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ABSTRACT

GROWTH BEHAVIOR OF FIBROBLASTS INFLUENCED BY SMALL CHANGES IN POLYMER SURFACE STRUCTURE

**by
Saraswathi Doddi**

Polymers are a promising class of biomaterials that can be engineered to meet specific end use requirements. The order and processing history of the polymer, which would alter the molecular orientation of the material could have a significant contribution towards cellular attachment and in turn, cell growth on the particular polymer. Surface properties of the material were considered to directly influence the properties of the adherent cells including cellular growth and reorganization. The present study is aimed at comparing cell growth on polyarylates with that of polylactic acid in their original state or by introducing small changes in the surface structure of the polymers, by adopting different processing techniques (i.e. drawn and undrawn forms). Though, it is the most widely used scaffold, polylactic acid has been found to degrade faster and produce acidic end products, making it unsuitable for many applications. The two polyarylates chosen for the study were poly (DTD) dodecandioate and poly (DTE) adipate taken from the two extreme positions of the combinatorial library developed by Prof.J.Kohn. Thermal analysis techniques were used to study the molecular structure of the material. Higher degradation rate, less water uptake in the aqueous environment and less acidic end products were obtained from the two polyarylates as compared to polylactic acid. There was a significant difference in the growth rate of the fibroblasts on the drawn and the undrawn forms of the (12,10)-polyarylate, suggesting that its behavior could be correlated to the number of structural conversions existing in the polymer.

**GROWTH BEHAVIOR OF FIBROBLASTS INFLUENCED BY SMALL
CHANGES IN POLYMER SURFACE STRUCTURE**

**by
Saraswathi Doddi**

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APPROVAL PAGE

**GROWTH BEHAVIOR OF FIBROBLASTS INFLUENCED BY SMALL
CHANGES IN POLYMER SURFACE STRUCTURE**

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Dedicated to all my beloved family members for their invaluable support during my studies. Special dedication to my uncle, Mr.P.Ramababu, who had played a part in one of the many steps of my academic career. A very special dedication to the best person in my life Sandesh Prakash

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CHAPTER 1

INTRODUCTION

1.1 Background

Biomaterials are substances other than food or drugs contained in therapeutic or diagnostic systems that are in contact with tissue or biological fluids. The interaction of cells with the laboratory designed materials is gaining considerable importance in implant technology and tissue engineering. Coaxing cells to form tissues in a reliable manner should be accomplished under constraints of cost, government regulations and societal acceptance. There are three ways in which a material can be used in tissue engineering. It should either be able to induce cell migration or tissue regeneration or they should have the ability to encapsulate cells and act as an immunoisolation barrier or they should act as a matrix to support cell growth and cell organization. The first approach was used in nerve and cartilage regeneration which involves the use of collagen constructs that stimulates healing and acts as an artificial skin¹. Polymers are being used for biomaterial encapsulation in three forms. Hollow fiber membrane is one of the forms which connect the cells to the body. Porcine hepatocytes used in the hollow fibers of polysulfone, acts as a bridge to transplant, for patients dying of liver failure².

Macrocapsules can be directly implanted in the body to act as an immunoisolation barrier. They can allow medium or small sized molecules to penetrate through and prevent immune cells from entering the cellular transplant. Polyacrylonitrile- polyvinyl chloride membranes was used to surround pain medication to form a macro capsule with which the cells are kept viable in aqueous environment³.

Alginates can be ionically cross linked to divalent calcium ions and can encapsulate cells, isolating them from the immune environment. This approach was pioneered by Lim and Sun in the treatment of diabetes in animal studies⁴. The polymer matrix acts as a scaffold in the third type of approach and it enables cellular proliferation and reorganization. The cells are allowed to grow and organize to form appropriate cellular constructs. Tissue regeneration occurs by the interaction amongst three components, which includes cells that restore tissue, scaffold that holds and creates tissue and signaling molecules that direct the cells to form the tissue. An ideal scaffold is three dimensional, highly porous with an interconnected pore network for cell growth and transport of nutrients and metabolic wastes. It should also carry a suitable surface chemistry for cell attachment. Biocompatibility and bioresorbability of the material play a major role in cellular differentiation. Controlled degradation rate of the scaffold is necessary to match the cell or tissue growth in-vivo. Mechanical properties of the material also play a major role as they have to match the properties of the tissue at the site of implantation.

Adsorption of proteins is the key event that takes place on the surface of a biomaterial, when it is exposed to protein containing fluids. The subsequent cell adhesion and growth will be dependent on the specific functional groups and the possible correlation between polymer composition and protein adsorption. This approach has been widely studied in the creation of artificial skin, in clinical trials for the creation of cartilage and also to create a variety of tissues in animal studies such as blood vessels, bone and urologic structures⁵.

1.2 Polymeric Biomaterials for Tissue Engineering

Metals and various industrial plastics are the most widely used biostable implant materials. They lack the molecular sequences and patterns necessary for normal cell function and often trigger aberrant cell responses on long term implantation. Poly-(lactic acid) is the most widely used polymer scaffold which is formed into fibrous systems or foams in desired anatomical shapes. However, PLA failed to perform adequately in many orthopedic applications. The significant water uptake by the polymer along with its faster degradation rate and the acidic end products has made the polymer unsuitable for long term applications.

Biocompatibility and biodegradability are the two main properties that have considerable importance on cellular growth and tissue regeneration. Biocompatibility refers to the reaction of polymers with blood and tissues, depending on the site and purpose of use. This includes toxicity and immunogenicity. The material should neither elicit a strong immunological response nor be toxic. Also, the material should degrade into non-toxic and non-immunological chemical species which are removed from the body by excretion or metabolism. A material may be biocompatible in one application but bioincompatible in another. Poly-(lactic acid) was considered to be fully biocompatible as it completely degrades into lactic acid, which is a naturally occurring metabolite in the blood stream. Biodegradability refers to materials which are enzymatically or chemically degraded in vivo into simpler chemical species. Poly-(lactic acid) has been used as a scaffold material in tissue engineering in various medical fields due to its superior biodegradability and mechanical property in vivo, controlled degradation rate and ease in forming pores.

1.3 Origin of Poly Amino Acids

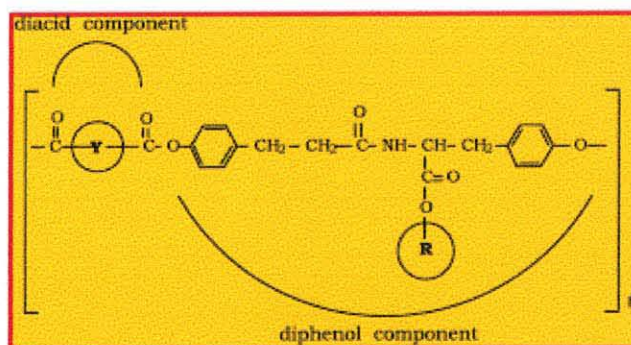
Recent approach in the development of the polymeric biomaterials is to custom design the polymer for in vivo use or for use in the proximity of the biological fluids. This is achieved by tailoring the properties of the polymer for the desired application. Poly(amino acids) have been the potential source of polymeric biomaterials in the early 1970's due to their structural resemblance with natural proteins, making them ideal for drug delivery both in vivo and in vitro. However, certain bioengineering and processing difficulties including immunogenicity, unfavorable mechanical properties, insolubility in common organic solvents, poor hydrolytic degradability, high thermal temperature transition ranges, unpredictable water permeability and swelling were identified.

Kohn and Langer⁶ made a recent attempt in the year 1984 to overcome the drawbacks faced with poly(amino acids). They have introduced specific non-peptide bonds alternating with peptide bonds in a natural amino acid based polymer backbone. The resulting polymers were either analogous or true structural isomers of poly(amino acids) with customizable degradation properties. The unique chemistry of these polymers had improved the properties of the polymer in a predictable and desirable way. Since, tyrosine is the only major, natural nutrient containing an aromatic hydroxyl group, the stiffness and mechanical strength of the poly(amino acids) was improved by the introduction of this non-cytotoxic aromatic backbone. This approach permitted the synthesis of biomaterials for drug delivery systems, sutures, artificial organs, which are usually derived from non-toxic metabolites.

1.4 Combinatorial Library of Polyarylates

Carefully designed monomers and polymerization strategies used by Prof.J.Kohn, led for the first time to create a combinatorial library in which material properties varied in a predictable fashion. The physio mechanical properties of the resulting polymers varied according to protecting groups attached to the carboxylic acid and the amino acid chain of the polymer backbone. This approach not only increased the number of available polymeric candidate biomaterials for medical applications, but also facilitated in identifying the correlation between polymer structure and its properties including glass transition temperature, air-water contact angle, mechanical properties and fibroblast proliferation⁷.

Combination of enzymatically degradable peptide bonds with hydrolytically degradable non peptide bonds in the polymer backbone, led to the formation of polyarylates. A series of pendant chains were attached to the monomer A and the systematic variation in the backbone structure was achieved with the B monomer. In this approach, copolymerization of fourteen different tyrosine diphenols and eighteen different aliphatic diacids in a permutationally designed monomer system led to the development of 112 distinct tyrosine derived polyarylates, which were strictly alternating A-B type copolymers consisting of an alternating sequence of a diphenol and a diacid. The properties ranged from amorphous to liquid crystalline in nature and a wide range of surface and bulk properties could be seen across the library. The number of methylene groups, substitution of oxygen for methylene groups and introduction of branched and aromatic structures were the only variations made systematically for the pendant chain and the backbone structures⁷.



- Nomenclature (R,Y) = length of alkane in sidechain, backbone

Figure 1.1 Desamino Tyrosyl Polyarylates⁸.

From the chemical structure, it can be understood that (2,4)-polyarylate has two methylene groups attached to its sidechain and four methylene groups on its backbone structure. Similarly, twelve methylene groups on the side chain and ten methylene groups on the backbone chain would constitute the chemical structure of (12,10)-polyarylate. These two polymers were compared in the present study due to the wide difference in their chemical structure.

1.5 Role of Fibroblasts in Wound Healing and Tissue Regeneration

An essential characteristic of connective tissue is its strength and elasticity. It supports anchors and connects various parts of the body. It is the most widespread tissue in the body whose adhesion and proliferation is a critical pre-requisite in wound healing and tissue regeneration. Fibroblast cells are elongated spindle shaped actively dividing cells present in the connective tissue. Disruption of tissue integrity associated with loss of substance results in a wound. Wound healing is the complex physiological process that is mediated by immune reaction and includes the restoration of tissue integrity by formation

of new structures, which can perform the same function. When a wound occurs, the anabolic activities are instigated in the connective tissue and major role of fibroblasts can be seen from the large quantities of collagen produced by them, which forms the main constituent of the extra cellular wound matrix and which are ultimately responsible for imparting tensile strength to the scar. When damaged, the orderly structure responsible for these properties will be disrupted and usually does not heal to yield the proper connective tissue.

Being the main cell type involved in protein production, fibroblasts are necessary for the regulation of extra cellular matrix. It is also necessary to control fibroblast growth over time to avoid biomaterial encapsulation.

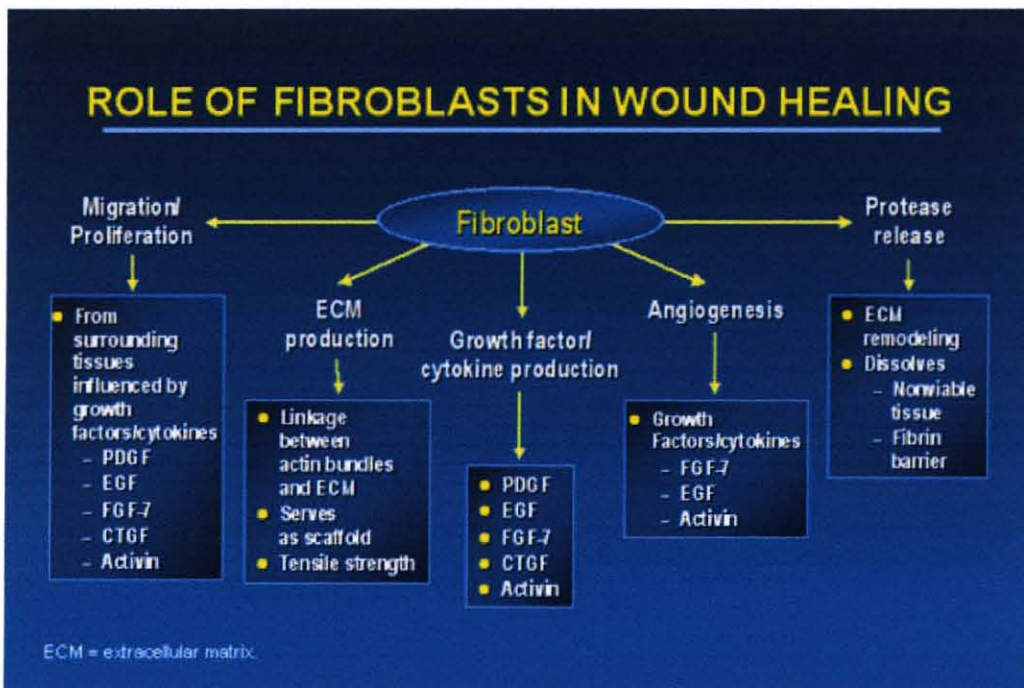


Figure 1.2 Role of Fibroblasts in Wound Healing⁹.

CHAPTER 2

POLYMERIC PROPERTIES INFLUENCING CELL GROWTH

Material surface properties influence the initial cellular events on the cell material interface. The intrinsic properties of the polymers combined with the knowledge of how such properties can be manipulated to achieve biocompatibility play a major role in assessing its influence on cell growth. The behavior of cells growing on a substrate can be explained by the phenomenon called Contact guidance. The cells tend to align themselves according to their preferred direction. This was explained by three main theories postulated¹⁰.

The first theory concentrates on focal adhesions, which are stiff rectangular transmembrane junctions, 10 micrometers long, and are usually the closest contacts between the extra cellular matrix of the cell and its substrate or cytoskeleton. The focal adhesions occur in an oriented way. Alignment of the entire cell is achieved by the actin filaments that originate from these points. Once the cell adheres to the surface in a definite direction, it will have the ability to grow and multiply on the substrate in the same way.

The second theory is based on the extracellular matrix protein adsorption. Cells produce proteins, which are adsorbed by the surface. These extracellular matrix proteins act as receptors for the cell to attach or adhere to the surface for further cell growth. The composition and the conformation of the adsorbed proteins depend on the surface properties of the substratum thus influencing cell growth. The third theory is mainly focused on the response of cells to mechanical local signals. The cytoskeleton of the cell

experiences various external forces from focal adhesions. The dynamic nature of the cytoskeleton will allow the cells to find an equilibrium state, to balance the internal and external forces favorable for their growth and differentiation. The equilibrium of forces is ultimately responsible for an aligned cell shape.

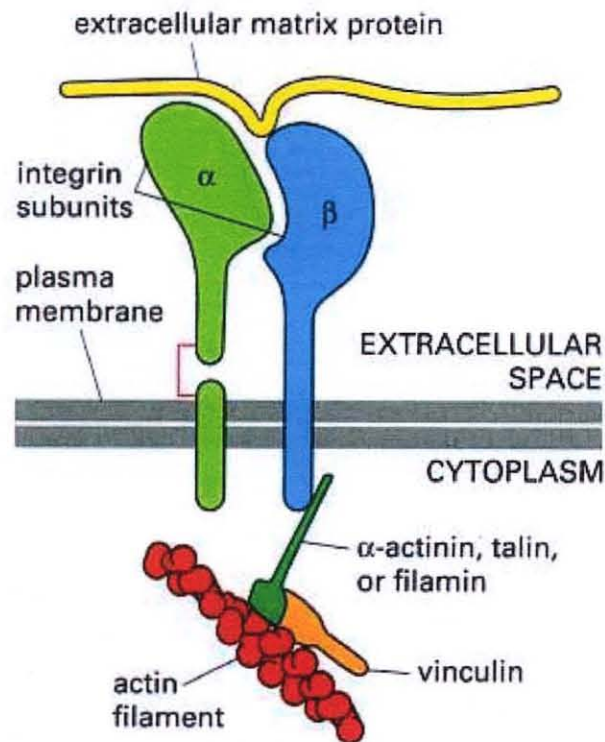


Figure 2.1 Focal Adhesions on the Extra Cellular Matrix¹¹.

In the mechanism of cell adhesion, actin filaments are broken down and elongated constantly in live cells. The actin microspikes result from the elongation of the filaments at the front edge of the cells or the cell lamellipodia. Cells tend to probe the substrate surface for suitable attachment places with these spikes. Once the required closest contact between the cells and the substrate is achieved, focal adhesion results. Mature actin fibers will be formed from actin polymerization at the site of focal adhesions. This process

continues throughout the entire cell until the cell reaches an equilibrium state, where the internal forces due to actin polymerization are balanced by the external forces due to focal adhesions. Since, the composition and the conformation of the proteins adsorbed by the extracellular matrix depend on the surface properties of the substrate, surface discontinuities might lead to unfavorable forces for focal adhesion. Walboomers et al. observed that a ridge on the surface acts as an unfavorable force for actin polymerization in the fibroblast cells¹⁰. Actin filaments tried to orient themselves along the groove direction to form focal adhesions by actin polymerization. Since the surface of the substrate is in immediate contact with the biological medium, the interface chemistry between biological systems and synthetic materials at the surface determines the nature of cell growth¹². Bulk properties determine the efficacy of the material in vivo, over its lifetime. The influence of processing techniques on the bulk, degradation and mechanical properties were well studied in the past. The present work assumes that, altering the order and processing history of the biomaterial should have a direct influence on the surface properties of the polymer.

Molecular orientation in the polymer, wettability, rate of degradation and the topographical features of the material were the four properties chosen to study the influence of process history. All these properties vary in the material according to the nature of the surface. The alignment of the polymer chains change with molecular orientation, thus affecting the nature of the surface. The wettability of the polymer in the physiological conditions varies according to the amount of surface exposed to the liquid. Degradation of the polymer in the saline environment takes place either by surface erosion or bulk degradation. In the former case, the integrity of the polymer bulk is

retained by the thinning of the material on the surface as the end products are washed off continuously from the surface. This is generally seen in hydrophobic materials. In the latter case, water enters the polymer bulk resulting in the hydrolytic degradation of the material. Hence the property of degradation is in part affected by the wettability property of the material and in turn, by the nature of the surface. The structure-property correlations in the polyarylate library were studied by Brocchini et al. Few of the material properties have been observed to follow a predictable fashion in the library⁷. Glass transition temperature was found to increase by 1°C intervals from polymer to polymer and air-water contact angle was found to increase by 0.5°C from polymer to polymer. Physio-mechanical properties were also found to follow a definite trend in the whole library. Fibroblast proliferation ranged from approximating that measured on tissue culture polystyrene to complete absence of proliferation.

2.1 Molecular Orientation

Polymers are large molecules with strong intermolecular forces and tangled chains existing in either crystalline or amorphous phase. The molecular orientation in the polymer can be related to the chemical composition and the structural details of the polymer. Regular arrangement of the monomers along the polymer chain, presence of polar and hydrogen bonding groups, small pendant groups and short polymer backbone chains have found to favor high degree of symmetry in the material, which would ultimately lead to better molecular orientation in the material.

Since, orientation of the polymer chains has a large effect on the degree of crystallinity existing in the polymer, stretching the bulk material either during its

synthesis or during its processing would likely affect the degree of orientation in the polymer. In semi-crystalline polymers, small crystals are connected to the soft amorphous regions by polymer chains. The nature of the semi-crystallinity is in between crystalline and amorphous polymers in which strength of the crystalline region is combined with flexibility of the amorphous region.

Variations in crystallinity may have the ability to change the roughness of the surface, on a nanometer scale. Washburn et al. showed that cells are exquisitely sensitive to these changes. It was shown that the rate of osteoblast proliferation on the smooth regions of the poly-(lactic acid) films is much greater than that on the rough regions and a monotonic variation in rate was observed as a function of roughness¹³. 3T3 fibroblasts were cultured on identical films of poly-(lactic acid) having different degrees of crystallinity and they have observed the growth rate to be lower on crystalline substrates than on amorphous substrates¹⁴. From the above findings, it can be inferred that processing conditions affecting the bulk or surface properties may have a direct influence on biological response.

2.2 Wettability

Wettability can be defined as an indication of biotolerance. It is a surface property which is unique for every material. Wetting is a thermodynamic process. The magnitude of free energy change involved determines the spontaneity of wetting, the rate at which the wetting process occurs, and how far it can progress against the external forces acting on it. Surface tension of the material is the unbalance of the molecular forces, when two different materials come in contact with each other to form an interface or a boundary. It

is the direct measure of the wettability of the material. Three interfacial boundary surfaces are involved in the wetting process. Each interface will have its own specific surface energy content. Wetting replaces an area of the solid- air interface by an equal area of solid- liquid interface and is generally accompanied by an extension of the liquid- air interface¹⁵. These surface relations vary progressively as wetting proceeds. Increase or decrease in the total surface energy takes place according to the extent of wetting in each interface. A hydrophilic polymer has high water vapor permeability. The extent of water absorption varies according to the degree of hydrophilicity. The surface chemistry of the polymer will allow the material to have high surface tension and have the ability to form hydrogen bonds with water. Depending on the chemical structure, the material either swells or shrinks due to water absorption.

The wetting property of the surface can be characterized by contact angle measurements. The angle made by the liquid on the interfacial boundary of the solid is measured as contact angle. A hydrophobic surface usually exhibits an angle greater than the angle exhibited by a hydrophilic surface. In case of water, zero contact angles can be seen at complete wetting. The contact angle values as measured by Brocchini et al. ranged from 64°C to 101°C and increased in about 0.5°C intervals from polymer to polymer. Structure property correlations in the combinatorial library clearly showed that there is decreased proliferation with increased hydrophobicity except for the polymers derived from oxygen containing diacids in their backbone, which had uniform cell growth irrespective of the nature of the surface⁷.

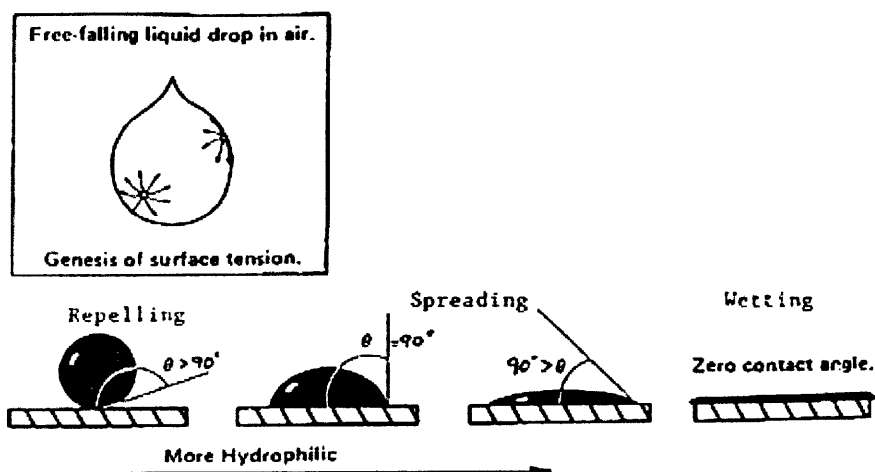


Figure 2.2 Contact Angle Measurement¹⁶.

Five different materials with different surface wettabilities were used by Georgi et al. to culture human skin fibroblasts¹⁷. They have observed decreased formation of actin stress fibers and focal adhesions with increase in hydrophobicity of the material, which indicates that fibroblasts are biased towards the hydrophilicity of the material. Since the polymers chosen for this study do not have any oxygen containing di-acids, fibroblast proliferation should be high on the most hydrophilic polymer. The interaction of different types of cells was studied on surfaces having different wettability gradient¹⁸. The maximum cell adhesion and growth of fibroblasts was observed on the surface having a contact angle with water at 55° . These results indicate that moderate hydrophilicity of the surfaces is required for cell growth.

2.3 Surface Morphology

The nature and morphology of the surface comes into the picture when the polymeric material is directly interacting with cells, tissues or the extra cellular matrix. Different

cells prefer different surfaces. Basement membranes are composed of extracellular matrix proteins serving as a substrate for overlying cellular structures. The topography of the basement membrane or the substrate surface is the complex network of pores, fibers and other micro dimensions. Hence, any synthetic surface with topographical features can regulate cellular behavior in a manner distinct from that of the chemistry of the surface. Specific interactions can be improved or suppressed by designing the material surface morphology. Cell surface interactions are usually desired with polymer scaffolds. Since, surface roughness increases the area of contact of the material with the surrounding tissue, cell surface interactions are not the same on rough and smooth surfaces. Surface roughness can enhance the cell ingrowth and tissue integration within the implants as long as precise control of the pores is maintained to control the interactions.

Fibroblasts and osteoblasts were cultured on poly-(hydroxybutyrate) films incorporated with different percentages of hydroxyhexanoate which resulted in films having varying degrees of roughness¹⁹. Smoothest surface was obtained by the PHB film having higher percentage of hydroxyhexanoate and the polymer surface roughness decreased with increase in the percentage of hydroxyhexanoate. Fibroblasts favored the smoothest surface obtained and osteoblasts favored the surface with appropriate roughness. Also, the down regulation of cell division on crystalline surfaces compared with the upregulation on phase separated polymer blends, observed by Washburn et.al suggested that the details of the topographical organization can have both positive and negative influences on cell proliferation.

CHAPTER 3

EXPERIMENTAL METHODS

3.1 Objective

The aim of the present study is in the area of forming new tissues by implantation of fibroblast cells on the polymer matrix and is specifically directed towards finding an appropriate polymer with desired properties. Poly-(lactic acid) is an already established promising biomaterial. Significant water uptake, faster degradation rate and formation of acidic end products by PLA were found to have adverse non-specific inflammatory effects in the physiological environment. In the context of such reports, the present study is aimed at the determination of the cell proliferation on polyarylates of the combinatorial library and comparing it with the poly-(lactic acid) in their drawn and undrawn conditions. The structure of the polymers was characterized using thermal analysis techniques to study and compare the physical behavior of the polymers. Poly(2,4) and poly(12,10) were chosen for the study because they differ in the length of the pendant chain and the backbone structure and they are located on the two extreme positions of the combinatorial library. The drawn and undrawn polymers differ in order and molecular orientation of individual units. It was assumed that a small change in the polymer surface structure might have a significant effect on the cell growth. Since the material properties of the polyarylate library vary in a predictable fashion, few of the properties that were studied for the chosen polyarylates can be used to predict the behavior of some of the remaining polymers in the library.

3.2 Method Adopted for Polymer Processing

Samples of the poly(DTD) dodecandioate and poly(DTE) adipate were synthesized in the laboratories of Advanced Material Design Co., and were supplied as powders of specified molecular weights in quantities ranging from 50 to several hundred grams. Poly-(lactic acid) was obtained from Boehringer Ingelheim. All the polymer samples were stored at 0°C and the films were processed in a carver press. The powdered form of the polymer of known weight was initially heated to a temperature of 120°C in a vacuum oven for around 12 hours to remove any traces of moisture that might have been absorbed. Once the moisture is removed, the powdered form of the polymer clustered into a lump, was removed carefully and placed on the kapton film surrounding the clean circular mold of thickness 0.2mm. The polymer along with the circular mold and the kapton film was placed in between the two hot plates of the carver press which was already heated to a temperature, slightly above the melting temperature of the polymer.

Table 3.1 Mold Temperatures suitable for Polymer Film Processing

| Polymer | Mold temperature (°C) |
|---------------------|-----------------------|
| Poly-(lactic acid) | 190 - 200 |
| (12,10)-polyarylate | 130 |
| (2,4)-polyarylate | 170 |

Pressure of about 15,000psi was applied for 10mins and the polymer was compression molded by maintaining the temperature of the plates. After extrusion, the film was rapidly quenched in water to prevent induction of crystallinity, irregularities and to obtain uniform thickness in the film. The drawability of the polymer might be

impeded, when the films are not rapidly quenched. Films of 0.2mm thickness were surrounded by kapton film and were stored at room temperature in a moisture free environment.

3.3 Polymer Drawing

The molecular orientation of the film is more regular, when the film is stretched or drawn. A temperature controlled heater was used to heat the polymer to their respective drawing temperatures and they were hand drawn or stretched to yield an average draw ratio of 5. The thickness of the film was definitely less than the undrawn film, but the specific strength and the molecular strength of the film would have definitely increased. The amount of orientation is mainly influenced by the draw ratio, temperature and the strain rate. Poly-(lactic acid) is a semi-crystalline material with a melting temperature in the range of 130°C to 180°C. Being amorphous in nature, poly(2,4) softens at around 80°C. Poly(12,10) was found to undergo molecular relaxation at around 40°C. Drawing the film pulls the individual chains into a roughly parallel organization making the oriented fibers strong and tough. In the case of crystalline polymers, the crystallite size was found to increase on drawing the polymer²⁰.

Table 3.2 Drawing Temperatures

| Polymer | Drawing temperature(°C) |
|---------------------|-------------------------|
| Poly-(lactic acid) | 100 |
| (12,10)-polyarylate | 55 |
| (2, 4)-polyarylate | 80 |

The amount of molecular orientation in the drawn and undrawn polymers, the temperature at which the thermal transitions takes place in the polymer was studied by Differential Scanning Calorimetric analysis. Thermogravimetric Analysis was utilized to study the amount of mass loss with temperature and the amount of water uptake by the polymer in the physiological environment. SEM images and the EPI-DIC images of the polymers have shown the difference in the structural arrangement of the fibers in the film. Shrinkage pattern of the poly(2,4) was measured in the saline conditions for a certain period to estimate the amount of shrinkage of the polymer in the saline media.

3.4 Cell Culturing Technique

The undrawn and drawn polymer samples of 6mm in diameter were chosen as they fit exactly at the bottom of the 96 well plates. The polymer is not supposed to float in the media as this might not allow sufficient nutrients to reach the cells growing on the polymer surfaces. Minimum essential eagle media with 10% fetal bovine serum and 1% antibiotic served as a growth media for the fibroblast cells. This was prepared by adding 56ml of fetal bovine serum and 6ml of antibiotic to 500ml of eagle media obtained from ATCC (American Type Culture Collection). The polymer samples were washed in ethanol to remove any traces of contamination. PBS was used to remove traces of ethanol from the samples. Poly(2,4) has shown sufficient shrinkage in the PBS solution, which led to the decrease in the surface area of the polymer.

Since poly-(propylene) well plates prevent cell migration, the polymer samples were transferred to the 96 well plates made of poly(propylene) to measure cell growth on the polymer samples at different time points. To minimize contamination, each prepared

cultured plate was exposed to UV light for 1 hour and they were preconditioned in the prepared media for a period of 24 hours. ATCC skin fibroblasts of second generation were cyro preserved. They were thawed and seeded at a density of 15000cells/cm². Cell growth was monitored for a period of 14 days.

Tissue culture plastic was used as a control. Tissue culture flasks were used to grow the remaining cells at the same fixed density. Nonadherent cells were washed off from the culture during biweekly feedings. When the cells are confluent, they were enzymatically detached with 0.25% trypsin containing 1mM EDTA for 2-4 min at 37°C. The detached cells were counted using heamocytometer and were subsequently replated for continued passaging. Media was changed every three days for the cells seeded on the polymers in the well plates and the proliferation rate of fibroblasts was measured using picogreen assay at four different time points. The remaining cells were cyropreserved at the end of second passage.

3.5 Differential Scanning Calorimetric Analysis

This technique can be used to measure the amount of crystallinity existing in the polymer. The amount of heat absorbed or evolved from the polymer can be measured in isothermal conditions. In this instrument, the heat flow into or out of the sample is measured as the sample is subjected to a programmed linear temperature range. The thermal properties of the sample are compared against a standard reference material which has no transition in the temperature range of interest, such as powdered alumina.

A balance is enclosed in an adiabatic enclosure. It has an empty aluminum pan, acting as a reference pan on one side and the polymer sealed in the aluminum pan is

placed on the other side. In this method, polymer is heated with reference to the reference pan. Both are heated at the same rate with a thermocouple monitoring the temperature of each holder. Heat is supplied electrically to maintain the temperatures of the two pans equal. The three main transition regions that can be observed on the DSC curve are glass transition temperature, crystallization temperature and temperature of melting.

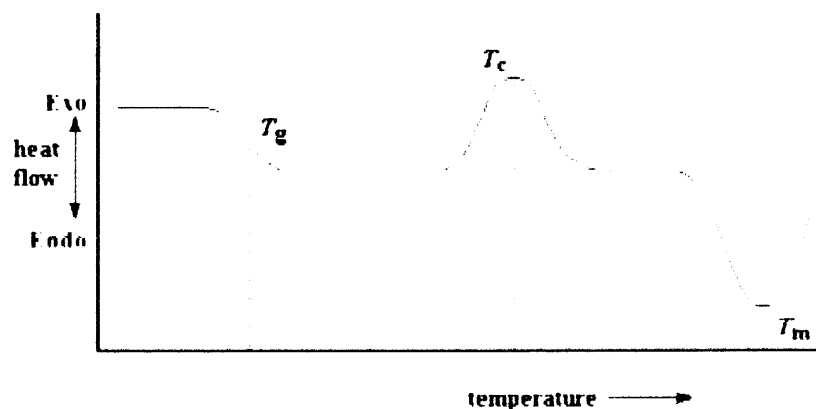


Figure 3.1 Transition Regions in the DSC Curve.

When the polymer is heated at isothermal conditions, transition regions are observed on the DSC curve as peaks and dips. Intermolecular relaxation processes in the tangled chain structure softens the polymer at the glass transition temperature, which is observed as huge dip on the curve. Addition of heat corresponds to its endothermal direction. Big peak is the curve that indicates crystalline temperature, where the polymer gives off large amount of heat to form a crystalline arrangement. When the polymer completely loses its orderly arrangement, it melts and absorbs lot of heat, which is shown as a huge dip or an endothermal peak on the DSC curve. At this stage, the crystallinity in the polymer will be totally destroyed. The breadth of the peaks can be related to the size

and degree of perfection of polymer crystals. The concept of glass transition is important to understand as it is the midpoint of the temperature range at which the polymer undergoes a phase change from brittle to rubbery state. Polymers are rigid and brittle with very little mobility below their glass transition temperatures. The motion of the individual segments becomes frozen with small scale molecular motion. Cooperative molecular motion is required for internal readjustments, which is achieved at temperatures well above T_g . The perfectly crystalline polymers do not show any glass transition temperature as the conformational rearrangement of the backbone cannot take place.

3.6 Thermogravimetric Analysis

Precise quantitative measurement of any mass changes in the polymer sample with increasing temperature at a controlled rate can be measured by this technique. It can directly measure dehydration, oxidation or degradation of the polymer with time and temperature. The change in mass may be due to formation or dissociation of various physical and chemical bonds at elevated temperatures. Hence, we can determine the chemical constitution of the material rather than the physical microstructure.

The sequence of physiochemical reactions that occur over specific temperatures and heating rates for a polymer are a function of its molecular structure and result in a characteristic curve in TGA analysis. The amount of water uptake by the polymer is measured by assuming that most of the water in the sample will evaporate at 100°C .

The sample is placed in an inert sample holder that hangs from the microgram balance during the entire experiment. An empty aluminum pan is placed in the sample holder. The weight of the entire sample holder configuration is electromagnetically

balanced, so that the balance weight can be zeroed before each run. Polymer sample is placed in the pan by properly aligning the sample to the groove of the hang wire and enclosing the whole arrangement in the nitrogen atmosphere. The sample was heated at a rate of $10^{\circ}\text{C}/\text{min}$. The amount of mass loss was measured as the weight was continuously monitored as a function of temperature. The amount of water uptake in the polymer was measured by comparing the mass loss in the dry polymer and the polymers soaked in the saline environment for a couple a days.

3.7 Aqueous Shrinkage

The shrinkage pattern of the material is extremely dependent on the process history. Since the shrinkage of the polymer in the saline environment reduces the surface area available for the cell growth, quantification of the shrinkage phenomenon is necessary. The origin of shrinkage lies in the amorphous regions. The amount of oriented and the non-oriented components in the amorphous phase and the molecular orientation of the material predominantly determine the amount of shrinkage in the material. Stronger materials are more prone to shrink as the shrinkage property is directly proportional to the amount of orientation existing in the material. Depending upon the polymer chemistry, the water may hydrolyse the polymer resulting in the degradation of its structure.

In the absence of polymer water reaction, plasticization of the polymer takes place resulting in the lowering of T_g and affecting the thermal and mechanical properties. When a semi-crystalline material is heated to a higher temperature, the typical shrinkage pattern in the material under zero load conditions can be shown in four distinct phases. The initial phase from room temperature to the glass transition temperature would be

characterized by small amounts of mass loss due to any traces of moisture in the film structure²⁰. Rapid shrinkage occurs due to relaxation of the oriented amorphous chains in the second phase. Hence, more the amount of oriented phase in the polymer, more it is prone to shrink. In the regions between the glass transition temperature and the melting temperature, irreversible shrinkage occurs due to chain folding and reorganization. Recrystallization takes place in this phase. Melting of the crystalline units in the structure would lead to sample failure in the final phase. This behavior is highly dependent upon process history and higher shrinkage results with greater orientation.

3.8 SEM and DIC Images

The surface morphology of the polymers can be viewed at a higher magnification by these methods. A finely focused electron beam scanned across the surface of the polymer generates the secondary electrons or some characteristic X-rays. The detectors collect these signals and form images on a cathode ray tube screen.

A differential interference contrast microscope works on the principle of transforming the phase shift of light induced by the refractive index of the sample into detectable amplitude difference. The light and dark areas of the image were obtained by the optical path differences caused by the differences in the index of refraction.

3.9 Contact Angle Analysis

The theory of contact angle is based on the equilibrium of an axisymmetric sessile drop on a flat horizontal, rigid surface. Contact angle on polymer surfaces is not only influenced by interfacial tensions, but also by many other phenomena including chemical

heterogeneity, surface roughness, molecular orientation, sorption layers, swelling and partial solubility of the polymer in the solution or presence of any low molecular constituents in the polymer.

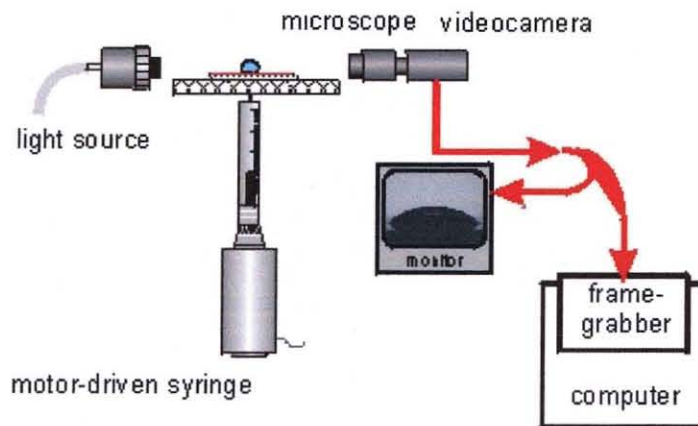


Figure 3.2 Contact Angle Apparatus²³.

The apparatus consists of a motor driven syringe, which places 10 μ l of water on the polymer sample of 6mm in diameter, placed in front of the light source on a sample. By careful observation, the microscope is focused to see the drop exactly on the center of the intersection of the two perpendicular lines, drawn on the lens. The air water contact angle made by the drop to the polymer surface is measured and the video camera was used to capture the pictures in the computer.

CHAPTER 4

RESULTS OBTAINED FROM THE STUDY

4.1 Fibroblast Proliferation Estimated using Pico Green Assay

The DNA content of the cells growing on the polymer is determined by the picogreen assay. The assay has a detection range from 25pg/ml to 1µg/ml which allows accurate quantitation of minimal amounts of widely variable samples. The double stranded DNA is quantitated by this ultra-sensitive fluorescent nucleic acid stain. Unlike, UV absorbance readings, the accuracy of the double stranded DNA quantitation is unaffected by the presence of single stranded RNA primers. Triton solution in required concentrations was used to expose the DNA content as it has the ability to dissolve the cell membrane. Microplate reader was used to detect the DNA content by performing fluorescence measurements at excitation detection of 485nm and emission detection of 528nm. The fluorescence measurements directly measure the DNA content in the cell which is directly proportional to the number of live cells growing on the polymer.

The results obtained from the DNA assay clearly show that the cell growth on the polymers is affected by various parameters. The influence of process history was evident from the difference in cell number as observed on drawn and undrawn polymers. Since, the trend followed by poly-(lactic acid) and the poly(2,4) is comparable, it is important to look into the common properties of the two polymers. The effect of polymer drawing on few of the surface properties was studied. The effect of polymer properties on the proliferation rate of the fibroblasts was previously studied for various applications.

Smoother surface, hydrophilic nature and less degree of molecular orientation was observed to favor fibroblast growth^{13, 18, 19}. The polymer having lower mass loss with less acidic end products favored the cell growth for long term applications.

4.2 Estimation of Hydrophilicity by Contact Angle Analysis

All the materials have the tendency to lower their surface area to attain lower entropy. The unbalance of the internal forces, which is the surface tension of the material, depends on the angle of contact at the interface of the solid-liquid boundary. High unbalances of the internal forces in the material provide more surface area to be available for the liquid at the interface boundary, thus making the material hydrophilic.

Polymer samples of uniform and flat surfaces were soaked in the PBS solution for a period of 7 days and the angle made by the water drop of 10 μ l was measured using contact angle apparatus. The angle of contact is high, when the area of contact at the interface boundary is low. This happens due to lack of active groups in the polymer to form hydrogen bonds with water, thus making the material hydrophobic.

Table 4.1 Contact Angle Measurements

| Name of the polymer | Undrawn polymer(deg) | Drawn polymer(deg) |
|---------------------|----------------------|--------------------|
| Poly-(lactic acid) | 57.521 | 61.814 |
| (12,10)-polyarylate | 89.216 | 87.148 |
| (2,4)-polyarylate | 44.630 | 48.561 |

The hydrophilic nature was the highest with poly(2,4) in its undrawn state. The drawn form of this polymer shows a higher contact angle value (less hydrophilic) as compared to its undrawn form. As there was reduction in the surface area with considerable amount of shrinkage in the drawn form of poly(2,4), the contact area at the interface boundary decreased and the angle of contact was higher.

The contact angle value obtained for the poly(12,10) clearly shows that the polymer is hydrophobic. Slight decrease in the angle for its drawn state should be due to the strong hydrogen bonds held between the amide groups of the polymer which was achieved by the orientation of the polymer chains on stretching.

Poly-(lactic acid) was observed to be less hydrophilic than poly(2,4) with its drawn form exhibiting a slight hydrophobicity as compared to its undrawn form. The hydrophilicity of the undrawn poly-(lactic acid) is due to the polar oxygen linkages in the polymer chain which bind to the water molecules by hydrogen bonds. The polymer chains are held together by hydrogen bonds between acid groups on stretching. The polar oxygen groups on the polymer chain bind to some of the hydrogen bonds resulted from stretching in the drawn form. Due to this reason, there are less number of oxygen linkages available for binding with water molecules in its drawn form, which makes the polymer less hydrophilic.

From the above observation, poly(2,4) is the most hydrophilic polymer. It was previously observed that fibroblasts prefer surfaces of moderate hydrophilicity with a contact angle of around 55° . Hence undrawn poly-(lactic acid) and poly(2, 4) in its drawn state should mostly favor fibroblast growth.

4.3 Water Uptake and Mass Loss by TGA Analysis

The hydrophilicity of the material can also be estimated from the amount of water uptake as observed from TGA analysis. The amount of water uptake in the polymer was estimated by performing TGA analysis on the dry film and on the film kept in the saline environment for a certain period.

4.3.1 Water Uptake in the Polymers

The bioadhesion potential of the polymer is governed by the amount of water uptake in the saline environment. Polymers undergoing degradation by bulk erosion mechanism have the tendency to absorb more water. Water behaves as a plasticizer by influencing the properties of the polymer.

Table 4.2 Water Uptake in the Polymers for a period of 7 days

| Polymer | Undrawn | Drawn |
|----------------------|---------|-------|
| Poly-(lactic acid) | 1% | <1% |
| (2,4)- polyarylate | <2% | 8% |
| (12,10)- polyarylate | <1% | <1% |

Undrawn and drawn polymers were incubated in the PBS solution for a period of 7 days at 37⁰C. As seen in the Figure A.1, there was no significant water uptake with the poly(12,10) in its drawn and undrawn states. This can be attributed to the hydrophobicity of the polymer evident from the values of the contact angle. In the Figure A.2, poly(2,4) showed less than 2% water absorption in its undrawn state and nearly 8% water uptake in its drawn form. The high amount of water uptake by the poly(2,4) resulted in shrinkage

of the polymer. From the Figure A.3, we can infer that poly-(lactic acid) did not show any significant water absorption in its drawn and undrawn state.

Since, poly-(lactic acid) was already known for its high water uptake, the undrawn polymers were incubated in the PBS for a period of 15 days at 37⁰C.

Table 4.3 Water Uptake by the Undrawn Polymers for a period of 15 days

| Polymer | Undrawn |
|----------------------|---------|
| Poly-(lactic acid) | 7% |
| (2,4)- polyarylate | 2% |
| (12,10)- polyarylate | <1% |

The level of water uptake increased with polylactic acid by nearly 6%, which is shown in the Figure A.4. The amount of water absorption by the poly(2, 4) and the poly(12,10) did not have any significant changes, showing less than 1% water uptake by (12,10) polymer and 2% by the (2,4) polymer respectively. As shown in the Figure A.5, poly-(lactic acid) has the highest water uptake among the undrawn forms and the amount of water absorption was found to increase with time. This might have an effect on its degradation rate as the polymer degrades hydrolytically. Hence, in terms of water uptake, higher proliferation rate could be seen on the undrawn and drawn forms of the poly(12,10).

Higher water uptake by the polymer might be a disadvantage in long term applications as it might lead to swelling and unpredictable water permeability, which might also cause reduction in mechanical properties. Shrinkage in the polymer causes

reduction in the dimensional stability and mechanical performance under biorelevant conditions²¹.

4.3.2 Mass Loss in the Polymers

The amount of mass loss in the polymeric materials was studied for a short period of time by performing TGA analysis on the polymer. The undrawn and the drawn form of the polymers were stored at room temperatures and were heated to a temperature of about 350⁰C at 10⁰C/min. The mass loss in the polymers was compared at around 300⁰C.

Table 4.4 Comparison of Mass Loss in the Polymers at 300⁰C

| Polymer | Undrawn | Drawn |
|---------------------|---------|-------|
| Poly-(lactic acid) | 7% | 5% |
| (12,10)-polyarylate | <1% | <1% |
| (2,4)-polyarylate | <2% | <2% |

As seen in the Figure B.1, there was about 7 % mass loss with the undrawn poly-(lactic acid) with 5% mass loss in its drawn form. From the Figures B.2 and B.3, 2% mass loss was observed with the undrawn and the drawn forms of the poly(2,4) and less than 1% mass loss in case of poly(12,10) respectively. The high mass loss in the undrawn PLA is due to the simple monomer groups. Structurally, the degradation rate in the polyarylates was improved by minimizing the number of inter-chain hydrogen bonding sites per monomer unit. This was achieved by the desaminotyrosine unit in the polymer⁶. The mass loss was slightly lowered in the drawn form of the poly-(lactic acid) as the polymer chains are more oriented. The hydrogen bond linkages across the polymer chains

formed due to stretching gives more stability to the structure. Figure B.4 shows the comparison of mass loss for all the three polymers in their undrawn state.

The mass loss was examined in the saline conditions by incubating the polymers for a period of 7 days in the PBS solution at 37⁰C. Though it is important for the implant to be degraded and resorbed in the body as early as possible in most of the applications, the release rate of the degradation products is high with the materials degrading at a higher rate. One of the example is poly-(lactic acid), which degrades faster and exceeds the clearance capacities of the surrounding tissue. Also, poly-(lactic acid) undergoes bulk degradation, which is achieved by the water entering the polymer matrix to hydrolyze the lactic acid monomers. The end products would be usually carboxylic acids, which might be responsible for the further drop in pH leading to inflammation and decrease in the mechanical properties at the earlier stages of degradation.

Thermogravimetric Analysis showed that poly-(lactic acid) did not have any considerable difference in the mass loss under saline conditions both in its drawn and undrawn form, which is shown in the Figure B.5. Though poly-(lactic acid) degrades hydrolytically by bulk mechanism, the mass loss was not high in a period of 7 days as it takes some time for the water molecules to seep into the polymer bulk. The extent of hydrolytic cleavage of the acid groups in the drawn poly-(lactic acid) is lowered due to less number of polar groups available for binding with water molecules. The effect of saline conditions on the amount of mass loss was negligible with the undrawn and drawn forms of the poly(12,10) and the undrawn form of the poly(2,4) which was evident from the Figures B.6 and B.7. Decrease in weight of less than 1% was observed with the drawn form of the (2,4) polymer. Hence, the degradation mechanism in the poly(2,4) may not be

due to bulk degradation. The water uptake by the polymer is not affecting the mass loss in the polymer. It is majorly responsible for the shrinkage of the material. The reduction in volume would have affected the density of the (2,4)-polyarylate.

Table 4.5 Comparison of Mass Loss for the Polymers in Saline Conditions

| Polymer | Undrawn | Drawn |
|---------------------|---------|-------|
| Poly-(lactic acid) | 5% | 3% |
| (12,10)-polyarylate | <1% | <1% |
| (2,4)-polyarylate | 2% | <1% |

The degradation rate was considered to be slow with materials having less crystallinity¹⁹. Increase in molecular orientation in PLA lowered the degradation rate to a certain extent which would also contribute towards simultaneous increase in the mechanical strength of the polymer. In terms of degradation, the drawn and the undrawn forms of the poly(12,10) was found to be the most favorable one. The poly(2,4) also exhibits favorable properties in terms of degradation towards fibroblast growth.

4.4 Determination of Transition Temperatures from DSC Analysis

DSC analysis of the drawn and undrawn samples was performed at isothermal conditions in the heat /cool/ heat cycle in the temperature range of 20⁰C-230⁰C. The characteristic curve obtained for the poly-(lactic acid) showed three transition temperature ranges including glass transition temperature, crystallization temperature and the melting temperature. There were no significant transition regions with the poly (2,4) apart for its glass transition temperature. Poly(12,10) showed multiple dips in the range of 50⁰C to

60°C due to enthalpic reorganizations occurring in the polymer. The DSC analysis was performed on the polymers at different temperature ranges to study the complicated behavior of the (12,10)-polyarylate. (2,4)-polyarylate and the poly-(lactic acid) did not show any significant difference on heating and cooling at different temperature ranges. Analysis on the (2,4) polymer is shown in the Figure C.1. The different arrangement of the peaks indicating the structural conversions in the polymer were observed with (12,10)-polyarylate, which is evident from the Figure C.2. These major peaks seem to appear in the same temperature range in every run indicating different modes of organization explaining its dependence on temperature and process history. This was the same case with poly(12,10) in its drawn form and the graph is shown in Figure C.3.

The transition regions in the drawn polymers had a slight shift as compared to undrawn polymers, which was characteristic to every polymer. The plots are shown in the Figures C.4 and C.5 for poly-(lactic acid) and poly(12,10) respectively. The amount of heat flow into and out of the polymer was slightly higher with the drawn form of the polymer. The endothermic and exothermic changes were high due to increase in the molecular orientation of the material. The polymer required more amount of heat absorption to break the extra hydrogen bonds formed due to stretching and the heat evolution was more on the reverse cycle to form these extra bonds.

In the drawn state, T_g was lowered for the poly-(lactic acid) and the poly(2,4). The amorphous nature of the poly(2,4) led to a slightly higher shift in the glass transition temperature as compared to the poly-(lactic acid). The arrangement of the polymer chains is more regular in the case of drawn polymers due to higher orientation of the molecular units resulted from the formation of hydrogen bonds. This arrangement will need a lower

temperature to attain molecular mobility in the polymer, leading to the reduction in the glass transition temperature.

The enthalpic reorganizations or the structural conversions in the drawn form of the (12,10)-polyarylate were initiated at a slightly lower temperature and they were found to last for a wider temperature range. This behavior of poly(12,10) was observed for different temperature ranges. It was previously observed that the (12,10) polymer exists in different modes of organization and these transformations are reversible as seen in enantiotropic polymorphism. The two modes of organization were observed at different temperatures. Higher temperature mode exists at a lower temperature range in a metastable state. The lower temperature mode is associated with ordered hydrogen bonding in its amide regions²³. Disordered hydrogen bonds were observed at the higher temperature mode of poly(12,10) by FTIR analysis. Also, the water absorption characteristics of the poly(12,10) at this mode were found to be similar with the lower amorphous polyarylates. This behavior of poly(12,10) led to the conclusion that the poly(12,10) is amorphous in the higher temperature mode. From the TSC analysis, it was observed in the previous study that poly(12,10) attains molecular mobility in the film form²³ at around 40⁰C. Hence two modes of non-crystalline organizations were observed in the structure of (12,10)-polyarylate.

4.5 Surface Morphology from the SEM and EPI-DIC Images

Drawing the polymers can have a significant influence on the surface topography of the material. The orientation of the molecular chains might align the fibers on the surface in one of the directions. The undrawn and the drawn polymer samples of the two

polyarylates of 6mm in diameter were punched out and stored at room temperature. Scanning Electron Microscopy was used to obtain the images at 2.5KX magnification.

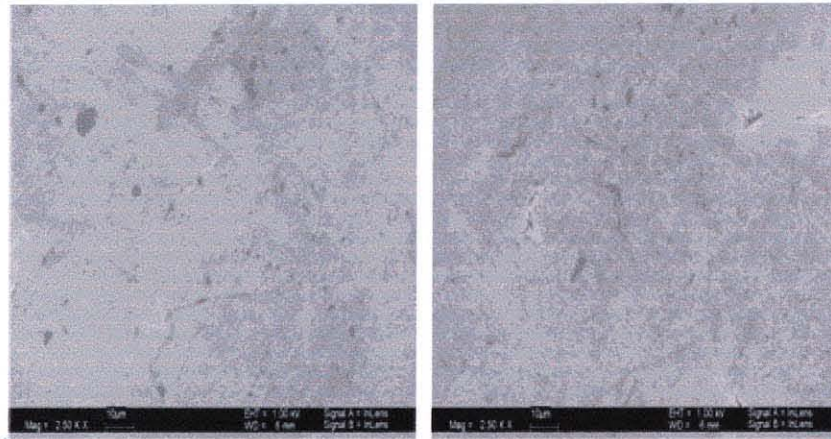


Figure 4.1 Poly(2,4) in its Undrawn and Drawn State at 2.5KX.

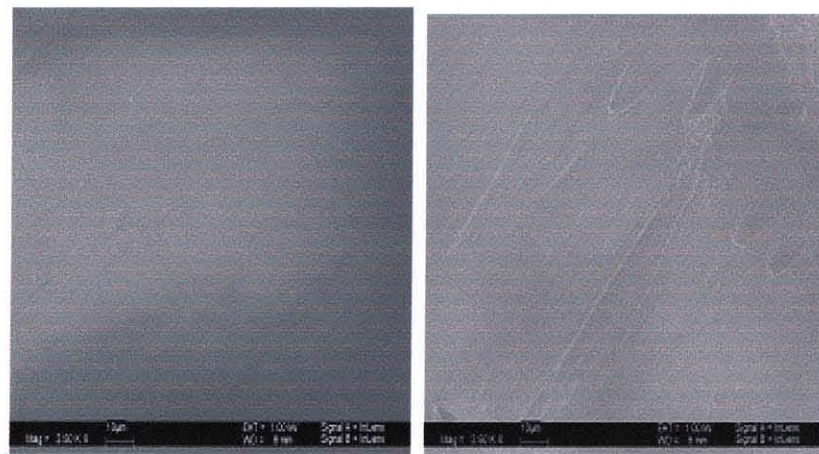


Figure 4.2 Poly(12,10) in its Undrawn and Drawn State at 2.5KX.

The difference in the refractive indices of the polymer samples led to the absorption of wavelengths of different amplitude. The influence of process history on the surface topography of the polymer can be observed from the difference in the color

intensities of the drawn and undrawn forms of the same type of the polymers.

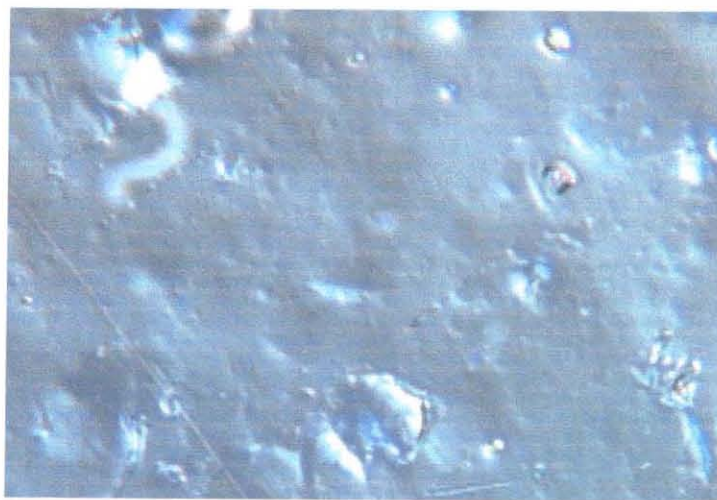


Figure 4.3 Poly (2,4) Undrawn at 50X.

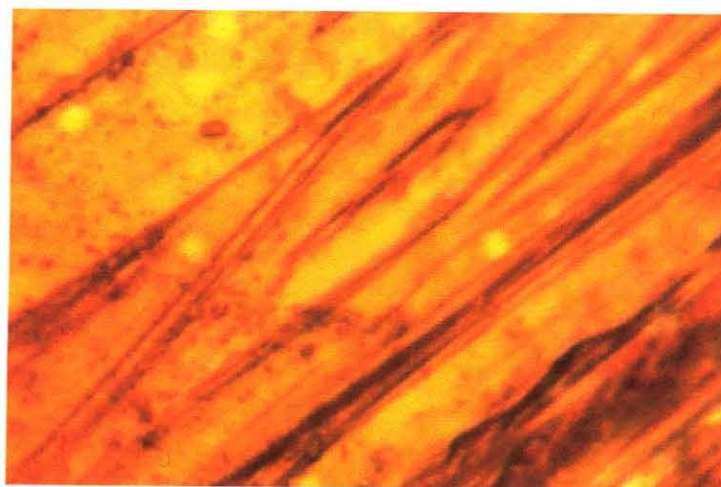


Figure 4.4 Poly (2,4) Drawn at 50X.

The alignment of fibers were clearly seen, when the image of the poly(2,4) film in its drawn state was focused in the cross direction.



Figure 4.6 Poly (12,10) Undrawn at 50X.

Poly(12,10) appears smoother as compared to poly(2,4). The alignment of fibers are visible only in the cross direction in all the drawn forms of the polymer, suggesting that the stretching of the polymer has resulted in this kind of orientation.



Figure 4.5 Poly (12,10) Drawn at 50X.

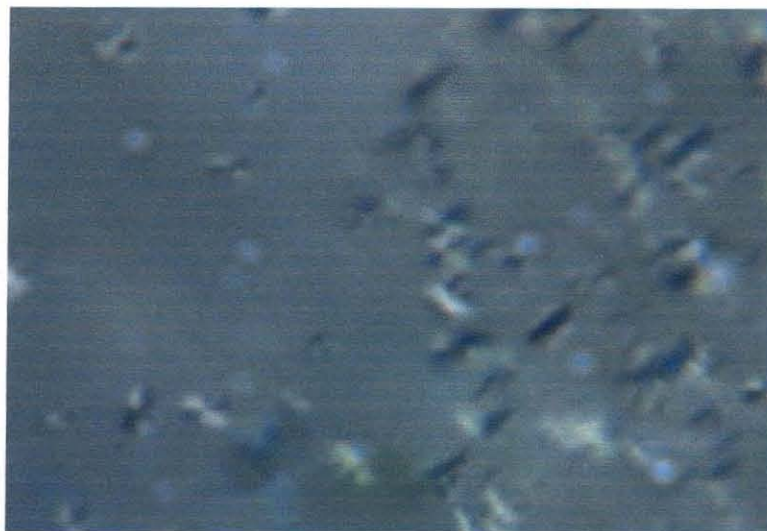


Figure 4.7 Undrawn Poly-(lactic acid) at 50X.



Figure 4.8 Drawn Poly-(Lactic Acid) at 50X.

The orientation of fibers in the drawn polymers was observed to a certain extent in the drawn images of the polymer samples as compared to their undrawn forms. The aligned fibers in the poly(12,10) appear thicker as compared to the other polymers.

4.6 Determination of the Shrinkage Behavior of the Polymers

The shrinkage of the material can be explained by a simple equation, given by

$$St = (1-X) Fa^{20}.$$

As explained before, the amount of shrinkage in the material is directly proportional to the (Fa) or the amount of molecular orientation existing in the material. X represents the crystallinity in the sample and the amount of shrinkage is high with less amount of crystallinity in the material. Amorphous content was found to favor the amount of shrinkage. There are two phases in the amorphous region- oriented phase and the non-oriented phase. Among the two phases, the oriented phase is likely to have more effect on the extent of shrinkage. The more the amount of oriented phase in the amorphous region of the polymer, more it is prone to shrink. It was observed that the polymer becomes more stabilized with the increase in the orientation of the material.

Polymers of fixed length were incubated in saline conditions for a period of 14 days. The decrease in length was measured to estimate the amount of shrinkage in all the polymers. Highest amount of shrinkage was observed with poly(2,4) in its drawn state.

Table 4.6 Percentage Shrinkage in the Polymer samples in Saline Conditions

| Polymer | Undrawn | Drawn |
|---------------------|---------|--------|
| Poly-(lactic acid) | -0.17 | -2.3 |
| (12,10)-polyarylate | -2.48 | -1.96 |
| (2,4)-polyarylate | -1.22 | -37.84 |

The shrinkage property can be considered negligible in the rest of the polymers. The high amorphous content and the increase in the oriented phase of the amorphous

regions by polymer stretching could be a major factor in the drawn (2,4)-polyarylate. The effect of molecular orientation on the property of shrinkage was clearly seen by comparing the values of the drawn and the undrawn forms of the (2,4)-polyarylate.

4.7 Results Obtained from DNA Assay

The proliferation of fibroblasts on the different polymers was compared by plotting the values obtained from the DNA assay at different time points. The error bars were graphically marked to show potential error in the data.

Poly-(lactic acid) favored the fibroblast growth in its undrawn and drawn state. From the characterization studies, it was observed that the polymer favors the cell growth mostly due to its hydrophilicity. The cell number on day 4 does not significantly differ for the drawn and the undrawn states. The effect of molecular orientation on the cell growth was not significant at this stage. This was also evident from the mass loss in the two states of the polymer. Insignificant water uptake by the two polymers on day 4 also contributed towards better cell growth on these polymers.

The cell number on day 7 increased considerably on the undrawn form of the poly-(lactic acid). As observed from TGA, the amount of water uptake was insignificant at this stage, which might be contributing towards better cell growth. Though the mass loss was higher with the undrawn form, there is a decrease in cell number with the drawn form of the polymer showing the effect of increase in the molecular orientation of the polymer. The cell number on day 11 was almost equal on both the states. This might be due to high water uptake, resulting in the swelling of the undrawn polymer. The mass loss also increased at this stage to a higher rate with the undrawn polymer in the saline

environment.

The cell proliferation on day 14 shows a drop in the cell number on both the states of the polymer with more drop in the undrawn form of the polymer. Comparing the properties studied in this work, the drop in the cell number with the undrawn poly-(lactic acid) can be clearly related to the high water uptake and high mass loss as observed from TGA. The tendency of the material to form acidic end products at higher mass loss is also one of the factors. Apart from all the properties of the polymer, the decrease in cell number on day 14 can be due to the high density of cell number achieved on the polymer surface at the previous time point. Sufficient space was not available for proper cell adhesion and differentiation.

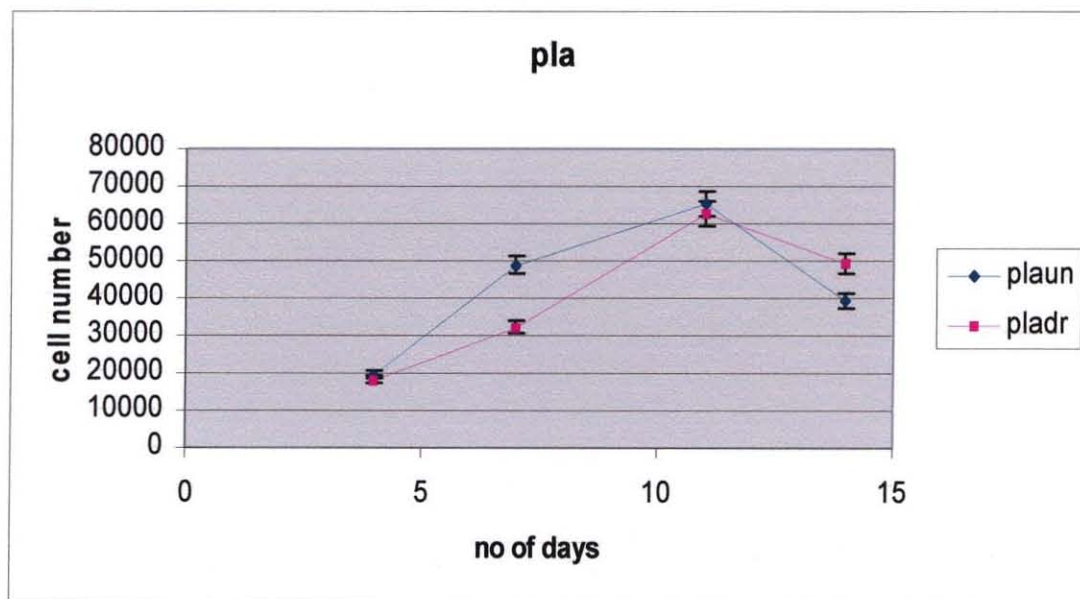


Figure 4.9 Cell Growth on Poly-(lactic acid) at Different Time Points.

From the characterization studies, the hydrophilic nature of the poly(2,4), its slow degradation rate and the amorphous nature can be the positive factors towards cell proliferation on these substrates. The shrinkage behavior of the polymer has reduced the

surface area of the drawn form to nearly 75%. The cell number observed on this form should be related to the area available for cell growth.

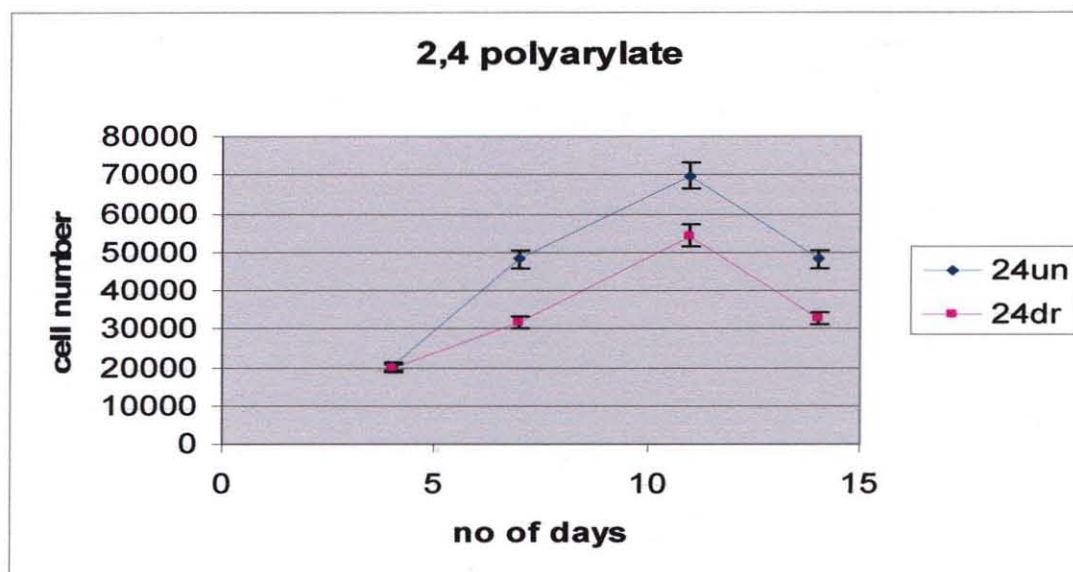


Figure 4.10 Cell Growth on (2, 4)-Polyarylate.

From the figure 4.10, the trend followed by the poly(2,4) could be compared to poly-(lactic acid). The cell number on day 4 was almost equal in both the forms. It was also observed visually, that the shrinkage in the drawn form was very less at this time point. Though, it was observed that the cell number on the undrawn form was higher on day 7, the reduction in the surface area of the drawn polymer by nearly 25% is important to be noted. From TGA, the level of water uptake has increased on day 7, which resulted in the shrinkage of the polymer. The mass loss of the drawn and the undrawn forms did not show much variation. Day 11 had higher level of shrinkage in the drawn polymer with the increase in cell growth. On day 14, the decrease in the cell number on both the forms can be attributed to the high cell densities achieved at previous time point. The drawn form of the poly(2,4) has a contact angle value closer to 55 degrees. The cell

number on the reduced surface area of the drawn form can be, in part due to the moderate hydrophilicity in the material, favorable for fibroblast proliferation. The drop in cell growth on the drawn form of the polymer might be due to saturation of the surface available for cell growth. Also, the comparable trend on the poly-(lactic acid) and the poly(2,4) must have some correlation with the short chain structures associated with these polymers.

Least mass loss and the insignificant water uptake by the poly(12,10) in addition to its hydrophobicity made the polymer follow a different trend towards cell growth as compared to the above two polymers.

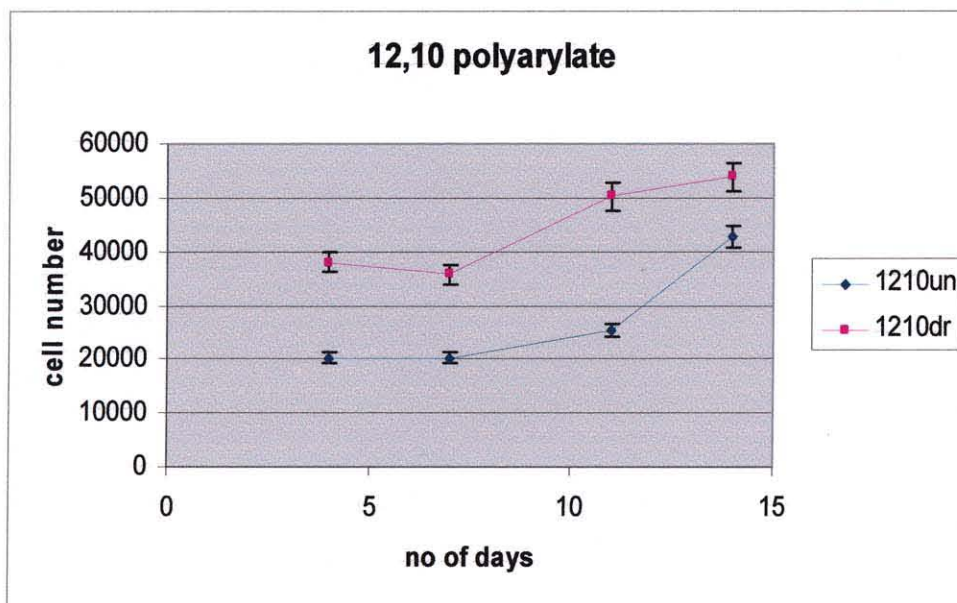


Figure 4.11 Cell Growth on (12,10)-Polyarylate.

The interesting observation was with the higher growth rate obtained by the drawn polyarylate, which is in contrast with the results obtained with poly-(lactic acid) and (2,4) polymer. The cell growth on the different time points increased with the number

of days, with the higher cell number on the drawn form of the polymer. The cell number remained almost the same on day 4 and day 7 in both the states of the polymer. As observed from contact angle analysis, the slight hydrophilic nature of the drawn form of the polymer resulted from the hydrogen bonds along the polymer chains should have a significant effect on the higher cell number observed on the drawn form. The ordered hydrogen bonds were observed for the poly(12,10) at a lower temperature range at around 40° . This property of the poly(12,10) was highly dependent on the thermal conditions and the processing history of the polymer. Since this temperature is closer to the saline temperature, the cell growth favored by the polymer might be due to some structural influence, especially on the drawn form of the polymer.

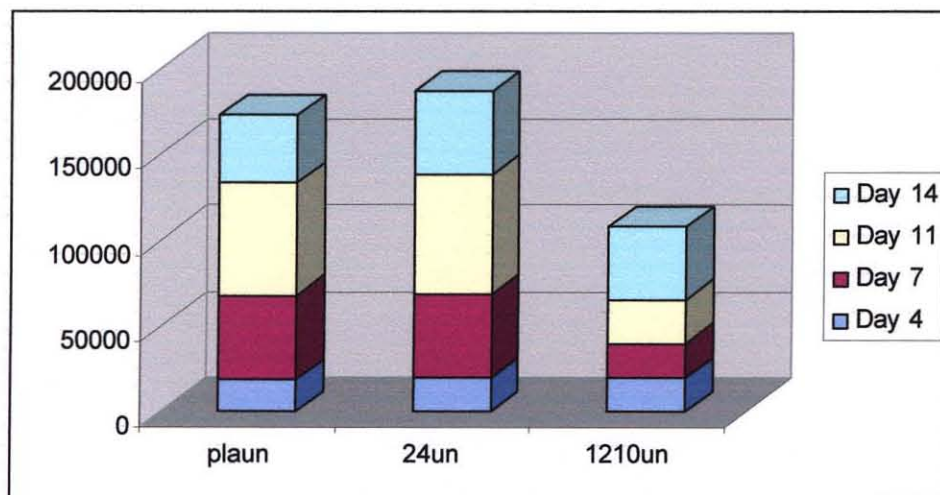


Figure 4.12 Fibroblast Proliferation on the Undrawn Polymers at Different Time Points.

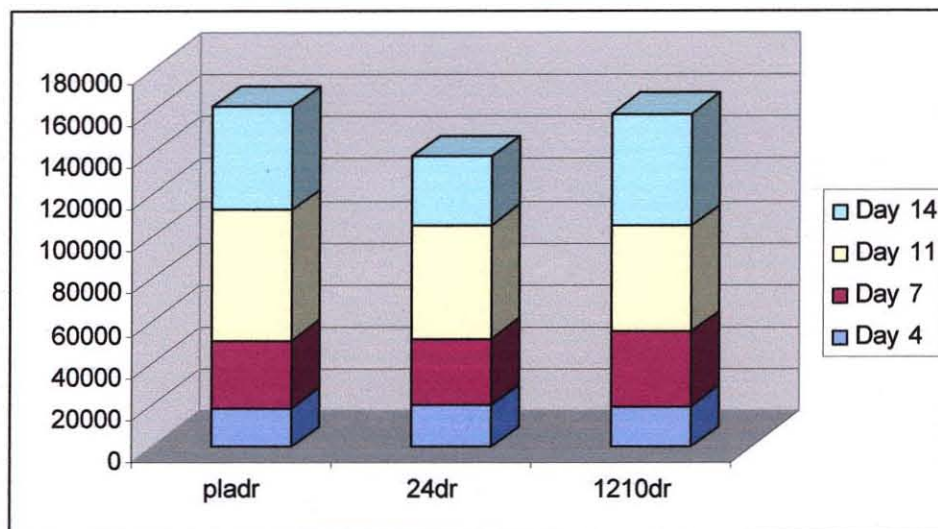


Figure 4.13 Fibroblast Growth on the Drawn Polymers at Different Time Points.

The cell number on the drawn and undrawn polymers were compared and shown in the Figures D.1 and D.2. The seeding of the cells on all the polymers was done in the same conditions of saline environment, irrespective of the properties of the polymer. The highest and the least cell growth seen among all the polymers was listed in the table below.

Table 4.7 Highest and Least Growth at Different Time Points

| Time points | Highest proliferation | Least proliferation |
|-------------|----------------------------|--------------------------|
| Day 4 | Poly(2,4) undrawn | Drawn poly-(lactic acid) |
| Day 7 | Undrawn poly-(lactic acid) | Poly(12,10) undrawn |
| Day 11 | Poly(2,4) undrawn | Poly(12,10) undrawn |
| Day 14 | Poly(12,10) drawn | Poly(2,4) drawn |

CHAPTER 5

CONCLUSION AND FUTURE SUGGESTIONS

The influence of processing conditions was clearly observed on every property studied, showing the interdependence of properties and their combined effect on cell growth. In the case of undrawn polymers, proliferation rate increased with increasing hydrophilicity. From the previous studies, fibroblasts were found to favor surfaces exhibiting contact angle in the range of 45°C - 60°C . Polystyrene is mostly used as control in tissue engineering as it favors good amount of cell growth on its surface. The contact angle of water on the surface of polystyrene was also found to be in the same range. From the contact angle data obtained in the study, the values in this range were obtained with undrawn poly-(lactic acid) and undrawn (2,4)-polyarylate, which showed good amount of cell growth. Hence, fibroblast growth on the polyarylates with lower values of R and Y should increase with increase in hydrophilicity as long as the values are in the range of 45°C - 60°C . Stretching the polymer favored polyarylates by increasing hydrophilicity in the case of poly(2,4) and decreasing hydrophobicity in the case of poly(12,10). Hence, depending upon the value of contact angle in its undrawn form, the processing conditions can be altered to obtain a polyarylate most suitable for fibroblast growth.

The amount of water uptake was low with poly(12,10), which should be the same case with the polyarylates with higher values of R and Y. The shrinkage of materials was seen with the lower members of the polyarylate library indicating the plasticization effects by water, which was high on increasing the molecular orientation.

Hence, changing the processing conditions altered the properties of the polymer, which were characteristic to every polymer. From the results of cell growth on these substrates, poly(12,10) was favored in its drawn state and poly(2,4) in its undrawn state. Since the polymers in the polyarylate library have high molecular weights and similar polydispersities, they should vary in a predictable fashion. Hence, polymers with lower values of R and Y should be favored in their undrawn state and the higher members of the library should be preferred in their oriented state. Thus, the present study showed that fibroblast cell growth was greatly influenced by the processing conditions. Depending upon the chemical structure of the polymer, the process history can be altered to favor cell growth. The influence of process histories on the fibroblast growth observed in the study can be used as a basis to find a suitable polymer according to the site and purpose of use. The comparison of cell growth at higher draw ratios would be a better way to understand the cell growth on the higher members in the polyarylate library. Different processing conditions like annealing techniques which can reduce the shrinkage in the lower members of the polyarylates would be a better way to improve cell growth on these polymers. Since the bioadhesion potential depends upon the nature of surface, observing the topographical features by measuring the roughness of the surfaces would be a better way to predict cell growth on the polymers. Microscopic observation of the attachment of cells on the surfaces at different time points can be used to study the surface details in a better way.

APPENDIX A

WATER UPTAKE BY THE POLYMERS

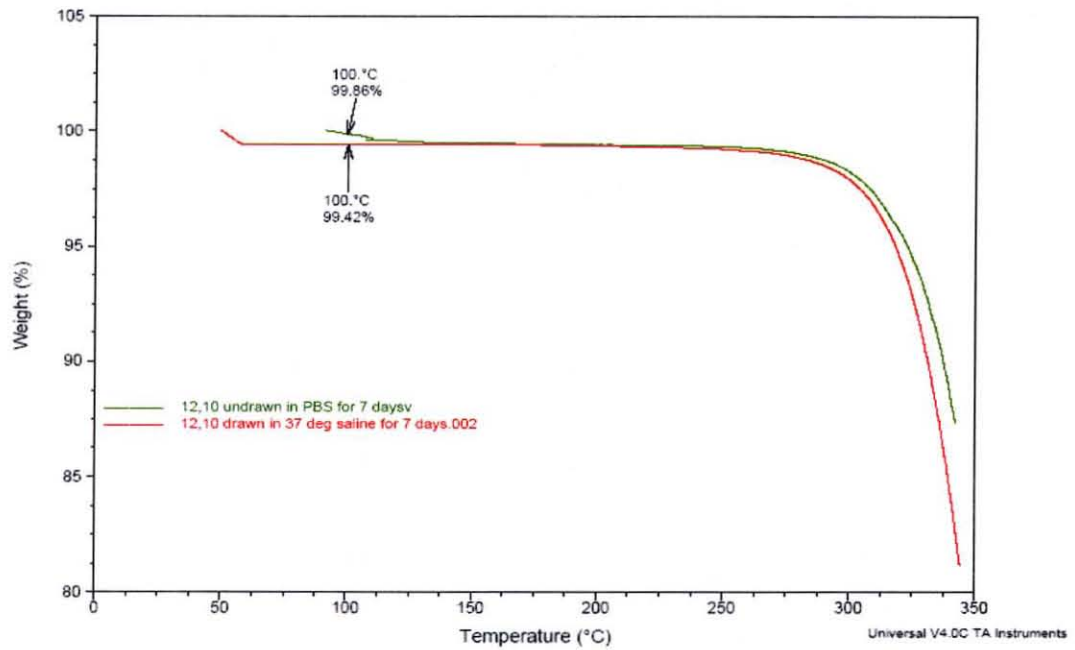


Figure A.1 Poly(12,10) in Undrawn and Drawn Forms after 7 Days in PBS.

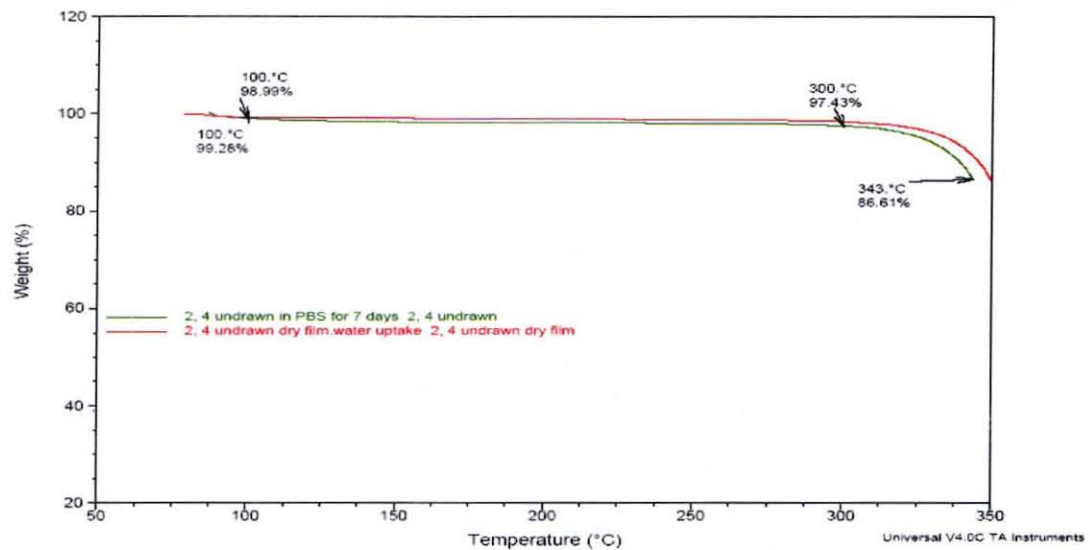


Figure A.2 Poly(2,4) in its Undrawn State at Room Temperature and after 7 Days in PBS.

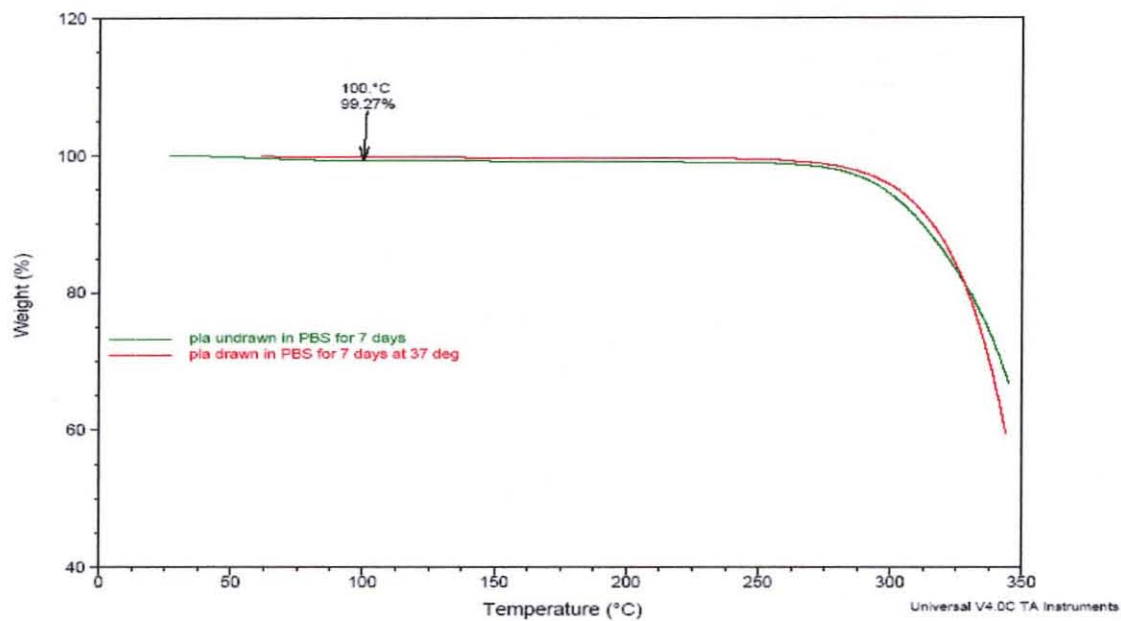


Figure A.3 Undrawn and Drawn Poly-(lactic acid) in PBS for 7 Days.

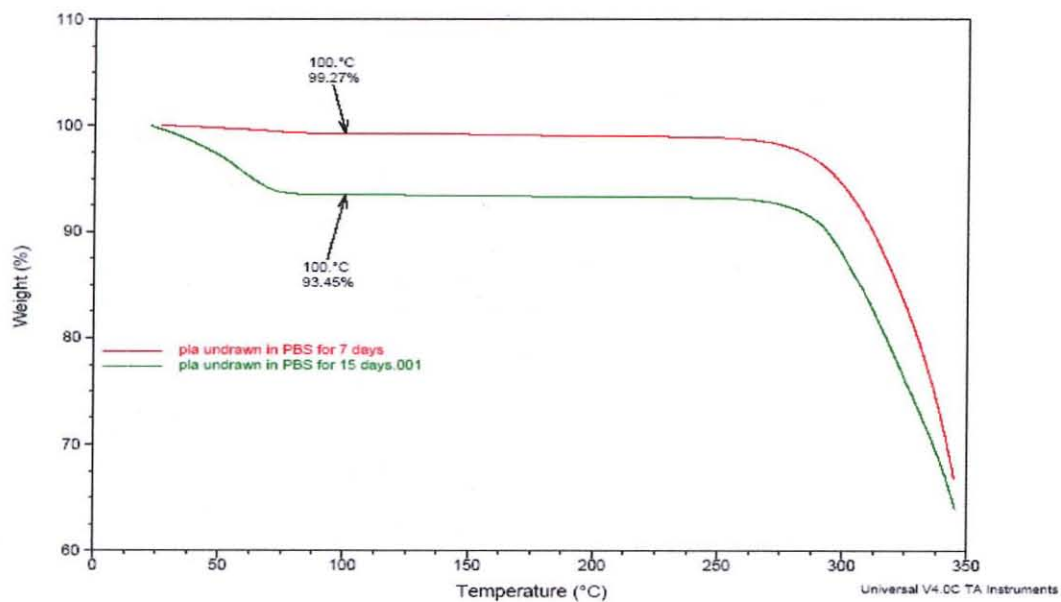


Figure A.4 Undrawn Poly-(lactic acid) in PBS for 7 Days and 15 Days.

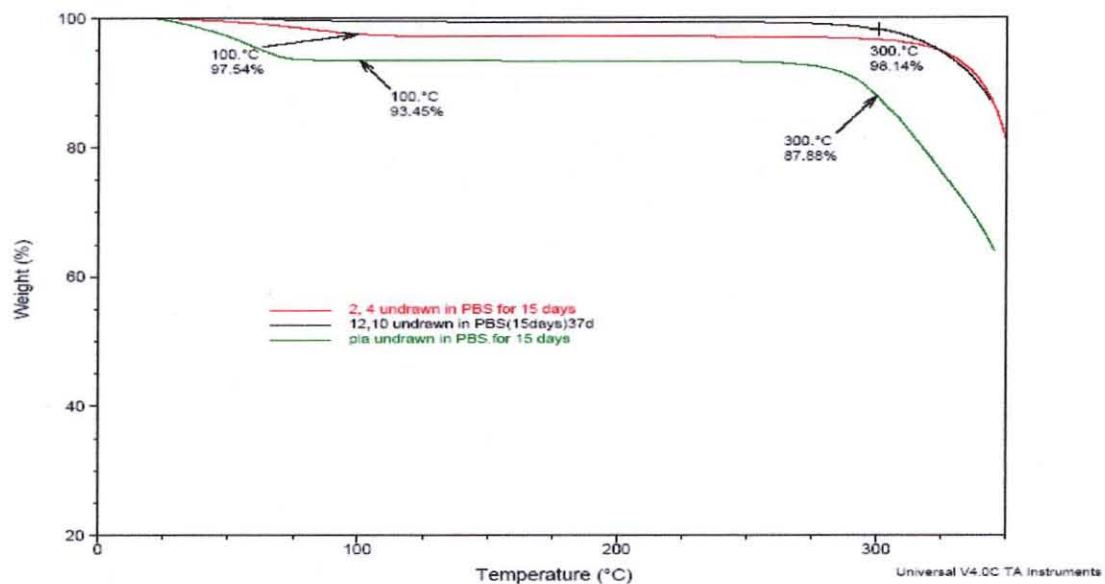


Figure A.5 Undrawn Polymers in PBS for 15 Days.

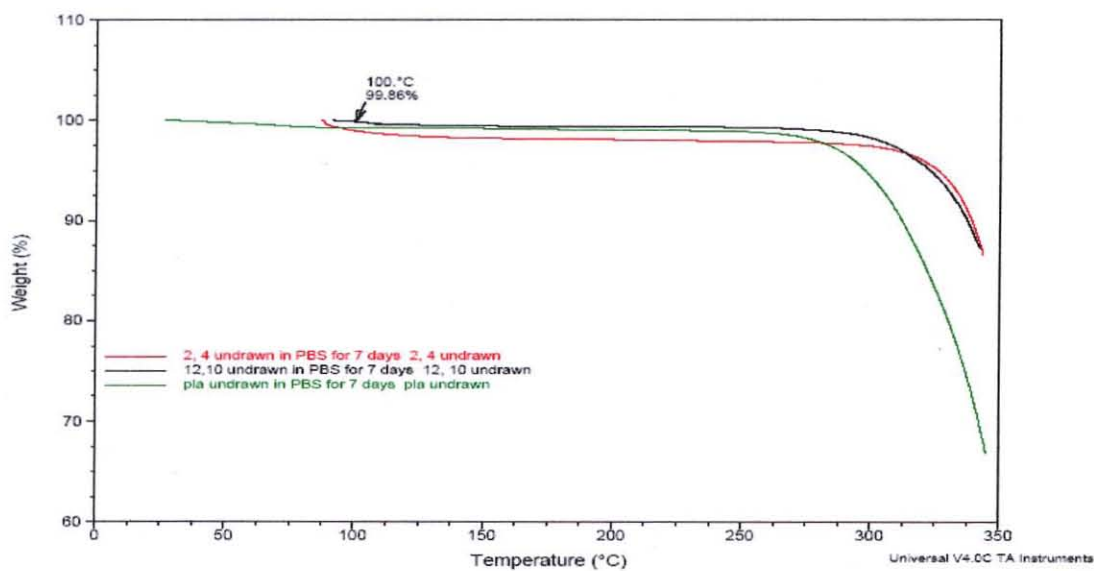


Figure A.6 Undrawn Polymers in PBS for 7 Days.

APPENDIX B

MASS LOSS IN THE POLYMERS

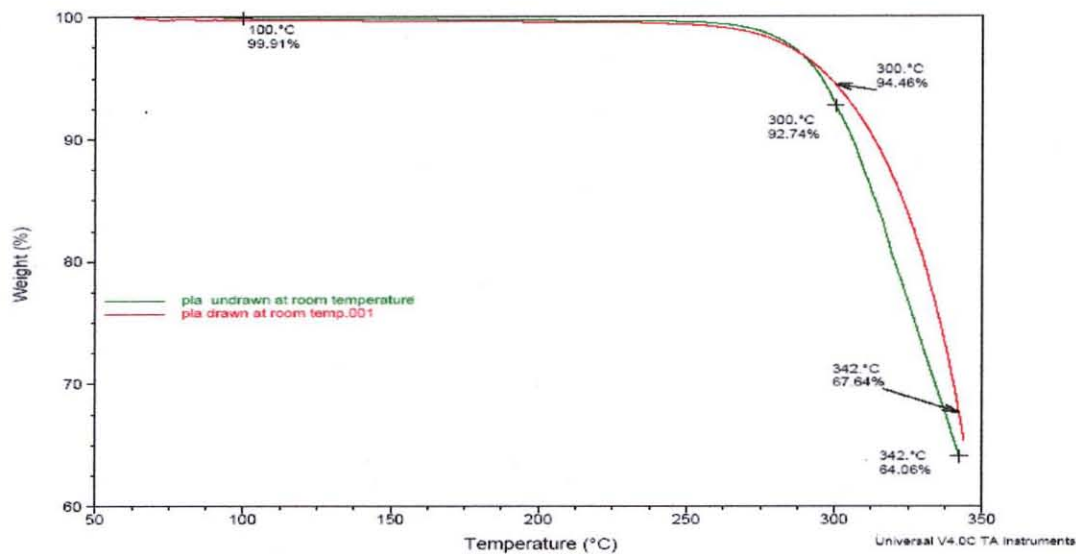


Figure B.1 Undrawn and Drawn Poly-(lactic acid) at Room Temperature.

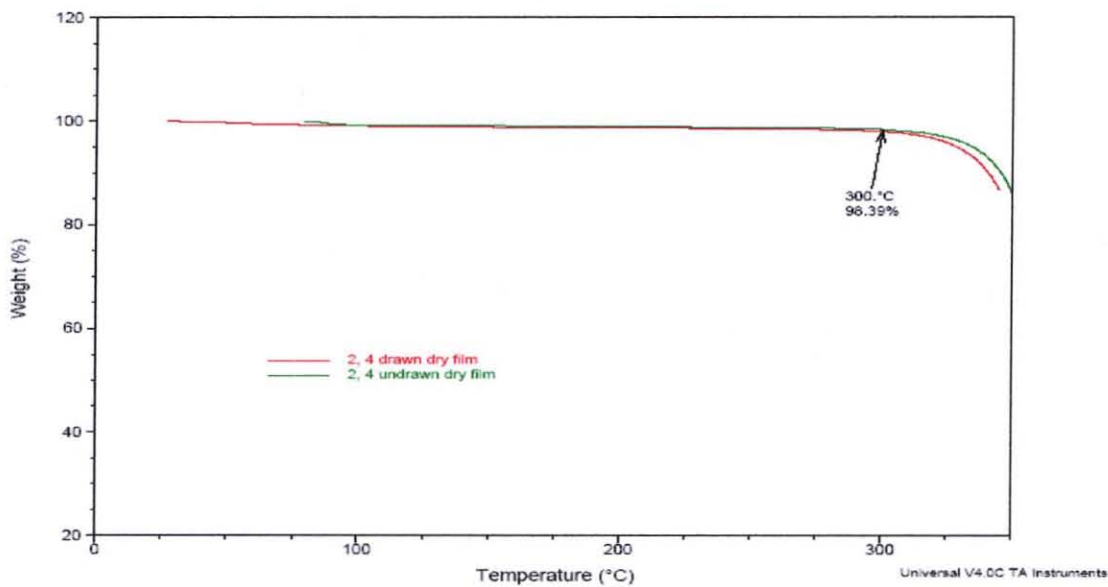


Figure B.2 Undrawn and Drawn Poly(2,4) at Room Temperature.

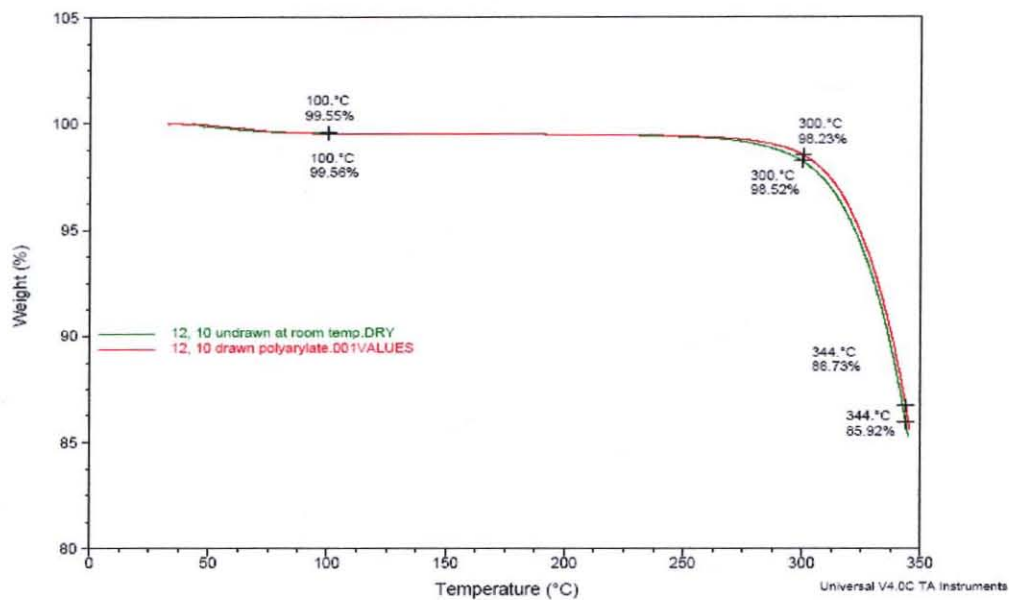


Figure B.3 Undrawn and Drawn Poly(12,10) at Room Temperature.

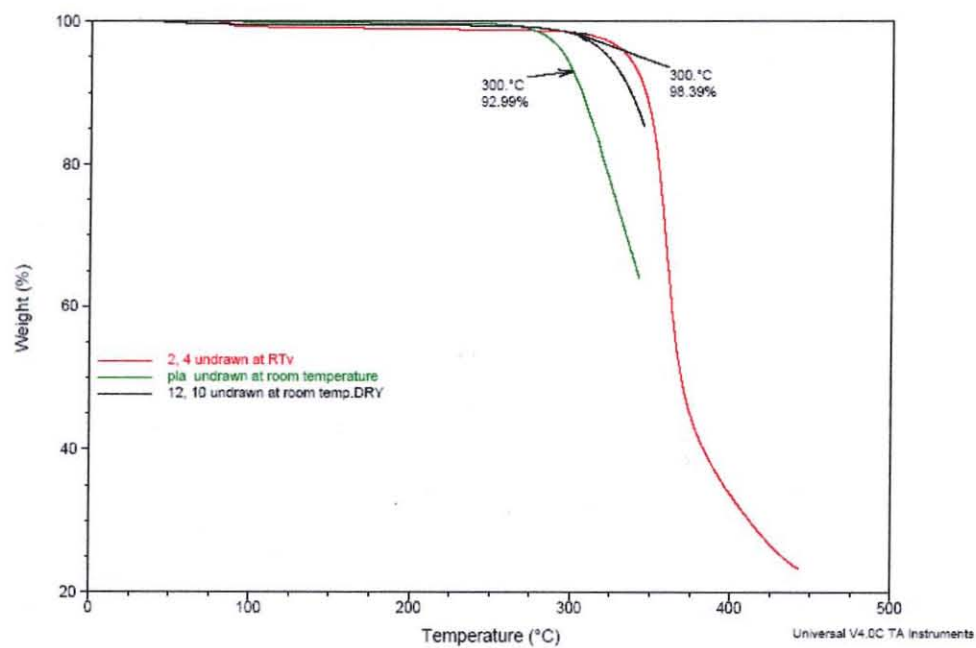


Figure B.4 Undrawn Polymers at Room Temperature.

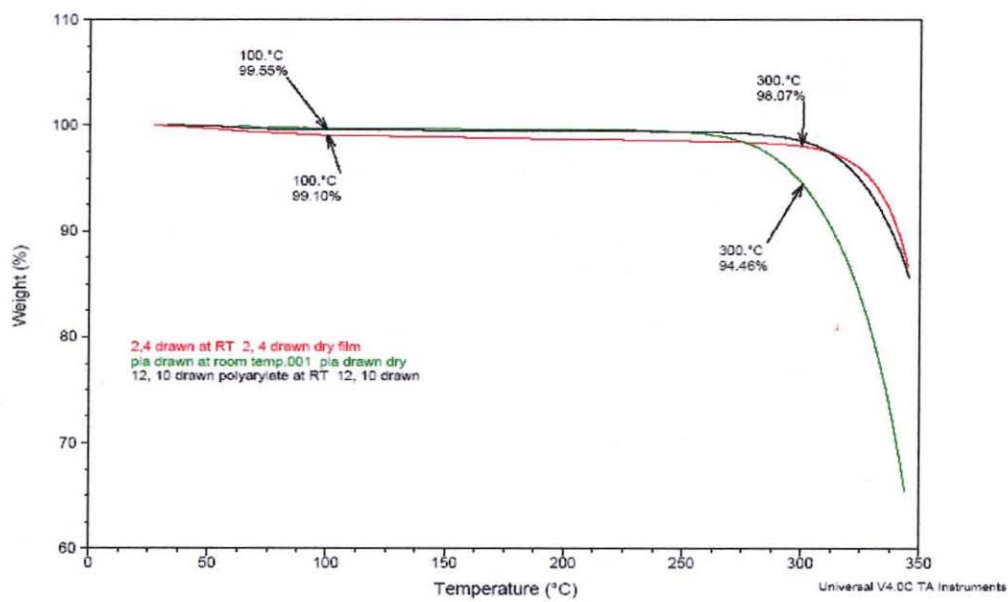


Figure B.5 Drawn Polymers at Room Temperature.

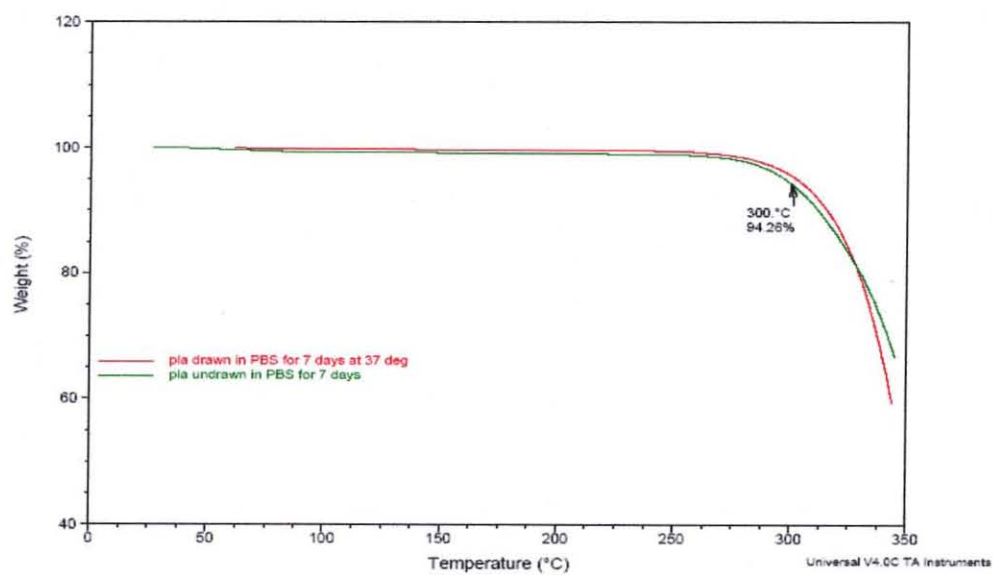


Figure B.6 Drawn and Undrawn Poly-(lactic acid) after 7 days in PBS.

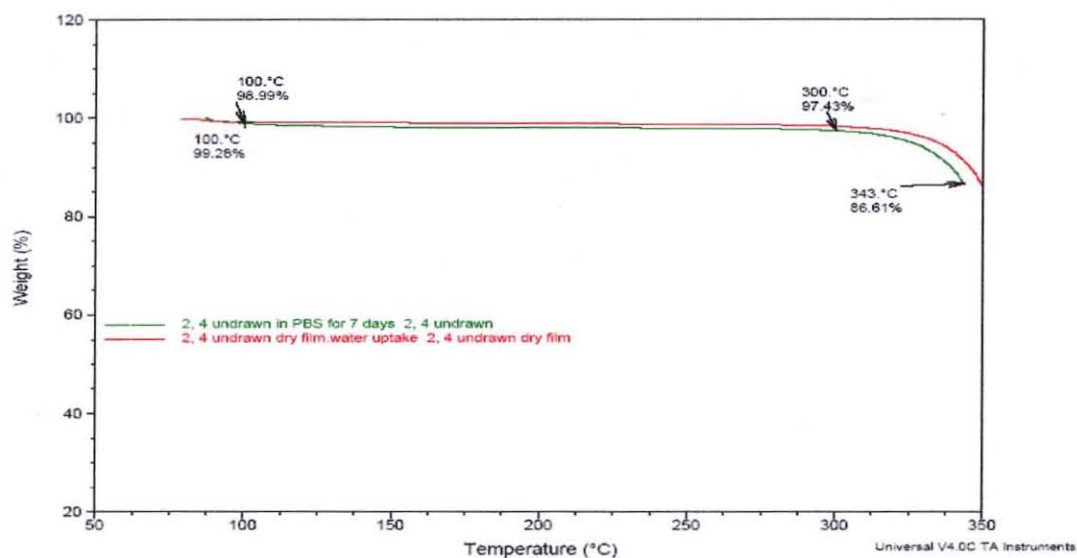


Figure B.7 Undrawn Poly(2,4) in PBS for 7 Days.

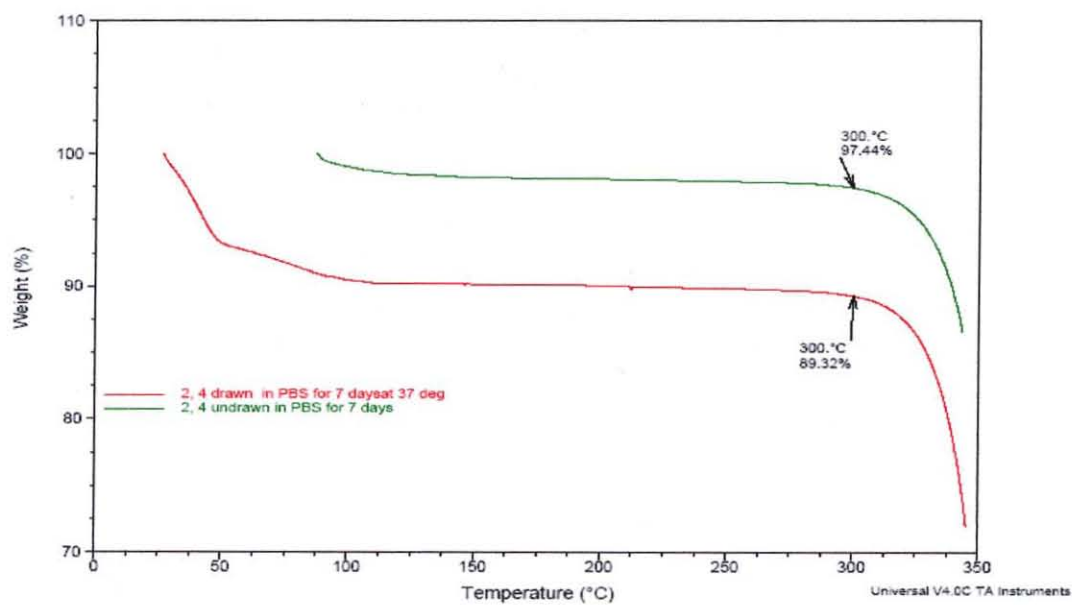


Figure B.8 Drawn Poly(2,4) in PBS for 7 Days.

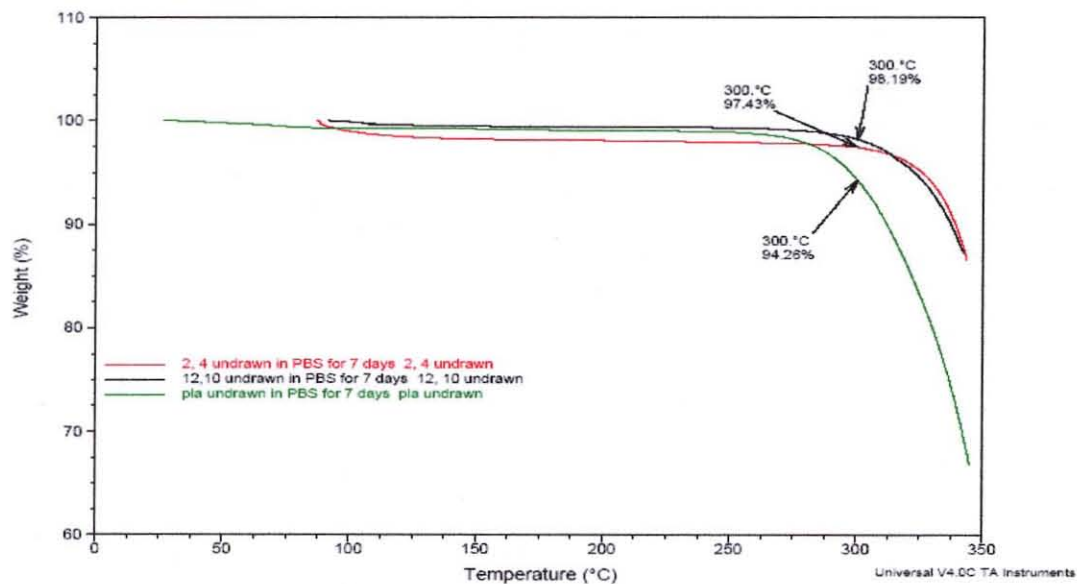


Figure B.9 Undrawn Polymers in PBS for 7 Days.

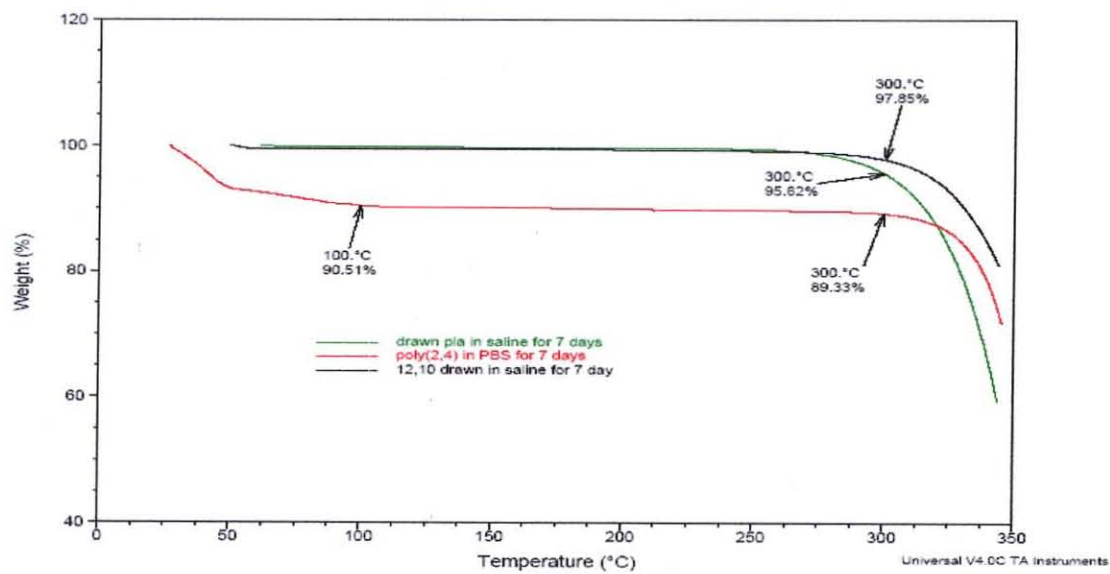


Figure B.10 Drawn Polymers in PBS for 7 Days.

APPENDIX C

POLYMER TRANSITION TEMPERATURE

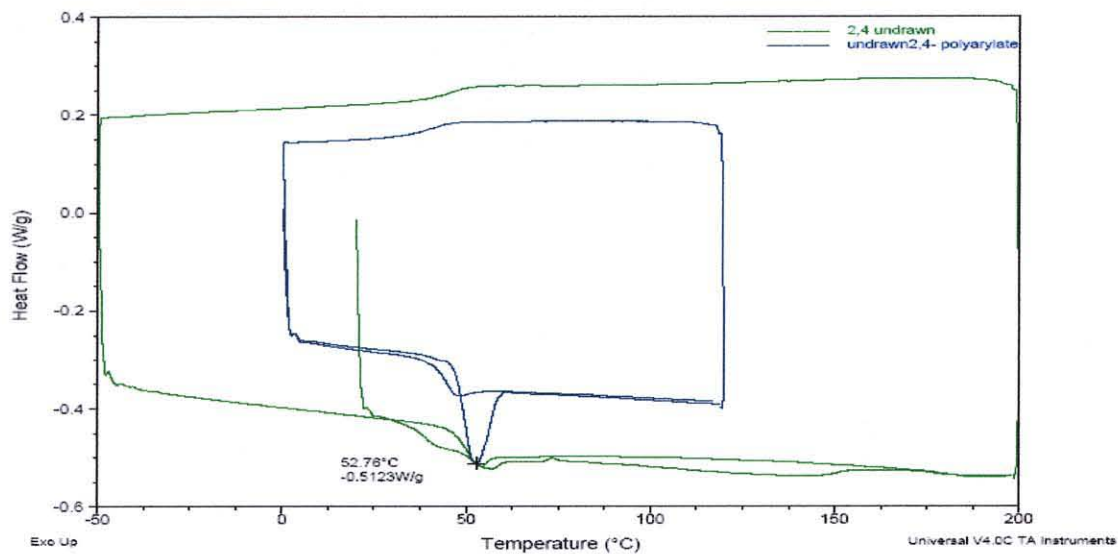


Figure C.1 Poly(2,4) in the Heat/Cool/Heat Cycle at Different Temperature Ranges.

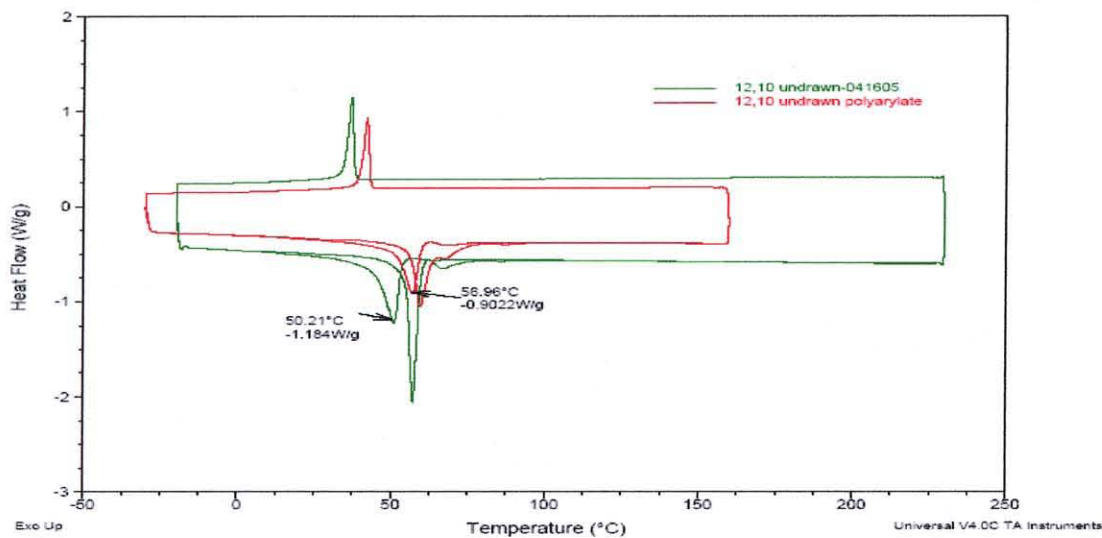


Figure C.2 Poly(12,10) Undrawn at Different Temperature Ranges.

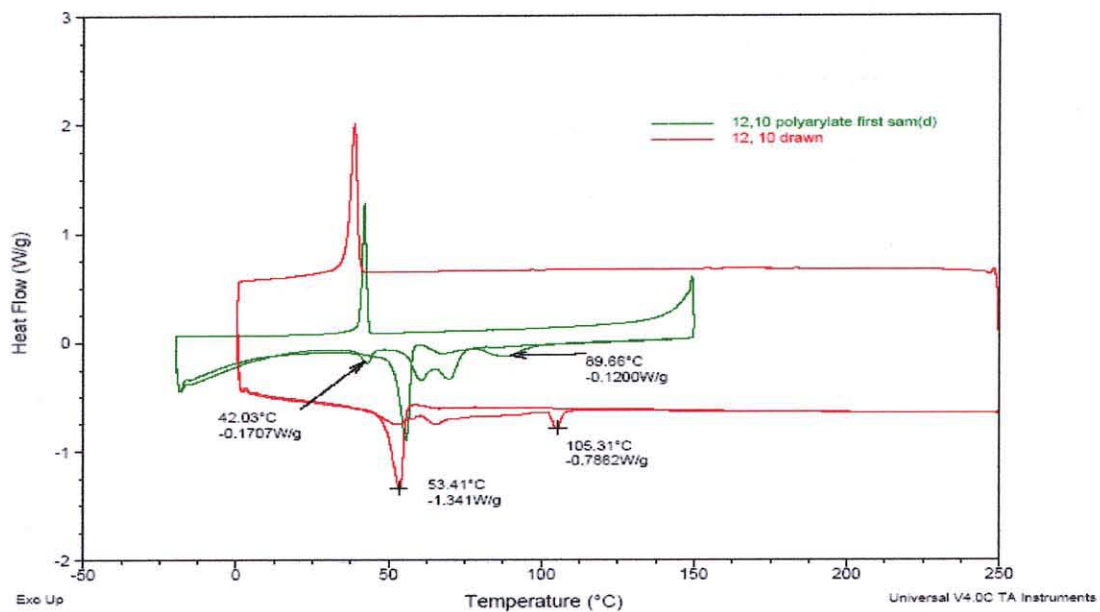


Figure C.3 Poly(12,10) Drawn at Different Temperature Ranges.

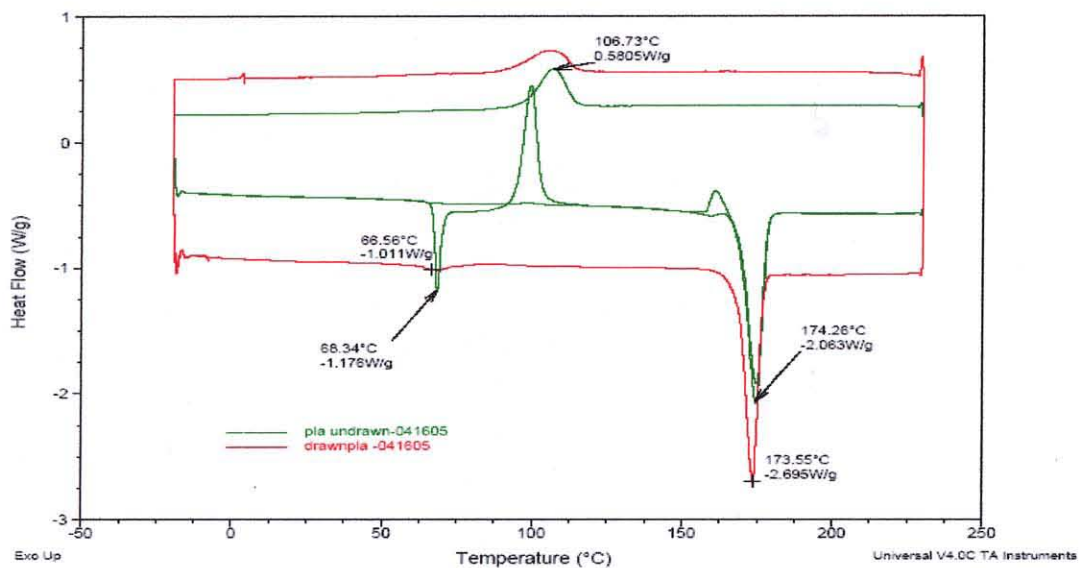


Figure C.4 Drawn and Undrawn PLA in the Temperature range of (20-230)°C.

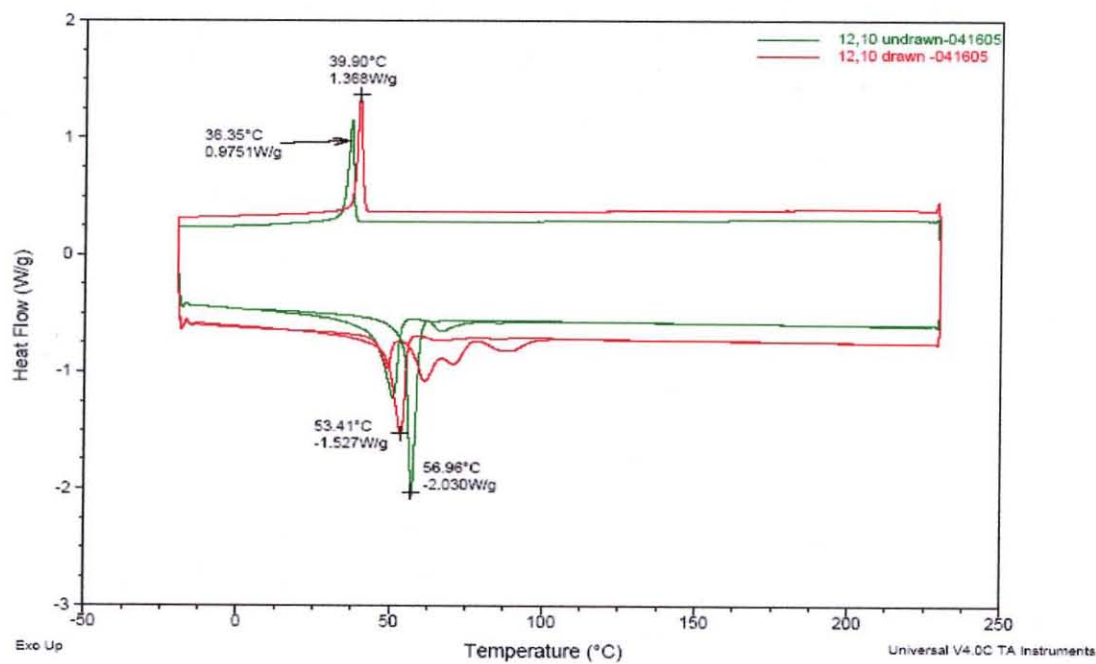


Figure C.5 Drawn and Undrawn Poly(12,10) in the Temperature Range of (20-230)⁰C.

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