrogen from the disubstituted urea with a free isocyanate group to form a biuret linkage.

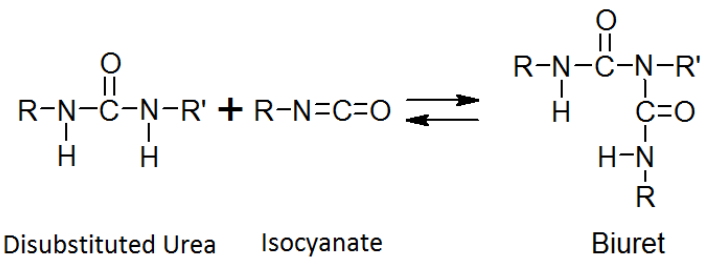


Figure 2.5 Biuret linkage forming reaction

Blowing can also achieved by the physical addition of a low boiling point nonreactive liquid to a foam formulation. The most generally used blowing agents are the chlorofluorocarbon, methylene chloride urethane grade, and trichloroethane. Vaporization of these liquids given gas molecules that diffuse into nucleated bubbles and contribute to foam expansion.

These are the most common ingredients and example used in the production of flexible polyurethane foam.

Table 2.1 List of the most common ingredients for polyurethane synthesis

Component	Example
Polyol	Polycaprolactone diol (PCL) Polyethylene glycol (PEG) Polypropylene glycol (PPG)
Isocyanate	Toluene diisocyanate (TDI) Methylene diphenyl diisocyanate (MDI) Hexamethylene diisocyanate (HDI)
Catalyst	Stannous octoate Triethylenediamine (TEDA) Dibutyltin dilaurate (DBTL)
Blowing agent	Distilled water Chlorofluorocarbon Methylene chloride Trichloroethane

The general role of each polyurethane component will be described in the next topic.

2.1.1 Polyol

The polyol are compounds of hydroxyl functional groups or isocyanate reactive groups available for organic reactions. They contain ester, ether, amide, acrylic, metal, metalloid and other functionalities. Processing and properties of the foam product can be influenced by the type of polyol structure. Nowadays flexible foam mostly produced from polyether or polyester polyol such as polyethylene glycol, polypropylene glycol, poly(tetramethylene ether) glycol, and polycaprolactone diol. Polyester polyol are composed of ester and hydroxyl group in one backbone. They are generally prepared from the condensation reaction of glycols, i.e., ethylene glycol, 1,4-butane diol, 1,6-hexane diol and dicarboxylic acid/anhydride (aliphatic or aromatic).

The properties of polyurethane also depend on the cross-linking degree and molecular weight of the starting polyol. Highly branched polyol give rigid polyurethane that have a good heat and chemical resistance, while less branched result in flexible foam with good resilience but low chemical resistance. Likewise, low molecular weight polyol give rigid polyurethane while high molecular weight polyol provide flexible polyurethane. Polyester polyol are sensible to hydrolytic degradation because of ester groups in their structure, this cause the inferior of mechanical properties of product. Polyether polyol are cheaper than polyester polyol. They are produced from the addition reaction between ethylene or propylene oxide and alcohol or amine. Polyurethane produced from polyether polyol result the high moisture permeable and low glass transition temperature (T_g) that limit their applications such as coatings and paints. Figure 2.6 show the most common used polyol in commercial.

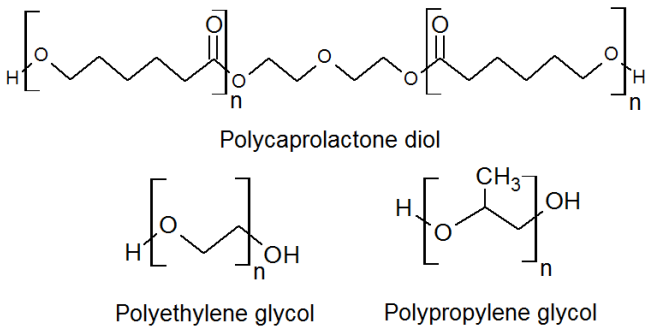


Figure 2.6 Examples of the most common polyol

Joanna L. Ryszkowska et al [1] synthesized porous polyurethane composite from polycaprolactone diol containing bioglass and fabricated it by polymer coagulation combined with salt particle leaching method. This polyurethane/bioglass composite foam with more than 70% porosity exhibit open pore size of 100 – 400 μm and contain micropores of less than 10 μm in pore wall. The mechanical properties and bioactivity of this product satisfies the requirements for bone tissue engineering applications. However the evaluation of cytocompatibility of this product addition on osteoblast cell attachment and proliferation has not been carried out.

2.1.2 Isocyanate

Isocyanate is the source of NCO groups reacted with functional groups such as hydroxyl group from polyol, cross-linker and water in the formation of polyurethane. Today the commercial grade isocyanate contains at least two groups of isocyanate per molecule called diisocyanate. These can be aliphatic, cycloaliphatic and aromatic such as toluene diisocyanate (TDI), methylene diphenyl diisocyanate (MDI), xylene diisocyanate (XDI), hexamethylene diisocyanate (HDI), butane diisocyanate (BDI), lysine diisocyanate (LDI). Figure 2.7 show some examples of the most common diisocyanate.

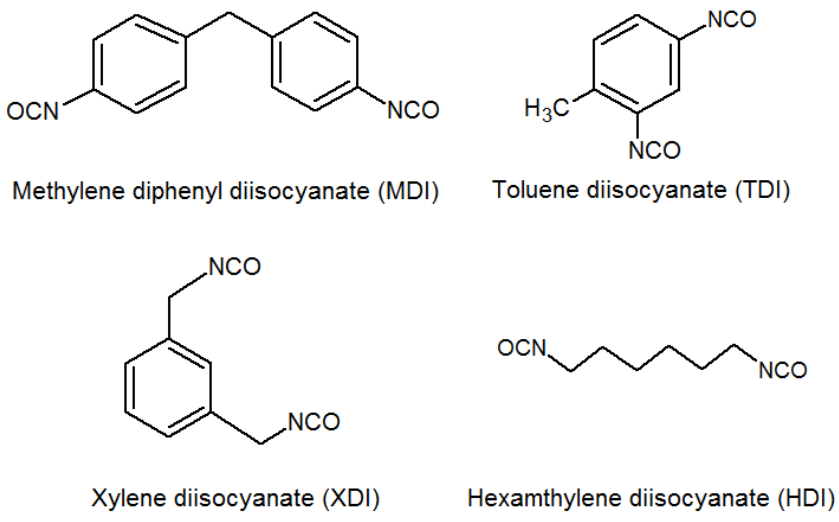


Figure 2.7 Examples of the most common diisocyanate

The two most common used isocyante in commercial are toluene diisocyanate (TDI) and methylene diphenyl diisocyanate (MDI). Both of them are usually used for foam production. For this research, we aim to synthesize the medical polyurethane that can degrade to the nontoxic product. But a major problem of aromatic diisocyanate is the release of carcinogenic compounds. So, we will avoid the toxicity of aromatic diisocyanate by using aliphatic diisocyanate such as lysine diisocyanate (LDI), lysine triisocyanates (LTI), butane diisocyanate (BDI) and hexamethylene diisocyanate (HDI) [12-16].

Spaans, C.J. et al [18] synthesized biomedical polyurethane from ε-caprolactone and 1,4-butane diisocyanate (BDI) with a high modulus. Using chain extender an ε-caprolactone with uniform-size hard segment was obtained. This product showed the excellent mechanical properties such as high modulus and tensile strength and released non-toxic products on degradation which suitable for meniscal prosthese application. However, this research did not study the cytotoxicity of product.

Guelcher, S.A. et al [6] synthesized polyurethane network using for fixing the fracture of bone defect that can integrate with host tissue and degrade to non-cytotoxic decomposition products from lysine polyisocyanate and polycaprolactone triol. This biomaterial showed mechanical properties comparable to commercially poly(methyl methacrylate) bone cement and biodegrade at a controlled rate to non-cytotoxic decomposition products. This product also supported the attachment and proliferation of MC3T3 cells deposit mineralized extracellular matrix. In conclusion, polyurethane network synthesized from lysine polyisocyanate have potential application as biodegradable cements, scaffold and composite for bone tissue engineering.

Hafeman, A.E.[19] et al synthesized polyurethane for scaffold and drug delivery systems for regenerative medicine. This degradable polyurethane was synthesized based on polyethylene glycol as well as lysine triisocyanate (LTI). The results showed that degradation of polyurethane synthesized from LTI produced α-hydroxy acid which have been shown to be non-toxic in vitro. Lysine and ethanolamine was also detected in degradation products of LTI, but it did not cause the cytotoxic because its concentration was not anticipated to reach toxic levels.

2.1.3 Chain extender

Chain extender (2 functional groups) and cross-linkers (more than 3 functional groups) are the group of hydroxyl and amine compounds that have low molecular weight and short chain. These structure effect on polyurethane morphology. The elastomeric properties of polyurethane derived from phase separation due to the non-polar, low melting point of soft segments incompatible with polar, high melting point of hard segments. The soft segments that formed from polyol are mobile and display in coiled formation, while the hard segments from isocyanate and chain extender are stiff and immobile because the hard segments covalently couple to the soft segments that inhibit plastic flow of polymer chain. This creates elastomeric resilience. During the deformation, a part of soft segments are stressed by uncoiling and the hard segments become aligned in the direction of stress. The orientation of hard segments and strong hydrogen bonding result in high tensile strength, elongation and tear resistance. The most favored chain extenders are ethylene glycol, propylene glycol, butane diol, propane diol, hexane diol, cyclohexane dimethanol and hydroquinone bis(2-hydroxyethyl) ether.

Tatai, L.et al [9] prepared biodegradable polyurethane (PU) from polycapro--lactone diol and aliphatic diisocyanate which are developed the hard segment degradation using degradable chain extender (DCE) synthesized from DL-lactic acid (LA) and ethylene glycol (EG). The results demonstrated that adding DCE may have yielded lower M_n because of the secondary hydroxyl group of DCE which is lower in reactivity compared to primary hydroxyl group of EG. Due to the lower M_n, non-DCE based PU showed higher modulus than DCE based PU but similar tensile strength. From thermogram, the presence of DCE appeared to have no significantly effect on PU morphology. Therefore, thermal properties of PU did not change with the addition of DCE. DCE based PU also show the grater mass loss than non-DCE based PU due to the ester linkage in urethane bond, the loss between series (various types of isocyanate) was not considerably different. So, PU degradation largely depends on the chain extender followed by the types of isocyanate.
In conclusion, the addition of DCE did not have a considerable effect on mechanical and thermal properties but, the molecular weight was lower due to its low reactivity.

2.1.4 Catalyst

Polyurethane foam productions are based on two competing reactions, to form polyurethane foam with good opened cell and physical properties, a balance of these two reactions are required. Suitable catalysts are used to carry out, accelerate and control these reactions. If the blow reaction is faster, foam possibly collapses. On the other hand, if the gelation reaction is faster, foam with closed cells were results and lead to foam shrinkage. To avoid these disadvantages, the appropriate amounts of each type of catalyst are carried on to sufficiently entrap gas produced in the polymer.

Catalysts used for catalyze reaction in polyurethane synthesis are usually choose from two major classes of compounds, tertiary amines and metal salts, mostly tin. Because of the different between activity and selectivity towards the polyurethane foam forming, the combination of these two catalysts are provided to balance blowing and gelation reaction, and to tune this reaction for desired production.

2.1.4.1 Tertiary amine catalyst

Tertiary amine is compounds containing a nitrogen atom that have three substituent groups and a free pair of electrons. This catalyst using for polyurethane synthesis, act as a blowing catalyst, they can catalyze gelation reaction as well. Activity of this catalyst depends on the effect of free pair electron of nitrogen atom; the donation of these electrons to the isocyanate groups is the most important effect to the formation of an intermediate complex. The steric hindrance of nitrogen atom and electronic effect of the substituent groups are the factor that influence on activity of tertiary amine catalyst. In general, requirements for good catalyst are

1) Nucleophile is strong enough to attack carbon atom of isocyanate group.

2) The catalyst capable of forming an active hydrogen amine complex.

3) The catalyst is soluble in water and form hydrogen bond with water.

For some polyurethane foam process, combinations of various amines are used in an attempt to balance blowing reaction and gelation reaction. The types of amine catalyst and concentration can be chosen to control the process requirements, for instance, cream time, rise time, gel time, curing time of the outer surface skin. These factor influence on the cell structure and physical properties of polyurethane foam.

2.1.4.2 Organometallic catalyst

Gelation reaction of polyurethane is promoted by organometallic catalyst. The most widely used of this catalyst is tin compound such as stannous octoate and dibutyltin dilaurate (DBTL). The catalytic activity is described by three complimentary mechanisms. The first mechanism describes the activation of polyol into a tin alkoxide which react with isocyanate to form urethane linkage. The urethane linkage increasingly reacts with polyol therefore propagating the polyol and regenerating the catalyst. The second one is the activating of isocyanate and in turn attacked by the polyol to further propagate the reaction and regenerate the catalyst. And the last one is the formation of tin and amine complex that accepts a polyol and more activate the complex to react with an isocyanate group to produce carbamate linkage. Unlike the tertiary amine catalyst, tin catalysts remain in the foam permanently. Moreover, stannous salt can oxidize into the stannic form promoting the oxidative degradation of flexible foams [11]. So, we avoid this factor by using DBTL as the catalyst.

2.1.5 Blowing agent

In many process, water is used as a chemical blowing agent. Water reacts with isocyanate to form primary amine and carbon dioxide. The increasing of water content gas the effect on cell structure and morphology of polyurethane foam, given the lower density of foam because of the blowing reaction. Furthermore, adding more amount of water which react with isocyanate provide the more hard segments and stiffness of the foam.

Although the carbon dioxide produced from the reaction between water and isocyanate, act as the blowing agent, some formulations also employ physical or auxiliary blowing agents. There are low boiling point solvents, inert toward chemical reactions. When the high temperature reaction has undergone, the low boiling point solvents were vaporized and provide supplementary gas for foam expansion. Result in the larger cell and more degree of opened cell, which cause a decreasing of foam density leading to the increasing of foam softness.

2.2 Biodegradable polyurethane

Biodegradation is the chemical dissolution of materials by bacteria or other biological methods. Materials were consumed by microorganism and return to the natural compound. Nowadays there are many synthetic polymers that can be broken down by microorganism such as polycarbonate, polyester and polyurethane, because their ester bond susceptible to attack by water.

A major motivation to develop the biodegradable polyurethane is the need of new materials suitable for biomedical applications, for instance, tissue engineering, wound covering, drug delivery system, bone adhesive and implantable devices [17-20]. Polyurethane is one of the most synthetic polymers that are generally used in medical devices to support the functions of organs and human body tissue, because of their excellent mechanical properties and good biocompatibility, and utility to design the polymer structure to meet the need of various biomedical applications.

Recently, there are some useful biodegradable polyurethanes applying instead of permanently implanting devices. It is a better way for patients provided that a temporary support is proceeded using the biodegradable polyurethane during the surgical healing period until the tissue and organs were regenerated. For instance, the solid and porous polyurethane composite were synthesized and characterized which carry out the in-vitro bioactive testing in simulated body fluid. These materials structure show the satisfy requirements for bone tissue engineering applications such as mechanical properties and bioactivity which appropriate for scaffold material [4]. Additionally, there is biodegradable polyurethane network that synthesized and designed to join with host tissue and degrade to the nontoxic decomposition products, useful as the biodegradable bone cement for fracture treating [7].

But, almost of the biodegradable polyurethane are the type of film, porous and composite materials that not applicable for all of biomaterial applications [4, 7-10, 12-15]. Therefore a major challenge to the polyurethane material and technology are the compatible of design, properties and requirement in technologies. The new materials must support and regenerate tissue and organs while providing a good mechanical support and degrade to the nontoxic or harmless product that not damage a human body.

In this research, we aim to synthesize the biodegradable polyurethane foam that suitable for biomedical device applications based on polyol (PCL, PPG), diisocyanate (BDI, LTI) and LA-EG chain extender, because of their mechanical properties, biocompatibility and availability.

2.3 In-vitro degradation

Due to the applications of these polyurethane foams, we have to prove the degradability of the product as well as study the degradation mechanism and degradation time to confirm that the amount of PPG, types of isocyanate and degradable chain extender have the effect on the degradability to get the appropriate condition for polyurethane foam synthesis: good morphology of foam with high compression properties and short time degradation.

In vitro degradation of polymer [20] was evaluated by weight loss and molecular weight changes overtime under the static culture conditions in

1) Distilled water for acceleration test at 70^{o}C

2) Phosphate buffer saline (PBS) solution 0.1M with enzyme at 37^{o}C

To study the effect of enzymatic degradation, the results from general test and acceleration test will be compared according to ISO10993 [21, 22] standard. Each polymer strip will be placed in 20 ml test tube with full fill of solution to perform the degradation test. The sealed test tubes will be placed in drying oven at 70^{o}C. This higher temperature will be used to accelerate the degradation rate. A well-established relationship with different temperature is available to convert the degradation profile to 37^{o}C. AT each time point, five of each type of foam will be sampled, rinsed with distilled water five times and dried for 24 h or until the constant weight was obtained before weight loss and molecular weight change analysis. Change in the weight average molecular weight and molecular weight distribution will be determined by gel permeation chromatography (GPC). The GPC data is calibrated with polystyrene standards with molecular weight in the range of 580 – 7,500,000 Da. DMF is used as an eluting solvent.

The degradation of polymer can be classified to 4 cases as follow:

1) Case 1 (No/No)

No change in mass balance and molecular weight/distribution. No degradation has been observed. The test is terminated; no real time degradation test is necessary.

2) Case 2 (No/Yes)

No change in mass balance, but molecular weight/distribution has changed. Check the bulk sample and debris for degradation products. Proceed with real-time degradation test, if necessary.

3) Case 3 (Yes/No)

Change in mass balance, but no change in molecular weight/distribution. Polymer is not degraded; fluid phase contains leachable which shall be assessed according to other relevant parts of ISO 10993. Proceed with real-time degradation test, if necessary.

4) Case 4 (Yes/Yes)

Change in mass balance and change in molecular weight/distribution. Identify and quantify leachable and polymer degradation product from the fluid phase and check the bulk sample and debris for degradation products. Proceed with real-time degradation test, if necessary. The degradation test procedure was shown in figure 2.8

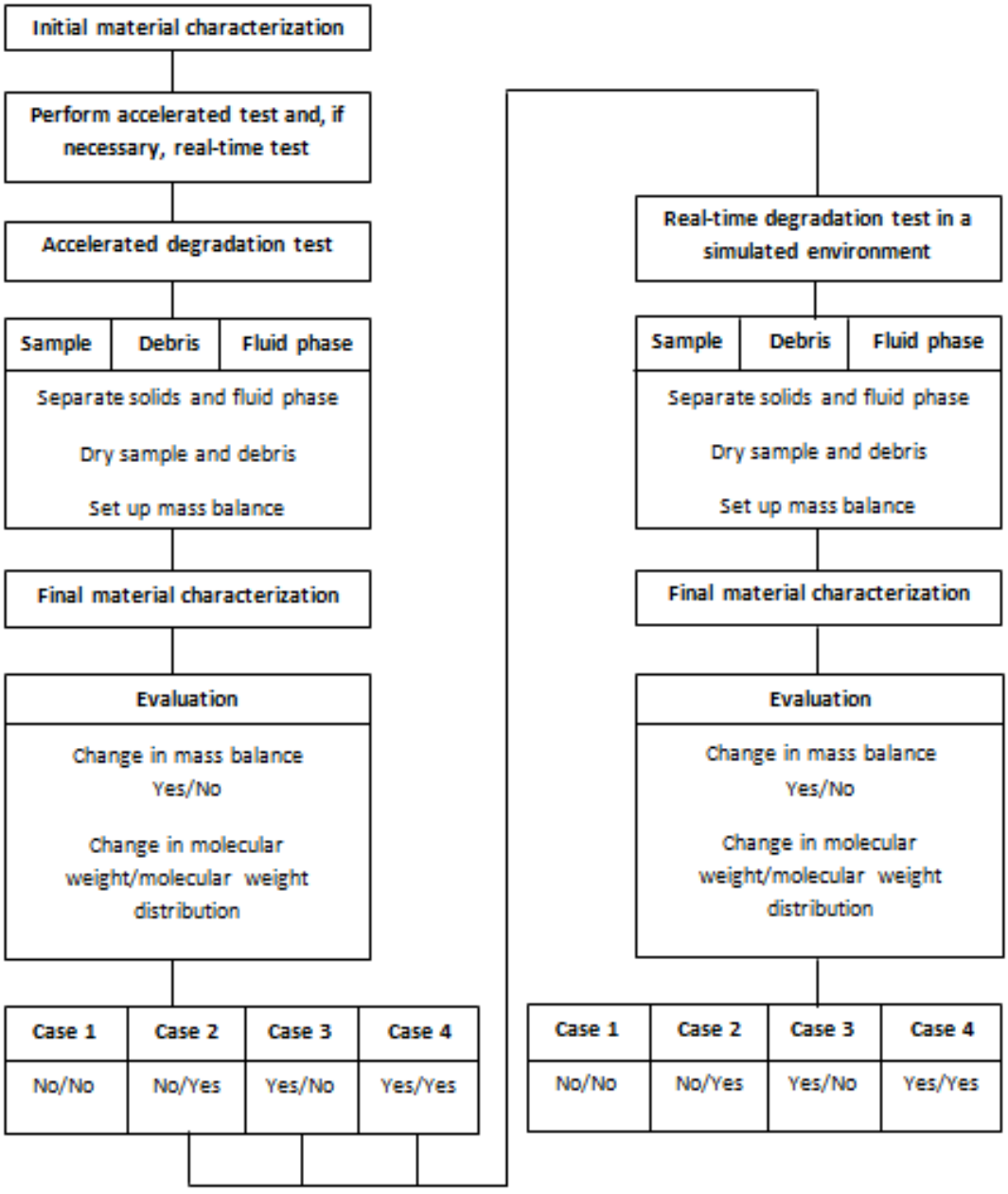


Figure 2.8 Flow chart test procedure [22]

2.4 Cytotoxicity of polyurethane foam

Polyurethane is the most suitable polymer for biomedical applications because of the various properties depends on the reactant such as polyol, polyisocyanate, chain extender, catalyst and blowing agent which can be produced specifically for each application. However, after the degradation of polyurethane foam, there are the degradation products from polyurethane. The toxic from polyurethane degradation is carcinogenic substance from aromatic of isocyanate and dibutyltin dilaurate catalyst using for polyurethane foam synthesis. Nevertheless, many research report that polyurethane obtained from aliphatic isocyanate show the nontoxic degradation product. But, toxic from catalyst still be concerned. So, we have to confirm that polyurethane foam in this research is the nontoxic product. If this foam has toxic, we must determine the toxic level of this product.

2.4.1 Determination of cytotoxicity

After the degradation results were obtained, the polyurethane foam product was extracted using centrifuge. The extracted product was placed into the cell and then cytotoxicity test was carried on. Determine of cytotoxic effects by either qualitative or quantitative means. Quantitative evaluation of cytotoxicity is preferable.

Qualitative and quantitative means are appropriate for screening purpose. In this research we use quantitative mean for cytotoxicity analysis.

Qualitative evaluation: Examine the cell microscopically using cytochemical staining if desired. Assess changes in, for instance, general morphology, vacuolization, detachment, cell lysis and membrane integrity. The change from normal morphology shall be recorded in the test report descriptively or numerically. A useful way to grade test sample is given in **Table 2.2**

Table 2.2 Qualitative morphological grading of cytotoxicity of extracts

Grade	Reactivity	Conditions of all culture
0	None	Discrete intracytoplasmatic granules, no cell lysis, no reduction of cell growth
1	Slight	Not more than 20% of the cells are round, loosely attached and without intracytoplasmatic granules, or show changes in morphology; occasional lysed cells are present; only slight growth inhibition observable.
2	Mild	Not more than 50% of the cell are round, devoid of the intracytoplasmatic granules, no extensive cell lysis; not more than 50% growth inhibition observable.
3	Moderate	Not more than 70% of the cell layers contain rounded cell or are lysed; cell layers not completely destroyed, but more than 50% growth inhibition observable.
4	Severe	Nearly complete or complete destruction of the cell layers.

The achievement of numerical grade greater than 2 based on **Table 2.2** is considered a cytotoxic effect.

Quantitative evaluation: Measure cell death, inhibition of cell growth, cell proliferation or colony formation. The number of cells, amount of protein, release of enzyme, and release of vital dye, reduction of vital dye or any other measurable parameter were quantified by objective means. Reduction of cell viability by more than 30% is considered a cytotoxic effect. Other criteria, including different cut-off points or an acceptable ratio of test-to-control result shall be justified for alternate cell lines or multi layered tissue constructs. The criteria shall be justified and documented. The Annex C protocol was used for quantitative determination of cytotoxicity of extract

The MTT cytotoxicity test of Annex C is based on the measurement of the viability of cell via metabolic activity [23-30]. Yellow water-soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) is metabolically reduced in viable cells to a blue-violet insoluble formazan. The number of viable cells correlates to the color intensity determined by photometric measurements after dissolving the formazan in alcohol

L929 cell was seeded into 48-well plates and maintained in culture for 24 hr to form a semi-confluent monolayer [31]. They are exposed to the test compound, extracted solution from polyurethane, over a range of concentration. After 24 hr exposure, the formazan formation is determined for each treatment concentration and compared to that determined in control cultures. For each treatment the percentage inhibition of growth is calculated

A decrease in number of living cells results in a decrease in the metabolic activity in the sample. The decreasing is directly relates to the amount of blue-violet formazan formed, as monitored by the optical density at 570 nm. To calculate the reduction of viability compared to the blank, Equation 2.1 is used.

$$\text{Viability (\%)} = \frac{100 \times OD_{570e}}{OD_{570b}} \quad \textbf{(2.1)}$$

Where

OD_{570e} is the mean value of the measured optical density of the 100% extracts of the test sample

OD_{570b} is the mean value of the measured optical density of the blanks.

The lower the percentage of viability, the higher cytotoxic potential of the test item is. If the viability of cells is reduced to less than 70% of the blank, it has a cytotoxic potential. The 50% extract of the test sample should have at least the same or a higher viability than the 100% extract, the test should be repeated.

CHAPTER III
EXPERIMENTAL

Chemicals

1) Polycaprolactone diol (PCL) MW 2,000 from S.M. chemical supplies Co., Ltd.
2) Polypropylene glycol (PPG) MW 2,000 from S.M. chemical supplies Co., Ltd.
3) Lysine ethyl ester triisocyanate (LTI) from Huahao industry Co., Ltd.
4) Butane diisocyanate (BDI) from Carbosynth limited .Co.Ltd.
5) Dibutyltin dilaurate (DBTL) from S.M. chemical supplies Co., Ltd.
6) DL-lactic acid (LA) from S.M. chemical supplies Co., Ltd.
7) Ethylene glycol (EG) reagent plus ≥ 99% from S.M. chemical supplies Co., Ltd.
8) Monosodium hydrogen phosphate (Na_2HPO_4) from Finechem Pty Ltd.
9) Sodium chloride (NaCl) from Finechem Pty Ltd.
10) Sodium dihydrogen orthophosphate (NaH_2PO_4) from Finechem Pty Ltd.
11) Lipase (porcine pancreas) 100 U/mg protein from S.M. chemical supplies Co., Ltd.

3.2 Biodegradable polyurethane foam polymerization

3.2.1 Synthesis of chain extender

1) LA was heated to 160 ^{o}C under nitrogen atmosphere for 6 hr in a 250 ml round-bottom flask (RBF) with a condenser and dean-stark to collect the water runoff.
2) A 5:1 molar ratio of EG was added to the polylactic acid (PLA) and heated to 180 ^{o}C for 21 hr to polymerize chain extender. Then distill excess EG from the RBF at 70 ^{o}C under vacuum (0.01 Torr) and collect it in liquid nitrogen trap.
3) Raise the temperature to 130 ^{o}C to distill the 2-hydroxyethyl 2-hydroxy propanoate (LAEG). The obtained LAEG chain extender structure was shown in **Figure 3.1**.

3.2.2 Synthesis of polyurethane foam

PU was synthesized with one step addition polymerization. PCL and PPG with various ratios (up to 50% of PPG) were poured into polystyrene cup and heated to 80 ºC until the precursor was completely melted. LAEG, DBTL catalyst and distilled water were added to the precursor and stirred vigorously. Then isocyanate (BDI or LTI at 1.5 NCO:OH molar ratio) were added to the precursor and fully stirred at 1,500 rpm for 10 s. Foam will be raised and cured at room temperature overnight and cut into a 3×3×3 cm^3 for mechanical testing. The PU structures based on BDI and LTI were shown in **Figure 3.2** and **3.3** respectively.

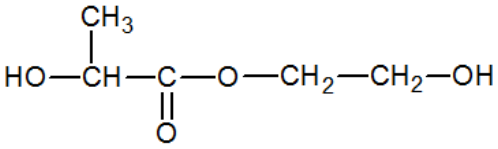


Figure 3.1 LAEG chain extender structure

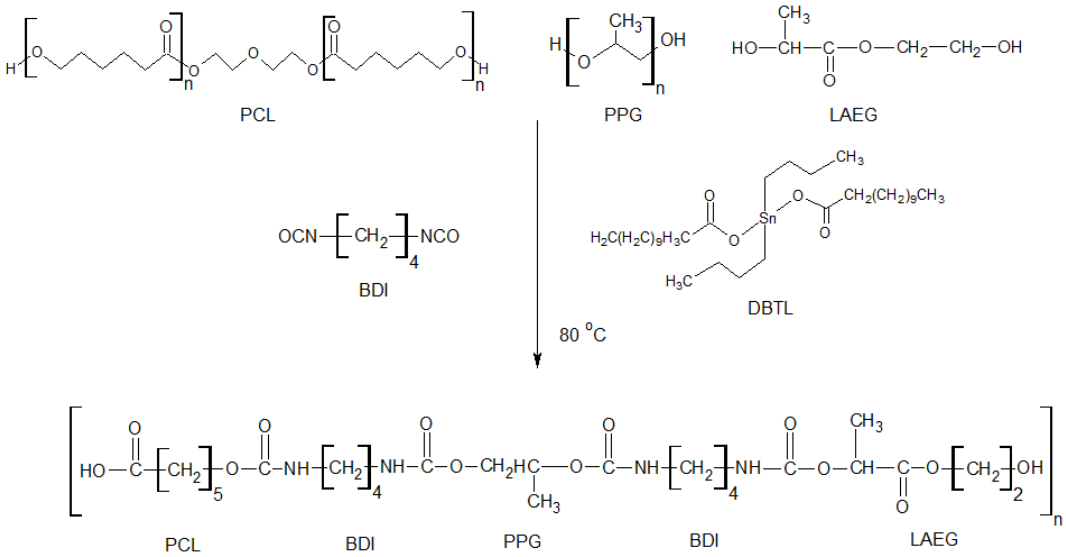


Figure 3.2 Polyurethane structure based on BDI

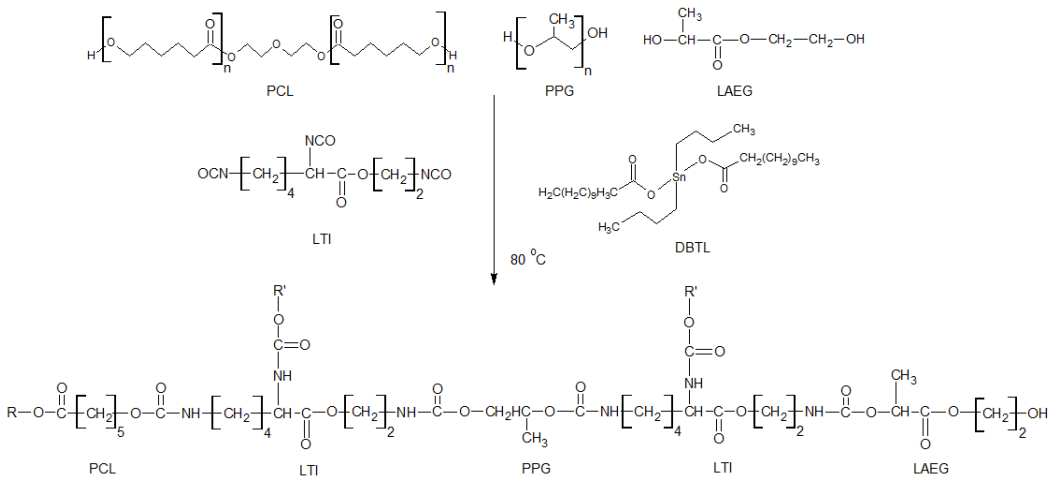


Figure 3.3 Polyurethane structure based on LTI

Table 3.1 Polyurethane synthesis conditions

Sample	Polyols molar ratio		Type of isocyanate
	PCL (%)	PPG (%)	
PU-BDI-0	100	-	BDI
PU-BDI-10	90	10	BDI
PU-BDI-30	70	30	BDI
PU-BDI-50	50	50	BDI
PU-LTI-0	100	-	LTI
PU-LTI-10	90	10	LTI
PU-LTI-30	70	30	LTI
PU-LTI-50	50	50	LTI

3.3 Water absorption measurement

The water absorption measurement was performed to study the effect of hydrophilicity of PPG on degradation rate of polyurethane according to ASTM D1037. Foams were cut into 1 cm^3 and three replicate for each sample and dried for 24 hr in oven at 60°C to remove moisture content in foam. The samples were immersed in distilled water for 24 hr and then weighed the initial weight. After the immersion, samples were dried and stored in sealed packed containing desiccant until the constant weight was obtained[32]. The water absorption was calculated as follow:

$$\text{Water absorption } (\%) = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100 \quad \textbf{(3.1)}$$

3.4 In-vitro degradation analysis

The obtained foams were cut into 1×1×1 cm^3, placed in 20 ml test tube (1 g:10 ml) and assess in-vitro by weight loss rate using phosphate buffer saline (PBS) solution with 0.1 M, pH 7.4 as the degradation medium in an oven that control temperature at 60°C. A 10 ml PBS solution that contains Na_2HPO_4 and NaH_2PO_4 was added to each test tube.

The hydrolytic degradation of polyurethane was performed using accelerated test to study the effect of hydrophilicity of sample on the degradation rate, sample was immersed in PBS solution and incubated in an oven at 60°C. After the end of each week, 5 samples of each condition was taken out, rinsed with distilled water for 5 times and stored in sealed packed containing a desiccant at room temperature until constant weight was obtained. This degradation test was carried out for 4 weeks. The percent weight loss was calculated as follow:

$$\text{Weight loss } (\%) = \frac{W_0 - W_t}{W_0} \times 100 \quad \textbf{(3.2)}$$

In equation 3.2, W_0 and W_t are the initial weight and weight after degradation respectively. The results of weight loss rate and water absorption are the average of five samples.

After studying the hydrolytic degradation test, the enzymatic degradation test with simulated condition was performed. Samples were immersed in enzyme solution containing 60 ml of PBS, 15 mg/ml of lipase from porcine pancreas in 30 ml of PBS and then incubated in oven at 37°C for 14 days. After day 3, 5, 7, 10 and 14 5 samples of each condition were removed from enzyme solution and rinsed with distilled water for 5 times and stored in sealed packed containing a desiccant at room temperature until the constant weight was obtained. The percent weight loss was calculated similar to hydrolytic degradation test.

3.5 Cytotoxicity test

3.5.1 Cell preparation

L-929 cell which is a cell of mouse fibroblast was used for the cytotoxicity test. This cell was seed in 25 cm^3 culture flask using DMEM supplemented with 10% horse serum and 1% penicillin-streptomycin and incubated at 37 °C in 5% CO_2 incubator for 24 hr until the cells had been grown as confluent monolayer before starting each cytotoxicity test. The flasks with confluent cell were trypsinished with 0.25% trypsin-versence and seeded in 48 well-plates with 300 µL of medium containing approximately 20,000 cells/well.

3.5.2 Reagent Preparation

1) Sample: A 30 µl of extracted sample was added to a 270 µl of DMEM in the final concentration as 1×10^{-4} g/ml equivalent to 100% of tested sample. The solution was filtrated using 0.22 µm syringe filter and then diluted to 1×10^{-5} g/ml to study the cytotoxicity at various concentrations
2) Control solution: positive control, zinc acetate was dissolved in 1 ml of 0.9% of saline solution and sterile with 0.22 µm syringe filter. This solution was then diluted with culture medium to get the concentration of 20 ppm. 300 µl of untreated sterile DMEM was used as negative control.

3.5.3 Cytotoxicity test

After seeding L929 cells in well plate for 24 hr, all of old medium was replaced with extracted solution in the concentration range at 1×10^{-4} and 1×10^{-5} g/ml comparison with 20 ppm of zinc acetate solution and blank in the sample plate. These have been done in quadruplicate and incubated in 37 ^{o}C incubator.

3.5.4 Evaluation: MTT assay

All samples were measured by using of 3-(4,5dimethylthaizol-2-yl) 2,5-diphenyltetrazoliumbromide (MTT) assay. The fibroblasts were put onto a 96-well micro titer plate at 20,000 cells/well. After 24 hr the culture medium of each well was removed and 200 μL of a 0.5 mg/ml MTT solution was added to each well, followed by incubation for 30 min at 37 ^{o}C. Purple formazan, occurred when MTT solution react with live cell, crystal was dissolved by adding 200 μL DMSO per well. The absorbance measurement of standard curve was performed using UV-Vis spectrometer with the number of cell in the range of 6,250 to 200,000 cells. The optical density was recorded on a multiwall micro plate reader (ICN, Birsfelden, Switzerland) at 570 nm, and normalized to the control optical density.

After perform the absorbance measurement, the correlation between optical density at 570 nm and number of cell was investigated and expressed in term of $y = mx+c$, when y is optical density at 570 nm, x is number of cell, m and C are the constant. If optical density was known then the number of cell was obtained indicating the cytotoxicity of extracted samples. According to ISO 10993-5, if cell viability is higher than 70% of the blank, the extracted solution is noncytotoxic.

3.6 Characterization

3.6.1 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectrometer was used for characterize polyurethane linkage and other functional groups such as carbonyl group, hydroxyl group, amine group, ester group and to ensure the disappearance of isocyanate group causing the toxic. So the polyurethane structure was confirmed by FTIR. Infrared spectra was recorded with Nicolet 6700 FTIR spectrometer and scanning range from 400 to cm^{-1} with scanning time of 64.

Figure 3.4 Fourier transform infrared spectrometer

3.6.2 Proton Nuclear Magnetic Resonance (^{1}H NMR)

NMR spectrometer was used for characterize the different types of hydrogen atom to identify the LAEG chain extender structure. Spectra were recorded with Bruker 400 Ultra ShieldTM NMR spectrometer and range of scanning from 0 to 14 ppm with number of scan of 64 and time domain of 64. Solvent using in this experiment is chloroform-deuterated ($CDCl_3$).

Figure 3.5 Nuclear magnetic resonance spectrometer

3.6.3 Differential Scanning Calorimetry (DSC)

The glass transition temperature (T_g), melting temperature (T_m), and crystallinity (X_c) of polyurethane was measured by means of DSC, using DSC 204 F1 Phoenix® operating at a heating rate of 20 ^oC/min from -100 ^oC to 150 ^oC. Sample was heated twice. In the first scan, samples were heated to 100 ^oC and then cooled to 0 ^oC to remove all impurities and ordered the structure. In the second scan, samples were heated from -100 ^oC to 150 ^oC. The overall crystallinity was calculated from the heat of fusion values using the formula $X_c = H_m/H_p$.

Figure 3.6 Differential scanning calorimeter

3.6.4 Thermogravimetric Analyzer (TGA)

The thermogravimetric analysis is a technique in which the mass of substance in monitored as a function of temperature or time as the sample specimen is subjected to a controlled temperature program in a controlled atmosphere. TGA detect the decreasing weight of sample as a function of temperature to determine the thermal degradation of polyurethane. This experiment was performed using TA Instrument SDT – Q600.

Figure 3.7 Thermogravimetric analyzer

3.6.5 Gel Permeation Chromatography (GPC)

GPC was performed on PU before in-vitro degradation test and after several time points of degradation to determine molecular weight and change of molecular weight using tetrahydrofuran (THF) as a mobile phase. PU was dissolved in THF and filtrated through a syringe filter. This equipment was calibrated with polystyrene standard in various molecular weights between 500 – 100,000 Da. Data were analyzed using Empower Pro software to determine the number average molecular weight (M_n) and weight average molecular weight (M_w) and polydispersity (M_w/M_n).

Figure 3.8 Gel permeation chromatography

3.6.6 Scanning electron microscopy (SEM)

The morphology of all samples such as pore size, pore size distribution was observed using SEM Hitachi S3400N with resolution 1-5 mm.

Figure 3.9 Scanning electron calorimeter

3.6.7 Mechanical test – Universal Testing Machine

A test method was based on ASTM D1621 for foam. Compression test was performed on the Instron at ambient temperature. For all samples, a 5 kN load cell was performed with a crosshead speed of 10mm/min to 20 % deformation. Data were measured by Blue Hill v 2.5 software. All PUs were cut into 3×3×3 cm^3 and then stored in desiccator for 3 days before testing.

Figure 3.10 Universal testing machine – Compression test

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Synthesis of degradable chain extender

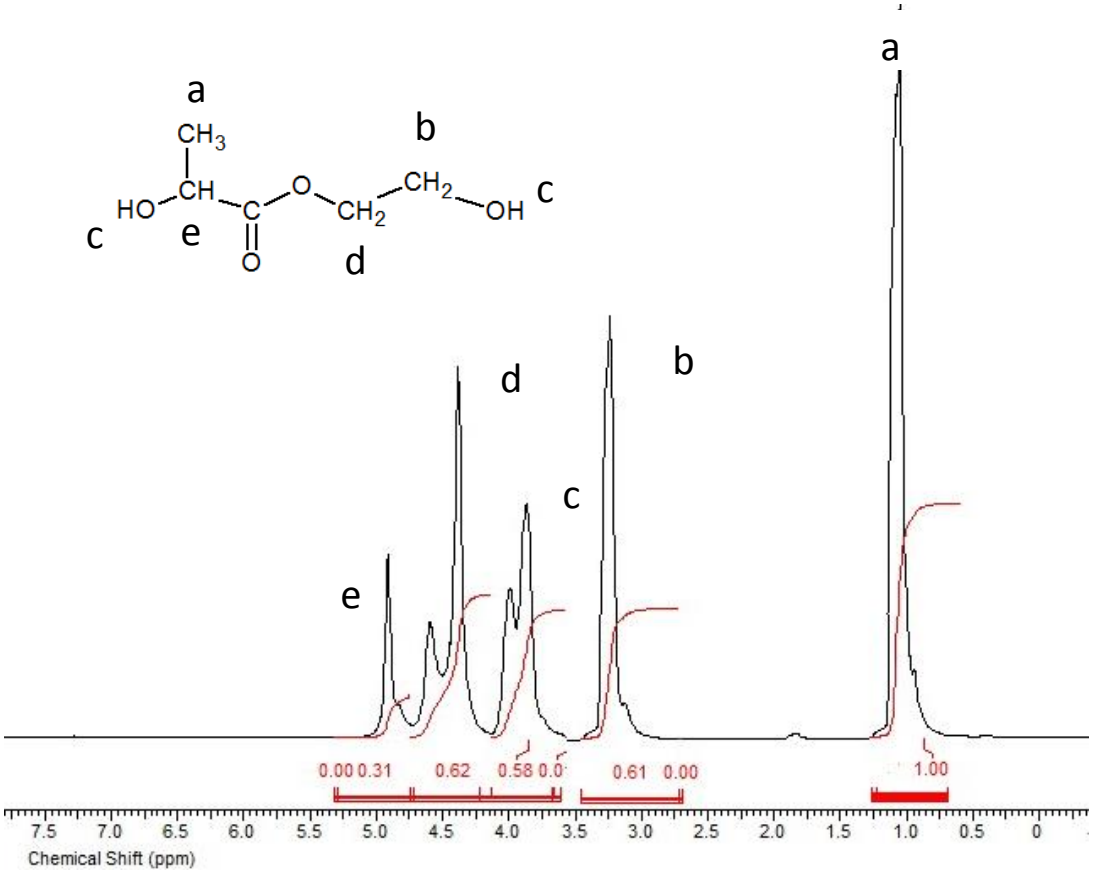


Figure 4.1 Chemical shift of H^1NMR of degradable chain extender (LAEG)

In this research, polyurethane was synthesized by using a degradable chain extender (hydroxyethyl lactate) via the esterification of DL-lactic acid and ethylene glycol to improve the degradability with more sensible to hydrolytic degradation of ester bond. Chemical shift of H^1NMR was shown in Figure 4.1. The chemical shift was found at 4.9 (H, CH), 4.4 (2H, CH_2), 3.8 (2H, OH), 3.3 (2H, CH_2-OH) and 1.2 (3H, CH3) corresponding to the structure of this chain extender. These showed that the degradable chain extender was synthesized [9].

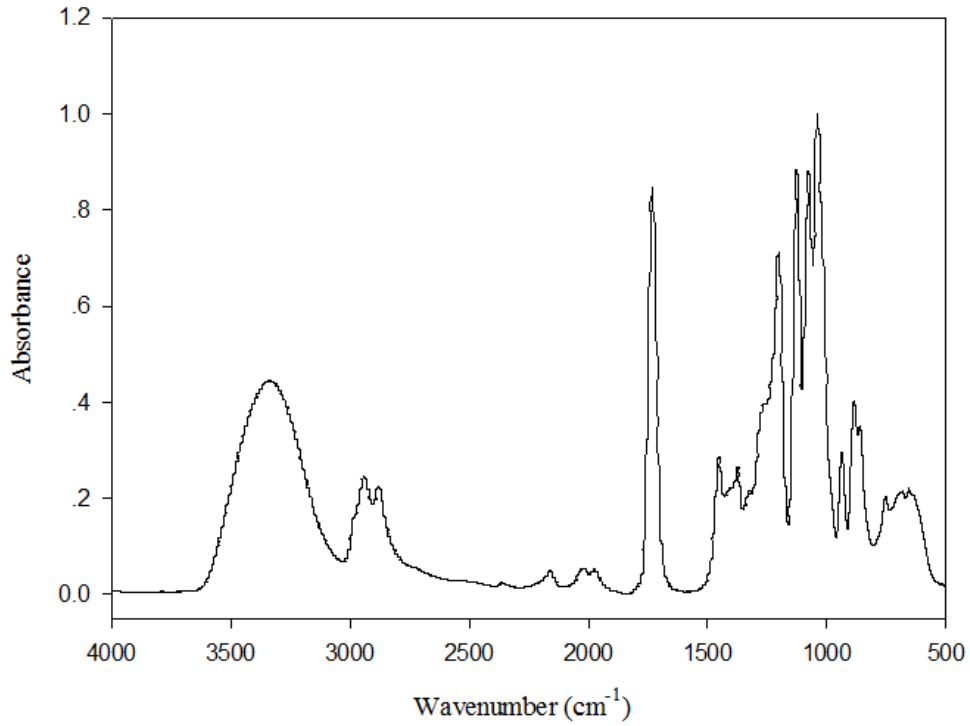


Figure 4.2 FTIR spectra of degradable chain extender (LAEG)

The chain extender was also investigated by fourier transform infrared spectroscopy (FTIR) to confirm the structure. The FTIR spectra of obtained product were shown in Figure 4.2. The broad band at 3336 cm^{-1} was hydroxyl group (OH). The absorbance band at 2940 and 2880 cm^{-1} indicated the peaks of asymmetrical and symmetrical CH_2 stretching respectively. The band of ether group (C-O) occurred at 1127 cm^{-1}. Interestingly, the band of ester group which refer to the first broken group when polyurethane reacts with water was observed at 1730 cm^{-1}. This result confirmed that the degradable chain extender was successfully synthesized.

4.2 Synthesis of polyurethane foam

4.2.1 Polyurethane structure

After synthesize the polyurethane foam through the reaction between polyol and polyisocyanate, the structure of polyurethane foam was confirmed by using infrared spectroscopy. This technique has been widely used to investigate and represent the characteristic vibration of hydrogen bonding. The broad band at 3340 – 3380 cm^{-1} is consistent with the stretching of NH bond of urethane and urea groups. Two bands at 2940 and 2860 cm^{-1} are the CH_2 stretching vibration, and it can be observed at 1460 cm^{-1}. Furthermore, it can be seen that the band of methyl group (CH_3) at 2970 cm^{-1} was appeared when the high content of PPG was used. The typical absorption band of ester bond (C=O stretching) was occurred at 1725 cm^{-1} and a shoulder at 1625 cm^{-1}, which is can be attributed to the urethane and urea carbonyl groups. The band located at 1530 cm^{-1} is C-N stretching (-C=O-NH-). The absence of absorption band at 2250 cm^{-1} indicated the disappearance of isocyanate group due to the complete reaction even the higher ratio of NCO/OH more than 1. It can be suggested that the excess isocyanate reacted with water to produce CO_2 which promoted the formation of polyurethane foam. It can be seen that C=O and C=O-NH bands of PU-LTI slightly shifted to the lower wavenumber in comparison with PU-BDI, due to more urethane linkage of LTI in polymeric bond. All of absorption bands were summarized as **Table 4.1**.

Table 4.1 Absorption bands of functional group in polyurethane

Band	Wavenumber	Assignment
N-H	3350	N-H stretching
CH_3	2970	Symmetrical stretching vibration of methyl
CH_2	2940 2860	Asymmetrical stretching vibration of methylene Symmetrical stretching vibration of methylene
N=C=O	2250	Free NCO group
C=O	1725	Ester stretching
C=O-NH	1530	C-N stretching N-H out of plane bending
CH_2	1460	Asymmetrical stretching vibration of methylene

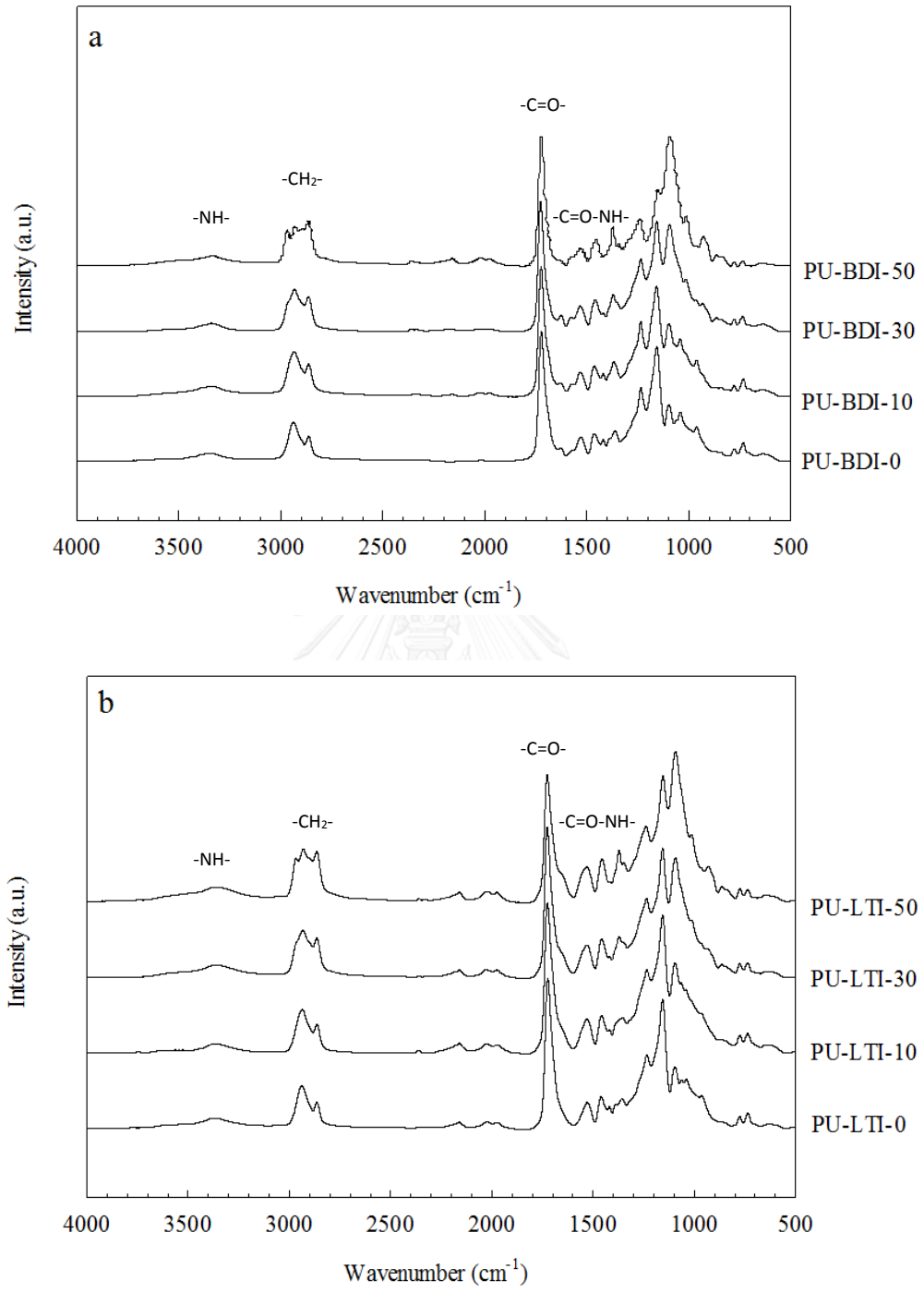


Figure 4.3 FTIR spectra of PU series with various ratios of polyol (a) BDI-based PU and (b) LTI-based PU

4.2.2 Thermal properties of polyurethanes

Thermal properties of polyurethane foam were measured by using differential scanning calorimetry (DSC). Glass transition temperature (T_g) of polyurethane was observed in the range of -54.20 to -62.82 ^oC which is varied depend on the types of polyisocyanate and the amounts of added PPG. Polyurethane synthesized from BDI and LTI have the T_g of -56.04 to -62.82 ^oC (**Figure 4.3a**) and -54.20 to -58.08 ^oC (**Figure 4.3b**) respectively. It can be seen that the T_g of polyurethane slightly decreased when adding high content of PPG because of the degree of branching of polymer. Branching has the two main effects on the mobility of polyurethane due to the reduction of local motion caused by the reduction of linear segmental length. the number of chain ends is increased and mobility of chain is increased [33]. Therefore, the decrease of T_g is due to the branched polymers that have more chain ends and large amount of free volume. Furthermore, T_g is also dependent on the types of polyisocyanate. The T_g of polyurethane synthesized from LTI is higher than that of BDI slightly. Because LTI has three functional groups in its structure so it can produce polyurethane with strong urethane linkage. The functional group in polymer decreased the mobility for the orientation of the polymer chain. The high energy is required to move polymer chain leading to the high obtained T_g of polymer. However, melting temperature (T_m) and crystallization temperature (T_c) was not observed in these DSC thermograms, it indicated that all of samples are amorphous. From the DSC results, it demonstrated that all of these polyurethane foams displayed the elastomeric behavior at room temperature.

Table 4.2 Glass transition temperature of polyurethanes with various ratios and different type of polyisocyanate

Sample	T_g (ºC)	Onset temperature (ºC)	Terminal temperature (ºC)	Region of glass transition
PU-BDI-0	-56.04	-59.17	-41.50	17.67
PU-BDI-10	-57.92	-61.24	-43.00	18.24
PU-BDI-30	-59.01	-60.49	-47.12	13.37
PU-BDI-50	-62.82	-59.98	-51.62	8.36
PU -LTI-0	-54.20	-61.16	-27.85	33.31
PU-LTI-10	-54.57	-61.05	-26.87	34.18
PU-LTI-30	-56.48	-60.80	-24.62	36.18
PU-LTI-50	-58.08	-59.14	-10.00	49.14

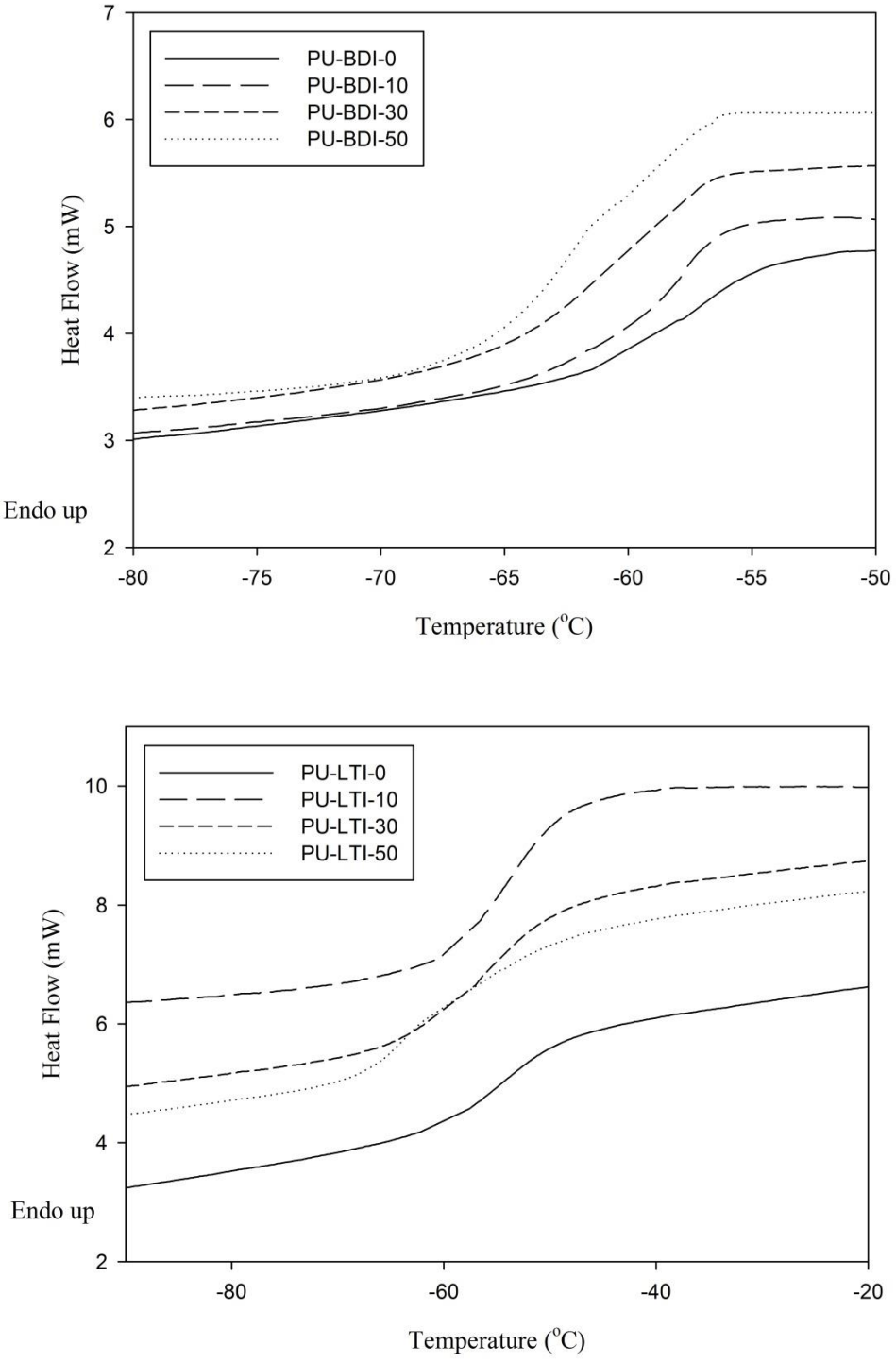


Figure 4.4 DSC thermograms of polyurethane (a) BDI-based PU and (b) LTI-based PU

4.2.3 Morphology of polyurethanes

Figure 4.5 shows SEM images of polyurethane foams. Polyurethane with various polyols molar ratios and types of polyisocyanate represent the different characteristic morphology of foam. Polyurethane synthesized from BDI with pure PCL and 10% of PPG showed the average pore size of 272 and 282 μm respectively. It is not significantly different. However, when PPG content were added higher than 10%, foam morphology was changed, and the average pore size of foam increase. At 30% and 50% of PPG content average pore size was increased to 2,236 and 1183 μm respectively. The increase of pore size occurred due to the low reactivity of PPG. The reaction of PPG with polyisocyanate to form urethane linkage was slower than PCL. The urethane reaction was not complete while the blowing reaction continuously occurred resulting in the agglomeration of bubble and internal surface collapse. The cell wall was obviously broken at 50% of PPG content resulting in the disordered and decrease in pore size. Therefore, the foam synthesized by using high content of PPG is denser leading to higher rigid foam. In the case of polyurethane synthesized from LTI, the average pore size decreased with increasing of PPG. The average pore size of these polyurethane foams with various ratios of 0, 10, 30 and 50% of PPG was 225, 227, 586 and 587 μm respectively. It can be observed that the average pore size increased with the high content of PPG. However, the average pore size of 0% to 10% of PPG, and 30% to 50% PPG are not significantly different, but the partially appearance of open cell structure can be found. These open cell have the effect on mechanical properties of polyurethane foam. Foam morphology of LTI based-polyurethane has smaller pore size and better orientation of the cell in comparison with BDI-based polyurethane because LTI has three functional groups that can form more urethane linkages leading to the stronger reactivity of chains. This different foam structure affects on the density and mechanical properties of polyurethane foam.

Table 4.3 Physical properties of polyurethane with various ratios and different type of polyisocyanate

Sample	Average pore size (μm)	Density (kg/m^3)
PU-BDI-0	272 ± 49	488 ± 50
PU-BDI-10	282 ± 40	463 ± 32
PU-BDI-30	2,236 ± 411	528 ±67
PU-BDI-50	1,184 ± 560	667 ±38
PU-LTI-0	225 ± 33	698 ± 36
PU-LTI-10	227 ± 38	623 ± 54
PU-LTI-30	586 ± 112	445 ± 28
PU-LTI-50	587 ± 119	372 ± 28

These foam structure provided oxygen permeability, bacterial barriers and fluid management, while minimizing surrounding skin maceration and irritation. The foam density played the important role as the parameter that affected on polyurethane degradation. Low density of foam means less amount of bulk polyurethane per volume and high surface area. So, polyurethane foam with high content of PPG might be degraded higher than that with low content of PPG because of high water adsorption and surface area.

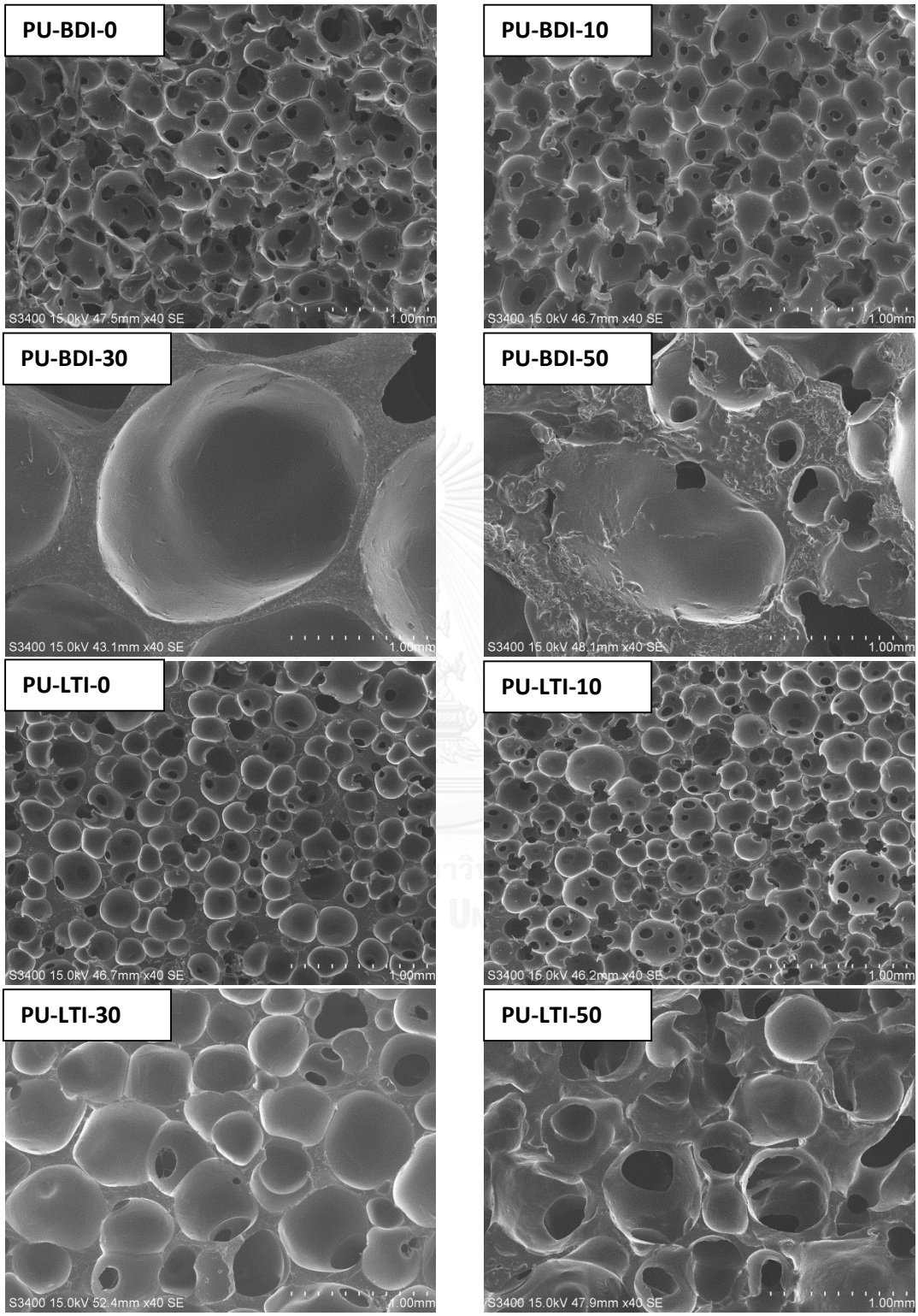


Figure 4.5 SEM images of polyurethane foams

4.2.4 Water absorption of polyurethane foam

The water absorption test was conducted according to ASTM D1037 to evaluate the effect of hydrophilicity from PPG content. Samples for the water absorption test were cut into 1×1×1 cm^3 and then dried at 70 ^oC in oven for 24 hr to remove moisture. The samples were immersed in distilled water for 24 hr and then weighed the wet weight. After immersion, samples were dried at 70 ^oC for 24 hr and then stored in sealed packed containing a desiccant. The final weight was obtained by weighing the dry weight of samples. Water absorption was calculated using the different weight between wet and dry sample. Table 4.4 shows ability of water absorption in polyurethane foam with various PPG content. After immersion for 24 hr the water absorption of BDI-based polyurethane and LTI-based polyurethane were 23-39% and 17-44% respectively. The results showed that the water content in polyurethane foam increased with the increasing of PPG which demonstrated the effect of PPG hydrophilicity on water adsorption. Furthermore, the water adsorption also depends on pore size of foam. The large pore size contributed to the high mass transfer diffusion and high density of foam resulting to the high surface area. From this result, it can be observed that the effect of high PPG content and large pore size was the important effect for high water adsorption.

Table 4.4 Water absorption of polyurethanes

Sample	PPG content (%)	Water absorption (%)
PU-BDI-0	0	23
PU-BDI-10	10	34
PU-BDI-30	30	37
PU-BDI-50	50	39
PU-LTI-0	0	17
PU-LTI-10	10	18
PU-LTI-30	30	41
PU-LTI-50	50	44

4.2.5 Mechanical properties of polyurethane foam – Compression test

To determine the effect of polyols molar ratios and types of polyisocyanate, samples were cut to 3×3×3 cm^3 and tested using compression mode with 1,000 N loading and compressed to 20% deformation of sample with according to ASTM-D1621.

Theoretically, the effect of more urethane linkage between polymer chains provides higher mechanical properties due to more interaction of urethane bonding. Furthermore, the branching of PPG affecting on morphology and density of polyurethane foam also has the significant effect on mechanical properties.

From **Figure 4.7** the stress-strain curves of polyurethane foam synthesized by BDI showed the compressive stress from 26.04 to 83.04 kPa and compressive strength range from 21.46 to 77.92 kPa. Compressive strength of polyurethane foam decreased with the decreasing of foam density which is related to PPG content. However, in case of PU-BDI higher amounts of PPG (>10%) provided polyurethane foam with higher density because of slow reaction of urethane linkage formation resulting in the very dense foam and large pore size. The mechanical properties of polyurethane synthesized fom LTI was shown in **Figure 4.7**. The compressive stress was in range of 21.42 to 68.00 kPa and compressive strength varied from 25.54 to 72.21 kPa. The compressive strength decreased with PPG increasing because high branching of polymeric chain increased the free volume in its structure leading to the low density of polyurethane foam.

The mechanical properties of BDI-based polyurethane were compared with LTI- based polyurethane to study the influence of polyisocyanate. At 0% and 10% of PPG molar ratios, the compressive stress and compressive strength of LTI-based polyurethane were higher than that of BDI-based polyurethane. On the other hand, the PPG content of 30% and 50% BDI-based polyurethane had higher compressive stress and compressive strength than that of LTI-based polyurethane. The increasing of mechanical properties of PU-BDI-30 and PU-BDI-50 due to the high dense foam observed by SEM. The highest compressive stress and compressive strength of BDI and LTI-based polyurethanes was found at 50% and 0% molar ratios of PPG respectively indicating that types of polyisocyanate had the effect on

mechanical properties of polyurethane foam with various ratios of polyols. Furthermore, PPG had the specific molar ratio that suitable for each type of polyisocyanate.

Table 4.5 Compression properties of polyurethanes with various ratios and different type of polyisocyanate

Sample	Compressive stress at 20% strain (kPa)	Compressive strength at 20% strain (kPa)
PU-BDI-0	45.18 ± 8.73	26.53 ± 2.89
PU-BDI-10	26.04 ± 3.01	21.46 ± 2.85
PU-BDI-30	70.79 ± 6.59	58.04 ± 5.92
PU-BDI-50	83.04 ± 5.97	77.92 ± 10.98
PU-LTI-0	68.00 ± 4.13	72.21 ± 6.04
PU-LTI-10	63.04 ± 2.07	62.91 ± 4.71
PU-LTI-30	49.49 ± 3.01	50.93 ± 3.30
PU-LTI-50	21.42 ± 3.18	25.54 ± 5.27

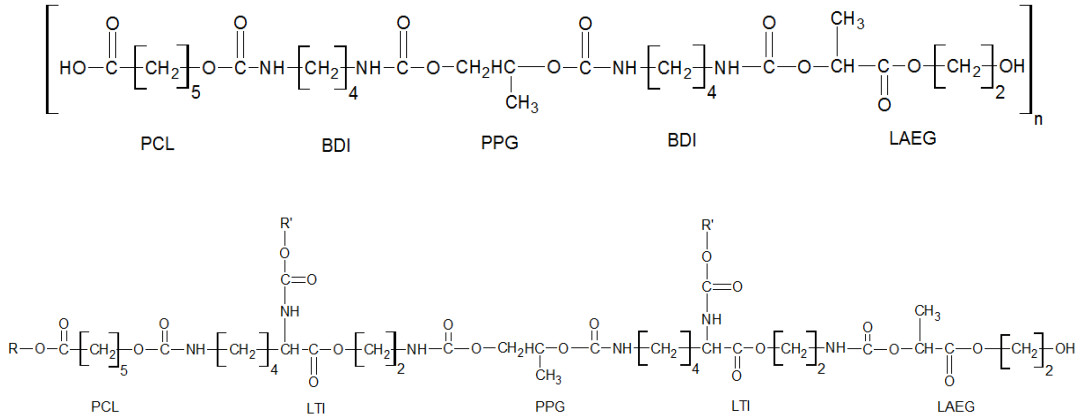


Figure 4.6 Polyurethane structure of PU-BDI and PU-LTI

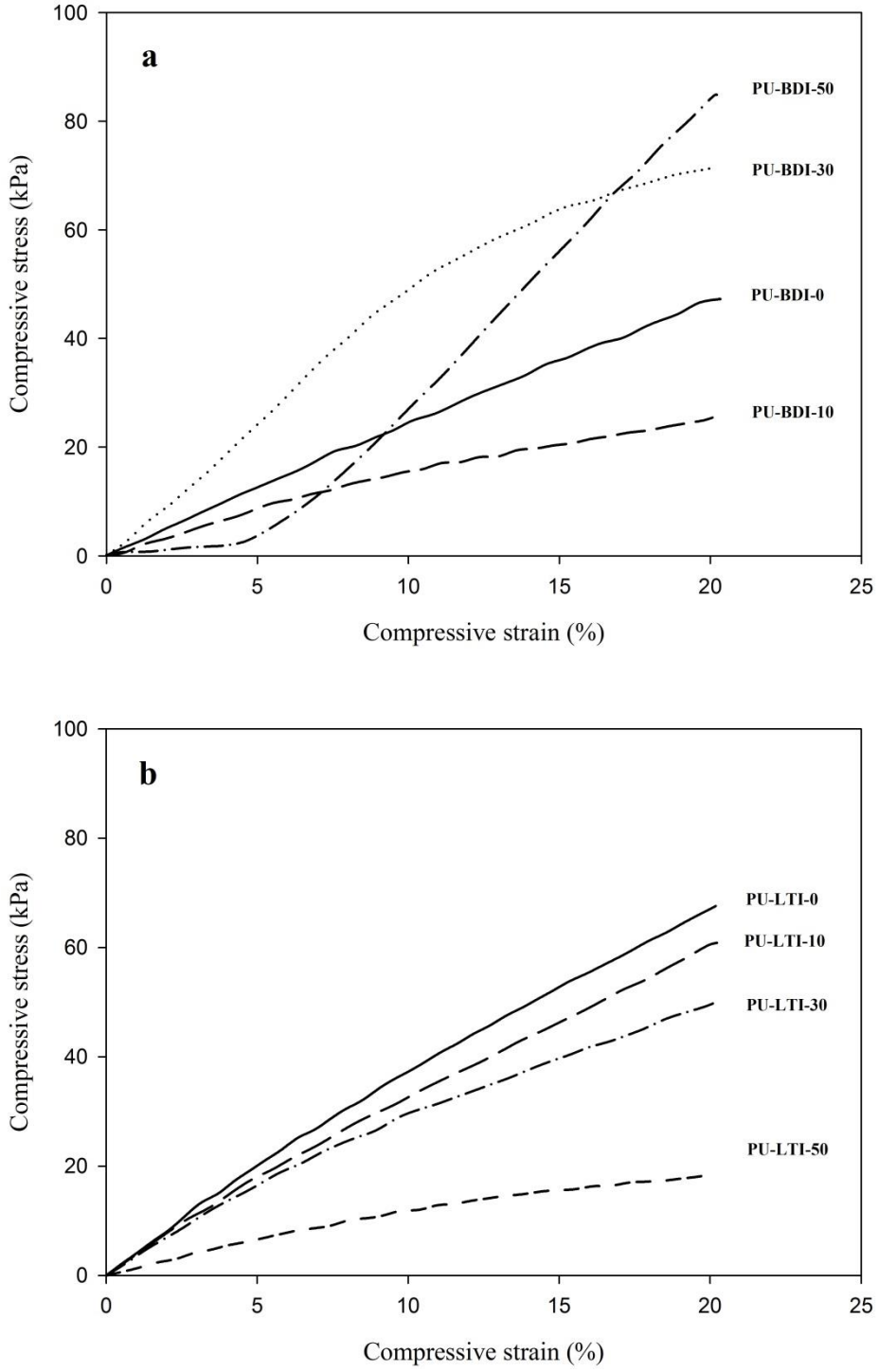


Figure 4.7 Stress – strain curves of polyurethane with various ratios and types of polyisocyanate

4.2.6 Hydrolytic degradation of polyurethane

The hydrolytic degradation of all polyurethane were performed in phosphate buffer saline (PBS) solution at pH 7.4 and 60^{o}C to study the influence of PPG content and type of polyisocyanate on the degradation time.

From **Figure 4.9**, it can be observed that the mass loss of polyurethane increase with the increasing of PPG because it is hydrophilic material resulting in the higher water absorption of polyurethane. The ester groups in polyurethane linkage were broken by hydrolytic degradation of water in buffer solution and all of samples had the slightly mass loss during one week (~5%). Polyurethanes were degraded along four weeks resulting in the increasing of mass loss with the longer degradation time. The significant difference mass loss of polyurethanes from week 1 to week 4 was analyzed by using one-way ANOVA test ($P<0.05$). The polyurethane synthesized from BDI can be degraded 14-38% and from LTI can be degraded 28-38%. The degradation rate of polyurethane was related to the high content of hydrophilicity of PPG as described above. From statistical analysis, mass change of polyurethane during 4 weeks is significant difference. Moreover, polyurethanes synthesized from LTI had the higher mass loss than that of synthesized from BDI significantly ($n = 4$, $P = 0.129$). This is due to the higher isocyanate group of LTI can form urethane bond containing ester group in its structure, which led to the high degradation rate of sample. In case of BDI-based polyurethane, the different mass loss between low hydrophilicity (PU-BDI-0 and PU-BDI-10) and high hydrophilicity (PU-BDI-30 and PU-BDI-50) was high in comparison with foam synthesized from LTI because they had very large pore size and disordered cell wall as shown in **Figure 4.5**. This parameter made polyurethane foam had the higher mass loss. It is demonstrated that hydrophilicity is an important factor that affects on the degradation rate of the sample and functionality of polyisocyanate also has the effect on the degradability of polyurethane [7].

The degradation products of polyurethane are alcohol (ROH), amine (RNH_2) and carbon dioxide (CO_2) that are not toxic for human body and can be removed from the body by circulated fluid such as blood and urination.

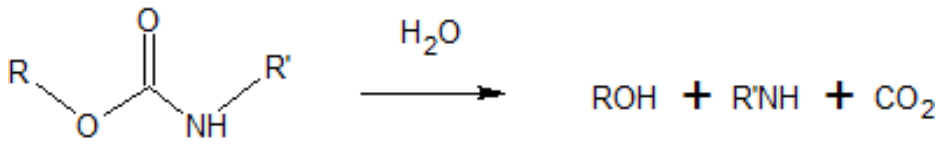


Figure 4.8 Hydrolytic degradation of polyurethane

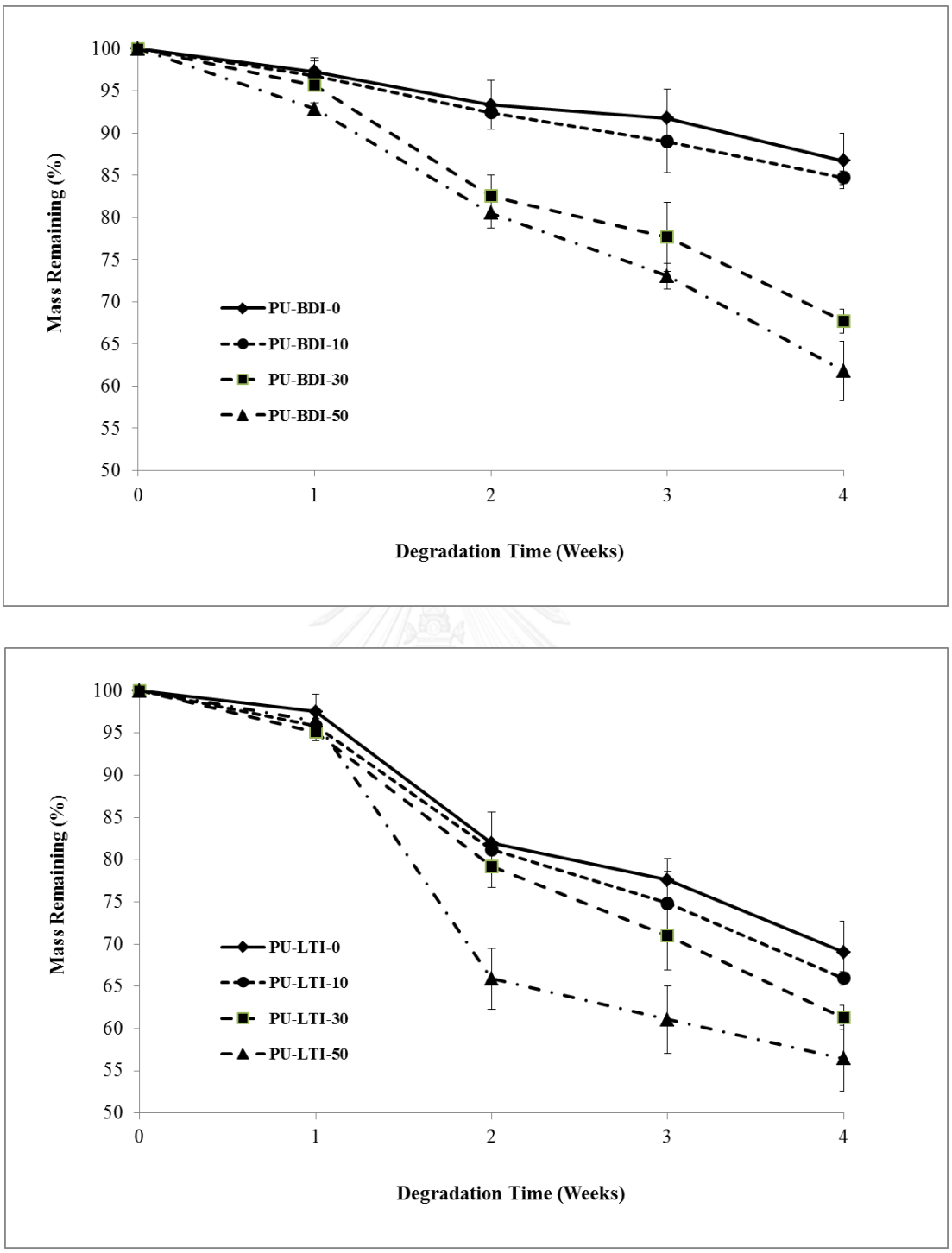


Figure 4.9 Percent mass loss of polyurethane during hydrolytic degradation at 60 ºC for 4 weeks.

Table 4.6 Percent mass loss of polyurethane during hydrolytic degradation for 4 weeks in PBS solution at 60 °C

Sample	Mass Remaining (%)			
	Week 1	Week 2	Week 3	Week 4
PU-BDI-0	97.29 ± 1.61	93.34 ± 2.87	91.74 ± 3.48	86.70 ± 3.31
PU-BDI-10	96.77 ± 1.74	92.44 ± 0.18	88.98 ± 3.72	84.71 ± 0.81
PU-BDI-30	95.66 ± 0.75	82.54 ± 2.51	77.66 ± 4.08	67.72 ± 1.43
PU-BDI-50	92.82 ± 0.78	80.51 ± 1.80	73.03 ± 1.54	61.81 ± 3.51
PU-LTI-0	96.22 ± 2.05	92.87 ± 3.73	84.88 ± 2.56	72.34 ± 3.70
PU-LTI-10	97.51 ± 2.46	81.94 ± 5.01	77.58 ± 4.23	69.04 ± 3.33
PU-LTI-30	95.83 ± 1.63	81.20 ± 2.58	74.85 ± 3.44	65.93 ± 4.41
PU-LTI-50	95.10 ± 0.36	79.22 ± 3.62	70.98 ± 3.97	61.30 ± 3.89

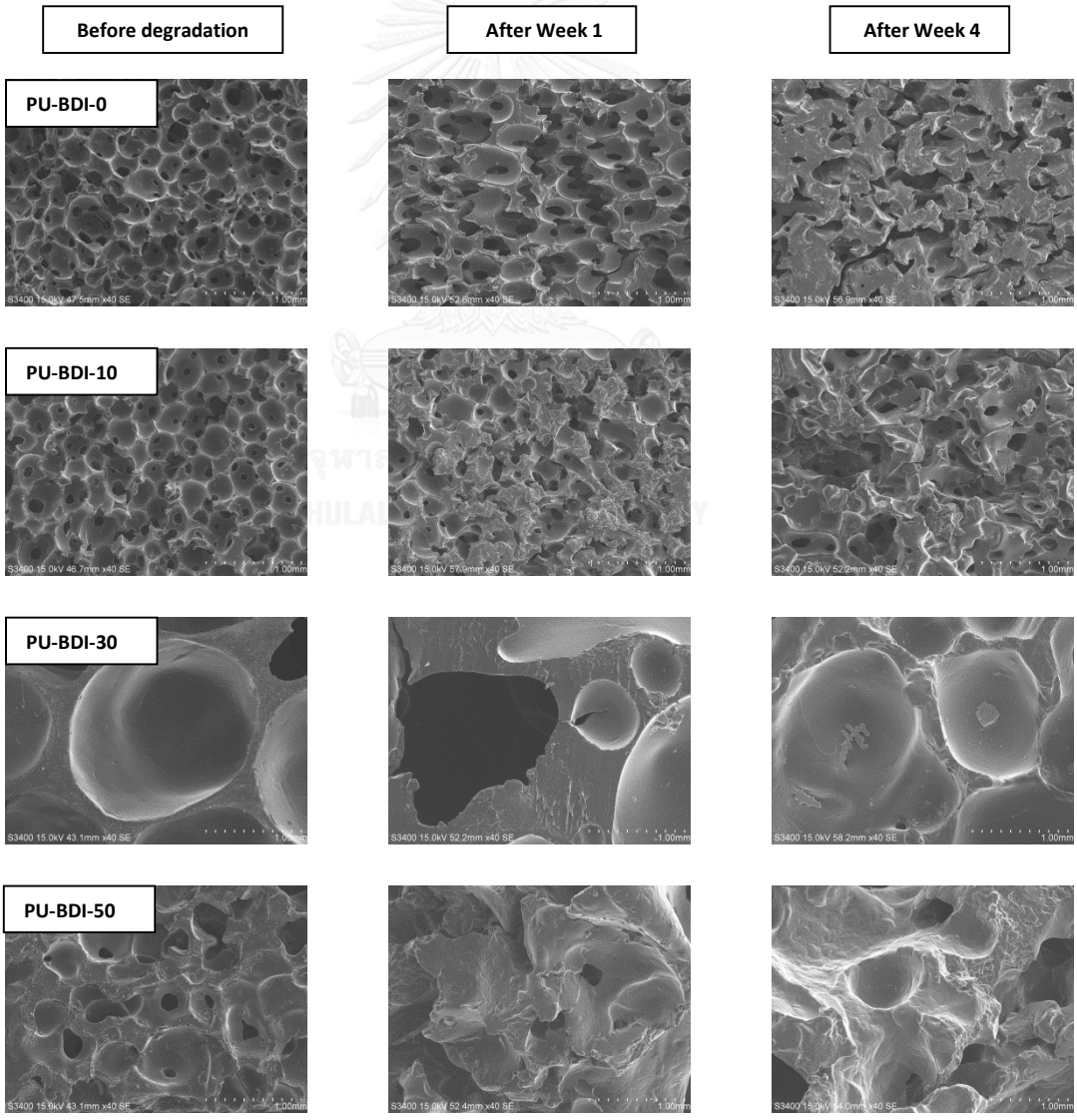


Figure 4.10 SEM images of PU-BDI after 1 and 4 weeks hydrolytic degradation

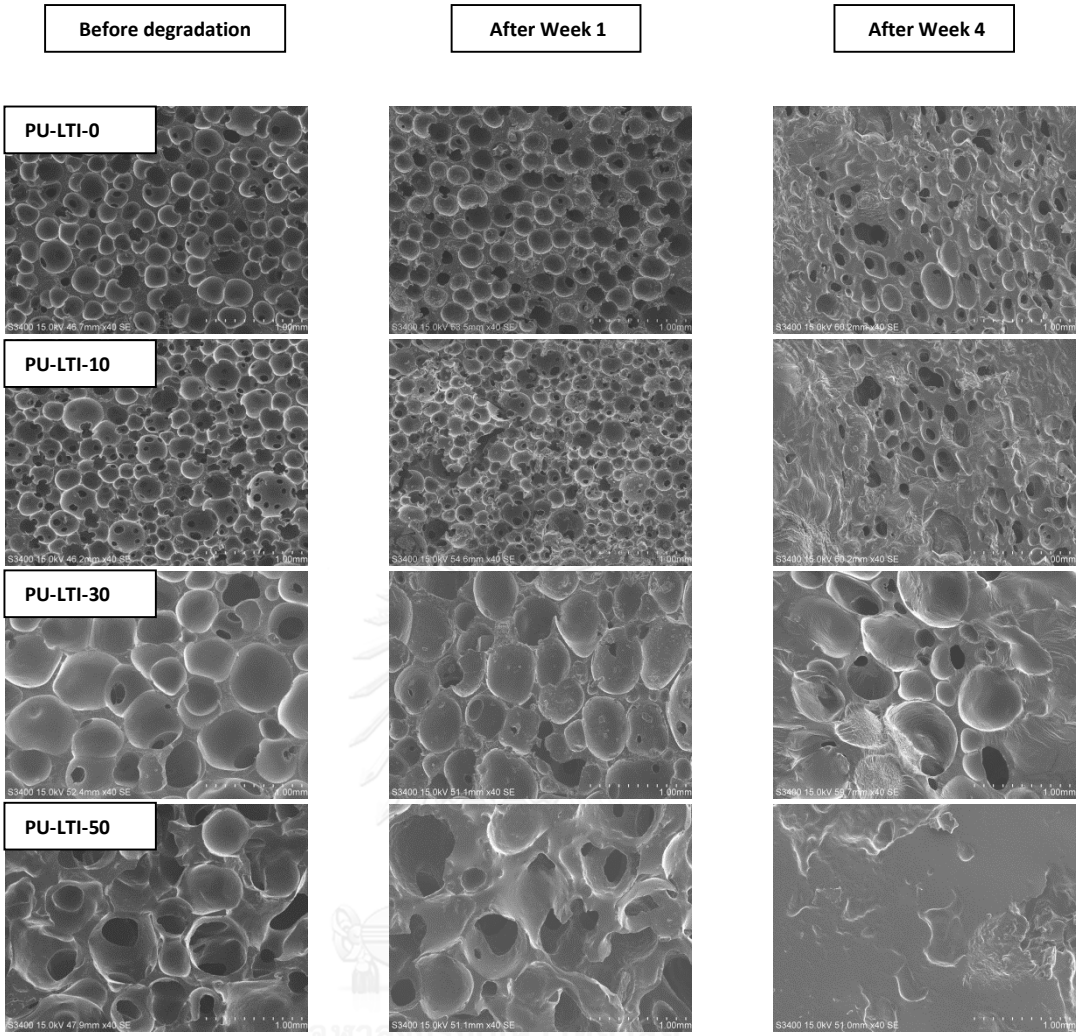


Figure 4.11 SEM images of PU-LTI after 1 and 4 weeks hydrolytic degradation

From SEM images of **Figure 4.10** and **Figure 4.11**, the degradation of polyurethane occurred at mass was slightly changed. After week 4, fine pores were disappeared and collapsed wall was observed due to the hydrolysis of ester bond, and this might contribute to the decreasing of T_m leading to melting of polymer. SEM images were used to confirm the bulk degradation of polyurethane foam. The pore and cross-sectional area of polyurethane was changed when PPG content in polymer was increased. This observation was related to mass loss during hydrolytic degradation as shown in **Figure 4.9** and **Table 4.6**.

4.2.6 Enzymatic degradation of polyurethane

The enzymatic degradation of all polyurethane were performed on phosphate buffer saline (PBS) solution with lipase enzyme from porcine pancreas at pH 7.4 and 37^{o}C to study the influence of PPG content and type of polyisocyanate with simulated degradation condition. Lipase is usually used for perform enzymatic degradation test due to lipase play the important role as the substance that can catalyze the hydrolysis of ester bond.

From **Figure 4.12** and **Table 4.7**, the slightly mass loss was occurred after 14 days of degradation but the significant effect of PPG content and types of polyisocyanate were not observed (n = 8, P = 0.001). The mass loss of polyurethane synthesized from BDI was in the range of 2 to 8 % while polyurethane synthesized from LTI was about 3 to 6 %. It can be seen that PU-LTI provided slightly higher the percent mass loss than that of PU- BDI because LTI had more functionalities than BDI that can form urethane linkage containing ester group and it also had ester group in its original structure. However, from the statistical analysis it can found that the mass loss between PU-BDI and PU-LTI was not significant difference (n = 4, P = 0.381). The effect of PPG content was not shown obviously. So, the effect of PPG content and types of polyisocyanate must be further studied for the longer degradation time.

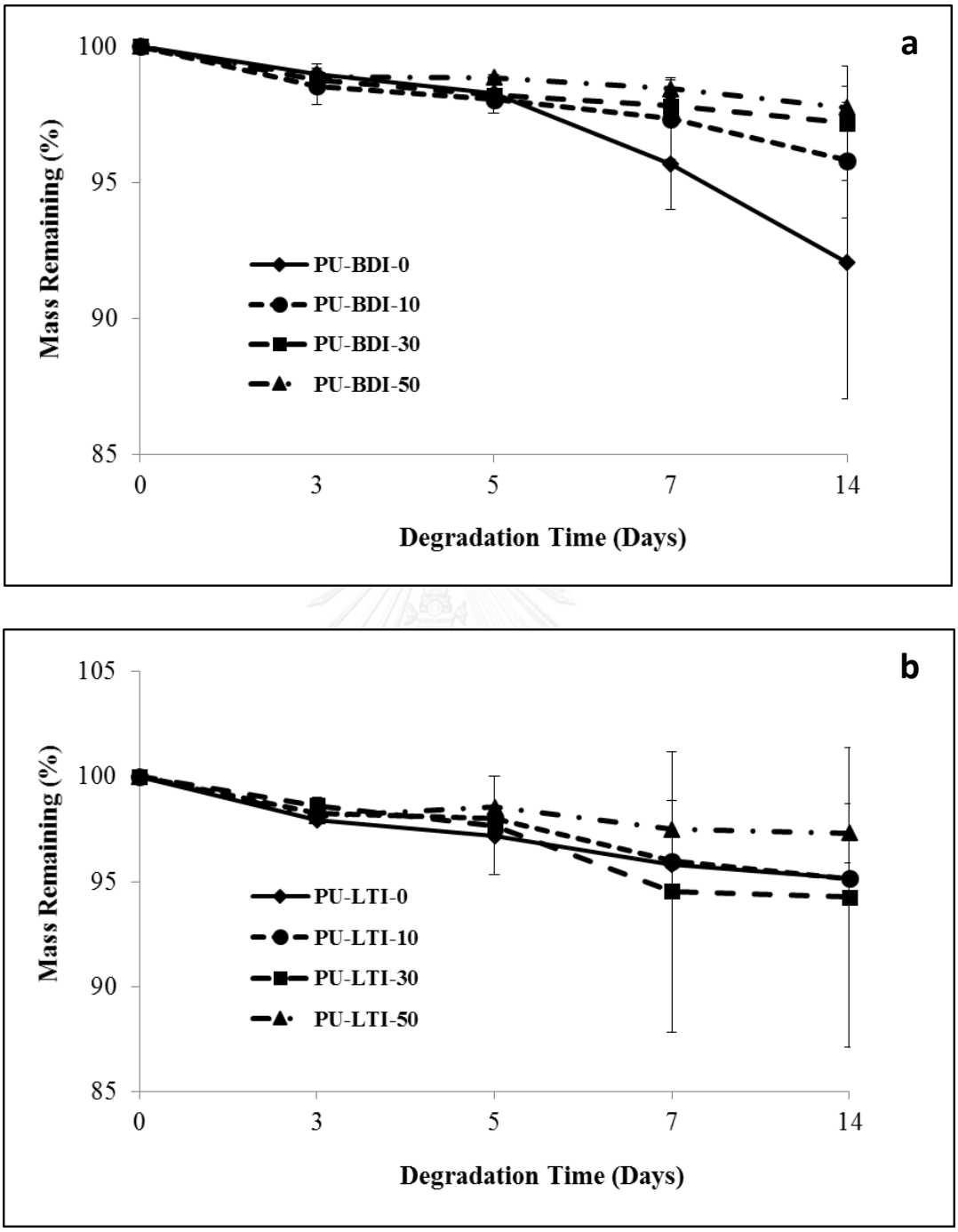


Figure 4.12 Percent mass loss of a) PU-BDI and b) PU-LTI during enzymatic degradation in PBS with lipase at 37 ºC for 14 days

Table 4.7 Percent mass loss of polyurethane during enzymatic degradation for 14 days in PBS solution with lipase from porcine pancreas at 37 °C

Sample	Mass Remaining (%)			
	Day 3	Day 5	Day 7	Day 14
PU-BDI-0	98.97 ± 0.04	98.26 ± 0.33	95.69 ± 1.68	92.04 ± 4.99
PU-BDI-10	98.53 ± 0.67	98.04 ± 0.51	97.34 ± 1.50	95.81 ± 2.11
PU-BDI-30	98.76 ± 0.28	98.21 ± 0.15	97.80 ± 0.25	97.17 ± 2.11
PU-BDI-50	98.87 ± 0.47	98.85 ± 0.11	98.45 ± 0.33	97.75 ± 0.79
PU-LTI-0	99.06 ± 0.21	99.09 ± 0.50	95.82 ± 1.78	95.16 ± 1.10
PU-LTI-10	97.92 ± 0.06	97.15 ± 0.55	95.96 ± 4.62	95.13 ± 4.93
PU-LTI-30	98.26 ± 0.41	97.66 ± 0.05	94.50 ± 6.69	94.25 ± 7.13
PU-LTI-50	98.61 ± 0.06	98.35 ± 0.10	97.50 ± 1.34	97.30 ± 1.42

Figure 4.13 and **4.14** showed SEM images of polyurethane foam during enzymatic degradation in PBS solution with lipase for 14 days. Due to the short time of degradation, the change in wall and pore of foam was not observed clearly. However, it can be observed that pore wall was partially collapsed and small pores occurred on the foam surface. The pore and cell wall of foam after 14 days enzymatic degradation were finer than that performed on hydrolytic degradation test in the similar period. This is because it did not have the thermal effect from high temperature condition as the hydrolytic degradation. This result contributed to the longer degradation time required for study the effect of lipase during enzymatic degradation test.

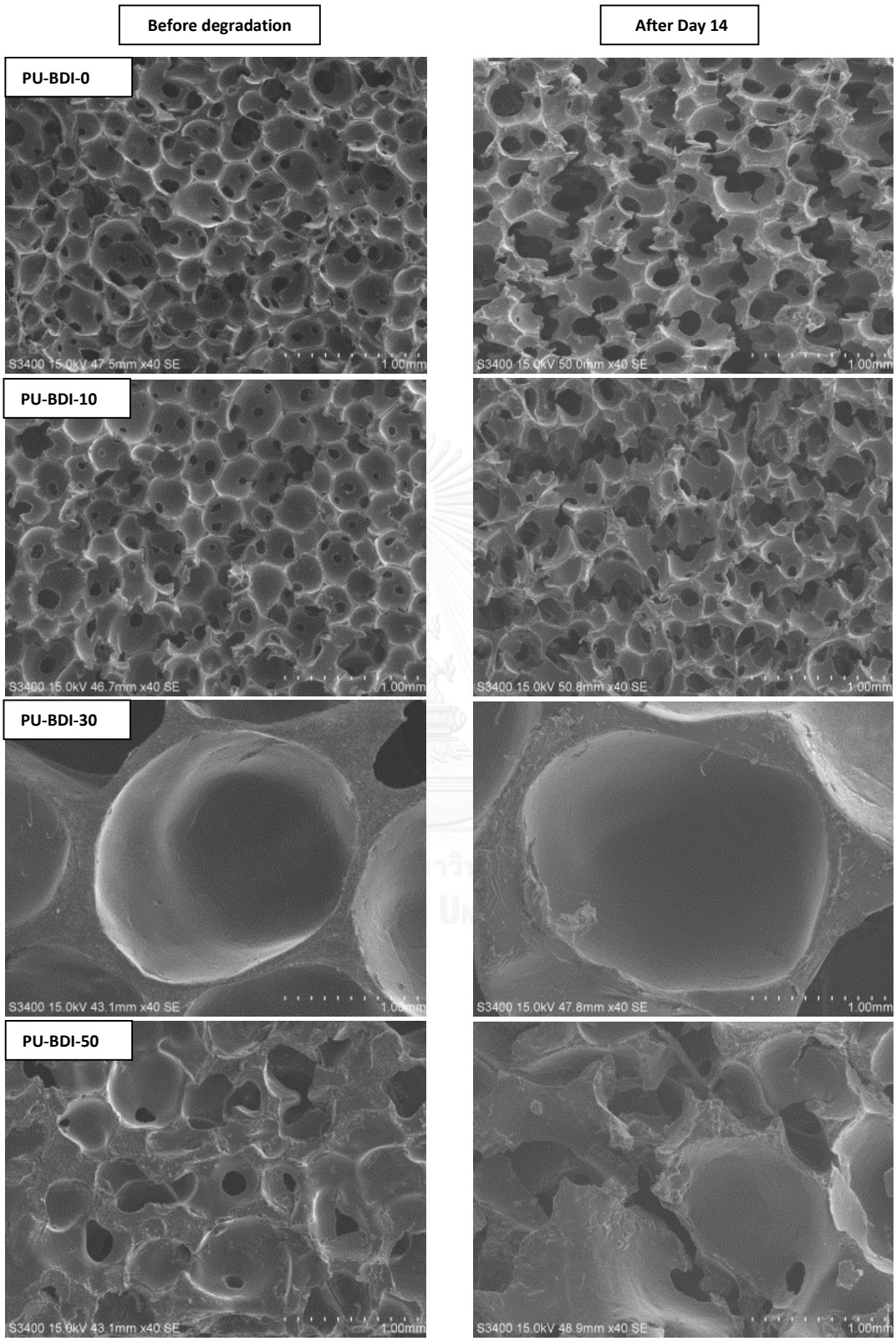


Figure 4.13 SEM images of PU-BDI after 14 days degradation in PBS with lipase

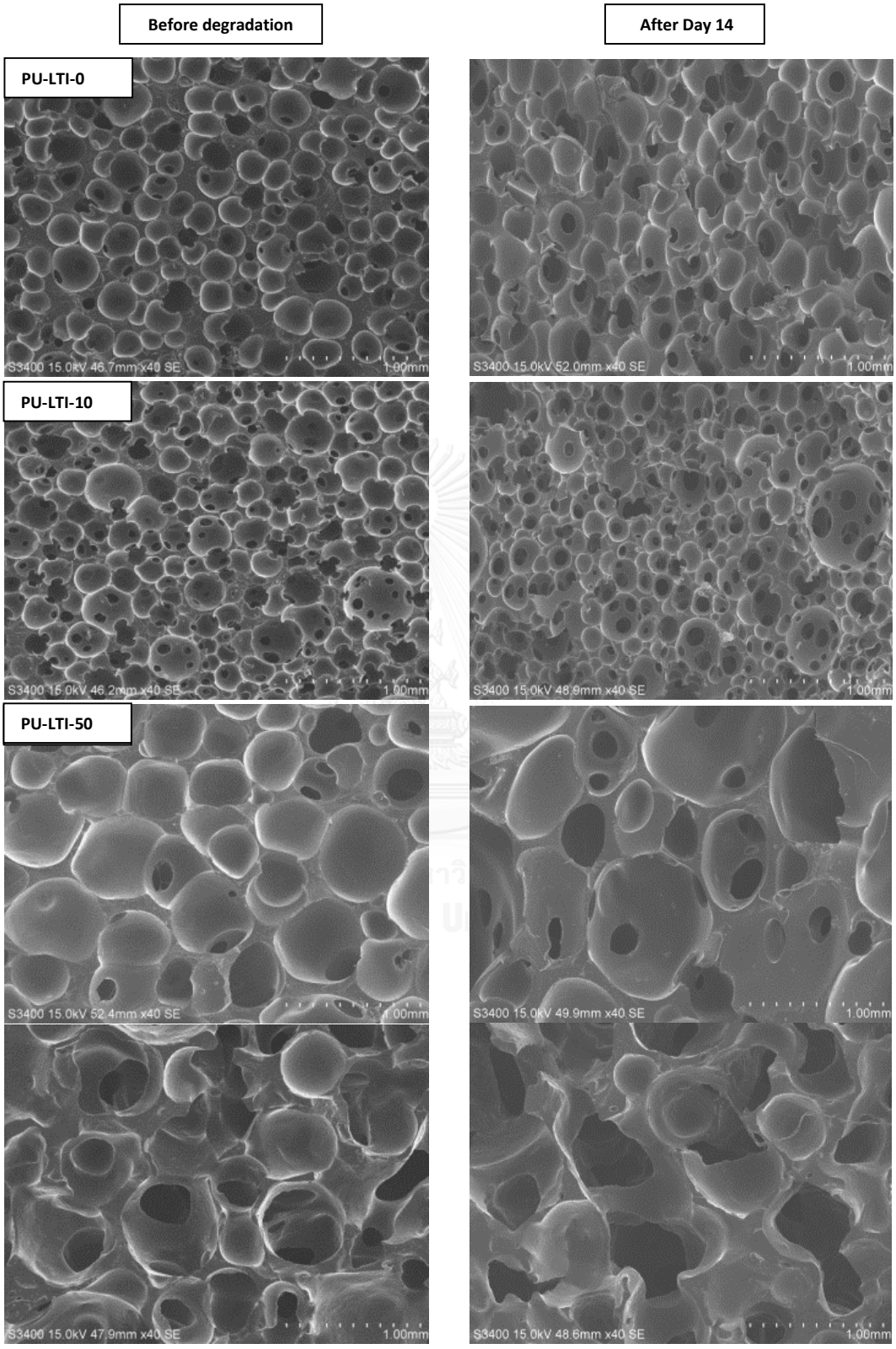


Figure 4.14 SEM images of PU-LTI after 14 days degradation in PBS with lipase

4.2.7 Cytotoxicity

Cytotoxicity of polyurethane foam was shown in term of percent cell viability that calculated from the number of cells seeding in various polyurethane degradation products. Calibration curve of MTT absorbance to number of cells was performed by measuring the absorbance (at 570 nm) of well-plate containing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) with various amount of L929 cell in the range of 6,250 to 200,000 cells. MTT was metabolically reduced in viable cells to a blue-violet insoluble formazan. The number of viable cells correlated to the color intensity determined by UV-vis spectrophotometer after dissolve the formazan in DMSO. The calibration curve was shown in **Figure 4.15**, higher in intensity mean more amount of viable cells.

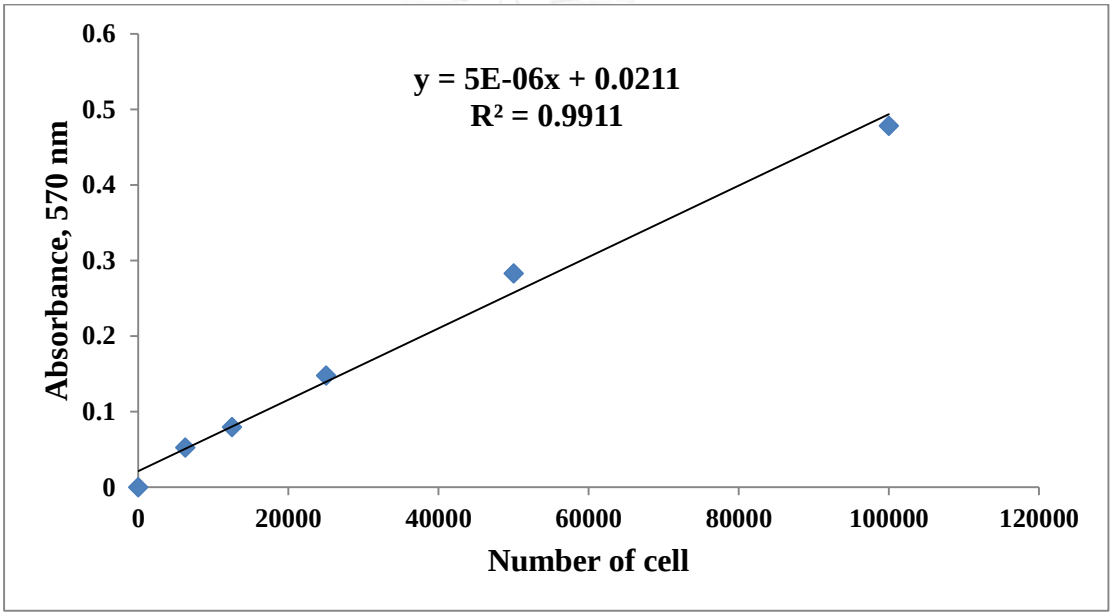


Figure 4.15 Calibration curve of MTT absorbance to number of cells

Cytotoxicity test of polyurethane foam was performed to evaluate the possibility for biomedical applications. Polyurethanes were immersed in sterile tube containing PBS solution (2 g of polymer: 1 ml of medium) and incubated at 70 ^{o}C for 24 hr. After the incubation, various concentration of extracted product was placed into well-plate containing L929 cell (20,000 cells). After seeding cell for 24 hr, the cytotoxicity of degradation product was investigated.

From **Figure 4.16** and **4.17**, the percent of cell viability and number of cell were shown. For control sample, cell viability in 20 ppm of zinc acetate (positive control) and DMEM (negative control) is 20.97% and 100% respectively. Zinc acetate cause the toxicity to cell resulting to the dead cell but DMEM is biocompatible to cell leading to the live cells. All polyurethane degradation products had higher cell viability than positive control demonstrated that this polyurethane can possibly degrade to nontoxic product. According ISO 10993,DMEM was used as reference to compare the cell viability between negative control and various degradation products. Cell viability in degraded PU-BDI is in the range of 70.92 to 92.99% and in degraded PU-LTI is 88.93-114.23% at concentration of 1×10^{-4} g/ml. A 78.5-96.62% and 89.99-115.27% is the range of cell viability seeding in PU-BDI and PU-LTI at concentration of 1×10^{-5} g/ml respectively.

The number of L929 cell was statistically analyzed using one-way ANOVA test ($P<0.05$) in MINITAB version 14.0 program for comparing the significant difference in number of cells. For concentration of 1×10^{-4} g/ml, the number of cell seeding in all extracted samples was investigated. It has the significant difference between all extracted polyurethane and positive control. The result also showed that the number of cell in extracted solution from PU-BDI was less than in extracted solution from PU-LTI ($n=4$, $P = 0.01$). It was demonstrated that LTI provided the polyurethane with less toxicity than BDI. For concentration of 1×10^{-5} g/ml, the result was similar to data from concentration of 1×10^{-4}g/ml ($n = 4$, $P = 0.01$).The extracted PU-LTI showed higher number of cell than extracted PU-BDI. However, according to the ISO 10993-5 sample is not toxic where its cell viability is higher than 70% in comparison with positive control. Furthermore, it was found that cell viability of extracted samples in concentration of 1×10^{-4} and 1×10^{-5} g/ml were not significant difference ($n = 8$, $P = 0.585$). It was demonstrated that less concentration than 1×10^{-4} g/ml did not have the effect on cell viability and cytotoxicity of polyurethanes.

In case of the dead cell was occurred, the toxicity from free isocyanate group was not appear because isocyanate completely reacted with urethane bond and water to form the other chemical structure, allophanate and carbon dioxide. This is consistent with the results of FTIR spectra in **Figure 4.3** which showed the disappearance of NCO group at 2250 cm^{-1}. Furthermore, the reactants using for synthesized polyurethane were carefully chosen. All reactants are biocompatible and have a very mild inflammatory with tissue except for catalyst. In this research, dibutyltin dilaurate was used as the catalyst to form polyurethane foam due to it can catalyze higher gelation reaction rate than tertiary amine catalyst. Therefore, this is one of the importance factors that cause the death of L929 cell. However, many researches reported that using less amount of dibutyltin dilaurate did not have the significant effect on brain neurotransmitter system and percent molarity of control rats [34-37]. In addition, the degradation products of degradable chain extender are ethylene glycol and lactic acid, this lactic acid might cause the change in pH that has the effect on cell viability. It can be observed that cell seeding in medium with extracted samples from LTI-based polyurethane have more cell growth than positive control. This is because LTI based-polyurethane can be degraded to lysine causing the growth of cell after 24 hr. Therefore, the cell viability was higher than 100% in comparison with positive control. Furthermore, it can be observed that cell viability in polyurethane degradation product which had concentration of 1×10^{-4} and 1×10^{-5} g/ml was not significant difference. The initial concentration (1×10^{-4}g/ml) using for cytotoxicity test was calculated from shape and size of polyurethane foam that applied to oroantral communication. If polyurethane foam can be instantaneously degraded in human body, the concentration of polyurethane degradation product is about 1×10^{-4}. Higher concentration of degradation product means higher toxicity from degradation product. So, if the higher concentration of degradation product (1×10^{-4} g/ml) was not had toxicity, the lower concentration (1×10^{-5}) must not had toxicity as well. It can be seen that biodegradable polyurethane foam can be applied to oroantral communication and can be used without toxicity from degradation product.

Table 4.8 Cytotoxicity results of extracted PU

Sample	Concentration (g/ml)					
	1×10^{-4}			1×10^{-5}		
	Cell viability (%)	Number of cells ($\times10^{-4}$)	Cyto toxicity	Cell viability (%)	Number of cells ($\times10^{-4}$)	Cyto toxicity
PU-BDI-0	71	3.58 ± 0.47	-	79	4.02 ± 0.71	-
PU-BDI-10	93	4.84 ± 0.44	-	88	4.54 ± 1.27	-
PU-BDI-30	73	3.72 ± 0.59	-	97	5.04 ± 1.98	-
PU-BDI-50	86	4.41 ± 0.60	-	81	4.13 ± 0.21	-
PU-LTI-0	114	6.04 ± 1.11	-	106	5.58 ± 0.34	-
PU-LTI-10	89	4.61 ± 0.86	-	100	5.24 ± 0.62	-
PU-LTI-30	103	5.41 ± 0.67	-	90	4.67 ± 0.49	-
PU-LTI-50	93	4.86 ± 0.14	-	115	6.09 ± 0.81	-
Zinc acetate	21	0.76 ± 0.39	✓	21	0.76 ± 0.39	✓
DMEM	100	5.23 ± 1.37	-	100	5.23 ± 1.37	-

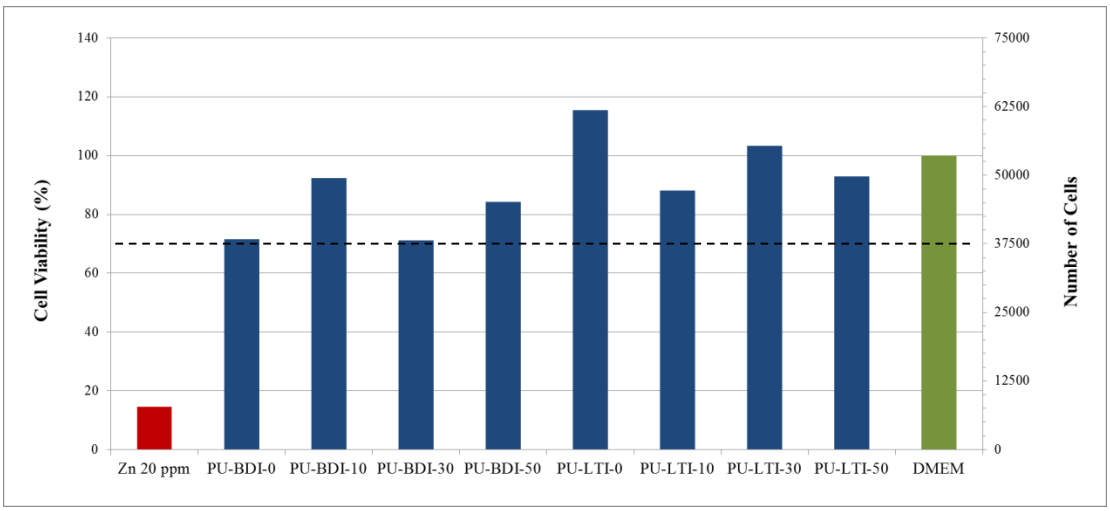


Figure 4.16 Cell viability and number of cells from MTT assay with various types of extracted PU (Concentration = 1×10^{-4} g/ml)

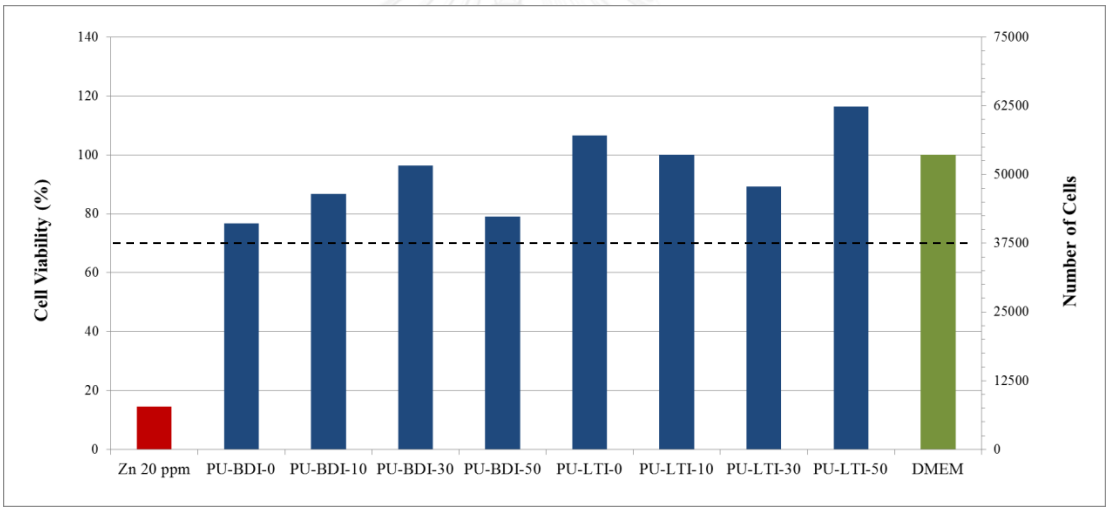


Figure 4.17 Cell viability and number of cells from MTT assay with various types of extracted PU (Concentration = 1×10^{-5} g/ml)

Figure 4.18 is the microscopic images of L929 cells seeding in positive and negative control. Cell image in DMEM showed the growth of cell while the dead cell occurred in zinc acetate like a spherical shape.

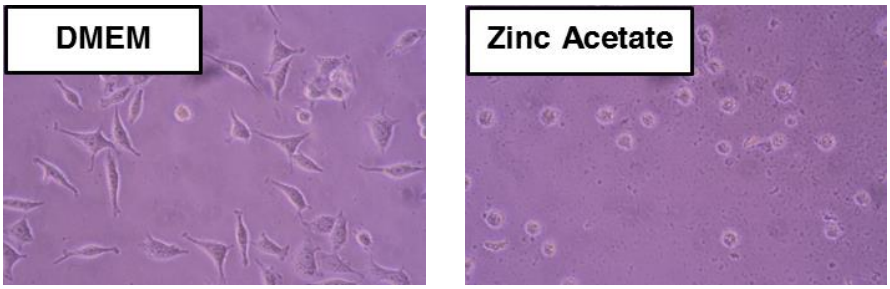


Figure 4.18 Images of L929 cells in positive (DMEM) and negative (zinc acetate) control

From **Figure 4.19** and **Figure 4.20**, the cell images in extracted polyurethane with various types and concentrations were shown. It can be observed that cells were live in all extracted samples though the dead cell was partially occurred. These results can confirm the non-cytotoxicity of degradation product from polyurethane foams.

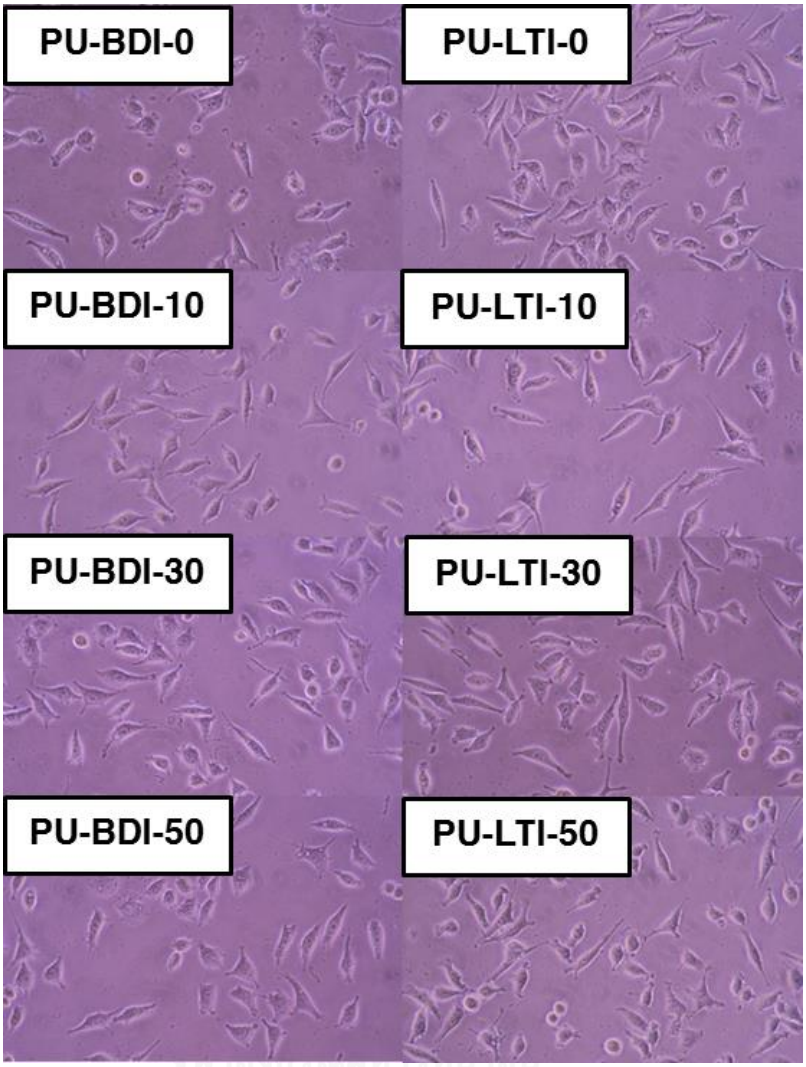


Figure 4.19 Images of L929 cells in extracted polyurethane at 1×10^{-4} g/ml

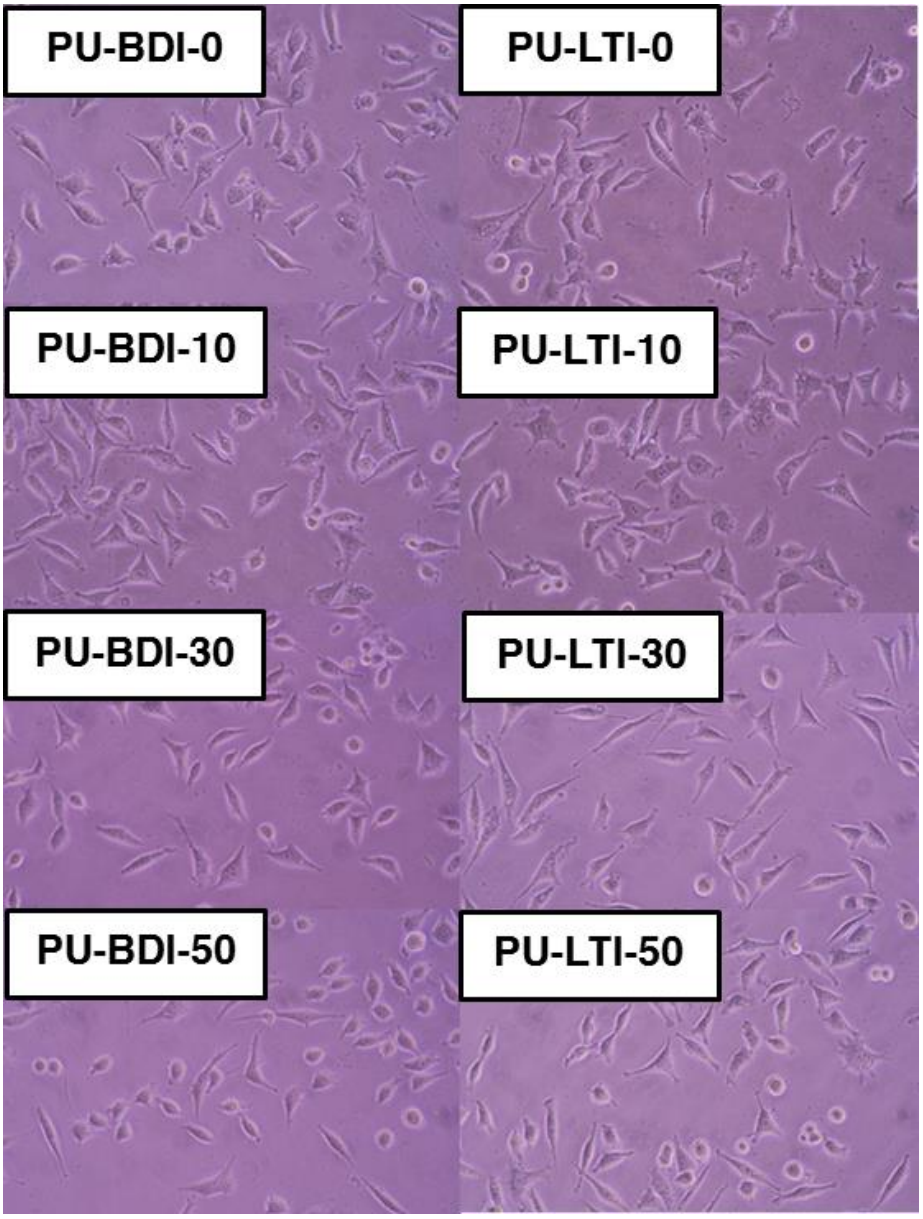


Figure 4.20 Images of L929 cells in extracted polyurethane at 1×10^{-5} g/ml

CHAPTER V
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this research, the synthesis of biodegradable medical PU foam using in the medical device application was performed. Polyurethane structure, mechanical properties, thermal properties and degradability were characterized using ^{1}H NMR, FTIR, DSC, SEM, universal testing machine and the cytotoxicity was investigated by using MTT assay with L929 cell.

We have successfully synthesized biodegradable PU foam from biocompatible material and enhance the degradability by using the degradable chain extender synthesized from DL-lactic acid and ethylene glycol. The structure of chain extender was confirmed by ^{1}H NMR and FTIR. Furthermore, FTIR was also used to confirm functional group of PU and especially the disappearance of free isocyanate.

PU was synthesized with the various ratios of polyols and types of polyisocyanate. From DSC thermograms, T_g of PU was slightly decreased with the increasing of PPG. PU-LTI showed the higher T_g than PU-BDI. The density of PU decreased with the addition of PPG which directly correlated with the compressive strength of PU foam except for PU-BDI-30 and PU-BDI-50. The water adsorption of PU increased with the higher content of PPG and played the important role as the parameter that effect on the degradability of PU foam during hydrolytic degradation. In addition, PU-LTI was more degraded than PU-BDI. However, the effect of PPG content and types of polyisocyanate was not observed during enzymatic degradation. Finally, the MTT assay data exhibited the less cytotoxicity of PU when performed on L929 cell in extraction test.

5.2 Recommendations

1. Study the effect of additive that can improve morphology and mechanical properties of polyurethane without toxicity from degradation product.

2. Use other catalyst instead of DBTL to avoid the toxicity from this reactant.

3. Use polyol with low molecular weight to improve degradability. However, the effect of low molecular weight polyol on PU processing and mechanical properties must be further studied.

4. Study the degradation of PU during enzymatic degradation for the longer time.

5. Investigate the molecular weight of these polyurethanes before and after degradation test to study the degradation mechanism.

6. Study the FTIR spectra after degradation test to confirm the degradation product

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APPENDICES

APPENDIX A
POLYOLS AND POLYISOCYANATE RATIO CALCULATION

The amount of PCL and PPG at various ratios, 1.5 NCO/OH molar ratios and the addition of chain extender was calculated using following equations.

$$n = \frac{m}{M_w} \quad \textbf{(A1)}$$

Where

n	=	Number of moles (mole)
m	=	Mass (g)
M_w	=	Molar mass (g/mole)

Equation A1 was used to convert mole to mass of reactant because the ratio of PCL and PPG, polyol and polyisocyanate was used in term of molar ratio

$$\rho = \frac{m}{V} \quad \textbf{(A2)}$$

Where

ρ	=	Density (g/cm^3)
m	=	Mass (g)
V	=	Volume (cm^3)

For example, in case of PU-BDI-30 synthesis the molar ratio between PCL and PPG is 7:3, molar mass of them is 2,000 g/mole. The amount of used polyols was calculated by following equation.

Based on 0.005 mole of polyol (PCL = 0.0035 mole, PPG = 0.0015 mole)

$$n_{PCL} = \frac{m_{PCL}}{M_{w_{PCL}}}$$

$$m_{PCL} = n_{PCL} \times M_{w_{PCL}}$$

$$m_{PCL} = 0.0035 \times 2{,}000$$

$$m_{PCL} = 7 \quad g$$

$$n_{PPG} = \frac{m_{PPG}}{M_{w_{PPG}}}$$

$$m_{PPG} = n_{PPPG} \times M_{w_{PPG}}$$

$$m_{PPG} = 0.0015 \times 2{,}000$$

$$m_{PPG} = 3 \quad g$$

So, PCL and PPG using for synthesize PU-BDI-30 are 7 and 3 g respectively. Polyisocyanate that used in this case is BDI (M_w = 140.14 g/mole) at 1.5 NCO/OH molar ratio.

LAEG chain extender (M_w = 134.15 g/mole) is one of the polyol and was used at 0.25 mole fraction for all conditions (effect of the amount of added chain extender was not studied in this research).

$$m_{LAEG} = 0.125(0.0035 + 0.0015) \times 134.15$$

$$m_{LAEG} = 0.1677 \quad g$$

Chain extender in form of liquid phase was used in term of volume, mass was converted to volume using Equation A2.

$$\rho = \frac{m}{V}$$

$$V = \frac{m}{\rho}$$

$$V = \frac{0.1677}{1.161}$$

$$V = 0.1444 \quad ml$$

Polyisocyanate, BDI, was used at 1.5 NCO/OH molar ratios

$$n_{BDI} = 1.5\ (0.0035 + 0.0015 + 0.00125)$$

$$n_{BDI} = 0.009375 \quad mole$$

Mole of BDI was converted to mass.

$$m_{BDI} = n_{BDI} \times M_{w_{BDI}}$$

$$m_{BDI} = 0.009375 \times 140.14$$

$$m_{BDI} = 1.3138 \quad g$$

Then, mass of BDI was converted to volume.

$$V_{BDI} = \frac{m_{BDI}}{\rho_{BDI}}$$

$$V = \frac{1.3138}{1.105}$$

$$V = 1.1889 \quad ml$$

The chemical data of all reactants including density and molar mass was shown as following table.

Table A.1 Chemical data base of starting materials

Chemical	Abbreviation	Molar mass (g/mole)	Density (g/cm^3)
Polycaprolactone diol	PCL	2,000	1.071
Polypropylene glycol	PPG	2,000	1.005
DL-lactic acid	LA	90.08	1.209
Ethylene glycol	EG	62.07	1.113
Synthesized chain extender	LAEG	134	1.161
Butane diisocyanate	BDI	140.14	1.105
Lysine triisocyanate	LTI	267.24	1.210
Dibutyltin dilaurate	DBTL	631.56	1.066
Water	H_2O	18	1.000

APPENDIX B
COMPRESSIVE STRENGTH CALCULATION

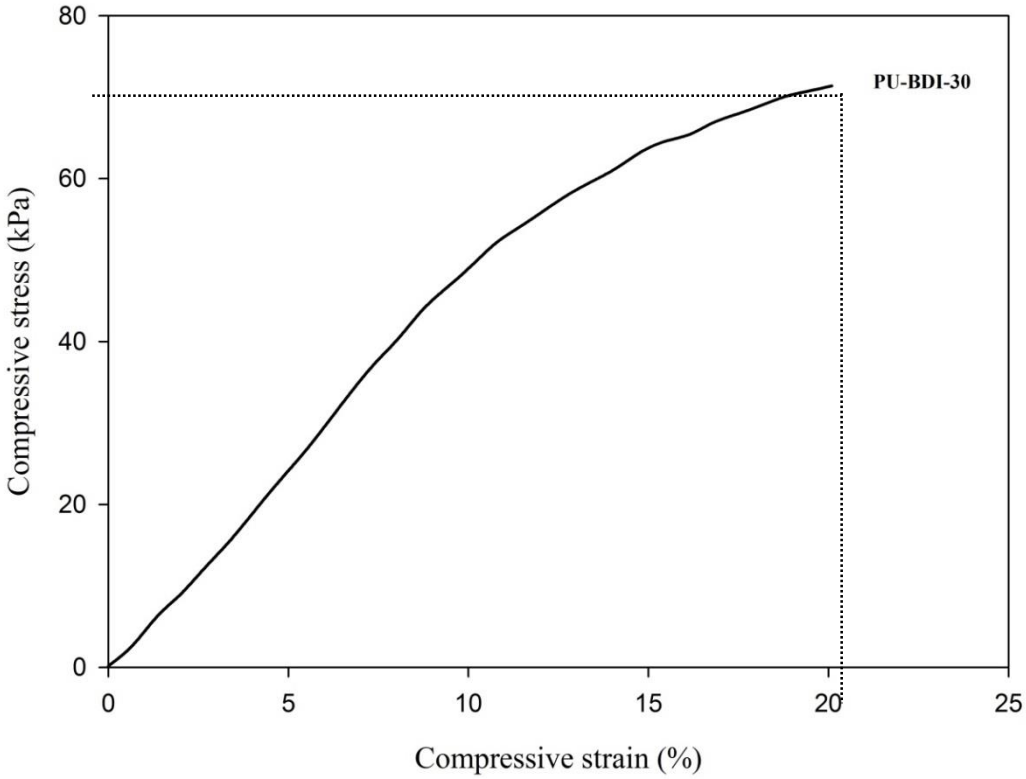

Figure B.1 Stress-strain behavior

For example, compressive stress-strain curve of PU-BDI-30 was shown and the following table is the raw data of compression test of this sample.

Sample with dimension of 3×3×3 cm^3 was compressed to 20% deformation, 5 kN compressive load with a cross head speed 10 mm/min.

At 20% compressive strain, the obtained compressive stress is 71.3924 kPa. The compressive strength was calculated using following equation.

$$\text{Compressive srength} = \frac{\text{Maximum compressive load}}{\text{Minimum cross-sectional area}} \quad \textbf{(B2)}$$

$$\text{Compressive srength} = \frac{54.1130\ \text{N}}{9\ \text{cm}^2}$$

$$= 6.0126\ \text{N/cm}^3$$

$$= 60.126\ \text{kPa}$$

Table B.1 Compressive stress-strain data

Time (sec)	Compressive stress (kPa)	Compressive strain (%)	Compressive load (N)
0	0.1677	0.0000	0.1273
1	2.6831	0.6725	2.0364
2	6.1997	1.3445	4.7056
3	9.0086	2.0163	6.8375
4	12.2508	2.6881	9.2983
5	15.4559	3.3605	11.7311
6	19.0423	4.0328	14.4531
7	22.6387	4.7043	17.1828
8	26.0572	5.3764	19.7774
9	29.8007	6.0484	22.6187
10	33.6780	6.7208	25.5616
11	37.2724	7.3929	28.2898
12	40.4285	8.0642	30.6852
13	43.9385	8.7365	33.3494
14	46.6537	9.4091	35.4102
15	49.2928	10.0811	37.4132
16	52.0524	10.7527	39.5078
17	54.0742	11.4250	41.0423
18	56.0683	12.0970	42.5559
19	58.0326	12.7690	44.0467
20	59.6739	13.4412	45.2925
21	61.3009	14.1128	46.5274
22	63.2479	14.7848	48.0052
23	64.5723	15.4573	49.0104
24	65.4087	16.1291	49.6452
25	66.8764	16.8011	50.7592
26	67.9402	17.4734	51.5667
27	69.0143	18.1454	52.3818
28	70.1046	18.8176	53.2094
29	70.7896	19.4898	53.7293
29.904	71.3924	20.0971	54.1869

APPENDIX C
MTT ASSAY CALCULATION

Firstly, the standard curve L929 cell must be performed, the correlation of number of cell and optical density of solution was expressed in term of equation

$$y = 5 \times 10^{-6}x + C$$

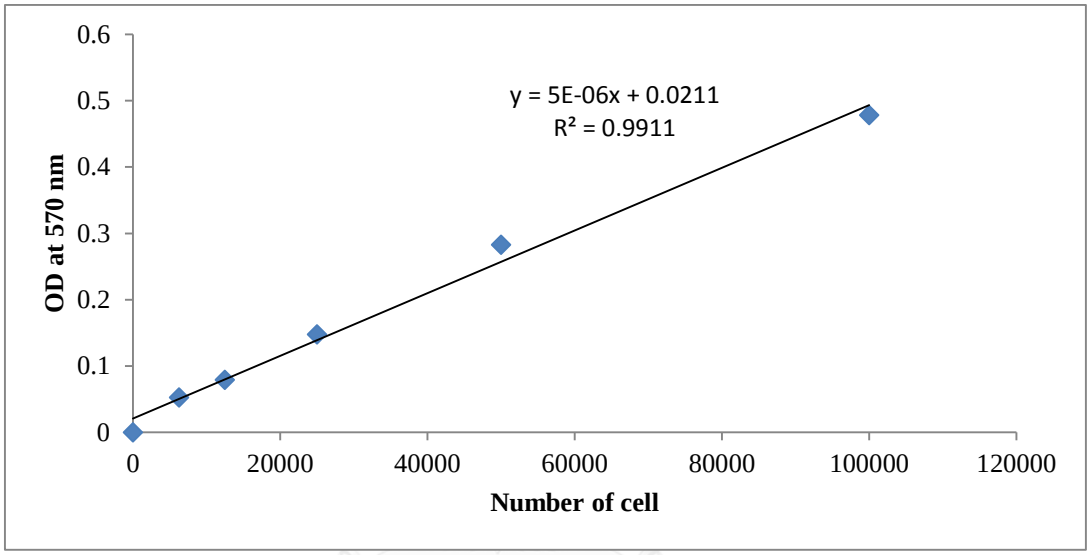

Figure C.1 Standard curve of L929 cell

$$\text{Number of cell} = \frac{\text{Optical density} - 0.0211}{5 \times 10^{-6}} \qquad \textbf{(C1)}$$

For example, the number of cell seeding in extracted solution of PU-BDI-10 at concentration of 1×10^{-4} was calculated using equation C1.

$$\text{Number of cell} = \frac{92.9945 - 0.0211}{5 \times 10^{-6}}$$

$$\text{Number of cell} = 48{,}380 \quad \text{cells}$$

APPENDIX D
ONE-WAY ANOVA ANALYZATION

ASSUMPTIONS

$H_0 = \mu_1 = \mu_2 = \mu_3 = \mu_i$ P = 0.05

$H_a = \mu_i = \mu_j$, difference

CASE: 1. P > 0.05 Accepted ⇨ 1.

2. P < 0.05 Denied ⇨ 2.

Table D.1 The significant (P) value of ANOVA analysis

Concentration (g/l)	Sample	P value
1×10^{-4}	PUs with positive and negative control	0
	PU-BDI series	0.038
	PU-LTI series	0.026
	PU-BDI and PU-LTI	0.001
1×10^{-5}	PUs with positive and negative control	0
	PU-BDI series	0.649
	PU-LTI series	0.033
	PU-BDI and PU-LTI	0.010

At concentration of 1×10^{-4} g/l for all sample (control and PUs)

One-way ANOVA: PU-BDI-0, PU-BDI-10, PU-BDI-30, PU-BDI-50, PU-LTI-0, ...

Source	DF	SS	MS	F	P
Factor	9	7662442500	851382500	14.45	0.000
Error	30	1767607500	58920250		
Total	39	9430050000			

At concentration of 1×10^{-4} g/l for PU-BDI series

One-way ANOVA: PU-BDI-0, PU-BDI-10, PU-BDI-30, PU-BDI-50

Source	DF	SS	MS	F	P
Factor	3	415577969	138525990	3.87	0.038
Error	12	429711875	35809323		
Total	15	845289844			

At concentration of 1×10^{-4} g/l for PU-LTI series

One-way ANOVA: PU-LTI-0, PU-LTI-10, PU-LTI-30, PU-LTI-50

Source	DF	SS	MS	F	P
Factor	3	604860000	201620000	4.40	0.026
Error	12	550460000	45871667		
Total	15	1155320000			

At concentration of 1×10^{-4} g/l for all PUs

One-way ANOVA: PU-BDI, PU-LTI

Source	DF	SS	MS	F	P
Factor	1	948301250	948301250	13.82	0.001
Error	30	2059098438	68636615		
Total	31	3007399688			

At concentration of 1×10^{-5} g/l for all sample (control and PUs)

One-way ANOVA: PU-BDI-0, PU-BDI-10, PU-BDI-30, PU-BDI-50, PU-LTI-0, ...

Source	DF	SS	MS	F	P
Factor	7	1431615000	204516429	2.18	0.074
Error	24	2255300000	93970833		
Total	31	3686915000			

At concentration of 1×10^{-5} g/l for PU-BDI series

One-way ANOVA: PU-BDI-0, PU-BDI-10, PU-BDI-30, PU-BDI-50

Source	DF	SS	MS	F	P
Factor	3	258140000	86046667	0.56	0.649
Error	12	1831900000	152658333		
Total	15	2090040000			

At concentration of 1×10^{-5} g/l for PU-LTI series

One-way ANOVA: PU-BDI, PU-LTI

Source	DF	SS	MS	F	P
Factor	1	741125000	741125000	7.55	0.010
Error	30	2945790000	98193000		
Total	31	3686915000			

At concentration of 1×10^{-5} g/l for all PUs

One-way ANOVA: PU-BDI, PU-LTI

Source	DF	SS	MS	F	P
Factor	1	741125000	741125000	7.55	0.010
Error	30	2945790000	98193000		
Total	31	3686915000			

APPENDIX E
CELL VIABILITY CALCULATION

Percent cell viability of L929 cell seeding in polyurethane degradation product was calculated by using positive control (DMEM) as reference. The percent cell viability was calculated from the ratio of number of cells in DMEM to number of cells in polyurethane degradation product

For instance, in case of PU-BDI-10 the cell viability of this degradation product was mentioned below.

$$\text{Number of cell in DMEM} = 52{,}343 \quad \text{cells}$$

$$\text{Number of cell in PU} - \text{BDI} - 10 = 48{,}380 \quad \text{cells}$$

$$\text{Cell viability of PU} - \text{BDI} - 10 = \frac{48{,}380}{52{,}343}$$

$$\text{Cell viability of PU} - \text{BDI} - 10 = 93 \quad \%$$

APPENDIX F

CONCENTRATION OF DEGRADATION PRODUCT CALCULATION

The concentration of polyurethane degradation product using for evaluate cytotoxicity test was calculated by using following information.

The polyurethane foam was used for oroantral communication in truncated cone shape as shown in **Figure F.1**.

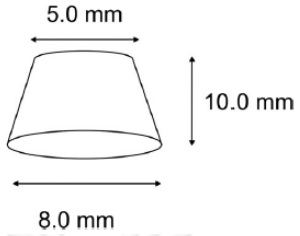

Figure F.1 Shape and size of polyurethane foam for oroantral communication

So, the mass of polyurethane (PU-BDI-10) using in this application is

$$m = \rho \times V$$

$$m = 0.4635 \times \left(\frac{1}{3} \times \pi \times 1\right)(0.25^2 + 0.4^2 + (0.25 \times 0.4))$$

$$m = 0.4635 \times 0.65155$$

$$m = 0.302 \quad g$$

The average volume of fluid in human body is approximately 4.5 liters. So, the mass of polyurethane can be converted to concentration.

$$\text{concentration} = m/V$$

$$\text{oncentration} = 6.71 \times 10^{-5} \quad g/ml$$

Concentrations of all polyurethane are in the range of 1×10^{-5} to 1×10^{-4}. So, the initial concentration that was used for cytotoxicity test is 1×10^{-4}.

VITA

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