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The Effect of the PLA Degradation Chemical on cell Proliferation

A Thesis Presented

by

Kuan-Che Feng

to

The Graduate School

in Partial Fulfillment of the

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Abstract of the Thesis

The Effect of the PLA Degradation Chemical on cell Proliferation

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Kuan-Che Feng

Master of Science

in

Materials Science and Engineering

Stony Brook University

2014

PLA is a material easy to manufacture. The biodegradability makes it a perfect material for tissue engineering.

Several conditions for biodegradability experiments for spin-coating Polylactic acid thin films were tried. Polylactic acid thin films were immersed in different solution for different times. Thickness, morphology and mechanical properties were analyzed after the Polylactic acid thin films immersing test.

Dermal fibroblasts were plated on the Polylactic acid thin films, culturing with conditioning medium. Thickness, morphology, mechanical properties and cell count were analyzed after the Polylactic acid thin films cell culture test.

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List of Abbreviations

Full term	Abbreviation/ Symbol
Atomic force microscope	AFM
Carbon dioxide	CO_2
4', 6-diamidino-2-phenylindole	DAPI
Dulbecco's Modified Eagle Medium	DMEM
Ethylenediaminetetraacetic Acid	EDTA
Fetal Bovine Serum	FBS
Hydrogen Peroxide	H_2O_2
Sulfuric Acid	H_2SO_4
Hydrofluoric acid	HF
Ammonium Hydroxide	NH_4OH
Phosphate buffered saline	PBS
Poly-carprolactone	PCL
Polydioxanone	PDS
Poly (ethyele glycol)	PEG
Poly (glycolic acid)	PGA
Poly (hydroxyalkanoates)	PHA
Poly (Lactide-co-Glycolide) Copolymers	PLGA

Polylactic acid	PLA
Penicillin-Streptomycin	PS
Ring-opening polymerization	ROP
Silicon dioxide	SiO_2
Scanning electron microscope	SEM
Glass transition temperature	T_g

Chapter 1 Introduction

1.1 A Brief Introduction of Biodegradable Plastic

Nowadays, approximately 140 million tons of petroleum-based plastic be produced every year. However, petroleum-based plastic cannot decompose by microorganism, they are not easy to break down in natural environment, so-called “white pollution”. [1] Scientists started to develop environment-friendly material, which can degrade in nature environment easily, cutting the dependence of human beings on fossil fuels. Figure 1 shows the major biodegradable plastics.

Although Biodegradable plastics have been invented for a long time. It has only been mass-produced in application areas such as food packaging, bag, and medical application for about ten years. However, nowadays many leading brands like Wal-Mart have started to use biodegradable plastic bags instead of petrochemical-based plastic bags. [2]

1.2 Mechanism of Biodegradation

Biodegradation is usually caused by biological activity. However, it sometimes needs to be triggered by non-biological reaction such as hydrolysis or photogradation. So biodegradable polymers are “bioerodable”, “hydrobiodegradable” or “photo-biodegradable”. [1]

In comparison with enzyme degradation, hydrolysis usually takes place at the cross-section instead of surface. Biological degradation usually cause by the enzymes or secretion of microorganisms. Microorganisms also eat or digest polymers causing mechanical aging.[2, 3]

Chapter 2 Materials

Lactic acid is hydroxycarboxylic acid, having a lot of application in food, chemical, and pharmaceutical industries. It is the monomer of polylactic acid, basic constitutional unit of polylactic acid.[4]

2.1 Lactic acids

Lactic acid is an organic acid that can be manufactured in two ways, chemical synthesis and carbohydrate fermentation.[5]

In the lactic acid production industry right now, approximately 90% of the lactic acid is produced by bacterial fermentation of carbohydrates and the remaining is synthetic by hydrolysis.[6, 7] Due to the resources is environmental friendly, using the renewable material instead of petrochemicals. And the bacterial fermentation production is highly specific. It can produce pure L-lactic acid or D-lactic acid. (See Figure 2.1) There is more advantages compare to chemical synthesis such as low cost of substrates, lower temperature requirement during production and low energy consumption.[4]

The fermentation process can be classified by different type of bacteria being used. The process needs carbon. Glucose, sucrose, lactose or sugar containing materials can provide the carbon. Sucrose-containing materials such as molasses are cheaper alternative raw materials for lactic acid production.

2.2 Synthesis of Polylactic acid

There are two main synthetic methods to produce Polylactic acid: direct polycondenstaion and ring-opening polymerization (ROP). (See Figure 2.2)

2.2.1 Direct polycondensation

The direct polymerization can happen by self-condensation because the lactic acid monomer has both –OH and –COOH groups. Solution and melt polycondensation are two methods to produce PLA. (See Figure 2.3)

Solution polycondensation can produce the PLA to achieve high molecular weight of over 200,000. Yet solution polycondensation has some disadvantages such as the impurities will affect the reaction cause the side reactions like racemization and trans-esterification. This reaction also creates a lot of organic solvents causing environmental pollution.

Compare to solution polycondensation, melt polycondensation can react without organic solvent when the reaction temperature is higher than the melting point of the lactic acid. (Gao et al. 2002). So it will not cause the pollution. However, the reaction is sensitive to several parameters such as temperature, time, catalysts and pressure. They are related to the molecular weight of the product, polylactic acid. Moreover, because the reaction temperature is over the melting point, it will cause the degradation of the polylactic acid, producing low molecular weight polylactic acid.[5]

2.2.2 Ring-opening polymerization

Using ring-opening polymerization can avoid the disadvantages of direct polymerization; so ring-opening polymerization is the main method to manufacture high molecular weight polylactic acid in industry. The only restriction is the reaction requires high purity of the monomer. By this way, it is easy to control the ratio of D-lactic acid and L-lactic acid in the final polylactic acid. [5](See Figure 2.4)

2.3 Advantage of Polylactic Acid

Polylactic acid (PLA) has a lot of advantages over petroleum-based plastics. First, PLA is eco-friendly. PLA is usually made from renewable resource such as corn and rice. So PLA is biodegradable, recyclable and compostable.[8-10]

Biocompatibility is another attractive advantage of PLA, especially to biomedical application. PLA will not produce toxic substance in tissues and PLA will not interfere with tissue healing. Furthermore, the degradation products of PLA are not toxic. That's the reason why PLA has been approved by US Food and drug administration (FDA) for human clinical applications. [8, 11-13]

Third, because of the low glass transition temperature (T_g) and low melting point, making PLA easy to process than other biopolymers such as poly (hydroxyalkanoates) (PHAs), poly (ethyele glycol)(PEG), etc. [14]

PLA manufacture is also energy saving. Producing PLA only needs half energy than manufacturing petroleum-based polymers. Scientists even predict that the energy demand will be reduced to less than 10% in the future. [15]

2.4 Polylactic acid in tissue engineering

The concept of the tissue engineering is providing a scaffold for cell to adhesive and regenerate the tissue part that can use in tissue and organ transplantation. So the material should be biocompatible and having good mechanical properties. In early age of tissue engineering, there are various materials have been tested. Metals are good choice, because metals have excellent mechanical properties, but they will not degrade in normal environment. Ceramic materials have great osteoconductivity, but they are hard to process. On the contrary, polymers are flexible and some of them are biodegradable. Therefore, biodegradable polymers become the nova in tissue engineering; polylactic acid is one of them. The scaffold that made from biodegradable polymers will degrade to product, which can absorb by human body after the new tissue or organ is formed. However, hydrophobicity and lack of functional groups are the drawbacks of polylactic being scaffold, which might affect the cell adhesion.[5]

2.5 3D printing in tissue engineering

Scientists are eager to make practical use of 3D printing technique in tissue engineering since it was invented by the Massachusetts Institute of Technology. Using 3D printing to build scaffolds to support the tissue or organ seems a great idea. But there are a lot of technical issues such as the speed of 3D printing, to overcome in order to applying the 3D printing technique in mass-produce.[16]

Chapter 3 Experiments

This study focused on the preparation of polylactic acid thin film and comparison of degradative level between different conditions, immersing into different solution for different time, before and after planted the human fibroblast cell.

3.1 Material Preparation

3.1.1 Polylactic acid

Polylactic acid was provided by Food and Drug Administration (FDA), manufactured by LACTEL from Birmingham, AL, USA. Product number is B6002-2. The Polylactic acid solid with the melting point of 173-178°C, glass transition temperature is 60-65°C. The Polylactic acid directly dissolved in chloroform without further purification.

The substrates for spin coating are silicon wafers. To form a suitable thin film, the polylactic acid must adhere to the silicon, and won't interact under incubation condition.

3.1.2 Preparation of the Silicon Wafer

Before using silicon wafers as a substrate, they must remove any interfering substance. At first, the silicon wafers were cleaved by diamond cuter and then they were sonicated in methanol solution for fifteen minutes to remove remaining particles.

Step two, in order to remove any organic substances, the wafers were immersed in mixed solution of De-ionized water, Hydrogen Peroxide (H_2O_2) and Ammonium Hydroxide (NH_4OH) in 5:1:1 ratios for fifteen minutes in slightly boiling condition.

Step three, in order to remove any ionic or metallic substances, the wafers were immersed in mixed solution of De-ionized water, Sulfuric Acid (H_2SO_4) and Hydrogen Peroxide (H_2O_2) in 5:1:1 ratios for fifteen minutes in slightly boiling condition. This step will also create oxide layer making the surface hydrophilic (Modified Shirake method).

Before using the wafers to do spin coating, the wafers were immersed in ten present hydrofluoric acid (HF) solution for 15 seconds. This step removes the oxide layer (SiO_2) that create in step two, the surface become hydrophobic and ready to do spin coating.

3.1.3 Spin Coating and Annealing

Silicon wafers (plane <100>) were cleaved to one centimeter by one centimeter. The polylactic acid was dissolved in chloroform in concentration 15mg/ml and spun coat onto Si wafer at 2000 rpm for 30 seconds using Headway Spin Coater. The coated wafers were moved to the oil trapped vacuum oven at a pressure of 10^{-3} torr for 2 hours at 80°C.

Annealing is essential for several reasons. First, make stable and uniform polymer thin film. Second, remove the additional solvent and residues from the polymer thin film, which might be toxic for the cell. Third, because the temperature is over glass transition temperature (the glass transition temperature of PLA is 50-60°C) the thin film will recrystallize after annealing. It also can simulate the situation that PLA cool down in room temperature right after 3-D printing. If the time to anneal is long enough, it will form a homogeneous surface and the diffusion net flow of matter will stop.

3.2 Cell culture

3.2.1 Cell culture parameter

Dermal fibroblast was provided by professor Marcia Simon form Department of Oral Biology and Pathology Stony Brook University for Medical Research. The cells were cultured with the Dulbecco’s Modified Eagle Medium (DMEM) (standard media for fibroblast culture) with 10% Fetal Bovine Serum (FBS) and 1% Penicillin-Streptomycin (PS) (full-DMEM) routinely.

The cells were plated on the Si wafers coated with PLA at a cell amount of 5,000 or 10^4 cells per well in 24 wells tissue culture plate. All the cells were incubated in a Napco 5430 Incubator at 37°C with 4.9% CO_2 and 100% humidity.

3.2.2 Fix and Stain

After incubated for specific time, the cells were fixed with 3.7% Formaldehyde, and permeabilize by 0.1% Triton solution, then stained the cell fiber with Alexa Flour 488 phallodium (Invirogen, Carlsbad, California) for at least 20 minutes, and stained the nucleus with 4', 6-diamidino-2-phenylindole (DAPI) for five minutes, respectively.

The morphology of the cells was visualized with a Leica TCS SP8X Configurable Confocal (Leica Micro-system Inc., Bannockburn IL).

In order to take picture using the optical microscope, the PLA was spin coating on the cover glass microscope slide with 1cm diameter, and annealed the film for 2 hours at 80℃ in a vacuum of 10^{-3} torr. Dermal fibroblast was plated on the PLA thin film with amount of 5,000 cells to 10^4 cells and cultured in the DMEM.

Dermal fibroblasts were plated from the flask onto the PLA surface by the following steps. In order to detach the cell from the flask, remove all the DMEM from the flask, and add 3 ml Trypsin with EDTA solution, an enzyme that cleave the peptide bonds of lysine and arginine residues, into the flask after rinse with Phosphate buffered saline (PBS). Shake the flask to make sure that Trypsin with EDTA solution covers the entire surface. Incubate the flask for 10 minutes at 37°C which is the reaction temperature of the Trypsin.

The cells were detached from flask and floating in the Trypsin solution. Add 6 ml of DMEM to neutralizing the enzymatic reaction of trypsin. Centrifuge the solution for 6 minutes in the speed of 1500 rpm. Aspirate the mixture of the Trypsin solution and DMEM. Remixing with DMEM and count the cell, add specific amount of the cell into the 24-well plate with 1ml DMEM.

3.3 Measure Method

3.3.1 Atomic force microscope

Atomic force microscope (AFM) is a type of high resolution scanning probe. Using the sharp tip as probe to scan the surface of the sample. The signals accept the laser reflection from the tip. Therefore, the signals change with the tip going up and down to detect the morphology of the surface. The probe also can use to measure the modulus of the surface by applying the force to the surface and measure the response of the tip. The basic structure shows in Figure 3.1

Compare to scanning electron microscope (SEM), AFM can provide the real 3D image, and AFM can do the measurement without special treatment such as gold or carbon coating. AFM also can work at normal pressure even in the liquid, so the AFM can work with living cell. Slow scan rate is the disadvantage of AFM.

3.3.2 Confocal microscope

Confocal microscope is type of optical microscopy with high optical resolution and contrast. Figure3.2 shows the basic structure of confocal microscope. The specimen is excited by the light source, laser or Mercury-vapor lamp, with specific wavelength, and emits the light. Compare to normal Fluorescence Microscopy, which will get the blur image from the focal plane. Confocal microscope is designed to get the image in specific focal plane. 3D image can be build with the image form different focal plane.

3.4 Processing

3.4.1 Immersing test

First, immersing the PLA thin film in Dulbecco's Modified Eagle Medium (DMEM), Phosphate buffered saline (PBS) and deionized water into tissue culture plate, 24 wells, for 7 days, 14 days and 28 days to simulating the environment inside the human tissue.

After immersing, use the ellipsometer and atomic force microscope (AFM) to measuring the change of the thickness, surface morphology and mechanical properties.

3.4.2 Cell culture test

Planting the fibroblast on the polylactic acid thin film and tissue culture plastic as control to simulating the situation that cell grow on the polylactic acid inside the human tissue or organ. The polylactic acid thin film with two kinds of substrates, silicon wafer and cover glass. Using cover glass as substrate is easy to observe and take picture during cell growth. Measuring the thickness, surface morphology, mechanical properties and counting the cell number every two days.

Because the PLA might degrade in the DMEM, and the effect of the PLA to the fibroblast is unknown. So two kinds of mediums are prepared. Normal medium with 10% with 10% FBS and 1% PS marks as minus (-). Minus medium started to immerse with PLA thin film when the cell plated on the PLA thin films and tissue culture plate to simulating the PLA degrade in the medium for the same time that the cell growth and storage in the incubator at 37°C with 4.9% CO_2 and 100% humidity, which marks as plus (+).

Chapter 4 Result and discussion

4.1 Morphology and thickness

Although the surfaces of the PLA thin film become rough after immersing in DMEM, PBS and deionized water for 7 days, 14 days and 28 days. The thicknesses of the PLA thin films do not have obvious difference between the original PLA thin film and the PLA thin film immersed in the solutions.

Figure 4.1 shows that the origin thickness is about 1500 to 1550Å. The thickness of the PLA thin films is around 1500Å after immerse in DMEM, PBS and deionized water for 7 days, 14 days and 28 days.

Figure 4.3 to 4.6 are the AFM images of the PLA thin film after immersed in DMEM, PBS and deionized water for 7 days, 14 days and 28 days. The surfaces become rougher as the time increase. The original PLA thin film roughness is about 70 nm. After immersed in DMEM, PBS and deionized water for 14 days, the roughness is over 200 nm.

Comparison between the PLA thin film in DMEM, PBS and deionize water. The thin films immersed in DMEM have the biggest change. (Figure 4.3 to 4.6)

4.2 Mechanical Properties of PLA thin film

However, the modulus test tells different story, the relative modulus of the PLA thin films change a lot, especially for the PLA thin film, which immersed in the DMEM.

Figure 4.2 shows that the average of the original relative modulus of the PLA thin films is 2.304 a.u. The PLA thin films become softer than the origin thin film after immerse in DMEM, PBS and deionized water for 7 days, 14 days and 28 days. The solution did change the mechanical properties of the PLA thin film.

Comparison between the PLA thin film in DMEM, PBS and deionize water. The thin films immersed in DMEM have the biggest difference in relative modulus (Figure 4.2), which has the same pattern in AFM image.

For cell culture test, the PLA thin films become softer than the origin PLA thin films after plated dermal fibroblast on the surface. Although the relative modulus drops fast in the first day no matter in the plus or minus medium, the relative modulus does not change a lot after first day, which shows in figure 4.7 and 4.8.

4.3 Cell growth

Figure 4.9 and 4.10 show that the fibroblasts grow slower on the PLA thin films than grow on the tissue culture plates. No matter which kind of medium is cultured. In control group, the doubling time is about 2 days. The doubling time for the cells on the PLA thin films is about 5 days. That is a significant difference.

Figure 4.9 and 4.10 also show that the samples, which cultured in the plus medium, grow slower than the cells on sample that was cultured with the minus medium. The amount of the cells has about 5000 margins between the cells culture in plus and minus medium. In test 1 the cells even stop growing after 5 days.

Figure 4.11 to 4.14 are another evidences that the fibroblasts grow slow in the first five-day on the PLA thin film, observing from the optical microscopy image. In the first day, many cells float in the medium not sitting on the PLA thin film (Figure 4.11). In the third day, the cells obviously grow slower on the PLA thin film (Figure 4.12). The cells on the tissue culture plate reach 100% confluent at fifth day (Figure 4.13). However, the cells on the PLA thin film reach 100% confluent at eighth day. (Figure 4.14)

4.4 Discussion

The mechanical properties of the PLA thin films are easily affect by the solution, especially the samples which immerse in the DMEM. The samples in PBS and deionized water have small difference which means the salt might not the major ingredient affect the mechanical properties of the PLA thin film.

The fibroblasts, which are cultured in the plus medium, grow slower than the fibroblasts were cultured with the minus medium. Although the cell growth rate back to normal at the eighth day in samples which are cultured with plus medium that is because the well was full of cells so the cells grow on the top of the other cells instead of contacting the surface of PLA thin film.

The reason might because the plus medium was storage at 37°C not in the 5°C environment. The medium might deteriorate in that storage condition, causing the cell growth rate drop.

Chapter 5 Conclusion

5.1 Summary

The thicknesses of the PLA thin films do not have significant change during the immersing test and cell culture test. However, the surface of the PLA thin film did become uneven after immersing test and cell culture test. Moreover, the mechanical properties have significant differences in both of the immersing test and cell culture test. It proves that DMEM, PBS, deionized water and dermal fibroblast will cause the PLA thin film changed.

The dermal fibroblasts do not grow as well as tissue culture plate might because of the PLA thin film surface is hydrophobic. The contact angle of the PLA thin film surface is approximately 80°. This results in low cell affinity even if the PLA is biocompatible.

5.2 Future works

3D printing might be the future star for tissue engineering to build scaffold. PLA is only one of the biodegradable materials suitable for tissue engineering. Poly (Lactide-co-Glycolide) Copolymers (PLGA), Poly (glycolic acid) (PGA), Polydioxanone (PDS) and Poly-carprolactone (PCL) are also biodegradable and able to process through 3D printing.

In the future, running the same experiments to analysis these materials to compare the advantages and disadvantages. In order to find the right material, applying the materials in tissue engineering through 3D printing.

Because of the surface of PLA thin film is hydrophobic, surface treatment might be one of the solutions. Surface treatment can control the hydrophobic, roughness and add some reactive group [8], helping the cells easy to grow on the surface.

There is another control test have to run in the future, because the plus medium used in cell culture test this time is storage in the incubator, which is not the normal storage condition. The ingredient in the plus medium might decay. So the next experiment will add another group that storage the normal DMEM in the incubator instead of refrigerator in order to compare the plus

medium that storage in the incubator. In order to figuring out which is the factor cause the cells grow slower than usual.

Figure

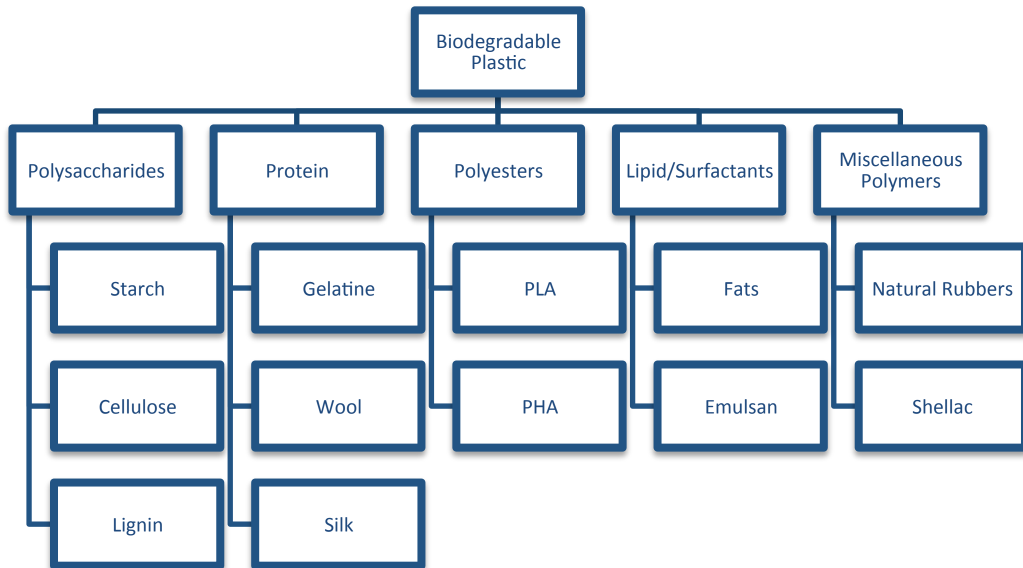

Figure 1.1 Type of biodegradable Plastic [1, 3]

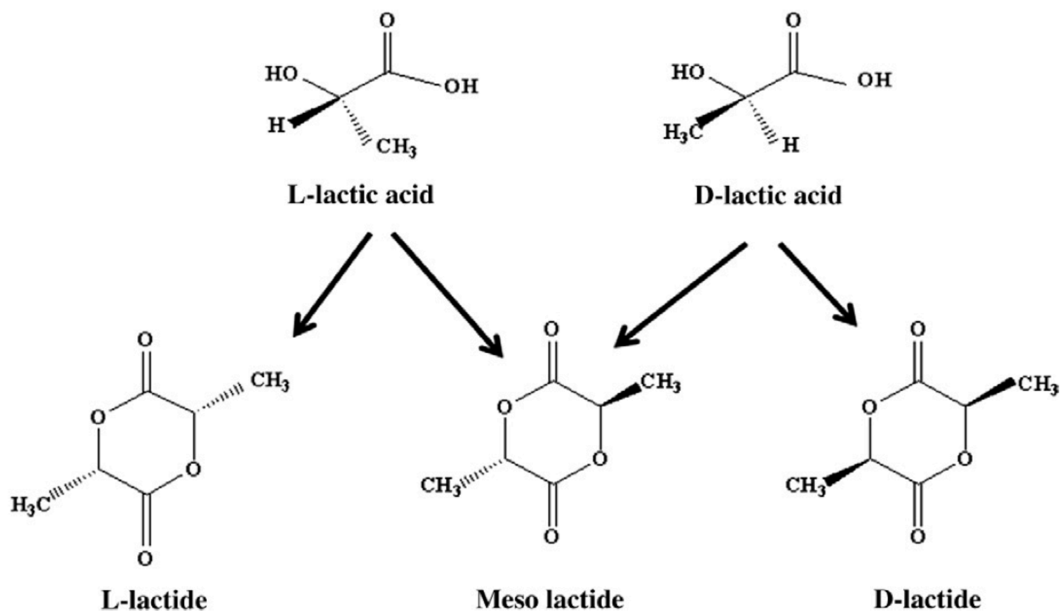

Figure 2.1 Stereochemistry of lactic acid and lactide molecules[1]

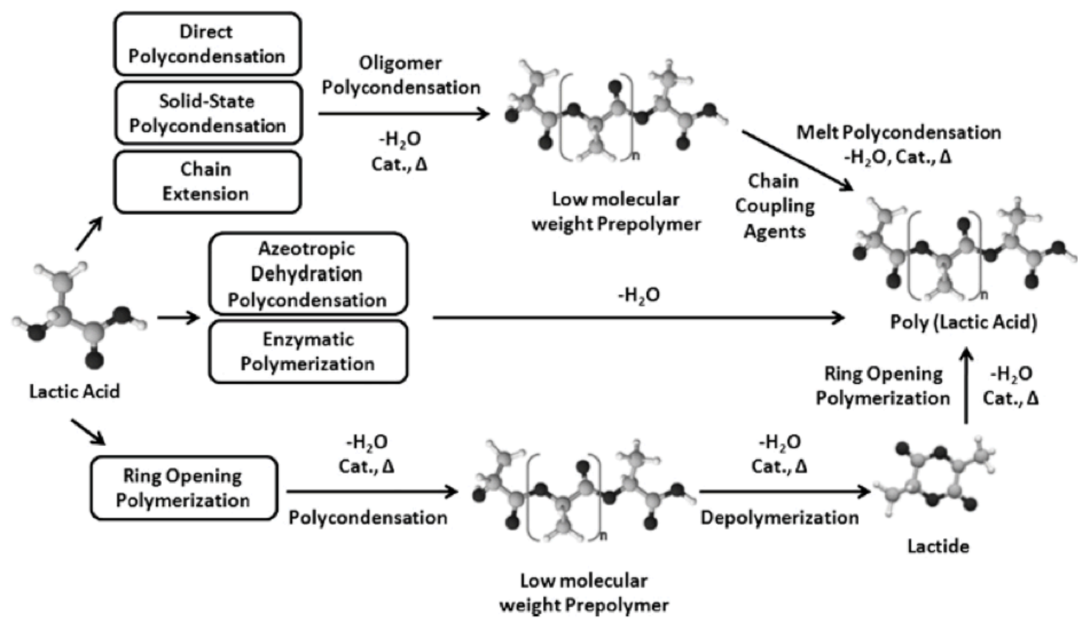

Figure 2.2 Synthesis of Polylactic acid [4]

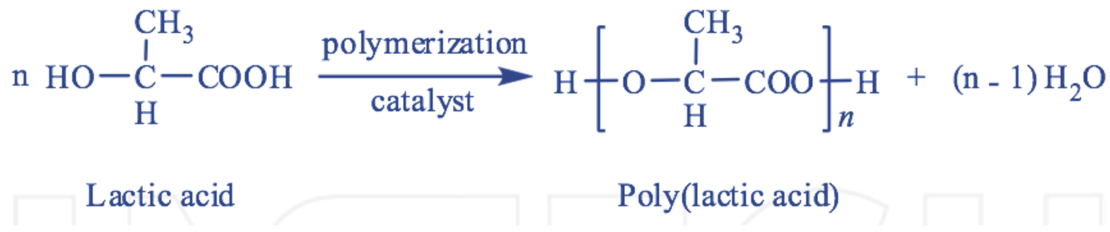

Figure 2.3 Direct polycondensation[5]

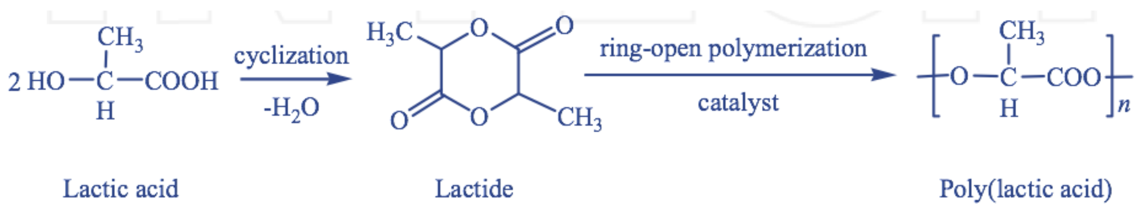

Figure 2.4 Ring-opening polymerization[5]

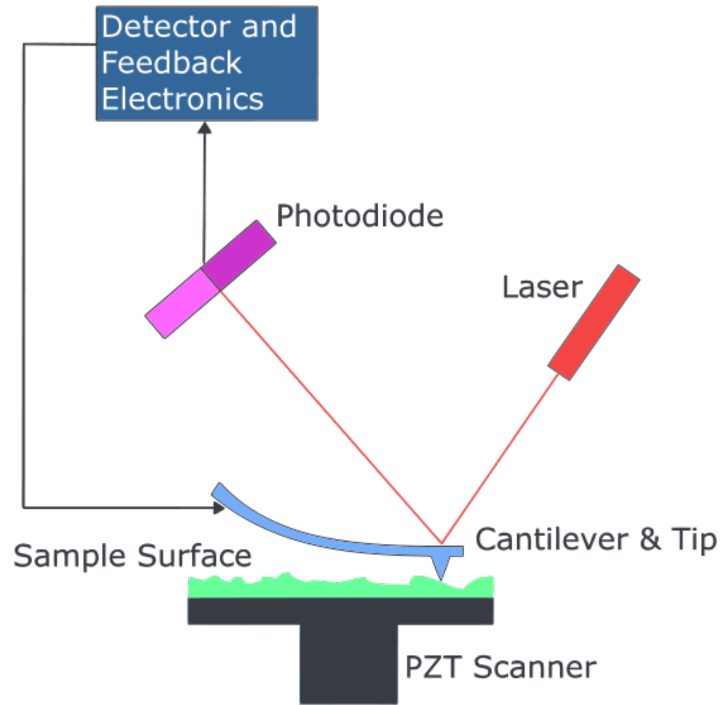

Figure 3.1 Principle of AFM [17]

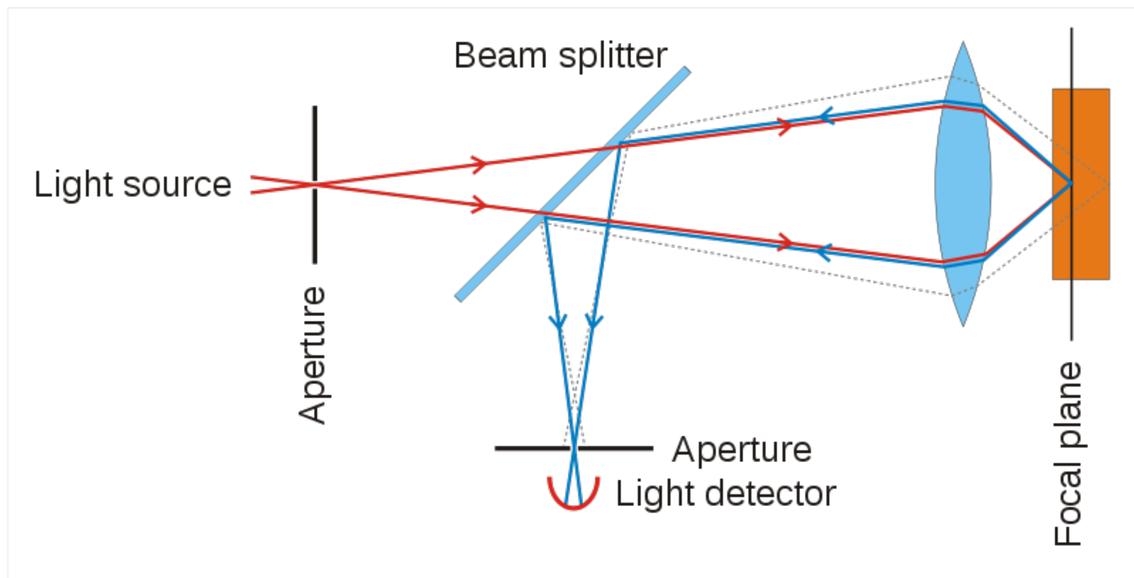

Figure 3.2 Principle of Confocal Microscope [18]

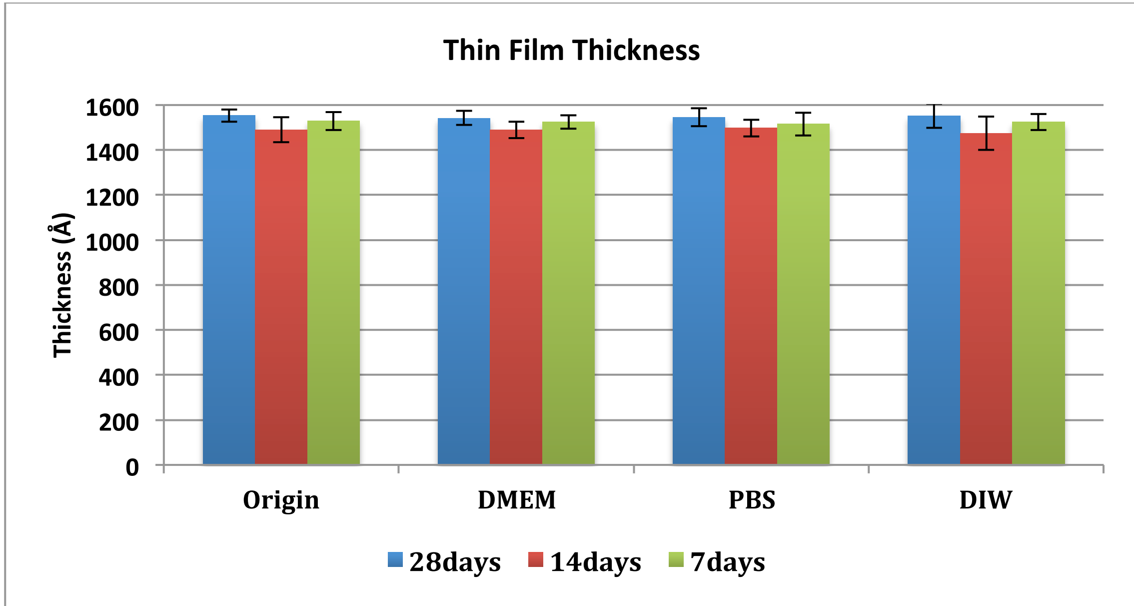

Figure 4.1 Thickness Change of PLA Immersing Test

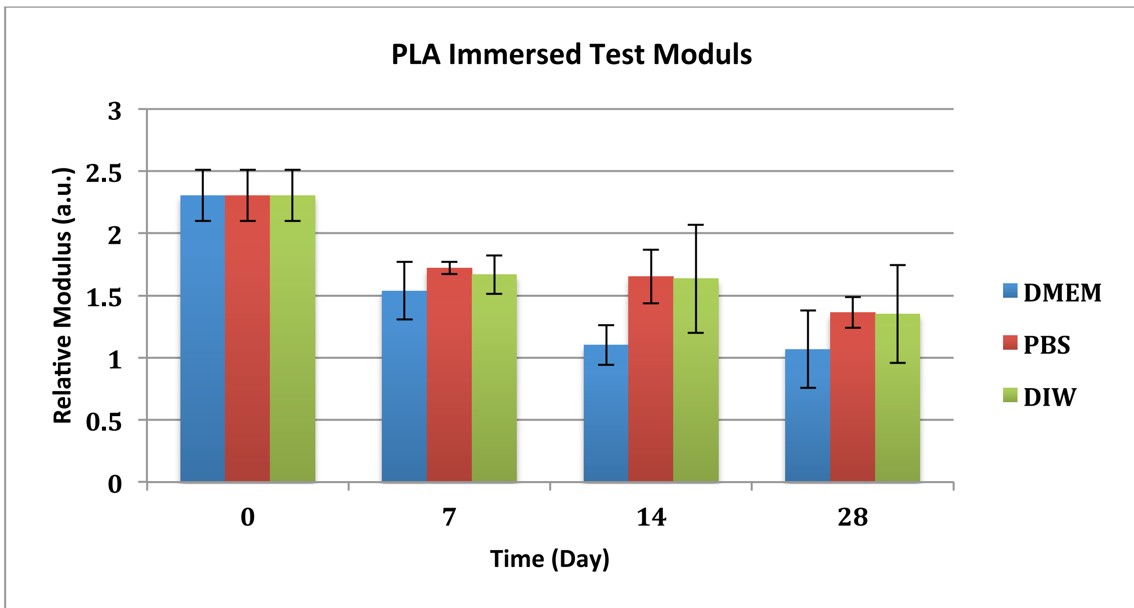

Figure 4.2 Relative Modulus of PLA Immersing Test

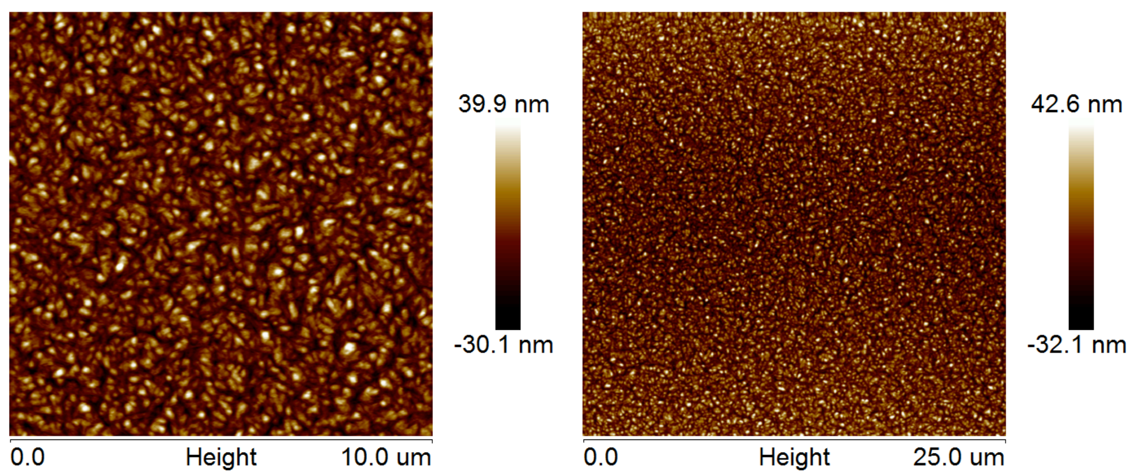

Figure 4.3 AFM Images of Origin PLA Thin Films

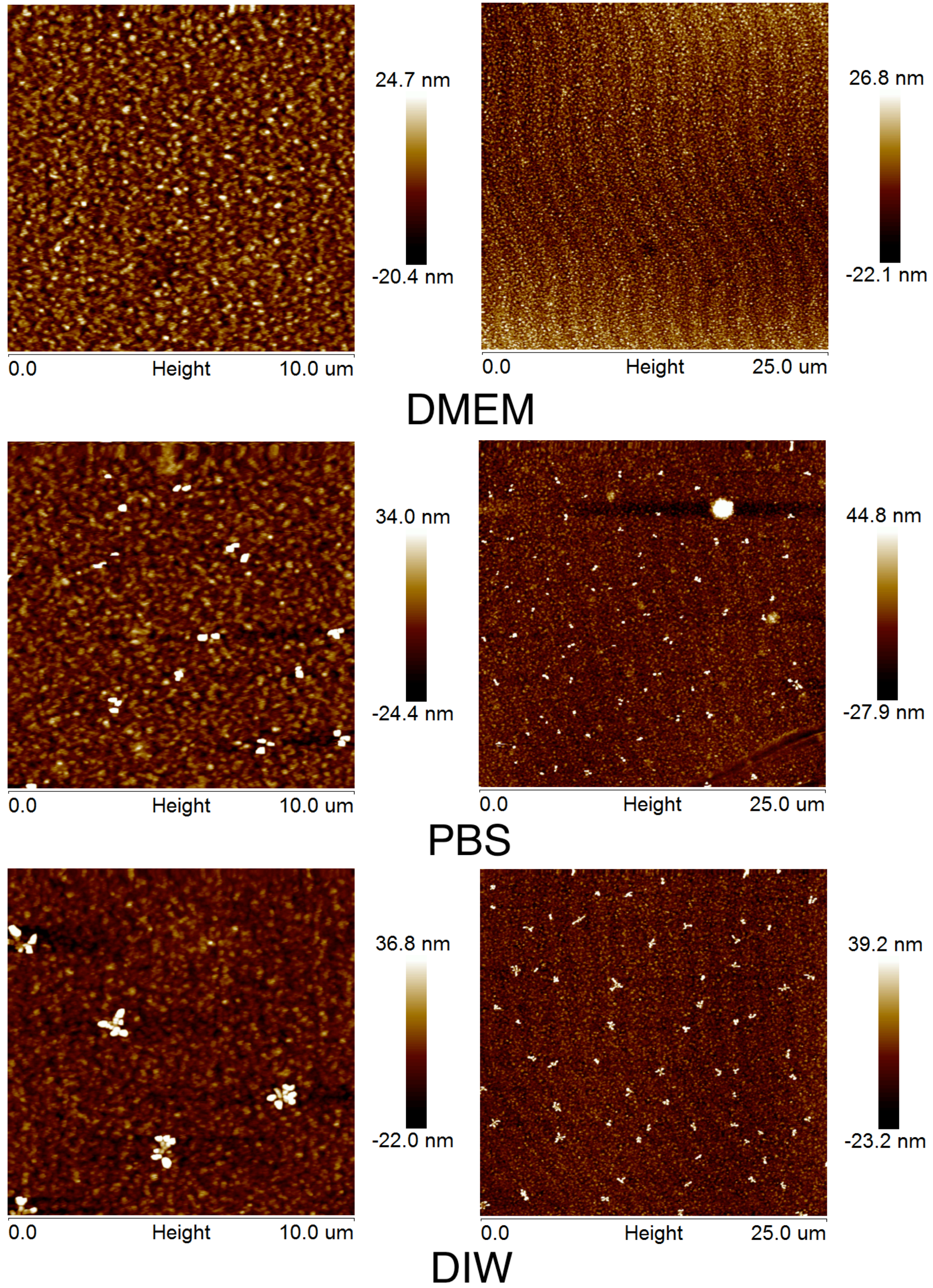

Figure 4.4 AFM Images of PLA Thin Films Immerse for 7 Days

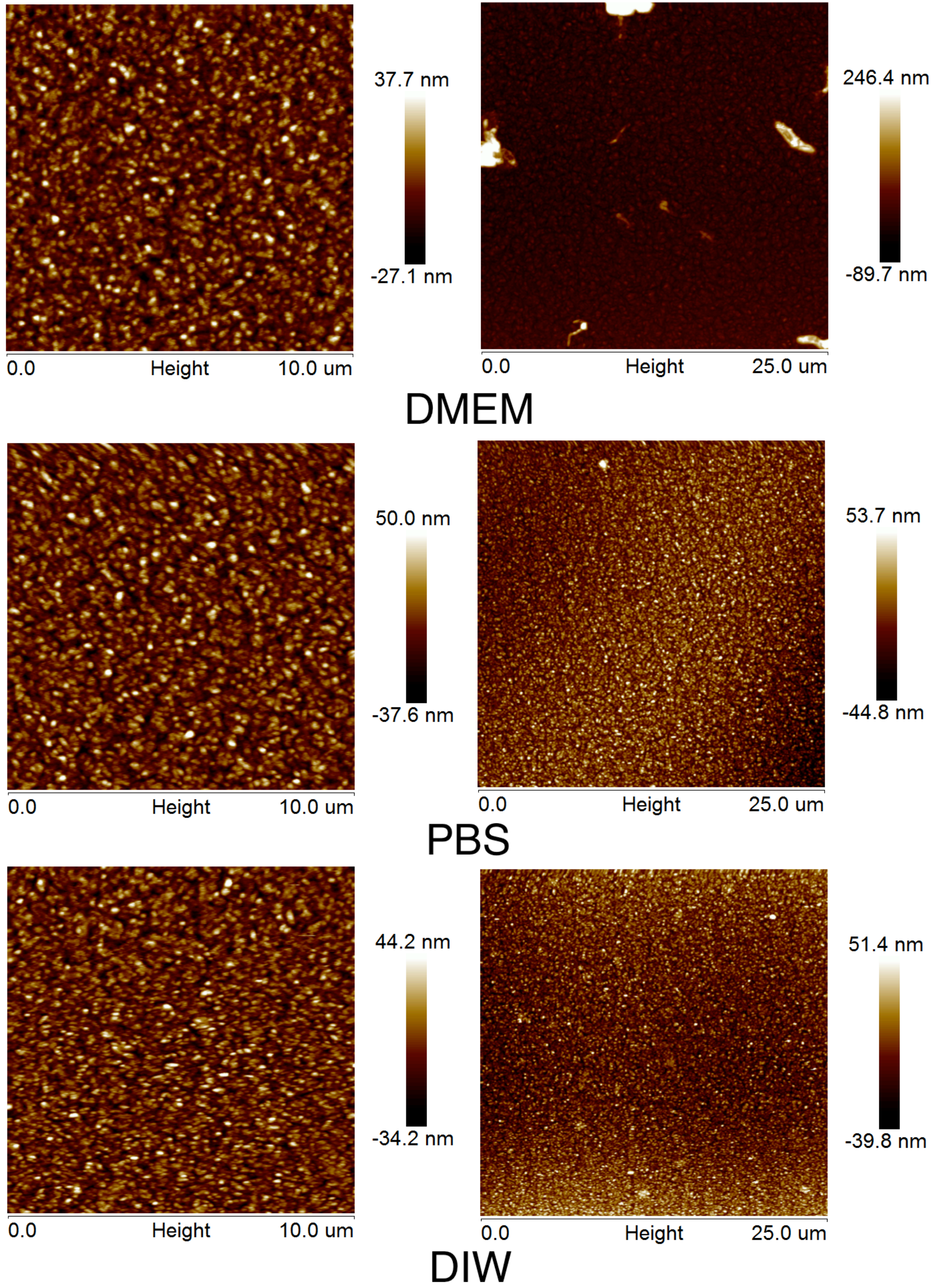

Figure 4.5 AFM Images of PLA Thin Films Immerse for 14 Days

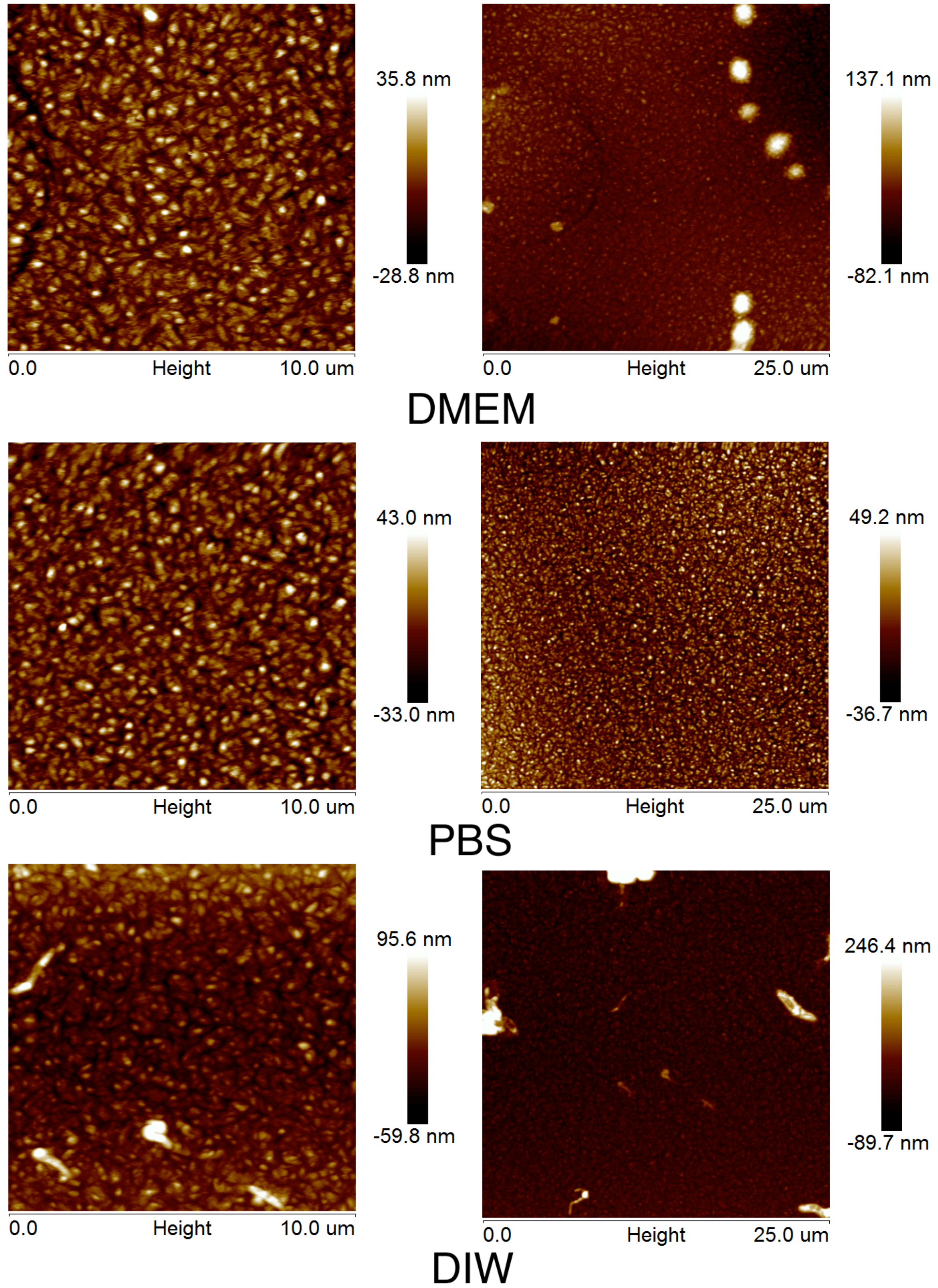

Figure 4.6 AFM Images of PLA Thin Films Immerse for 28 Days

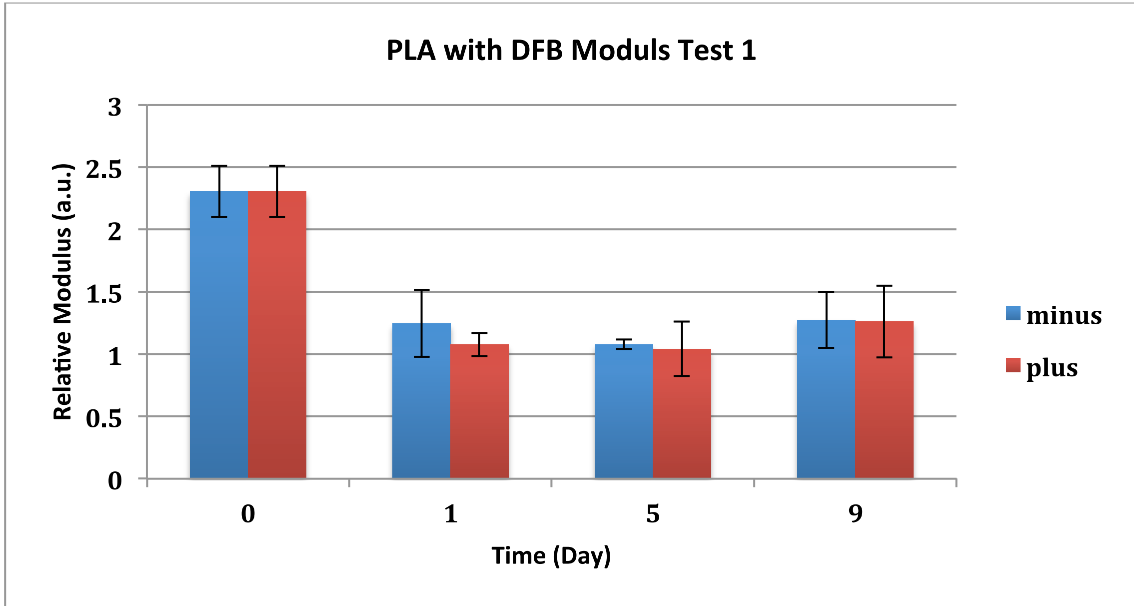

Figure 4.7 Relative Modulus of PLA of Cell Culture Test 1

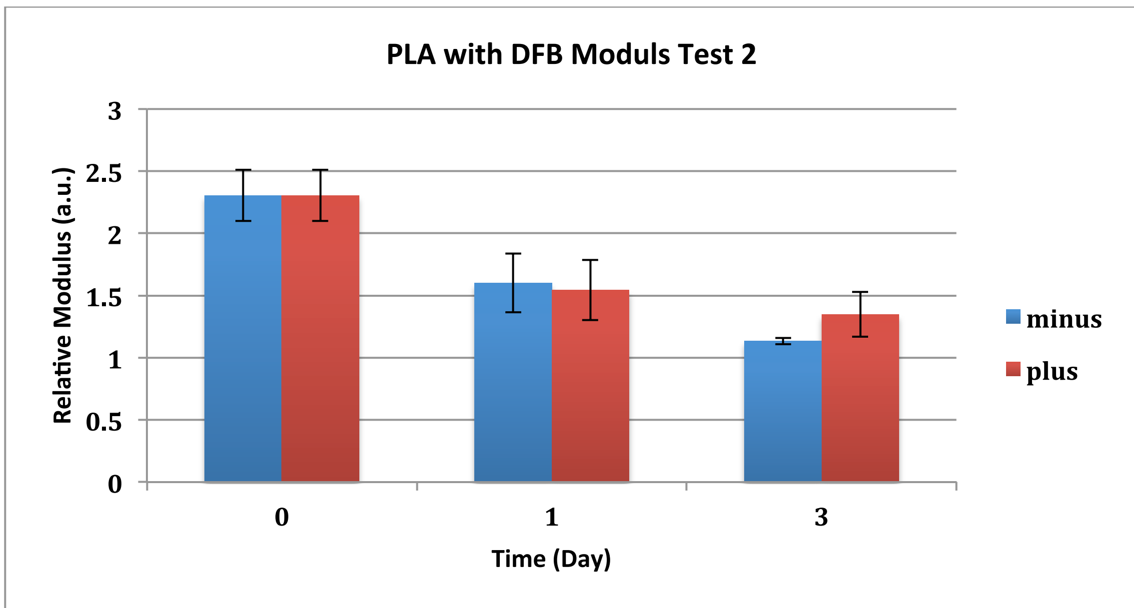

Figure 4.8 Relative Modulus of PLA of Cell Culture Test 2

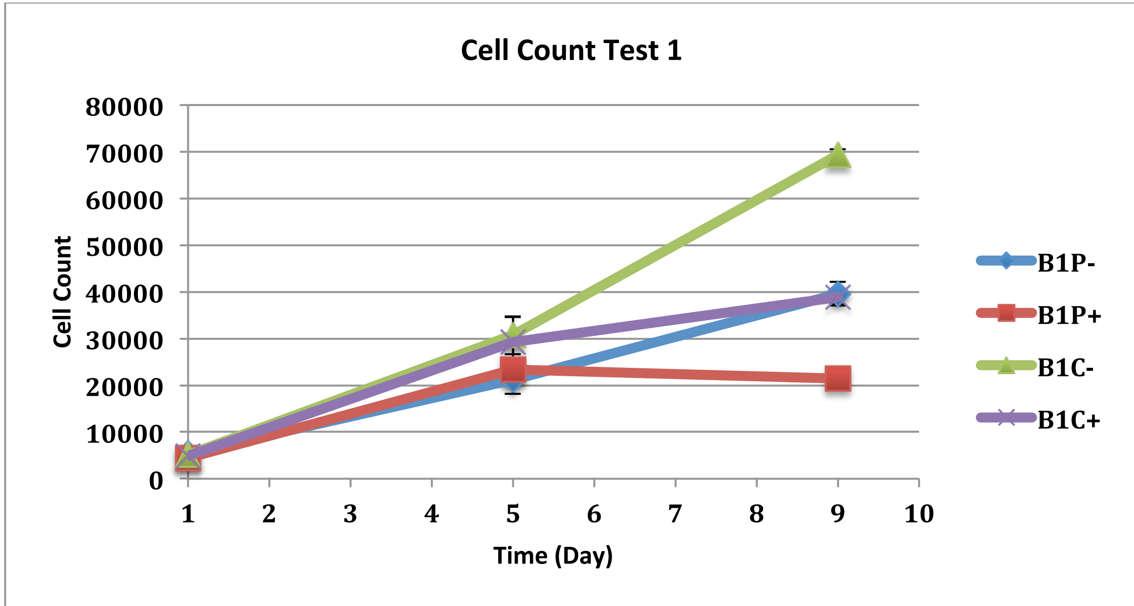

Figure 4.9 Cell Count of Cell Culture Test 1

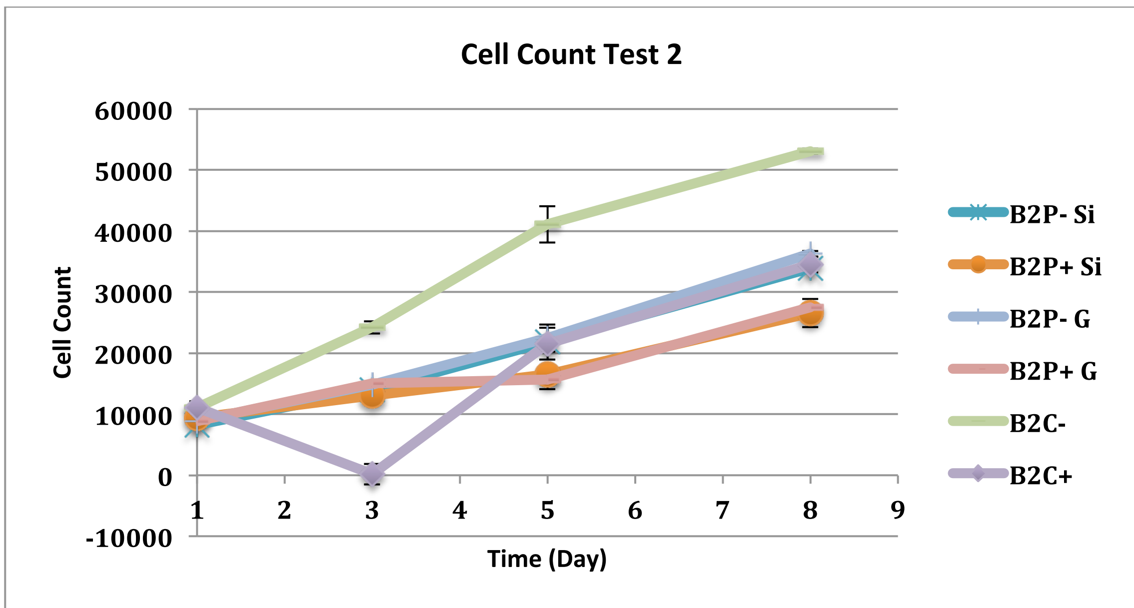

Figure 4.10 Cell Count of Cell Culture Test 2

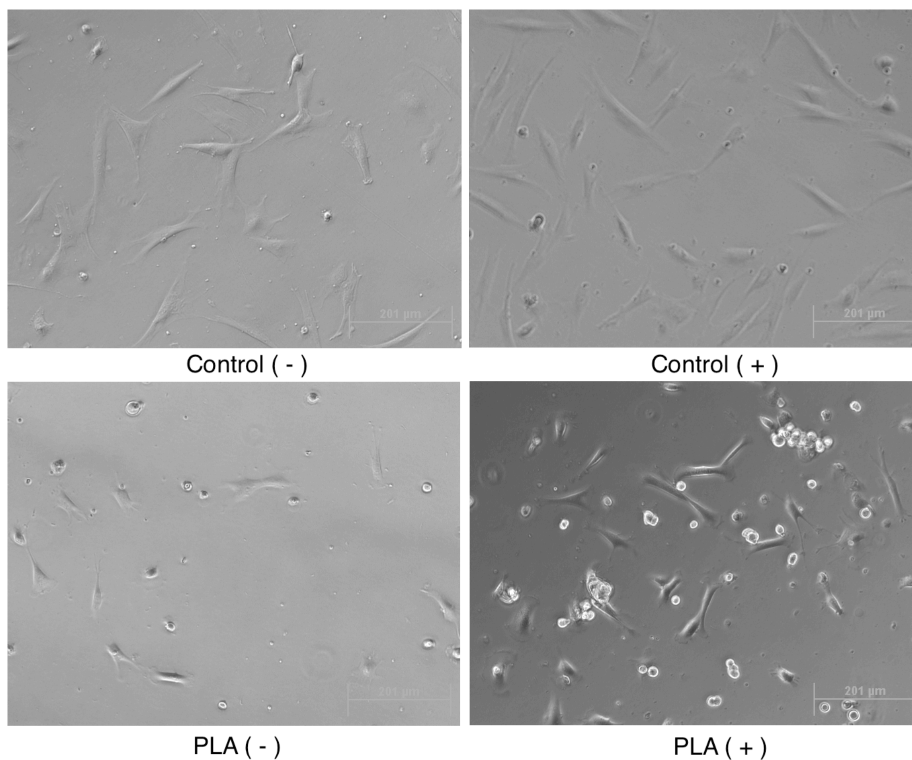

Figure 4.11 Optical Microscope Image of Cell Culture Test 2 Day 1

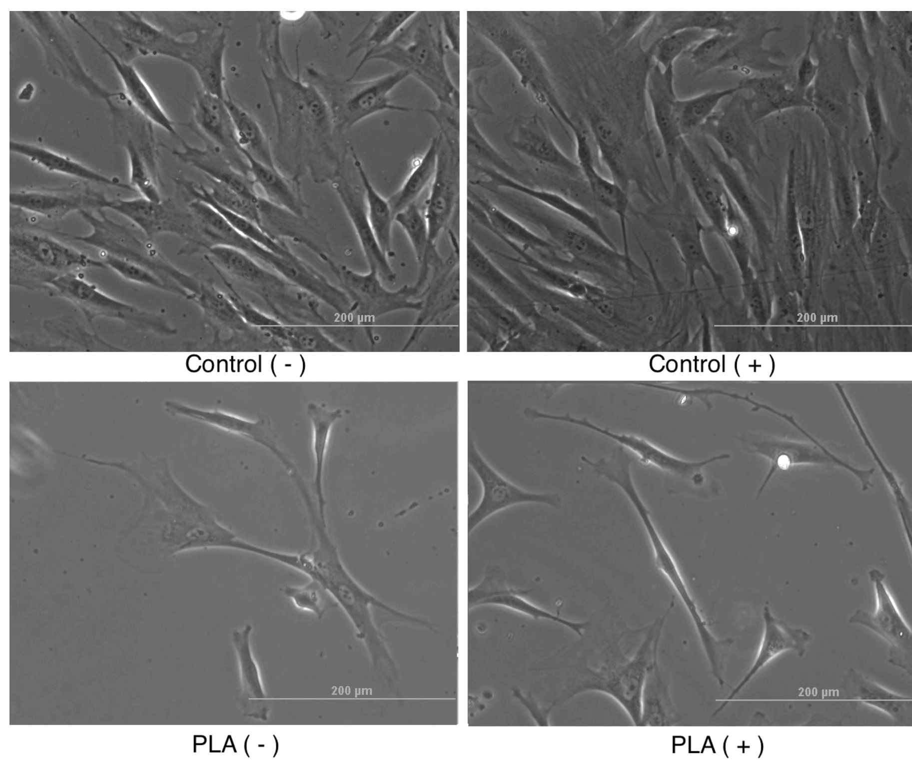

Figure 4.12 Optical Microscope Image of Cell Culture Test 2 Day 3

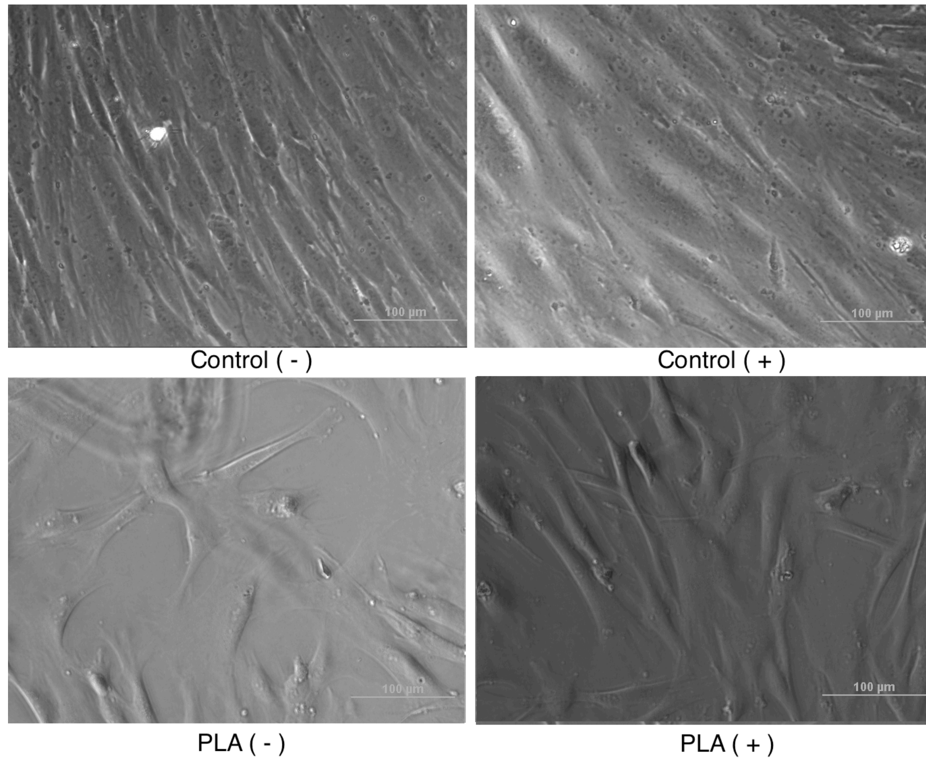

Figure 4.13 Optical Microscope Image of Cell Culture Test 2 Day 6

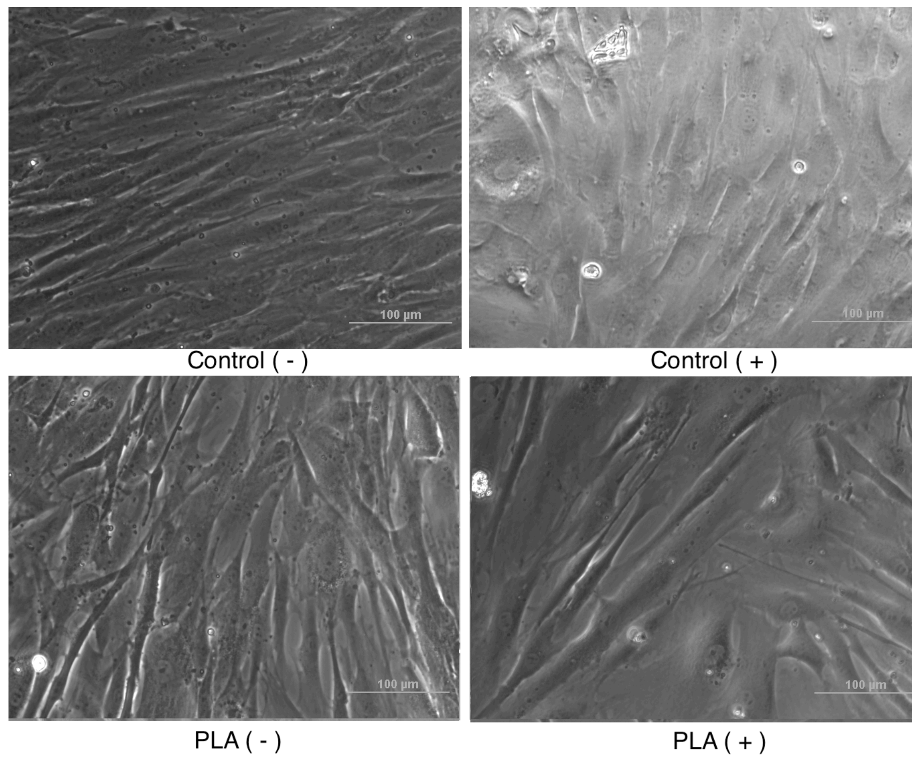

Figure 4.14 Optical Microscope Image of Cell Culture Test 2 Day 8

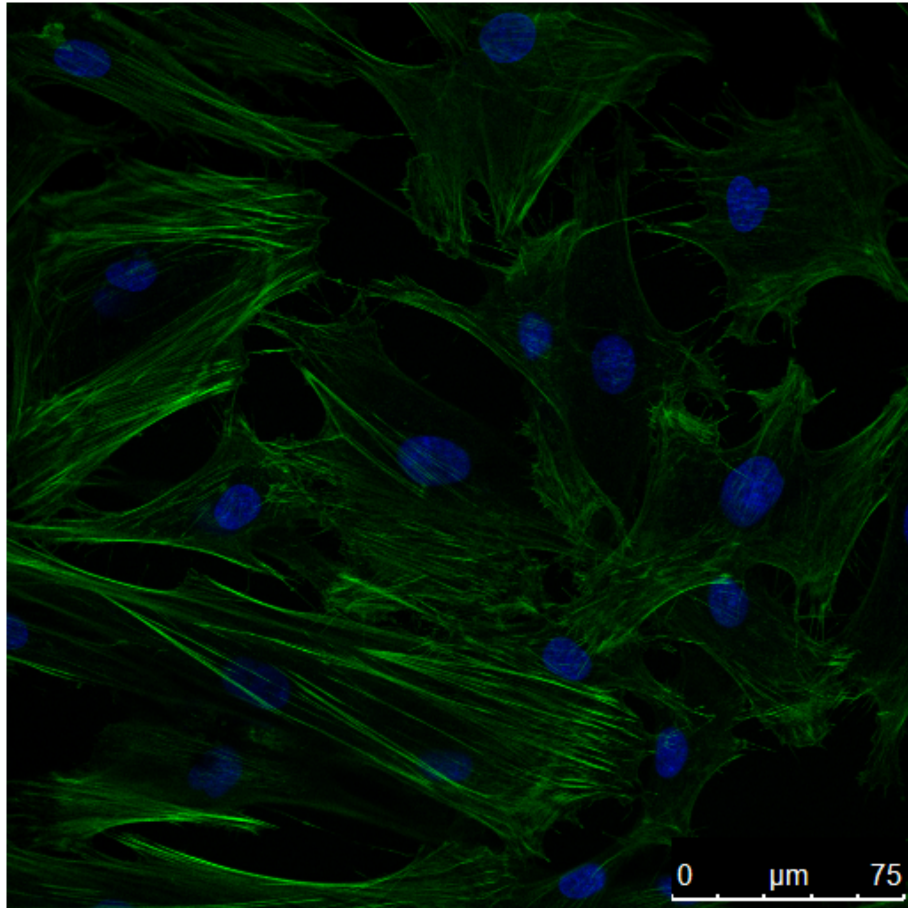

Figure 4.15 Confocal Microscope Image of Cell Culture Test 2 Day 8

Reference

[1] Madhavan Nampoothiri K, Nair N, John R. An overview of the recent developments in polylactide (PLA) research. Bioresource technology. 2010;101:8493-501.
[2] Platt DK. Biodegradable Polymers Market Report2006.
[3] Kaplan DL. Biopolymers from Renewable Resources1998.
[4] Lopes MS, Jardini AL, Filho RM. Poly (Lactic Acid) Production for Tissue Engineering Applications. Procedia Engineering. 2012;42.
[5] Xiao L, Wang B, Yang… G. Poly (Lactic Acid)-Based Biomaterials: Synthesis, Modification and Applications. … Science. 2012.
[6] Mukund GA, Anjani JV, Digambar VG. Lactic acid production from waste sugarcane bagasse derived cellulose. Green Chemistry. 2007;9.
[7] Ramesh PB, Kevin OC, Ramakrishna S. Current progress on bio-based polymers and their future trends. Progress in Biomaterials. 2013;2.
[8] Rahul MR, Amol VJ, Douglas EH. Poly(lactic acid) modifications. Progress in Polymer Science. 2010;35.
[9] Kojo AA, Laurent MM. Impact Modification of Polylactide with a Biodegradable Ethylene/Acrylate Copolymer. Macromolecular Materials and Engineering. 2010;295.
[10] Ma P, Hristova-Bogaerds DG, Goossens JGP, Spoelstra AB, Zhang Y, Lemstra PJ. Toughening of poly(lactic acid) by ethylene-co-vinyl acetate copolymer with different vinyl acetate contents. European Polymer Journal. 2012;48.
[11] Hideko TO. Super-tough poly(lactic acid) materials: Reactive blending with ethylene copolymer. Polymer. 2009;50.
[12] Ma P, Spoelstra AB, Schmit P, Lemstra PJ. Toughening of poly (lactic acid) by poly (β-hydroxybutyrate-co-β-hydroxyvalerate) with high β-hydroxyvalerate content. European Polymer Journal. 2013;49.
[13] Velde KVd, Kiekens P. Biopolymers: overview of several properties and consequences on their applications. Polymer Testing. 2002;21.
[14] Chen C, Chueh J, Tseng H, Huang H, Lee S. Preparation and characterization of biodegradable PLA polymeric blends. Biomaterials. 2003;24:1167-73.
[15] Takeshi S, Kazuo K, Umaru Semo I, Hiroyuki H. The effect of crosslinking on the mechanical properties of polylactic acid/polycaprolactone blends. Journal of Applied Polymer Science. 2006;101.
[16] Barry B. 3-D printing: The new industrial revolution. Business Horizons. 2012;55.

[17] Atomic force microscope block diagram.svg
http://upload.wikimedia.org/wikipedia/commons/7/7c/Atomic_force_microscope_block_diagram.svg

[18] Danh, Diagram of confocal principle
http://upload.wikimedia.org/wikipedia/commons/thumb/d/dc/Confocalprinciple_in_English.svg/800px-Confocalprinciple_in_English.svg.png