Three-dimensional printing of biodegradable polymeric scaffold

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Three-dimensional Printing Of Biodegradable Polymeric Scaffold

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Abstract

A degradable multi-functional scaffold was developed to provide mechanical support for tissue regeneration and to deliver various genes/ proteins/ drugs with respective release profile at different stages of implantation. An indirect binding approach with three-dimensional printing (3DP) was accomplished in this study to solve the limitation of solvent printing for polymeric scaffold. This method enables the printing of different polymers with water and the fabrication of highly versatile scaffold for either soft or hard tissue implantation. The mechanical properties were determined by uni-axial tensile testing, and the morphology was studied with scanning electron microscope (SEM), BET (Stephen Brunauer, Paul Hugh Emmett, and Edward Teller theory) and density bottle based on Archimedes' Principle. Significant high strength (ultimate tensile strength (UTS) 1.9 MPa, modulus 420.6 MPa, and strain at UTS 11.89 %) was attained for the 3DP L-poly(lactide/ L-poly(lactic acid (PLLA) scaffold using high concentration poly(ϵ -caprolactone) (PCL) infiltration (35 %), as well as annealing. This scaffold was made up of a mixture of low and high molecular weight PLLA. The porosity obtained was about 40 % with average pore size of 82 μm .

The synthesis of low molecular weight PLLA (< 80,000 Da) was studied with different catalyst systems and reaction parameters. Three catalyst systems, namely a binary catalyst system stannous chloride/ toluene sulphuric acid (SnCl_2/TSA), and two single catalyst systems, TSA and stannous(II) octoate (Sn(II) Oct), were studied. The reaction parameters investigated included oligomer molecular weight, ramping sequence, reaction time, and reaction temperature. The polymers obtained were characterized using gel permeation chromatography (GPC), Fourier transform infrared (FTIR), modulated differential scanning calorimetry (MDSC) and nuclear magnetic resonance (NMR). The degree of racemization was also determined quantitatively using ^{13}C NMR. The correlation between the catalyst systems and the degree of racemization, and crystallizability were studied. In this study, PLLA with molecular weight of up to

54,000 Da, with good crystallization behaviours and stable thermal properties was obtained through melt polycondensation. This demonstrated the potential of using melt polycondensation to synthesize higher molecular weight PLA, with the use of a binary catalyst system. The in-vitro hydrolytic degradation of the synthesized PLLA, in phosphate buffered solution at pH 7.4 and 37 °C was studied; where the changes, in terms of mass loss, molecular weight, morphology and thermal properties were investigated. It was found that mass loss occurred as early as the first week of degradation, with the decrease in molecular weight. Hence, the synthesized of about 30,000 Da PLLA may be considered as a suitable candidate as a porogen for in-situ pores formation in the 3DP scaffolds. Due to its early mass loss, this PLLA could also be used as a fast controlled release drug carrier.

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Chapter 1. Introduction

1.1. Overview

To date, there are several strategies that have evolved to deal with tissue loss. These strategies included organ transplantation from one individual to another; tissue transfer from a healthy site to an affected site in the same individual; and replacement of tissue functions with synthetic-material devices such as prosthetic valves joints and dialysis machines [1]. While these strategies incorporate significant advances in the field of medicine, they have a number of inherent limitations. Tissue engineering (TE) seeks to provide a new solution to tissue loss, through replacement or restoration of tissue or organ function with constructs that contain specific population of living cells. This method holds the promise to one day replace living tissue with living tissue that is designed and constructed to meet the needs of each individual patient.

Scaffolds with porous architecture are commonly used in TE. The basic functions of the scaffold are to preserve tissue volume, provide temporary mechanical support, and deliver cells. It is essential to produce a scaffold with suitable mechanical strength to sustain any in vivo stresses and loading without the support of neotissue especially at the early stages of surgery [2, 3]. It is critical that scaffolds have high porosity, interconnectivity and the correct pore size to facilitate cell adhesion, in-growth and mass transportation. This is particularly vital at the later stage of implantation when the cells started to migrate deep into the scaffold [4].

A successful scaffold should balance mechanical function with cell in-growth and mass transport. Unfortunately, mechanical strength of the scaffold is negatively correlated to the porosity and pore size. The balance often presents a trade-off between a denser scaffold providing better mechanical function and a more porous scaffold providing better cell in-growth and mass transport [5]. It is rather challenging to strike a good balance.

Scaffold plays an important role in TE. However, the cell regenerative capability, which varies widely with cell types and the circumstances of injury, is not controlled solely by scaffold design and fabrication. The local presences of certain biofactors (genes and/ or proteins), such as growth factors (GF), antibiotic, and anti-inflammatory drugs, play key roles to determine the success of the regenerative process. Conventional delivery method, through direct injection [6], has its limitations. It is usually difficult to attain target delivery and is ineffective due to the short half-lives of most drugs. In general, although scaffold delivery is able to reach the target site more readily, delivery is usually restricted to only one type of biofactor each time. The scaffold release profile is through diffusion, thus it is normally too fast for ample control and not in zero order [7-12]. The engineering of complex tissue, involving the transformation of neotissue into a functional replacement tissue, occurs in a series of steps. Each step involves different biological signals [5]. A controlled release system, which can deliver different types of biofactors with desired concentration profile at different times for the differentiated states of cells, is thus desired.

The objective of this project is to develop degradable multi-functional scaffold. The mechanical and degradation properties of this scaffold will be adaptable to suit either soft or hard tissue implantation.

As adequate mechanical support is more critical at the beginning stage of implantation [3], and high porosity is desired for cell in-growth and mass transport during the later stages [4], a dense scaffold that can form pores in-situ is preferred. This scaffold can be constructed from a mixture of polymer(s), with high strength but slow degradation rates, as the matrix material; and a fast degrading polymer as the porogen. A dense scaffold will first be constructed from a mixture of polymers with fast and slow degradation rates to attain the high strength required. Upon implantation, the polymer with the fast degradation rate will be dissolved, leaving behind pores in-situ in the matrix for cell in-growth. As

porosity increases, the scaffold strength will be reduced. However, this loss of strength will likely be compensated by the growth of cell/ neotissue that will give certain level of mechanical support [2, 3].

By proper material selection and combination, the degradation and the mechanical properties of the scaffold can be controlled and adapted to the specific tissue requirement. Furthermore, the degradation characteristics of the scaffold should be designed appropriately to provide a sequential transition in which the regenerated tissue assumes function as the scaffold degrades. Various biofactors can be incorporated into the polymers of the scaffold and be released into the surrounding tissue as each polymer degrades or come into contact with body fluid at different periods of time.

1.2. Scope

1.2.1. Materials selection

A material that can be used as a scaffold in TE must satisfy a number of requirements. These include biocompatibility, biodegradation to non-toxic products within the time frame required for the application, ability to support cell growth and proliferation, appropriate mechanical properties and maintaining mechanical strength during most part of the tissue regeneration process. In addition, the material should be able to be processed to complicated shapes with appropriate porosity.

In this project, PLLA (L-poly lactide/ L-poly lactic acid) was selected as the polymer to form the multi-functional scaffold matrix. This polymer is biocompatible and is among the few synthetic polymers that have gained FDA approval for human clinical use. The scaffold made by this polymer indicated positive cell attachment and growth for both soft [13- 17] and hard tissue regeneration [18]. Two different molecular weights PLLA were used to construct

the multi-functional scaffold with different degradation rates. PLLA is generally rigid and brittle due to its high crystallinity.

PCL (poly(ϵ -caprolactone)) is another FDA approved synthetic biodegradable linear polyester [19]. PCL was suggested to be more appropriate for cell growth and the formation of ECM than PLLA due to its lower rigidity [20]. Though PCL is flexible, the strength of PCL is low and not sufficient for load-bearing application [21].

The mechanical drawbacks of these two polymers are expected to be compensated and improved, by the incorporation of PCL into PLLA scaffold matrix through infiltration process. According to Zhao et al [20], the incorporation of PCL effectively reduced the rigidity of PLLA. Furthermore, since the degradation rate of PCL is slower than PLLA, drugs that require a later release into tissue can be included into PCL.

1.2.2. Synthesis of PLLA

The synthesis of PLLA served two purposes in this project. The first purpose was to study the feasibility of using a simpler and cheaper synthesis process - polycondensation to produce PLLA with similar properties as synthesized by a more tedious and expensive commercial synthesis process - ring opening process (ROP). Secondly, PLLA within low to medium average molecular weight (M_w) range was required for the fabrication of multi-functional scaffold. Due to low market need, various M_w within this range were not available commercially. Thus PLLA with M_w falling within this range was synthesized.

PLLA with M_w at about 10,000 to 50,000 Da (was required in this study) was synthesized in the laboratory.

PLLA is commonly produced by a two-step ROP process. The ROP process is highly successful in producing PLA with high molecular weight, good mechanical

properties and crystallization behaviour. However, the major disadvantage using the ROP method is the complicated purification process of the lactide, which is time consuming and involves high cost [22- 25]. In addition, the initiator bound to the polymer synthesized is hard to be removed by purification. This reduces the polymer thermal stability [26] as well as changes the hydrolysis and mechanical properties.

A cheaper and more direct route to produce PLLA is through melt polycondensation of lactic acid. The tedious lactide purification can be avoided in this method. The catalyst used does not bind to the polymer after synthesis and can be readily removed by purification. However, the range of PLA M_w obtained is generally less than 10,000 Da. In addition, the high reaction temperature and low pressure level will cause side reactions such as racemization, discoloration and thermal/ thermal oxidative degradation. These side reactions affect the molecular weight, crystallization behaviour and yield of the polymer. In this study, various types of catalysts and reaction parameters were studied and optimized in order to inhibit the side reactions and raise the molecular weight. PLLA with M_w more than 10,000 Da, good crystallization behaviour and stable thermal properties was desired. Furthermore, the possibility of synthesizing high molecular weight PLLA using melt polycondensation was explored.

1.2.2.1. Catalyst systems

Three catalyst systems were used for PLLA melt polycondensation. The catalyst systems included a binary-catalyst system (SnCl_2 / TSA), and two single-catalyst systems TSA and Sn(II) Oct respectively. The properties of the PLLA synthesized by these three catalyst systems were investigated. TSA was used as a single catalyst, in order to gain a better understanding of its effect, and subsequently the role of this catalyst as a component in the SnCl_2 / TSA binary-catalyst system. Sn(II) Oct is widely used for the synthesis of PLA by ROP, and is proven to be one of the most effective ROP catalysts. Sn(II) Oct also has good

solubility in many lactones, low toxicity and is FDA approved [27]. Here, the effectiveness of this catalyst in melt polycondensation was investigated.

To study the impact of different catalyst systems on the PLLA properties, the polymers produced were characterized with respect to the molecular weight, crystallinity, racemization, discoloration, thermal properties and structural properties.

There are many reported studies on the racemization effect of PLLA synthesized by ROP. Only a few reports discussed the racemization effect of PLLA synthesized by melt polycondensation [28, 29]. The low racemization obtained from the PLLA synthesized using SnCl_2 / TSA binary-catalyst system was reported by Kimura and Gao [23, 24]. Here, the degree of racemization of PLLA synthesized using different catalyst systems were studied qualitatively and quantitatively using NMR. By determining the degree of racemization quantitatively, a better understanding of the impact of catalyst types on the degree of racemization would be obtained. Subsequently, the correlation between the degree of racemization and polymer crystallizability was also investigated.

1.2.2.2. Process optimization

The synthesis process consists of two steps, whereby the oligomer of L-lactic acid was first produced in the oligomerization step, and subsequently polymerized with the catalysts in the polymerization step. The influences of the various reaction parameters on the final polymer molecular weight and thermal properties were studied. The reaction parameters investigated were the oligomer M_n obtained in the first step; the ramping sequence, reaction temperature and reaction time in the polymerization step.

1.2.3. Scaffold fabrication

One of the Rapid Prototyping technologies, three dimensional printing (3DP) was chosen to fabricate the scaffold. 3DP technique requires shorter object building time than other RP technologies. Complex scaffold architectures with through channels and overhanging features can be produced without any support structure [30]. This allows the scaffold to be tailor made individually to fit the tissue defect of an individual patient [31]. No heating and melting are needed throughout the 3DP process, and this allows it to be used for thermal sensitive polymeric materials, such as PLLA [30, 32]. In addition, this enables the 3D printing process to incorporate various protein, gene and cells, which denature at elevated temperatures.

One of the limitations of 3DP is the binder used. For printing of the organic polymer, specific printing solvents are required for each material. This limits the polymer selections for 3DP. Most important, polymers that dissolve in different solvents could not be printed together as a scaffold. In this case, the fabrication of the multi-functional scaffolds consisting of different polymers with versatile mechanical and degradation properties is hindered. PLLA is generally printed with chloroform [18, 33], which is the few organic solvents that can readily dissolve and bind the powder particles. However, chloroform is toxic and the residue in the scaffold will likely cause adverse effect to the cells. Hence, an indirect binding approach was proposed in this study to overcome this restriction.

Planar distortion is often encountered especially for thin and small 3DP scaffold. In this study, a series of printer parameters, including layer thickness and the amount of solvent deposited, were investigated to minimize planar distortion and raise the tolerance of the scaffold.

1.2.3.1. Post-printing processes

One of the general drawbacks of 3DP scaffold is its poor mechanical properties. The inferior mechanical properties are the result of weak binding strength

between the particles, and the high porosity of the scaffolds due to the nature of process. Therefore post-printing processes, such as cold isotactic pressing [33], and infiltration [30], are normally applied to enhance the mechanical properties, and to prevent the disintegration of scaffold in the water.

Infiltration increases the mechanical strength of the scaffold by filling in some of the pores and increasing the strut thickness of the structure, thereby reinforcing the scaffold. The difficulty to be addressed for the infiltration process is the comprehensive penetration through the entire the scaffold. Two new infiltration methods were proposed in this study, in order to achieve uniform penetration of infiltration even with high concentration infiltrant.

Annealing is another method which serves to increase the strength of a porous material by increasing the adhesion between particles in the scaffold. In this study, respective infiltration and annealing, as well as a combination of both were attempted. The effect of the treatment(s) to the scaffold mechanical properties and water resistance was studied.

The possibility to obtain a higher strength 3DP scaffold was explored in order to determine the potential of this fabrication process for high strength implant application. The investigated post-printing process conditions included different types of infiltrant, infiltrant concentration, annealing environment, annealing temperature and duration as well as the sequence of these two treatments in combination. Infiltration efficacy (IE) was used to associate the post treatment effect to scaffold mechanical properties.

1.2.3.2. Incorporation of PLLA_{low}

The incorporation of synthesized PLLA of 15,000 Da M_w (PLLA_{low}) with the high M_w PLLA at different ratios was investigated for scaffold printing. The PLLA_{low}, due to its lower melting temperature, would melt at the annealing temperature and enhance the inter-particle binding. At the same time, the high M_w PLLA in the

scaffold would remain in solid state and prevent the scaffold structure from collapsing. The optimized annealing condition and mixing ratio of the PLLA were developed with respect to the scaffold mechanical properties.

The hydrolytic degradation behaviour of the lower M_w PLLA was studied to serve as a guideline for this polymer functioning as a porogen for in-situ pore formation and at the same time as a drug carrier for fast controlled release. There are limited studies on the hydrolytic degradation of PLLA with M_w above 10,000 Da and below 80,000 Da. It is known that the mass loss patterns and the degradation rates of PLLA with different molecular weights are different. A delay in mass loss of several weeks was always observed for the high M_w (>80,000 Da) PLLA synthesized by ROP [34- 38]. Here, the hydrolysis of medium M_w PLLA (~30,000 Da); synthesized using the binary-catalyst system was investigated. The changes of the sample mass; M_w and M_{wD} with degradation time were recorded and compared with the degradation pattern of PLLA with high and low M_w . The variations of morphology, thermal properties and crystallinity during degradation were discussed.

Chapter 2. Literature Review

2.1. Tissue Engineering (TE)

TE seeks to provide a new solution to tissue loss, through replacement or restoration of tissue or organ function with constructs that contain specific population of living cells. Unlike traditional approaches for the treatment of lost tissue or organ function, TE holds the promise to one day replace living tissue with living tissue that is designed and constructed to meet the needs of each individual patient [1, 4]. In effect, cells could be isolated from a healthy site in the patient, expanded in vitro/ vivo and/ or modified by gene therapy to replace a defective gene, and then implanted back into the patient in the appropriate location under appropriate conditions to correct the functional defect. And there, the surgeon can potentially adapt these fundamental techniques TE with the tools of minimally invasive surgery to bring the whole concept of disease treatment to a new higher and modern technological level [1, 114].

Three avenues have been explored in TE: the injection of isolated cells, the placement of encapsulated systems, and the implantation of cells in matrices. The last method is to seed the cells with/ without gene/ protein onto a porous degradable material known as scaffold. The scaffold will guide the cell organization and growth and allow diffusion of nutrients to the transplanted cell. Ideally, the cell-scaffold is prevascularized or would become vascularized as the cell mass expands after implantation. Vascularization could either be a natural host response to the implant or may be artificially induced by sustained release of angiogenic factors from the scaffold. The fundamental toolkit for TE includes cells and the scaffold.

Cells used in TE can be autologous, allogenic or xenogenic. They can be used as primary culture, expanded in vitro or cryo-preserved. The cells used are generally mature differentiated cells; however, cells from neonatal tissues can also be used [1]. The other possibility is to use stem cells, which can be

proliferated through multiple generations and made to differentiate into appropriate cell types. The critical issue of using stem cells is to learn how to control the stem cells and make them permanently differentiate into the desired cell type [1, 115].

2.1.1. Scaffold for TE

Far from being a passive component, scaffold material and porous architecture play a significant role in tissue regeneration by preserving tissue volume, providing temporary mechanical function, and delivering cells and/or protein/gene [5]. The basic requirements of a scaffold for TE are listed in **Table 2.1** [1, 3, 114].

Table 2.1 Basic requirements and design parameters for TE scaffold

Requirement	Design parameter	
Not to provoke inflammatory response or toxicity in vivo.	Must be biocompatible, non-toxic and non-carcinogenic.	Materials selection
To assist in the growth of 3D tissue and organs.	3D scaffold of specific shape.	Scaffold shape and size design
Give a way to a uniform high cell seeding density. To promote cell proliferation and migration leading to tissue in-growth throughout the scaffold. To allow for the movement of nutrients and waste in and out of the scaffold. To allow significant cell-surface interactions such as cellular attachment	High porosity with optimum pore size. High interconnectivity between pores. High surface area to volume ratio.	Scaffold fabrication techniques
To provide the appropriate surface for cell attachment proliferation and differentiation of function.	Optimum polymer surface chemistry and topography.	Materials selection
The scaffold may degrade to leave only natural tissue.	Rate of degradation to match rate of tissue formation. Polymer degradation products must not be toxic or promote inflammation in vivo	Materials selection
Processes sufficient structural integrity to retain shape in vivo, with enough mechanical strength to support developing tissue and withstand in vivo forces.	Scaffold should have equal mechanical properties of developing tissue.	Materials selection Scaffold fabrication techniques Scaffold shape and size design

2.1.1.1. Mechanical properties and porosity

The scaffold must provide adequate mechanical support for wound contraction forces at the initial stage, and later for the tissue remodelling. If the scaffold

cannot provide a mechanical modulus in the range of 10-1,500 MPa for hard tissues or 0.4- 350 MPa for soft tissues [5], then any nascent tissue formation will probably also fail due to excessive deformation. In addition, the degradation profile of the scaffold should be designed appropriately such that it retains sufficient structural integrity until the newly grown tissue has replaced the scaffold supporting function [39].

Scaffold must possess an open-pore geometry with a highly porous surface and microstructure that allows cell in-growth and reorganization, and provides the necessary space for neovascularization from surrounding tissues in vivo. The highly porous microstructure with interconnected porous networks is critical in ensuring spatially uniform cell distribution, cell survival, proliferation and migration [39]. The scaffold's pore interconnectivity and permeability directly affect the diffusion of physiological nutrients and gases to, and the removal of metabolic waste and by-product from cells that have penetrated the scaffold [39]. The diverse nature of tissue architectures requires different microenvironment for their regeneration that includes the employment of scaffolds with optimum pore sizes. Scaffolds with pore sizes over 50 nm are called macroporous scaffolds and often are used when an integrated tissue structure is desirable [17]. It has been reported that calcified tissue in-growth can occur at a minimum pore size of 100-150 μm , and 200-250 μm for soft tissue in-growth [15]. When the pores employed are too small, pore occlusion [39] by cells will prevent cellular penetration and matrix elaboration within the scaffolds. In addition, high internal surface area to volume ratio is essential in order to accommodate the large numbers of cells required to replace or restore tissue or organ function(s). Since pore size and internal surface area are inversely related, a compromise between the two parameters has to be determined depending on the application.

A successful scaffold should balance mechanical function with cell in-growth and mass transport, by providing a sequential transition in which the regenerated tissue assumes function as the scaffold degrades. This balance often presents a

trade-off between a denser scaffold providing better mechanical function and a more porous scaffold providing better cell in-growth and mass transport [5]. It is also important to note that changing the porosity or permeability of the scaffold can have a significant impact on the degradation characteristics of the scaffold. It has been shown that low porosity and permeability scaffolds degrade faster [40]. It is speculated that this may be due to the inhibition of autocatalytic degradation.

2.1.2. Biofactors delivery in TE

Scaffold plays an important role in TE. However, the cell regenerative capability, which varies widely with cell types and the circumstances of injury, is not able to be controlled solely by proper scaffold design and fabrication. In this situation, the local presence of certain biofactors (genes or proteins), such as growth factors, antibiotics and anti-inflammatory drugs, which are capable of instructing the transplanted cells, will likely become the key to the ultimate success of the regenerative process.

There are several important considerations in the delivery of biofactors. First, given the quantity and complex actions of growth factors, it is essential to identify the key biofactor(s) to deliver for particular tissue regeneration.

Second, the rate of change of the biofactors concentration with time and spatial position should be controlled for specific tissue application. The engineering of complex tissue architectures will probably require methods for introducing and changing molecular signals during process of neotissue development. The transformation of a neotissue into a functional replacement tissue occurs in a series of steps, in which each step potentially involves different biological signals [5]. The changes of the types of biofactors, its respective concentration with time can influence the differentiated state of cells within tissue.

Finally, the mode of biofactor delivery must target the desired cell population and minimize signal propagation to non-target tissues and cells. The earliest reported

applications of the growth factors as therapeutic agents involved intravenous injection, but this delivery method is not localized to the target tissue and is also ineffective because of the growth factors' short half-lives. Although very small quantities of growth factor are necessary to generate a cellular response, growth factors are rapidly degraded once secreted [6]. Direct therapeutic application of growth factors therefore requires substantial systemic doses at levels that can generate undesired effects [6].

2.2. Materials

A material that can be used as a scaffold in TE must satisfy a number of requirements. These include biocompatibility, biodegradation to non toxic products within the time frame required for the application, ability to support cell growth and proliferation, appropriate mechanical properties and maintaining mechanical strength during most part of the tissue regeneration process. In addition, the material should be able to be processed to complicated shapes with appropriate porosity [116]. Each polymer is selected according to its properties to meet specific TE requirements. Sometimes, composite materials are used in order to attain better performance compared to their individual components.

A variety of materials have been used for scaffold fabrication [32]. These materials include metals, ceramics, polymers and their composites. The major disadvantages of metals and ceramics for TE application are their non-biodegradability and limited processability. In contrast, polymers are more versatile. The composition and structure of the polymers can be modified and tailored to meet specific application.

Many types of polymeric materials have been used for TE. They can be simply categorized as naturally derived materials, such as collagen and fibrin; and synthetic polymers, such as polylactides (PLA), polyglycolide (PGA), and their copolymers polylactide-co-glycolide (PLGA). Naturally derived materials have the

potential advantage of biological recognition that may positively support cell adhesion and proliferation. However, they may exhibit immunogenicity and contain pathogenic impurities. There is also less control over their mechanical properties, biodegradability, and batch-to-batch consistency. Many of them are also limited in supply and can therefore be costly [32]. An advantage of synthetic polymers is reproducible large scale production with controlled properties such as strength, degradation rate and microstructure. Therefore, synthetic biodegradable polymers have been widely used as vehicles for cell transplantation and scaffolds for TE.

The most commonly used biodegradable polymers consist of PLA, PGA and the copolymers. The other synthetic polymers commonly used for TE scaffolds include PCL, polyanhydrides, polycarbonates, polyphosphazene, polyfumarates, and poly(butylenes terephthalate)/ poly(ethylene oxide) [32]. **Table 2.2** and **Table 2.3** lists the mechanical and thermal properties of the most commonly used biodegradable polymers respectively.

Table 2.2 Mechanical properties of PGA, PLLA and PCL

Polymer/ M _w (Da)	UTS (MPa)	Tensile modulus (MPa)	Elongation at break (%)
PGA	NA	NA	NA
PLLA (50,000)	47	1720	3
PDLLA (48,000)	35	1930	2
PCL (10,000)	15	250	>100

Tensile testing samples were injection moulded. PLLA and PDLLA were moulded at 200 °C, PCL moulded at 120 °C [41]

Table 2.3 Thermal properties of PGA, PLLA and PCL

Polymer	T _m (°C)	T _g (°C)	Complete degradation time (Months)	Degradation products
PGA	225~230	35~40	~ 6-12	Glycolic acid
PLLA	173~178	60~65	~ >24	L-lactic acid
PDLLA	NA	49	~ 12-16	D-lactic acid/ L-lactic acid
PCL	58~63	-65~-60	~ >24	Caproic acid

2.2.1. PLA, PGA and copolymers

2.2.1.1. Biocompatibility

Although there are a variety of biodegradable polymers available now for medical applications, PLA, PGA and the copolymers remain the most popular and widely used among these materials. These polymers are biocompatible and are among the few synthetic polymers that have gained FDA approval for human clinical use. Scaffolds made from this group of polymers have indicated positive cell attachment and growth for both soft [13, 14, 15, 16, 17] and hard tissue regeneration [18].

2.2.1.2. Hydrolytic degradation

The degradation of these polymers generally involves random hydrolysis of their ester bonds. The degradation product of PLA is lactic acid, which can enter the tricarboxylic acid cycle and be excreted as water and carbon dioxide. PGA is broken down into glycolic acid and can be excreted in the urine. The rate of degradation is determined by factors such as configuration, copolymer ratio, crystallinity, molecular weight, morphology, stresses, amount of residual monomer, porosity of the scaffold produced, and site of implantation. In general, PLA is more hydrophilic than PGA, and is more resistant to hydrolytic attack than PGA.

The hydrolytic degradation of semicrystalline high molecular weight PLLA generally proceeds via random bulk hydrolysis in two distinct stages. The first stage is characterized by the preferential attack of the ester linkages in the more accessible amorphous regions, while the second stage is characterized by the attack of the less accessible crystalline regions [76, 96]. Uncatalysed and autocatalysed degradation of semicrystalline PLLA have been observed by different researchers [76, 83- 87, 95- 99]. The number average molecular weight (M_n) has been used for the modeling of these two mechanisms. The uncatalysed model was proposed by Chu [98] and Anderson [95], with a kinetic relationship as follow:

$$\frac{1}{M_{nt}} = \frac{1}{M_{no}} + kt \quad \text{(Equation 2.1)}$$

Where $M_{nt} = M_n$ at time t ; M_{no} is the initial M_n , k is the rate constant and t is the degradation time. This statistical approach does not account for the possibility of autocatalysis accelerating the polymer degradation rate.

The second model, which is the autocatalysed model, was proposed by Pitt et al [99]. He derived a relationship based on the kinetics of the ester-hydrolysis reaction, accounting for autocatalysis by the generated carboxylic acid end groups and it is as follow:

$$\ln M_{nt} = -kt + \ln M_{no} \quad \text{(Equation 2.2)}$$

The assumption used in both the models was that the extent of degradation is small. Hence, a linear relationship should exist between $1/M_n$ versus time for uncatalysed model and $\ln M_n$ versus time for autocatalysed model, up until the point of mass loss.

2.2.1.3. Mechanical properties

Mechanical properties are critical for load bearing applications such as bone fixation, scaffold implantation. These applications require the polymer to maintain certain strength level for its entire usage life span. The mechanical properties of PLA, PGA and the copolymers can be varied to a large extent ranging from soft and elastic plastics to stiff and high strength materials.

The mechanical properties of PLA are mainly determined by the polymer molecular weight, crystallinity and polymer stereoregularity [27]. The molecular weight of the polymer has significant effect on the polymer's mechanical properties. It has been shown that the tensile strength and modulus of PLLA increased by a factor of 2 when the weight average molecular weight increased from 50,000 to 100,000 Da. However, a further increase in molecular weight to 300,000 Da did not influence the mechanical properties in any significant way [42]. According to Moon et al [24] and Anders et al [26], the mechanical

properties were known to become saturated when the molecular weight was more than 70,000 Da. The mechanical properties of the polymer are also enhanced by degree of crystallinity. Annealed PLLA exhibited higher tensile strength and impact resistance than PLLA without annealing [22, 26]. The increment of crystallinity induces crosslinking and enables more polymer chains to align better.

The effect of the polymer chains stereoregularity to the mechanical strength of PLLA and PDLLA was studied [27]. PDLLA (50:50 D/L-lactic acid) lost all tensile strength after two months of implantation, whereas the strength of pure PLLA was unchanged during that period of time. This was because the former, with higher D-lactate unit had lower stereoregularity, thus lower mechanical strength.

PGA, which has higher degradation rate, tends to lose their mechanical strength rapidly, typically over a period of 2- 4 week after implantation [42]. However, PLA scaffold or implant took months or even years to lose the mechanical integrity whether in vitro or in vivo [43]. In general, PGA, with higher crystallinity (46-50%), is more rigid, brittle and has higher strength than PLA. However, due to the higher degradation rate of PGA, it is only applicable for short term load bearing usage.

2.2.1.4. Processability

The solubility of PLA, PGA and its copolymers is affected by many factors. Polymer composition and crystallinity play an important role in the solubility of the polymers. The semi-crystalline homopolymer PGA only dissolves in very strong solvent, such as hexafluoroisopropanol. Thus, PGA is not favourable for various solvent related processing techniques, such as solvent casting, and solution spinning. The solubility of PLA is affected by the polymer crystallinity and molecular weight. PLA with higher crystallinity and molecular weight is harder to dissolve than PLA with lower molecular weight and crystallinity [22, 26, 27]. Good solvents for PLLA include dichloromethane (DCM) and chloroform [26].

PLA is susceptible to thermal degradation at temperatures above 200 °C, causing molecular weight and viscosity changes. These will result in the compromise of the mechanical properties of injection moulded PLA [44]. Conventional processing methods, like injection moulding, extrusion and melt-spinning, will normally subject the polymer to processing temperatures 10-15 °C above its melting temperature. Anders et al [26] concluded that melt spinning of PLLA had drastic thermal degradation. The decrease in molar molecular weight during the melt spinning could go up to 70 %. The same polymer, when subjected to solution-spinning, showed higher tensile strengths (27 % higher) and modulus (72 % higher). The better mechanical properties yielded were explained by the minimal degradation encountered by solution-spinning. New processing methods like RP must be sought when processing parts like scaffolds, where mechanical properties are vital to performance.

2.2.2. PCL

2.2.2.1. Biocompatibility

PCL is believed to be a suitable implant material due to its high biocompatibility with osteoblast and is primarily aimed as bone substitute material for maxiofacial reconstructive surgery [44]. PCL is less rigid than PLLA, and has been suggested to be more susceptible for cell growth and matrix formation [20].

2.2.2.2. Hydrolytic degradation

PCL degrades through hydrolysis under physiological conditions [42]. PCL degrades at a much slower rate than PLA. It has a degradation time of 2 to 3 years [45]. For example, PCL with an average molecular weight of 50,000 Da takes about 3 years for complete degradation in-vitro [45]. PCL is useful in the development of long term implants and controlled release [40] applications.

2.2.2.3. Mechanical properties

PCL has been used to make fixation of rabbit humeri osteotomies. The results showed that PCL fixation caused less stress shielding than stainless steel. Nevertheless, the malunion rates reported indicated PCL mechanical strength was not sufficient for load-bearing applications [40]. According to Williams et al [46], scaffold constructed by pure PCL with SLS had compressive modulus and yield strength values ranging from 52- 67 MPa, and 2.0- 3.2 MPa, with a porosity of 37 %. This scaffold only possessed mechanical properties within the lower range of trabecular bone. However, biocomposites made up of PCL may possess the appropriate mechanical properties and resilience to serve as scaffolds. Much research is currently focused on the use of PCL biocomposites and its copolymers with both natural and synthetic polymers to enhance the strength of the PCL products [46]. The limitation of PLLA in application, attributed to its brittleness, can be improved by copolymerization or polyblending with PCL [20]. According to Zhao et al [20], the incorporation of PCL effectively reduced the rigidity of PLLA. However, the strength of the scaffold was not reported. PCL in combination with reinforcing filler has been proposed for load bearing implants such as intramedullary pins [44].

2.2.2.4. Permeability

One of the attractive points of PCL is its low glass transition temperature (T_g) of about -60 °C. PCL is always in rubbery state at room temperature, which undoubtedly contributes to the very high permeability of PCL for many therapeutic drugs [42].

2.2.2.5. Processability

One of the attractive points of PCL is its low cost, and is readily available in large quantities, as compared to other biodegradable polymers such as PLA and its copolymers [7]. This allows PCL to be processed using conventional processing methods, which usually require large amount of materials. PCL has a larger processing window, thus thermal degradation of this polymer can be avoided,

unlike PLA. No reduction in chain length was reported for PCL injection moulded samples of up to 160 °C [44].

In addition to the above, PCL can dissolve more readily in organic solvents as compared to PGA and PLLA. The most common solvents used include DCM [7], THF [20] and acetone [47]. Another interesting property of PCL is its propensity to form compatible blends with a wide range of other polymers [42].

PCL scaffolds have previously been created with a variety of RP techniques [46] including FDM, SLS, photopolymerization of a synthesized PVL macromer, shape deposition modelling, precision extruding deposition, 3DP, low-temperature deposition, and multi-nozzle free form deposition.

2.3. Synthesis of PLA

LA can be manufactured by different polymerization routes, as shown schematically in **Figure 2.1**. Low molecular weight PLA (<10,000 Da) is commonly synthesized by melt polycondensation with or without catalyst. High molecular weight PLA (>80,000 Da) can be attained through three routes: condensation/ coupling, solution polycondensation or ROP.

Figure 2.1 Synthesis of PLA by different methods

In the condensation/ coupling method, the bifunctional end groups (-OH and -COOH) of low molecular weight prepolymer are modified by chain-coupling agents into monofunctional end group (all -OH or all -COOH). The modified prepolymer is then further linked with chain extending agents to attain higher molecular weight. The polymer produced has good mechanical properties. This method is economically feasible and has the flexibility to manufacture copolymers with different functional groups. However, the final polymer may still contain unreacted chain-coupling and chain-extending agents, which are not biodegradable and biocompatible [48].

Another method to obtain high molecular weight PLA without the use of chain-extending agents is through solution polycondensation. This method was first developed by Ajioka et al [49] in 1995. However, the use of solvent increases the difficulty of process control and polymer purification. This in turn increases the process cost and limits the process to small-scale production [49, 50].

Recently, a sequential melt/ solid polycondensation method was developed by Moon et al [51, 52] to produce PLLA with high molecular weight. A low molecular weight PLLA was first prepared by conventional melt polycondensation. The PLLA produced was then heated at a temperature of around the crystallization point for a week for solid polycondensation. A PLLA with high molecular weight of up to 100,000 Da and of high crystallinity was obtained. However, the process of solid polycondensation is time consuming.

Two-step ring opening polymerization (ROP) process is the most commonly used method to produce high molecular weight pure PLA, without any chain-extending agent. The lactides are produced through depolymerization of LA oligomer in the first step. The second step involves ring opening process and polymerization of lactides to form PLA (**Figure 2.1**). The catalysts/ initiators used are transition metal compounds of stannous, aluminium, lead, zinc, bismuth, iron and yttrium. Stannous(II) octoate (Sn(II) Oct) is the most effective initiator for this process.

ROP can be carried out at low reaction temperatures (110-130 °C) to produce high molecular weight PLA [53]. This process is highly successful in producing pure PLA with good molecular weights and mechanical properties because side reactions are suppressed at low reaction temperatures. However, the cost of producing PLA using ROP is high due to the complicated process of lactide purification [22]. In addition, the types of monomer available for ROP process are limited. Hence the flexibility to manufacture copolymers with different functional groups is also greatly reduced. However, after the synthesis process, the initiators attached to the polymer chain ends are hard to remove. This may change the properties and increase the toxicity of PLA, especially for the lower molecular weight polymers. Furthermore, the attached initiators are reported to enhance the thermal degradation of PLA at elevated temperatures [26].

2.3.1. Melt polycondensation

The cheapest way to produce PLA is through melt polycondensation of lactic acid. The PLA produced by this method has low molecular weight ($<10,000$ Da) and is brittle. This PLA is appropriate for drug delivery and not for any load bearing applications [54- 57]. It is difficult to produce high molecular weight PLA by conventional melt polycondensation, even under high temperature and long reaction duration.

The PLA melt polycondensation reaction is generally carried out at high reaction temperatures ($\sim 150-200$ °C) to enhance the polymerization rate. In the initial stage of polycondensation, polymerization reaction is autocatalyzed by carboxyl end groups, without the presence of catalyst [58]. As the molecular weight of the polymer increases with reaction time, the number of carboxyl end groups reduces significantly [24]. The reduction in functional groups inhibits the autocatalytic effect and the polymerization reaction. Catalysts are needed in order to attain higher molecular weight PLA. The reduction of the functional groups causes the polarity of the reaction medium to decrease. It is advisable to add the catalyst at this low polarity stage instead of the initial high polarity stage to maintain the effectiveness of the catalyst [24]. As the reaction continues and the molecular weight increases, the reaction medium becomes increasingly viscous. The occurrence of polymerization is now determined by the probability of end groups to meet and bind with each other [58].

Two main reaction equilibriums are involved in PLA polycondensation, namely dehydration equilibrium and ring-chain equilibrium. The dehydration equilibrium involves polymerization reaction through the condensation of lactic acid bifunctional end groups to give the polymer and the by-product water (**Figure 2.2**).

Figure 2.2 Dehydration equilibrium for polymerization of PLLA

In order to shift the dehydration equilibrium towards polymerization, the by-product water has to be removed continuously and efficiently through distillation. As the reaction proceeds, the polymeric melt viscosity increases and the removal of water becomes increasingly difficult with time. Failure to remove water instantaneously during this stage causes the reaction to remain at equilibrium. In a worse case scenario, ester hydrolysis may take place and causes the reduction in molecular weight. Thus, high vacuum is generally applied throughout the reaction to facilitate the removal of water [58- 60].

Although, high reaction temperature and high vacuum are needed to promote polymerization, these conditions at the same time cause side reactions such as ester-interchange reaction, oxidative degradation and formation of lactide. The lactide formed has low boiling point and is easily distilled off together with the by-product water. However, the removal of lactide displaces the ring-chain equilibrium, as shown in **Figure 2.3**, towards the formation of more lactide. This induces depolymerization of the aliphatic PLA chain formed and reduces the molecular weight.

Figure 2.3 Ring-chain equilibrium for depolymerization of PLLA into lactide ring structure

To obtain high molecular weight PLA, the dehydration and ring-chain equilibria must be controlled simultaneously. The polymerization step is desired and should

be enhanced, while the lactide formation process, which causes depolymerization, should be inhibited to attain high molecular weight polymer [22, 24].

The high reaction temperature also causes the PLA to undergo thermal degradation and oxidative thermal degradation. Thermal/ thermal oxidative degradation causes polymer chain scission and deactivation of functional groups. This degradation is always accompanied by the formation of CO, CO₂ and carbon. The formation of carbon is believed to be one of the main reasons causing PLA discoloration. Melt polycondensation is carried out under vacuum to prevent oxidative thermal degradation. However, it is difficult to completely remove oxygen from the reaction system and oxidative thermal degradation will occur [22, 24, 27].

Ester-interchange reaction is another side reaction in polycondensation of PLA. It involves the interchange of ester-ester, hydroxyl-ester and carboxyl-ester. It is reportedly the main factor that causes racemization, where a small amount of L-lactate units in the PLLA chains transforms to D-lactate units [28, 61, 62]. The presence of D-lactate units in the polymer chains hinders chain alignment and reduces the crystallinity of the polymer [28].

In summary, the high temperature and vacuum needed for polymerization bring about certain undesirable side reactions at the final stages of polymerization which induce depolymerization and inhibit the increment of polymer molecular weight. Hence, it is necessary to search for suitable catalyst candidates, which will maximize the polymerization reaction while minimizing the side reactions.

2.3.1.1. Catalysts

The catalysts used for polycondensation can be divided into two categories based on their catalytic mechanism, namely proton acid and metal ion (Lewis acid). Proton acid catalysts give out protons and promote protonation of the

carboxyl group. The protonized carboxyl group enhances the addition reaction of nucleophilic hydroxyl group and therefore the polymerization [63, 58]. The reaction mechanism catalyzed by proton acid is illustrated as below:

(1) The oxygen in the polymer carboxyl end group (-COOH) is protonized.

(2) The protonized carboxyl end group is then attacked by the oxygen of -OH end group of another polymer chain to form a tetrahedral intermediate. This step is the rate-determining step.

(3) Water molecule is released after the tetrahedral intermediate undergoes proton transfer.

(4) Finally, the proton catalyst is regenerated. The two initial shorter polymer chains are now bounded with each other to form a longer polymer chain.

It is generally accepted that metal ion catalyst provides ligands for the exchange of carboxyl and hydroxyl groups in the melt medium. The detailed reaction mechanism is however rather controversial. A reaction scheme of the metal ion catalyst was proposed by Kim et al [50], using stannous (II) chloride dihydrate ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) as an example.

(1) Self-condensation of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ into an activated hydrate with ligands (L).

(2) The $-\text{COOH}$ end group of PLA attacks the $-\text{OH}$ group of the catalyst site and forms a catalyst complex. Water is released.

(3) The $-\text{OH}$ end group of PLA coordinates with the catalyst complex.

(4) The polycondensation reaction between the positively charged -COOH group and nucleophilic -OH group is induced around the catalyst site to form the polymer.

Another catalyst reaction scheme was suggested [64]. According to this scheme, the catalytic effect arose from the complexation of carboxyl group on the metal atom in the second step. The complexation induced a positive charge on the carboxyl group. This encouraged the nucleophilic hydroxyl end groups of PLA to react with the carboxyl group.

The catalytic activity of metal ion catalysts is dependent on the composition of the reaction medium and the reaction conditions. The presence of water, which is unavoidable in melt polycondensation, may lead to the formation of metal-oxygen-metal bonds and of condensed forms of metal alkoxides. These formations decrease the number of active exchangeable sites and, therefore, lower the catalytic activity [58].

Hiltunen et al [28] reported the effects of different single catalysts and polymerization temperature on the molecular weight and crystallinity of PLA. The

catalysts used include various kinds of metal ion and proton acid catalysts. By varying different catalysts and polymerization conditions, the range of molecular weights obtained was from 3,000 Da (without catalyst) to more than 20,000 Da (with catalyst). The crystallinity of PLA was also varied from totally amorphous to highly crystalline (52.3 %). It was reported that sulfuric acid was the best catalyst at every temperature used (180- 220 °C) producing the highest molecular weight and crystalline PLA. Other possible catalysts include antimony (III) oxide, dibutyltin dilaurate, zinc chloride, and Sn(II) Oct.

The effect of binary catalysts systems on PLLA melt polycondensation, were studied by Kimura et al [24, 25] and Gao et al [23]. The two types of binary-catalyst systems reported to produce PLLA with high molecular weight and crystallinity in a relatively short reaction time were stannous(II) chloride dihydrate/*para*-toluene sulphonic acid monohydrate (SnCl_2/TSA) and stannous(II) chloride dehydrate/ germanium(IV) tetraethoxide ($\text{SnCl}_2/\text{Ge}(\text{Oet})_4$). The binary-catalyst systems effectively promoted the dehydration reaction and minimized various side reactions. The increment of dehydration reaction enhanced polymerization, while the reduction of side reactions slowed down the depolymerization reaction. Thus, the molecular weight of the polymer produced by the binary-catalyst systems was higher than that produced by single-catalyst system in a relatively short reaction time. With racemization restrained, the crystallinity of PLLA was increased. In addition, the binary-catalyst systems minimized the polymer discoloration, which was probably caused by thermal oxidative degradation.

2.4. Scaffold Fabrication Techniques

Traditional techniques of scaffold fabrication comprise of fiber bonding, solvent casting and particulate leaching, membrane lamination, melt moulding and gas foaming. However, these techniques are inadequate to produce a multi-functional scaffold suitable for both TE and controlled release. The drawbacks for these techniques include the extensive use of highly toxic organic solvents, incomplete

removal of residual particulates in the polymer matrix, irregularly shaped pores, and insufficient interconnectivity of pores. The incorporation of the gene/protein is normally limited to one type, without spatially patterning for release sequence, time or concentration control. The rest of the disadvantages of traditional techniques include long fabrication periods, labour-intensive processes, poor repeatability, and thin structures [32, 39, 40, 43]. Moreover, most of these methods bear a restriction on shape control and fail to produce individual tailor-made TE scaffold. This is because these traditional methods lack the capability of computer designing and fabricating external and internal shapes [39, 32, 43].

2.4.1. Rapid prototyping

The technologies developed recently, culminating in rapid prototyping (RP) or solid free form (SFF), provide more options to fabricate scaffolds, and have overcome certain problems faced in traditional techniques. The basic concept for combining RP and TE is illustrated in **Figure 2.4** [65].

Figure 2.4 Concept of the fabrication of individual tailored made scaffold by the combination of RP and TE techniques

RP is a generic term for a number of technologies that enable components to be made with neither the need for conventional tooling nor the services of skilled model-makers [65- 67]. These RP technologies enable the fabrication of objects with complex architectures according to the computer-aided design (CAD) without the preparation of moulds. The computer converts the virtual object, created by CAD into the corresponding real three-dimensional (3D) objects. This object is built layer by layer of slices in sequence. As the scaffold design is based on a computer software model, the desired 3D interconnectivity and structure can be accurately controlled by the highly repeatable computer-controlled fabrication process. This combination of computed design and computer controlled fabrication of complex 3D architectures is the key feature of RP technologies [31]. In addition, the complicated scaffold architecture can be tailor made individually to fit into the tissue defect of an individual patient [31].

Stereolithography apparatus (SLA), selective laser sintering (SLS), laminated object manufacturing (LOM), fused deposition modelling (FDM), and three-dimensional printing (3DP), are examples of available RP techniques [66]. Each RP technique has its own advantages and disadvantages. The way in which the layers are produced, and the materials in which parts can be built; vary significantly between the different RP processes. It is necessary to understand the conditions of each RP techniques before an RP process is selected in order to fulfil the requirements of the end-application.

2.4.2. Three-dimensional printing

Three-dimensional printing (3DP) belongs to the binding process. The 3DP machine consists of a feed chamber, built chamber, a feed roller and binder cartridge (**Figure 2.5**) [66].

Figure 2.5 Three-dimensional printer

The feed chamber contains a quantity of specially prepared raw printing powder. The vertical position of the powder upper surface can be varied by raising or lowering the feed chamber piston. The adjacent build chamber operates in a similar manner. The vertical piston of the build chamber determines the height of the chamber. The horizontally reciprocating feed roller transfers the raw powder from the feed chamber to the build chamber, and spreads the powder evenly to a single layer. Excess powder for each layer is swept down to an overflow chute. The binder cartridge travels over the surface layer of the build chamber powder to deposit a binder solution matching the current slice of CAD data. The build chamber piston then descends a predetermined distance. This distance is the layer thickness and can be varied between 0.100- 0.250 mm [65]. New layer of powder is then spread from the feed chamber over the build chamber surface and the printing process is repeated. When all the layers have been printed, the

untreated powder is cleaned from the printed part and this printed part is then removed from the build chamber.

3DP process can be applied to build parts derived from different materials including polymer, metal and ceramic powders. Typical binders for the metal and ceramic parts are polymer emulsions (e.g. acrylic copolymer) and inorganic copolymers (e.g. colloidal silica) [68, 69]. Organic polymers are printed with the certain corresponding solvents. PLA and its copolymers are generally printed with chloroform, which is the only few organic solvents that can readily dissolve and bind the powder particles. However, chloroform is toxic and the residue in the scaffold will cause adverse effect to the cells. According to Giordano et al [33] the amount of chloroform that remained in PLLA scaffold was 0.5 wt% after 1 week of drying, whereas the amount of residual chloroform permitted in drugs, defined by the US Pharmacopoeia, was 0.006 wt% [4]. Recently, residual chloroform extraction using liquid carbon dioxide has been investigated [18]. This technique reduces the level of chloroform below 0.005 wt%. Another option is to use a non-toxic solvent as binder. Lam et al [30] used water as a binder with 3DP to produce starch-based polymer (cornstarch, dextran and gelatine) scaffolds. However, heat treatment and infiltration of these scaffolds with PLLA and PCL solution in dichloromethane were required to prevent the dissolution of the scaffold under in vivo conditions.

3DP technique requires shorter object building time than other RP technologies. Complex scaffold architectures with through channels and overhanging features can be produced without any support structure [30]. The key advantage of 3DP is that no heating and melting are needed throughout the process, unlike normal large-scale processes such as injection moulding, blow moulding, thermoforming and extrusion [64]. This allows 3DP to be used for thermal sensitive polymeric materials, such as biodegradable polymers PLA [30, 32]. In addition, this enables the 3D printing process to incorporate various protein, gene and cells, which denature at elevated temperatures. Water based binder or powder blends can be

formulated to allow the incorporation of these biological and/ or pharmaceutical agents into the scaffolds, with predetermined control release profile. The use of multiple print heads with 3DP will permit such agents to be dispensed into the scaffolds during fabrication [36].

One of the drawbacks for this powder-filled 3D structure is the difficulty in removing internal unbound powder. The resolution of the 3D printed scaffolds is also limited by the nozzle size, the precision of the print head movement and the powder particle size. In the context of TE scaffolds, base biomaterials are not usually available in powder form and thus require grinding [2].

A scaffold microstructure, including pore shape, pore size, porosity, and interconnectivity, plays a significant role in scaffold-tissue interaction, degradation control and mechanical support. Conventional scaffold fabrication methods are not capable of fabricating interconnected porosity throughout the scaffold. More importantly, their geometric parameters cannot be consistently controlled and easily reproduced. However, 3DP can fabricate complicated regionally architectures into the scaffold specifically, such as engineered macroscopic channels and microscopic porosity [5, 30].

The microstructure of the scaffold can be tailored by varying the printing speed, the flow rate, drop position of the liquid binder and the particle size/ layer thickness [39]. The scaffold fabricated by 3DP is porous in nature. The pores of the 3DP scaffold are irregular in shape with a wide range of sizes, from few μm to few hundreds of μm . More consistent pores in size, shape and orientation can be generated by mixing porogens into the powders prior to scaffold fabrication [39]. However, the application of porogens might lead to residues within the scaffolds due to incomplete leaching. In addition, the bindings of the particles formed by 3DP are relatively weak. The inclusion of particulates leaching method generally increases the porosity and significantly reduces the strength of the scaffold [30]. In order to improve the mechanical properties of the scaffold, small pore size and

low porosity are desirable. Nevertheless, high porosity and good pore interconnectivity are the two critical conditions to enhance cell growth [13, 14]. The mechanical properties of 3DP scaffolds with optimum porosity are one of the main considerations that need to be addressed.

Giordano et al [33] succeeded in fabricating dense PLLA scaffolds with chloroform as binder. Test bars were fabricated from low and high molecular weight PLLA powders. Cold isotactic pressing (CIP) was performed after printing to improve the mechanical properties for the printed bars. The maximum measured ultimate tensile strength (UTS) was 17.40 MPa (53,000 Da), elastic modulus 450.19 MPa with strain at UTS 3.41 %. However, due to the densification of PLLA powder with the use of chloroform and post-printing process CIP, the scaffolds made were dense.

Sherwood et al [14] has fabricated discrete phase composite scaffolds from PDLGA/ PLLA in one phase and PLLGA/ TCP in the second phase. Pores were generated using salt leaching method. The maximum measured UTS and modulus of the PLLGA/ TCP phase was 5.7 MPa and 200 MPa, with 25 % porosity. However, when the porosity increased to 55 %, UTS and modulus reduced to 1.6 MPa and 83 MPa.

The mechanical properties and porosity of the starch based polymer scaffold produced by Lam et al [30] had a maximum compressive yield strength and modulus of 1.77 MPa and 55.19 MPa respectively. The corresponding porosity was about 43 %. The porosity decreased from 50 % to 43 % after infiltration, while the strength increased from 1.12 MPa to 1.77 MPa. A linear and inversely proportion relationship between the compressive stiffness and porosity was suggested.

A few research groups have demonstrated that 3DP scaffold support cell attachment in vitro [5, 13, 14, 18, 70, 71] and single tissue regeneration in vivo

[72- 74]. Kim et al [71] employed 3DP, with salt leaching technique, and created porous PLGA scaffolds. The pore size ranged from 45-150 μm and the porosity was 60 %. To improve pore interconnectivity and porosity, the scaffolds were constructed by printing and horizontally stacking 800 μm diameter longitudinal channels that were arranged in parallel throughout the scaffold's height. Electron microscopy examinations conducted after 2 days of in vitro cell culture with hepatocytes (HCs) revealed the successful attachment of large numbers of HCs to the scaffolds outer surfaces and internal channels. Histological sections showed the in-growth of HCs into the pore structures.

Zeltinger et al [13] investigated the pore size and porosity of 3DP scaffold constructed using scaffold leaching method on cell adhesion, proliferation, and matrix deposition. The scaffolds had two different porosities (75 % and 90 %) and four different pore size distributions (<38, 38-63, 63-106, 106-150 μm). Three different types of cell, canine dermal fibroblasts, vascular smooth muscle cells and microvascular epithelial cells were cultured on the scaffolds. Cell culture results demonstrated the suitability of 3DP scaffolds in supporting tissue development for different cell types.

Roy et al [72] analyzed the in vivo performance of composite PLGA/ TCP 3DP scaffold in a rabbit calvarial defect model. The scaffold was printed with designed macroscopic channels, controlled porosity gradient (87.5- 80 %) and pore size (125-150 μm). This scaffold had a higher percentage of new bone growth as compared to scaffolds without the designed channels. This work suggested that channel size, porosity and pore size can be controlled and used to influence new bone formation and calvarial defect healing.

Wu et al [75] explored the possibility of 3DP techniques to fabricate scaffold with controlled release of multiple drugs in rhythmic cycles and approximate zero order release kinetics. Several construction methods were demonstrated to test the drugs control the release. The result showed that the release rate and

release time could be controlled by either specifying the position of the drugs within the device or by controlling the local composition and microstructure with the 3DP process. In addition, the mechanism of resorption could also be controlled by manipulating the composition and microstructure of the device during construction.

As mentioned earlier, one of the major limitations of 3DP biodegradable polymeric scaffolds is the low mechanical properties [14, 30, 33]. Here, 3DP scaffolds of higher strength with suitable porosity and pore sizes were manufactured and characterized. Another limitation of 3DP of PLLA scaffolds is the use of chloroform as the binder. Here, an indirect binding approach was investigated to replace the toxic chloroform. This approach also enables 3DP to be used on a wider selection of materials. By proper materials selection and combination, the degradation and the mechanical properties of the scaffold can be controlled and adapted to the specific tissue requirement. This provides a sequential transition in which the regenerated tissue assumes function as the scaffold degrades. In addition to the above, this approach also enables the incorporation of different biofactors into the scaffold. As the scaffold degrades, controlled release of multiple biofactors at different periods of implantation can be obtained.

Chapter 3. Materials and Methods

3.1. Materials

L-LA 80 wt% aqueous solution, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$, TSA, Sn(II) Oct , PCL (80,000 Da), and chloroform-d were purchased from Sigma-Aldrich Chemical Co. Arabic gum (AG), chloroform, diethyl ether, DCM and tetrahydrofuran (THF) were purchased from Merck. All the reagents were used as received. High molecular weight PLLA (130,000 Da), purchased from Cargill Dow, were spherical granules in about 3 mm diameter. The polymer was purified using the same method used for the purification of synthesized PLLA.

3.2. Synthesis of PLLA

Three different catalyst systems were used in the polycondensation of L-LA and their effects on the polymer molecular weight, racemization and discoloration were studied. The two single-catalyst systems included Sn(II) Oct and TSA. The binary-catalyst system used consisted of the combination of SnCl_2 and TSA.

The polycondensation process consisted of two steps, which were oligomerization and polymerization. In the oligomerization step, L-LA was first heated to 150 °C, at 760 torr atmospheric pressure in a 250 ml three-neck flask for 2 hours to remove the free water. The three-neck flask was equipped with a mechanical stirrer and connected to a vacuum system through a cold trap. The pressure of the reacting system was then reduced to 100 torr and maintained at 100 torr for the next 2 hours. This was followed by another pressure reduction to 30 torr for 4 hours. The reaction temperature was kept at 150 °C throughout the entire oligomerization process. The product was a transparent viscous oligomer gel. The initial oligomer was further reacted under different reaction conditions to produce oligomers with different M_n .

In the polymerization step, catalyst(s) was added into the oligomer, and the resulting mixture was mechanically stirred. The polymerization step was divided

into two stages: the ramping stage and the constant stage. During the ramping stage the temperature of the system was increased while the pressure was reduced to meet the optimized reaction conditions. Four different ramping sequences were conducted as detailed in **section 4.4.2.1**. In the constant stage the temperature and pressure were maintained at a fixed reaction conditions for a predetermined period of time. Two different polymerization temperatures, 180 °C and 220 °C were studied. The reaction time investigated were 8, 12, 18 and 20 hours for 180 °C, and 0.5, 2, 4 and 8 hours for 220 °C. The reaction pressure was maintained at 10 torr, which was reported optimum pressure for PLLA melt polycondensation [24].

The colour of the final product, before purification, was noted in order to determine the discoloration effect of the polymer. The final product was dissolved in chloroform and precipitated in diethyl ether. The polymer was filtered out from diethyl ether, and dried under vacuum at room temperature to yield the purified PLLA, which was white in colour. The percentage yield was determined by the weight ratio of the purified PLLA to the initially charged reagent.

3.3. Preparation of Films

PLLA films were prepared by a simple solvent casting method. The synthesized PLLA was dissolved in DCM at a concentration of 0.35 g/ml. The PLLA solution was then spread over a glass plate using a film applicator. The wet thickness of the film was set at 300 μm by controlling the thickness gap of the film applicator.

The film was air dried at room temperature for 2 days to allow the solvent to evaporate slowly in order to prevent the formation of air bubbles. The film was then kept under vacuum at room temperature for a week to evaporate any remaining solvent. The dried film with a thickness of around 45 μm was cut into dimensions of 3 cm x 1 cm.

3.4. In Vitro Hydrolytic Degradation

The in vitro degradation of PLLA was conducted by degrading the films in phosphate buffered solution (PBS) at 37 °C for a fixed period of 135 days. The changes to the film properties, such as mass, molecular weight, molecular weight distribution and surface morphology, were monitored throughout the whole degradation process. The changes to the thermal properties and degree of crystallinity were also examined.

The initial mass (m_0) of each film was measured and recorded before it was degraded. The films were placed in 10 ml screw top bottles containing PBS at pH 7.4. The bottles were then incubated in a water bath at 37 °C without agitation. The pH of the buffer solution was monitored over time to ensure that a stable pH of 7.4 was maintained at all times.

At fixed time intervals, the films were removed from the PBS solution, rinsed with distilled water and surface dried using water absorbent paper. The samples were then vacuum-dried at room temperature for three days before analysis. After drying, the dried mass (m_d) of the film was recorded. The mass loss of each film was calculated using **Equation 3.1**:

$$\text{Mass loss (\%)} = 100 \times \frac{(m_0 - m_d)}{m_0} \quad \text{(Equation 3.1)}$$

3.5. Scaffold Fabrication

3.5.1. Powder preparation and scaffold printing

A 3D printer, developed and commercialized by Z-Corp (Z400) for water-based ink printing was used. The standard materials and binder provided by Z-Corp were meant for non-tissue engineering purposes. In this study, a mixture of PLLA with two different molecular weights and a selected solid phase binder AG powder were used. The purchased PLLA with higher molecular weight, M_w of 130,000 Da was cryogenic milled (SPEX CertiPrep 6850 Freezer/Mill) to smaller

sizes after purification. The synthesized PLLA had a lower M_w of around 15,000 Da was in fine powder form after purification, and no milling was required. Sieves with micro mesh sizes were used to separate the PLLA particles into size range of 106-212 μm . PLLA powder coarser than 212 μm was reground to achieve the desired particle size. The PLLA and AG powder were mixed in 80: 20 weight ratio using turbula mixer for 1 day to ensure homogeneity. All the ground powders were stored at all times, before and after milling, in a vacuum oven at 30 °C. Distilled water was used as the 'ink' instead of the generally used solvent chloroform.

During printing, a thin layer of powder mixture was spread on a piston plate and the print head rastered above the powder bed and deposited water droplets in selective areas to create the scaffold. The water deposited dissolved the AG, binding the PLLA particles together. After one layer was completed, a new layer was spread, followed by the deposition of water. The lamination process was repeated until fabrication of the scaffold was completed. Printing parameters, layer thickness and saturation level, were studied to minimize the scaffold degree of planar distortion. The plate of the printed parts was dried overnight at room temperature and the loose powder was removed to reveal the final scaffolds. Residual water was removed with further drying in vacuum at 30 °C for another 3 days.

Both rectangular bars (12 mm X 7.2 mm X 2.4 mm) and dog bone shape tensile bars were printed. The tensile bars were printed based on the dimensions stated in ASTM standard D638-91 [33], with the length of the gripping portions at each side of the tensile bar reduced by 20 mm. The modification in dimensions was made to reduce the amount of powder required per sample, due to the low yield from the cryogenic process.

3.5.2. Post-printing processes

Post-printing processes were necessary to enhance the strength of the scaffolds and also increase its resistance to disintegrate in water. In this study, the post-printing processes involved were infiltration and annealing. The effectiveness of these post-printing processes was evaluated separately and jointly. The process parameters and the sequence of these two post-printing were also optimized.

3.5.2.1. Infiltration

Four different infiltration methods were experimented, in order to select the most appropriate method which ensured uniform penetration of infiltrant through the entire scaffold. In the first and second methods, scaffolds were soaked in the infiltrant without/ with ultrasonic bath for various lengths of time. The third and fourth methods' simple set-up are shown in **Figure 3.1 (a)** and **(b)**, whereby 20 ml of infiltrant were forced through the scaffold via a vacuum pump or by a plunger. The infiltrants were prepared by either dissolving PLLA (M_w 130,000 Da) or PCL (M_w 80,000 Da) in DCM and THF in a predetermined ratio. The infiltrated scaffolds were dried in an enclosed space to allow for slow solvents evaporation. This was to prevent any stress built up and warpage of the scaffolds. The scaffolds were then dried in vacuum at 0.0005 torr, 50 °C for 1 week to remove any residual solvents.

Figure 3.1 Infiltration set-up for (a) vacuum method (b) plunger method.

Infiltration efficacy (IE) was calculated using **Equation 3.2** to determine the ability of the infiltrant to penetrate and be retained in the scaffold.

$$IE (\%) = \frac{m_{IF} - m_0}{m_0} \times 100\% \quad \text{Equation 3.2}$$

where m_0 was the mass of the scaffolds before infiltration. m_{IF} was the mass after infiltration and drying for one week. The effect of different infiltrant materials, annealing conditions, mixing ratios of PLLA_{high}/ PLLA_{low}, and infiltrant concentrations to the IE were studied.

3.5.2.2. Annealing

Annealing was another method which served to increase the strength of the porous material by increasing the adhesion between particles. The effect of annealing environment, duration and temperature on the mechanical properties were studied. Scaffolds were annealed in a furnace oven in both vacuum and nitrogen environments for 1 to 15 hours at temperatures ranging between 100 and 190 °C.

3.5.2.3. Evaluation of post-printing processes

The infiltration techniques and annealing conditions were evaluated based on the samples' resistance to 10 minutes in an ultrasonic bath. Each scaffold was halved and placed in an ultrasonic bath for 10 minutes. The effect of water combined with ultrasonic dissolved and removed the AG from the scaffold. With AG no longer contributing to the scaffold strength, the remaining structural integrity was dependant on the inter-particle strength brought about by infiltration or annealing. Samples which crumbled meant that the infiltrant was unable to penetrate through the middle of the scaffold; or the annealing conditions were not sufficient to form inter-particle bonding. As a result, that particular infiltration method or annealing condition was eliminated. Only methods or conditions which produced scaffolds that did not crumble were considered and used in mechanical testing.

3.6. Characterization Techniques

The properties of the polymer synthesized with different reaction conditions and catalysts were studied using gel permeation chromatography (GPC), modulated differential scanning calorimetry (MDSC), Fourier-transform infrared spectroscopy (FTIR), ^1H NMR and ^{13}C NMR. The hydrolytic degradation of the polymer was also studied. The mechanical strength, porosity and morphology of the PLLA scaffold fabricated using 3DP with different printing parameters and post-printing processes were investigated. The characterization study allowed a good understanding and justification on the effectiveness of the synthesis process, and the scaffold fabrication process.

3.6.1. Gel permeation chromatography (GPC)

GPC was used to study the molecular weight of the synthesized polymer, the molecular weight change of the polymer film during degradation and the molecular weight change of the scaffold fabricated before and after annealing.

Agilent series 1100 gel permeation chromatograph (GPC) was used. A combination of two polystyrene gel columns, PLGel Mixed Bed 5 μm , 300 x 7.5 mm and a guard column 50 x 7.5 mm, were used at 35 $^{\circ}\text{C}$ with THF and DCM in a volume ratio of 80:20 as the mobile phase. The molecular weights of samples were obtained relative to polystyrene standards.

3.6.2. Modulated differential scanning calorimetry (MDSC)

MDSC was used to study the thermal properties and crystallinity of the synthesized PLLA, films, and scaffold.

Thermal studies were performed using a modulated DSC (DSC 2920, TA Instruments, USA), equipped with refrigerated cooling system (RCS). The polymer was first heated from -20 $^{\circ}\text{C}$ to 180 $^{\circ}\text{C}$ and cooled back to -20 $^{\circ}\text{C}$, followed by a second heating scan to 180 $^{\circ}\text{C}$. A heating rate of 5 $^{\circ}\text{C}/\text{min}$ was used for both the heating and cooling scans.

To avoid oxidative degradation, the sample and reference pans were purged with nitrogen at a constant flow rate of 48 ml/min. The amount of each sample used for analysis was about 5 mg. The modulated mode allows the heat capacity of the sample to be measured in one run with an error of 1 % or less. The glass transition temperature (T_g), melting temperature (T_m), crystallization temperature (T_c) and degree of crystallinity (DOC, %) of the polymer were measured.

The T_m and T_c were taken as the temperature corresponding to the maximum of the endothermic and exothermic peak respectively. The DOC was calculated from the difference in the melting enthalpy (ΔH_m) and crystallization enthalpy (ΔH_c) from the first heating scan as shown in **Equation 3.3** [22]. DOC determined the crystals formed originally in the polymer sample before the first MDSC heating scan.

$$DOC(\%) = \frac{\Delta H_m - \Delta H_c}{(\Delta H_{m100\%})} \times 100\% \quad \text{(Equation 3.3)}$$

ΔH_m and ΔH_c were taken as the total area under the endothermic and exothermic peaks respectively. $\Delta H_{m(100\%)}$ was the enthalpy of melting for a perfect PLLA crystal. The value of $\Delta H_{m(100\%)}$ for PLLA was taken as 93 J/g. [22, 26]

The crystallizability of the polymer was the intrinsic ability of the polymer to crystallize. Crystallizability was directly affected by the stereoregularity of the polymer chains. Polymer with higher stereoregularity presents higher crystallizability [22, 26, 76].

The first heating scan cancelled the thermal history of the polymer. Thermal history affected the degree of crystallinity of the polymer. The first heating scan melted all the crystals previously formed during synthesis process. In the second heating scan, a melt-crystallization peak was observed followed by a melting peak. The polymer recrystallized during the heating process of the second heating scan and hence the melt-crystallization peak. These crystalline regions

then melted with further heating, which gave rise to the melting peak observed. The area under the melt-crystallization peak (ΔH_c) was equal to the area under the melting peak (ΔH_m).

Crystallizability of the polymer was determined by either the ΔH_m or the ΔH_c presented in the second heating scan of polymer MDSC. In this case, ΔH_m was used to determine the crystallizability according to **Equation 3.4** [28, 76, 77]:

$$\text{Crystallizability (\%)} = \frac{\Delta H_m}{(\Delta H_{m100\%})} \times 100\% \quad (\text{Equation 3.4})$$

3.6.3. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra of the synthesized polymer were obtained using the Perkin Elmer system 2000 Fourier Transform Infrared Spectrometer. The polymer sample was first thoroughly grounded and mixed with KBr powder before compressing into discs. A total of 16 scans per sample at a resolution of 4cm^{-1} were obtained over the mid IR region of 4000 to 400 cm^{-1} .

3.6.4. ^1H NMR and ^{13}C NMR spectroscopy

^1H NMR and ^{13}C NMR spectra were used to study the structure of the polymer synthesized. The ^1H NMR and ^{13}C NMR spectra of the polymers produced were acquired at room temperature using a Bruker 400 MHz NMR Spectrometer, at 400.1 MHz and 100.6 MHz respectively. Chloroform-d was used as the solvent. Chemical shift (ppm) was measured using tetramethylsilane (TMS) as an internal reference. ^{13}C NMR spectrum was also used to inspect the stereoregularity of the synthesized polymer quantitatively and qualitatively [28, 29, 78, 79]. Inverse gate decoupling mode was applied for quantitative measurements of the polymer stereoregularity.

3.6.5. Scanning electron microscopy (SEM)

The surface morphology and inner structure of the scaffolds with different post-printing processes were observed by SEM 6360. The samples were first

mounted on a metal holder using double-sided carbon tape and gold coated using a sputter coater model SPI-model. Samples were gold coated for 160 s, with the gas pressure at 20 mbar and the current adjusted to approximately 18 mA. Voltage used to observe the scaffolds was 15kV.

The diameters of 50 macro-pores and micro-pores were measured respectively by image analyzer SPOT from the secondary electron images taken with magnification of x70 and x1000. The average of the diameters of these two types of pores was calculated respectively. The measurement of pores size was carried out three times on independent samples from each batch of scaffolds.

3.6.6. Mechanical test

The tensile properties of the scaffolds with different post-printing conditions were evaluated. Tensile tests were conducted with an Instron Microtensile Tester using a 1 kN load cell and pneumatic grips to hold the specimens in place. Scaffolds were tested at a cross head speed of 0.25 mm/ min at room temperature. The tensile modulus, UTS, strength at break and their corresponding strains were determined from the stress-strain graph in MPa. The energy at maximum load, calculated by integrating the area below the stress-strain curve up to UTS, was also measured. A total of five tensile test bars were tested for each experimental group.

3.6.7. Scaffold architecture characterization

The porosity of the scaffold was measured according to the method adopted by Yang et al [17] based on Archimedes' Principle. The weight of a density bottle filled with ethanol (density ρ_e) was measured as W_1 , and the weight of the dry scaffold was measured as W_s . The scaffold was then placed inside the density bottle and degassed under vacuum for more than 20 min to remove any air bubbles trapped inside the pores. The density bottle was filled with ethanol and weighed as W_2 . The ethanol saturated scaffold was removed from the density bottle and the weight of the density bottle was recorded as W_3 . The density (ρ_s),

porosity and pore volume/ mass (V_p/ W_s) of the scaffold were calculated as follows:

$$V_p \text{ (Pores volume)} = \frac{(W_2 - W_3 - W_s)}{\rho_e} \quad \text{(Equation 3.5)}$$

$$V_s \text{ (Scaffold volume)} = \frac{(W_1 - W_2 + W_s)}{\rho_e} \quad \text{(Equation 3.6)}$$

$$\rho_s = \frac{W_s}{V_s} = \frac{W_s \rho_e}{(W_1 - W_2 + W_s)} \quad \text{(Equation 3.7)}$$

$$\text{porosity} = \frac{V_p}{(V_p + V_s)} = \frac{(W_2 - W_3 - W_s)}{(W_1 - W_3)} \quad \text{(Equation 3.8)}$$

The surface areas per mass (SA/ M) of the porous scaffolds were determined by ASAP 2020 Micromeritics BET method, at -196 °C, 100 % nitrogen. Samples were degassed at 50 °C for 5 hours prior to measurements.

Chapter 4. Polymer Synthesis

4.1. PLLA Melt Polycondensation

The dual challenges to synthesize PLLA by melt polycondensation were firstly to promote polymerization by concurrently enhancing dehydration reaction, and then to minimize various side reactions inherent with this process. It was necessary to remove the by-product water continuously and efficiently so as to promote dehydration. Failure to do so would result in the reaction remaining at the equilibrium stage or even ester hydrolysis, both of which would produce low molecular weight polymer [58].

The major side reactions to be suppressed include lactide formation, racemization and thermal/ thermal oxidative degradation. The formation of lactide causes depolymerization, and thus reduces the yield and molecular weight of the polymer [23-25, 53, 58, 80, 81]. Racemization prevents crystallization and thus the deterioration of mechanical properties [22, 26, 27]. Thermal/ thermal oxidative degradation lead to deactivation of functional groups, thermal chain scissions and the formation of impurities, such as carbon, CO and CO₂. Discoloration was believed to be caused by the carbon formation. Therefore, the colour of the polymer product could be used to determine the extent of thermal oxidative degradation during the synthesis process [23-25, 58].

In this synthesis study, three areas: the experimental system set-up, catalyst system and process condition were investigated and optimized in order to fulfil the criteria stated above—to promote dehydration and suppress various side reactions.

4.2. Synthesis System Set-up

Figure 4.1 shows the synthesis set-up of PLLA melt polycondensation. A three-neck flask was used for PLLA synthesis reaction. The flask was connected to

three different systems: gas supply, mechanical stirrer and vacuum control. The gas supply system consisted of the nitrogen inert gas source and oxygen remover. The vacuum control system was connected to the three-neck flask through a reflux condenser and cold trap.

Figure 4.1 Synthesis set-up of PLLA melt polycondensation.

4.2.1. Dehydration system

The vacuum system aided the removal of by-product water generated during polycondensation of PLLA. The polymeric melt was also mechanically stirred in order to assist the diffusion of water and prevent regional over-heating during the polymerization reaction [27, 59, 58].

The pressure of the system was kept at 10 torr to minimize excess lactide formation and depolymerization [23-25, 58, 81]. A pressure detector and a valve

were connected to the vacuum pump itself in order to maintain the system pressure. Whenever the pressure level went up to 11 torr, the valve would open and the vacuum pump would be activated to reduce the pressure level. Once the vacuum level dropped back to 10 torr, the valve would close and the pump would stop. Meanwhile, the side product of the reaction, namely water vapour, would be removed by the suction of the pump when the valve opened.

4.2.1.1. Effect of nitrogen gas flow

In order to increase the valve-opening rate, a flow of inert nitrogen gas was introduced into the reaction flask during the constant stage of the reaction. This flow of gas enhanced the increment of pressure level and the valve opening-rate to an average of 16 times/min (from 4 times/min) to maintain the pressure level at 10 torr.

The properties of two PLLA polymers produced by the same oligomerization and polymerization processes with and without the flow of nitrogen gas were tabulated in **Table 4.1**. The pressure level of both reaction systems was maintained at 10 torr.

Table 4.1 Properties of PLLA synthesized with and without the flow of nitrogen gas

Nitrogen Flow	Polymer	M_w (Da)	M_{wD}	Yield (%)
No	PLLA _{noN2}	13,000	1.56	40
Yes	PLLA _{N2}	30,000	1.59	42

With the presence of nitrogen gas flow, the PLLA produced has a molecular weight which is significantly higher than that of PLLA without nitrogen gas flow. The presence of nitrogen gas flow increased the valve-opening rate and water removal. This in turn, increased dehydration and polymerization. However, the increase of valve-opening rate did not significantly affect the process yield or the molecular weight distribution (M_{wD}).

4.2.2. Lactide formation control system

The lactide formed during synthesis had low boiling point and was easily distilled off with the by-product water. The removal of lactide disturbed the ring-chain equilibrium and therefore generated more lactide. This in turn caused depolymerization and lower yield [22- 26, 58]. In order to inhibit the generation of more lactide, the lactide formed was refluxed back into the reaction system to maintain the ring-chain reaction at an equilibrium stage, thus preventing the displacement towards the formation of lactide.

A reflux condenser was connected to the reaction flask as shown in **Figure 4.1**. The inner tube of the condenser was heated up to 55 °C by a ribbon heater. The molten temperature range of lactide is around 50°C to 70 °C. The liquid lactide formed in the condenser would then flow back into the reaction flask, thus minimizing the loss of lactide from the system.

4.2.3. Thermal oxidation control

In order to reduce thermal/ thermal oxidative degradation, oxygen gas must be removed from the reaction system. A flow of inert nitrogen gas was introduced into the system followed by the application of vacuum to remove any oxygen gas before the synthesis process. This oxygen gas removal step was repeated three times in order to thoroughly remove any residual oxygen thoroughly. A substance called Oxyclear was also used to remove any oxygen gas residue in the nitrogen gas. However, slight discoloration was still observed during the synthesis despite the precautions taken. This could be due to minor leakage of oxygen gas into the vacuumed system during the synthesis process.

4.3. Catalyst Systems

The maximum PLLA molecular weight synthesized by melt polycondensation without any catalyst was about 3,000 Da [37, 55]. The carboxylic auto-

catalyzation inducing polymerization was retarded with the reduction of carboxylic functional group concentration [23-25, 58]. In this case, catalyst was needed to promote further polymerization.

In this present work, three different catalyst systems: a binary-catalyst system and two single-catalyst systems were studied. The binary-catalyst system was made up of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ and TSA. The two single-catalyst systems were pure TSA and Sn(II) Oct . The quantities of the catalyst added were in terms of weight ratio with respect to the weight of PLLA oligomer used. The ratio of TSA to SnCl_2 of the binary-catalyst system was kept at 1:1 molar ratio. This ratio was found to be the optimum by Gao et al [23], and Moon et al [24]. The total weight of the binary catalysts used for reaction was 0.7 wt% with respect to the oligomer weight. The properties of PLLA synthesized using two different ratios of single TSA catalyst were also studied. This was to gain a better understanding of the respective effect of TSA on the polymerization, and subsequently the role of TSA as a component in the binary-catalyst system.

Table 4.2 Properties of PLLA synthesized using different catalyst(s) systems

	Catalyst(s)	Catalyst/ Oligomer (wt%)	M_w (Da)	M_{wD}	Appearance	Yield (%)
PLLA _{TSA1}	TSA	0.7	16,000	1.62	Light beige	60
PLLA _{TSA2}		0.5	13,000	1.58	Light beige	58
PLLA _{SnOct}	Sn(II) Oct	0.7	19,000	1.49	Reddish brown	45
PLLA _{bin}	SnCl ₂ /TSA	0.7	30,000	1.59	Beige	42

4.3.1. Molecular weight, yield and discoloration

The synthesis results (**Table 4.2**) showed that binary-catalyst system was effective in raising the molecular weight. The high molecular weight obtained was believed to be mainly attributed to SnCl_2 in the binary-catalyst system. SnCl_2 , as

a metal ion (Lewis acid) catalyst, was able to introduce multiple protons to protonize the carboxylic end groups and enhance polymerization [28]. A screening of different single-catalyst was conducted by Moon et al [24]. The single catalysts used included germanium oxide (GeO_2), aluminium oxide (Al_2O_3), zinc oxide (ZnO), stannous oxide (SnO) and $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$. Among all the catalysts investigated, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ produced PLLA with the highest molecular weight. Unlike metal ion catalyst, proton acid catalyst can only introduce a single proton to protonize the PLLA end groups, and was less effective in promoting polymerization. This was depicted by the insignificant increase of molecular weight as TSA amount increased from 0.5 wt% to 0.7 wt%. Molecular weight of PLLA was generally affected by both dehydration/ polymerization reaction and lactide formation reaction; whereas the yield obtained was determined solely by the latter. The high yield obtained for both PLLA_{TSA} illustrated that TSA catalyst was able to inhibit lactide formation. Thus, the low molecular weight produced by TSA was due to the ineffectiveness in promoting dehydration/ polymerization reaction, and not due to high lactide formation.

TSA, as a proton acid catalyst, successfully minimized side reactions including lactide formation and discoloration. Both PLLA synthesized by TSA had high yield and minimum discoloration. The colour of PLLA_{bin} was slightly darker than PLLA_{TSA} , but no significant discoloration was observed. PLLA produced using SnCl_2/TSA binary-catalyst system had lower yield than that produced by single catalyst TSA. This was due to the smaller amount of TSA in the binary-catalyst system as compared to the single catalyst TSA.

The combination of metal ion catalyst SnCl_2 , and proton acid catalyst TSA in the binary-catalyst system, gave the combined desirable effects of both catalysts in producing PLLA with higher molecular weight and little discoloration and racemization. The effect of binary-catalyst system on racemization will be discussed in **section 4.3.3**. A model was proposed by Moon et al [24] to explain the reduction of side reactions by using binary-catalyst system. His model states

that terminal groups of PLLA (-OH or -COOH) are coordinated with the ligands of the catalyst center Sn(II). As polymerization progresses, the polymer molecular weight increases and the concentration of -OH and -COOH end groups decreases. This in turn, increases the concentration of the open coordination sites on Sn(II), which are responsible for side reactions such as discoloration and racemization. In the binary-catalyst system, TSA molecules fill these open coordination sites, thus reducing the side reactions. However, if the ratio of TSA to Sn(II) catalyst is too high, the activity of Sn(II) catalyst will be hindered by the TSA molecules. Hence it was determined that TSA: Sn(II) molar ratio of 1:1 was required for optimum reaction [24].

Sn(II) Oct, a metal ion catalyst (Lewis acid catalyst), is widely used in the synthesis of PLLA by ring opening polymerization (ROP) [26, 22, 27, 80, 82- 87]. This catalyst is one of the most effective catalysts with in terms of achieving high molecular weight with low level of racemization (< 1 %) for ROP. It has been reported [84- 86] that through ROP, the molecular weight of PLLA synthesized using Sn(II) Oct was more than 500,000 Da even at low reaction temperature of 120°C. Sn(II) Oct is lightly soluble also in many lactones, has low toxicity and is FDA approved [26, 22, 27, 80, 82- 87].

In the present study, the effectiveness of Sn(II) Oct as a melt polycondensation catalyst was investigated. The molecular weight of PLLA_{SnOct} (19,000 Da) was lower than PLLA_{bin}, but slightly higher than PLLA_{TSA}. Sn(II) Oct was not that effective in promoting dehydration reaction in PLLA polycondensation, as compared with the binary-catalyst system. Nonetheless, this metal ion catalyst was still better than the proton ion catalyst TSA in obtaining higher molecular weight PLLA. PLLA_{SnOct} suffered severe discoloration and was reddish brown, unlike the other polymers. Hence, Sn(II) Oct was not effective in minimizing discoloration and possibly enhanced thermal/ thermal oxidative degradation as the reaction temperature increased. On the whole, the performance of Sn(II) Oct

was less satisfactory than the binary catalyst system as a polycondensation catalyst.

4.3.2. Thermal properties of synthesized PLLA

Figure 4.2 illustrates MDSC heating and cooling thermograms for PLLA_{bin}. The heating and cooling rate used were both 5 °C/min. In the first heating scan, a glass transition step and a single endothermic melting peak were observed at 40 °C and 143 °C respectively. During the cooling scan, no exothermic crystallization peak was observed suggesting that PLLA_{bin} remained amorphous throughout this stage. It is possible that the cooling rate of 5°C/min was too fast for the crystallization of the polymer [77, 88]. In the second heating scan, the amorphous polymer recrystallized giving rise to the melt-crystallization peak observed at 107 °C. With further heating, these crystals melted giving rise to the double endotherms around 140 °C to 149 °C.

Figure 4.2 MDSC heating and cooling scans of PLLA_{bin}

According to the melt-recrystallization model [77], the two endothermic peaks at T_{m1} 141 °C, and T_{m2} 149 °C, represents the melting of the crystals at these two temperatures. The exothermic peak at around 145 °C is attributed to recrystallization. During the heating scan, melting first occurred at around 140 °C, followed by a melt-recrystallization at 145 °C and finally the melting of all the

crystal at T_{m2} 149 °C. Both melting and recrystallization occurred simultaneously during the initial stage of the melting and were competitive in the process. The endothermic peak appeared when the rate of melting was larger than the rate of recrystallization, while the exothermic peak appeared when the rate of recrystallization was faster than the rate of melting. The model also suggested that the small and imperfect crystals changed successively into more stable crystals during the melt-recrystallization process and melted at a higher temperature, T_{m2} .

Figure 4.3 displays the MDSC second heating scans of PLLA synthesized using the three different catalyst(s) systems. In PLLA_{bin}, a glass transition step (T_g 49 °C), a crystallization peak (T_c 107 °C) and double melting peaks at 140 °C and 149 °C were observed. The MDSC thermograms of PLLA synthesized by TSA showed a glass transition step, a crystallization peak and a single melting peak. As for PLLA_{SnOct}, only a T_g at 48 °C was observed. This indicated the latter was amorphous and was not able to crystallize during the heating scan at 5 °C/min from 20 °C to 170 °C, unlike PLLA_{bin} and PLLA_{TSA}.

Figure 4.3 MDSC thermograms of the second heating scans of synthesized PLLA

The T_g of the four PLLA are shown in **Table 4.3**. The T_g of the polymers were all around 50 °C and were not significantly affected by the types of catalyst used. PLLA was generally semi-crystalline at appropriate annealing condition. The semi-crystalline polymers would be in a glassy state when used in-vivo, since the T_g of these polymers were above body temperature, 37 °C. These glassy polymers degrade slower and exhibit higher strength than the same polymers in a rubbery state.

Table 4.3 MDSC reheating scan thermal properties of synthesized PLLA

	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_m^a (J/g)	Crystallizability (%)
PLLA _{bin}	49.0	107.5	140.5 (T_{m1})	28.6 ^a	30.8
			149.4 (T_{m2})		
PLLA _{TSA1}	52.0	93.0	154	47.4	55
PLLA _{TSA2}	47.0	84.7	153	51.2	51
PLLA _{SnOct}	48.0	NA	NA	0	0

^a ΔH_m of PLLA_{bin} was taken as the area under the double melting peaks

The thermal properties of the PLLA synthesized by different catalysts are shown in **Table 4.3**. PLLA_{TSA} had lower T_c and higher T_m as compared to PLLA_{bin}. Crystallizability (%), the intrinsic ability of the polymer to recrystallize, was calculated using **Equation 3.4** as shown in chapter 3. The PLLA synthesized using the three different catalyst(s) systems displayed different crystallization behaviours. PLLA synthesized using binary-catalyst system and TSA single-catalyst system were semi-crystalline; whereas PLLA_{SnOct} did not exhibit any recrystallization and was amorphous. The low T_c in both PLLA_{TSA} suggested that the polymer chains needed less energy to rearrange and crystallize; and the high T_m indicated that the crystals formed during melt-crystallization had less defects, thus requiring more energy to melt [76]. The effect of the catalysts on the crystallization behaviour of PLLA is further discussed in the next section.

4.3.3. Racemization of synthesized PLLA

4.3.3.1. ^{13}C NMR spectra

The degree of racemization (DOR, %) of PLLA_{bin} , $\text{PLLA}_{\text{TSA1}}$, $\text{PLLA}_{\text{TSA2}}$ and $\text{PLLA}_{\text{SnOct}}$ were studied by inverse gate decoupled ^{13}C NMR spectroscopy. The typical ^{13}C NMR spectrum of PLLA is shown in **Figure 4.4**. The peak located at around 169.6 ppm is the carbonyl signal of PLLA, whereas the methine signal and methyl signal are at 69.0 ppm and 16.8 ppm respectively [23, 25, 89]. The stereosequence configurations (isotactic(i) and syndiotactic(s)) in the polymer chains were studied by enlarging the ^{13}C NMR spectra of the carbonyl signal (169.7 to 169.2 ppm) of PLLA_{bin} , PLLA_{TSA} and $\text{PLLA}_{\text{SnOct}}$ (**Figures 4.5a, 4.5b, and 4.5c**). The multiple peaks observed in the carbonyl region at different chemical shifts (ppm) were due to different stereosequence configurations, where the stereosequence configurations of up to six-lactate units could be recognized by ^{13}C NMR [23-25, 29 78, 79, 90- 92]. The configurations for each corresponding peak were identified and labelled (isotactic(i) and syndiotactic(s)), as shown in **Figure 4.5**. The most significant peak at 169.63 ppm was mainly contributed by isotactic(i) stereosequence configuration of the L-lactate unit in the polymer chains. In addition to the main peak, there were also four smaller peaks located at 169.47, 169.39, 169.28 and 169.24 ppm. These smaller peaks were due to the small amount of syndiotactic(s) stereosequence configuration of the D-lactate units in the L-polymer chains. The details of the stereosequence configurations corresponding to each peak are listed in **Table 4.4**.

Figure 4.4 ^{13}C NMR spectrum of PLLA

Figure 4.5 Enlarged carbonyl signal of ^{13}C NMR spectrum of PLLA produced with (a) binary-catalyst system (b) single-catalyst system TSA and (c) single-catalyst system Sn(II) Oct

Table 4.4 Stereosequence configurations of the peaks

	Chemical Shift (ppm)	Isotactic/ syndiotactic stereosequence	L/ D-Lactate unit stereosequence
Main peak	169.63	iiii	L-L-L-L-L-L
		siii/ iiis	D-L-L-L-L-L L-L-L-L-L-D
		siis	D-L-L-L-L-D
Small peaks	169.47	iiisi	L-L-L-L-D-L
	169.39	iisis	L-L-L-D-L-D
		iisii	L-L-L-D-L-L
		sisii	L-D-L-D-L-L
		sisis	D-L-L-D-L-D
	169.28	isiii	L-L-D-L-L-L
	169.24	isisi	L-L-D-L-D-L

L: L(+)-lactate unit; D: D(-)-lactate unit

The presence of D-lactate units in PLLA was due to the occurrence of racemization. During ester-interchange reaction, a small amount of L-lactate units were transformed into D-lactate units. As the number of D-lactate units increased, more syndiotactic stereosequence configurations were detected. Hence the increase in the corresponding peaks area [24, 25, 28, 29, 78]. The more D-lactate units formed, the higher the DOR.

4.3.3.2. Degree of racemization

DOR of the PLLA synthesized was calculated by the ratio of the area under the main peak to the areas under the small peaks in the carbonyl region [23, 29, 78, 92].

Table 4.5 DOR and crystallizability of PLLA synthesized with different catalyst(s) systems

	Integral Area Ratio (isotatic peak : syndiotactic peaks)	DOR (%)	Crystallizability (%)
PLLA _{bin}	1: 0.24	24.0	30.8
PLLA _{TSA1}	1: 0.091	9.1	55
PLLA _{TSA2}	1: 0.168	16.8	51
PLLA _{SnOct}	1: 0.378	37.8	0

The catalysts used showed a significant effect on the polymer DOR, ranging from 9 % to 38 % (**Table 4.5**). In this study, TSA was the most effective catalyst in inhibiting racemization, producing both PLLA_{TSA} with the lowest DOR. By increasing the TSA amount from 0.5 wt% to 0.7 wt %, the DOR reduced by 7.7 %. The presence of TSA in the binary catalyst was believed to have played an important role in inhibiting racemization. The higher DOR of PLLA_{bin} compared to the two PLLA_{TSA} was possibly due to the smaller amount of TSA in the binary catalyst, and/ or the presence of metal ion catalyst SnCl₂ that did not inhibit racemization. Sn(II) Oct, another metal ion catalyst, was not effective in reducing racemization and produced PLLA_{SnOct} with the highest DOR.

Figure 4.6 DOR vs. crystallizability of PLLA synthesized with different catalyst(s) systems

Figure 4.6 shows that the crystallizability reduced significantly with the increase of DOR. PLLA_{SnOct} with the highest DOR at 37.8 % was not able to recrystallize and remained amorphous. This was due to the presence of high amount of D-lactate units in the polymer chains that reduced stereoregularity and thus hindered the chains alignment [22- 29, 79, 92].

4.3.3.3. Mechanism of racemization

Racemization mainly occurred during the bond breaking process of ester-interchange reaction [28]. The probability is small for L-lactate unit to transform into D-lactate without ester-interchange reaction. During the ester-interchange reaction, the ester bond in the polymer chain is first broken and a new ester bond is reformed. The ester bond can be broken at two different points, the carbonyl-oxygen bond or the alkyl-oxygen bond (**Figure 4.7**). Only the cleavage of alkyl-oxygen bond will cause racemization.

Figure 4.7 The two points in the PLLA chain which can be broken during ester-interchange reaction

PLLA_{TSA} had the lowest degree of racemization among the polymers synthesized. This was because the proton acid catalyst did not enhance the cleavage of alkyl-oxygen during the ester-interchange reaction. In contrast, the metal ion catalyst enhanced alkyl-oxygen cleavage during ester-interchange reaction and therefore the degree of racemization. PLLA_{SnOct}, produced by the strong ester-interchange

catalyst Sn(II) Oct, had the highest degree of racemization among all the polymers synthesized. Hence, this catalyst enhanced the ester-interchange reaction through the cleavage of alkyl-oxygen bond.

In conclusion, the effect of the binary-catalyst system on the molecular weight, yield, discoloration, crystallization and racemization behaviours was studied. Metal ion catalysts, such as SnCl₂ and Sn(II) Oct, were effective in promoting dehydration reaction, but were not able to inhibit various side reactions including lactide formation, discoloration and racemization. On the other hand, TSA a proton acid catalyst exhibited opposite effects. Hence, by combining the metal ion catalyst (SnCl₂) with proton acid catalyst (TSA), a complement effect in promoting dehydration reaction and inhibiting side reactions was obtained.

4.4. Process Optimization

The synthesis process consisted of two steps, which were the oligomerization step followed by the polymerization step. In the oligomerization step, the monomer lactic acid would first undergo self-polycondensation to form oligomer. No catalyst was added in this step to prevent the deterioration of the catalytic activity by the high polarity of the reactant. However without catalyst, the oligomer would produce molecular weight in the hundreds of Da, Therefore catalyst was added in the beginning stage of the polymerization step. The process conditions were optimized for high molecular weight PLLA synthesis using binary catalyst, SnCl₂/TSA.

4.4.1. Oligomerization

The initial oligomer (OLLA0) was produced according to the reaction conditions suggested by Gao et al [23] and Kimura et al [24-25] as stated below.

The initial oligomer (OLLA0, M_n 545 Da) was further reacted under different reaction conditions (**Table 4.6**) to produce oligomers with different number average molecular weight (M_n) (**Table 4.7**).

Table 4.6 Additional reaction conditions during oligomerization step

Oligomer	Additional Reaction Conditions
OLLA1	90 °C, 100 torr, 2 hours
OLLA2	150 °C, 100 torr, 2 hours
OLLA3	120 °C, 100 torr, 2 hours, followed by 150 °C, 100 torr, 2 hours

All the oligomers produced were further polymerized at 180 °C, 10 torr for a predetermined duration. The ramping sequence used was RS2, which was discussed in **section 4.4.2.1**. The catalyst used was SnCl_2/TSA , 0.7 wt% with respect to the weight of the oligomer.

Table 4.7 Properties of PLLA synthesized with different oligomers

	Oligomer	M_n (Da)	M_{wD}	Polymer	M_w (Da)	M_{wD}	Appearance	Yield (%)
1	OLLA0	545	1.54	PLLA0	26,000	1.55	Yellow	40
2	OLLA1	582	1.57	PLLA1	30,000	1.59	Beige	42
3	OLLA2	745	1.70	PLLA2	27,000	1.54	Milky white	39
4	OLLA3	810	1.71	PLLA3	19,000	1.6	Beige	83

Table 4.7 shows the final polymer M_w obtained with respect to the initial oligomer M_n . Polymers synthesized from OLLA0, OLLA1 and OLLA2 had similar molecular weight of around (26,000 to 30,000 Da). The M_w of PLLA synthesized from OLLA3 was the lowest at 19,000 Da. The highest yield obtained was 83 %, which was with OLLA3. OLLA0 to OLLA2, which produced polymers with $M_w \geq 26,000$ Da had lower process yield. In contrast, OLLA3, which gave the lowest molecular weight polymer, had the highest process yield.

Generally, the polymers did not suffer serious discoloration irrespective of the oligomer M_n used. The colour of PLLA0 was yellow and slightly darker than the rest of the polymers.

4.4.2. Polymerization

Polymerization is the second step of the two-step synthesis process, which involved the polymerization of the low molecular weight oligomer to a higher molecular weight polymer in the presence of the catalysts SnCl_2/TSA , 0.7 wt%. The second polymerization step can be divided into two stages: the ramping stage and the constant stage. During the ramping stage the temperature of the system was increased while the pressure was reduced to meet the optimized reaction conditions. In the constant stage the temperature and pressure were maintained for the optimized condition duration.

4.4.2.1. Ramping stage of polymerization

The influence of the ramping stage on the final polymer properties was investigated. Four different ramping sequences were conducted in order to discover the optimum ramping sequence in terms of the molecular weights obtained. The four ramping sequences used were as follow:

RS1: The temperature of the system was increased from 25 °C to 180 °C and the pressure was decreased from 760 torr to 10 torr simultaneously in 4 hours.

RS2: The pressure was first decreased to 10 torr in 30 min at 25 °C. The temperature was then increased gradually to 180 °C in 2 ½ hours.

RS3: The temperature was first increased from 25 °C to 180 °C in 2½ hours, at 760 torr, with the flow of nitrogen gas. The pressure level was then decreased to 10 torr in 30 min.

RS4: Similar to RS1, the temperature was increased to 180 °C while the pressure was decreased to 10 torr simultaneously in 4 hours. The pressure was further decreased to 1 torr and maintained at 1 torr for 2 hours. This was followed by raising the pressure back to 10 torr.

The time taken for the whole RS1 to RS3 ramping process was 4 hours, except for the last ramping sequence, RS4, which took 6 hours. The ramping stage was followed by the constant stage, which was at 180 °C, 10 torr for a predetermined duration. The final polymer molecular weight and yield were determined and as shown in **Table 4.8**.

Table 4.8 Properties of PLLA synthesized with different RS

	Ramping sequence	M _w (Da)	M _{wD}	Appearance	Yield (%)
1	RS1	23,000	1.52	Dark yellow	71
2	RS2	26,000	1.56	Brown	45
3	RS3	16,000	1.49	Light Brown	48
4	RS4	31,000 ^a	1.69	Dark reddish brown	52

^a Repeated: M_w gained 28,000 Da

The PLLA synthesized using RS4 had the highest M_w among the four ramping sequence. As both RS4 and RS1 had the same initial temperature increase with pressure decrease conditions, the higher M_w obtained from RS4 was attributed to the additional two hours reaction at 1 torr, 180 °C. The reduction of the pressure to 1 torr effectively enhanced the removal of by-product water and thus the dehydration reaction. However, a lot of lactide was generated when the pressure level was maintained at 1 torr. Hence, the pressure level was increased back to 10 torr after 2 hours to prevent excessive lactide formation, which blocked the vacuum pathway of the reaction set-up. The effects of RS1 and RS2 produced polymer of comparable M_w. In RS3, the temperature was first raised to 180 °C followed by a decrease in pressure. The increment of temperature alone in the first 2½ hours was not enough to effectively remove the water formed. This restrained the increment of the molecular weight.

The yields of the processes using different RS were also determined. The highest yield obtained was from RS1, 71 %, followed by RS4, 52 %. The lowest yield was obtained from RS2, 45 %. From the yields of RS1 and RS4, it was deduced that simultaneously increasing the temperature while decreasing the pressure improved the yield. However, in RS4, the additional 2 hours reaction at 1 torr increased the lactide formation, which led to the lower yield. Even though RS1 and RS2 produced polymers with similar molecular weights, the yield of RS1 was 71 %, while the yield of RS2 was only 45 %. RS2 was effective in enhancing dehydration effect but not the yield. This showed that decreasing the pressure followed by increasing temperature did not inhibit lactide formation. As for RS3, it was not effective in producing polymer with high molecular weight or yield.

Among the four polymers produced by different ramping sequences, the colour of PLLA produced by RS4 was the darkest, which was dark reddish brown. This serious discoloration was due to the side reaction caused by the additional 2 hours at low pressure (1 torr).

4.4.2.2. Constant stage of polymerization

The change in temperature and reaction time during the constant stage on the properties of PLLA was also investigated. Two different polymerization temperatures, 180 °C and 220 °C were studied. The reaction time investigated were 8, 12, 18 and 20 hours for 180°C, and 0.5, 2, 4 and 8 hours for 220 °C. The reaction pressure was maintained at 10 torr, which was reportedly the optimum pressure for PLLA melt polycondensation [24].

Effect of different reaction times at 180 °C

The properties of PLLA synthesized for different reaction times were investigated (**Table 4.9**), while the reaction temperature and pressure were maintained at 180 °C and 10 torr.

Table 4.9 Properties of PLLA synthesized at different durations at 180 °C

Polymerization duration (hours)	M _w (Da)	M _w D	Appearance	Yield (%)
8	18, 000	1.5	Yellow	46.7
12	30, 000	1.6	Yellow	41.9
18	54, 000	1.5	Brown	55.3
20	30, 000	1.5	Brown	40.1

Figure 4.8 M_w of PLLA synthesized at different reaction time at 180 °C

Figure 4.8 shows the M_w obtained with different polymerization times. The degree of polymerization increased with the reaction time up to 18 hours. However, the M_w decreased to 30,000 Da when the polymerization time was 20 hours. This was due to depolymerization, which could be attributed to a few factors. Firstly, as the polymerization time increased, the removal of water became less efficient due to the increase in reactant viscosity. The failure to instantaneously remove water as it was formed caused the polymerization reaction to remain at equilibrium stage, where depolymerization through ester hydrolysis could take place [58]. Secondly, prolonged heating enhanced thermal/thermal oxidative degradation causing depolymerization by thermal chain scission. The increase in lactide formation with prolonged heating would also promote the scission of PLLA chain and the reduction of molecular weight.

In general, the yield obtained did not show a significant decrease with reaction time. Even after 20 hours, the lowest yield obtained was at 40 %. The effect of PLLA discoloration increased with polymerization time, changing from yellow to brow. This could be due to the formation of carbon by thermal/ thermal oxidative degradation with prolonged heating.

Effect of different reaction times at 220°C

PLLA were synthesized at different polymerization times at 220°C, 10 torr. The properties of the PLLA obtained are as shone in **Table 4.10**.

Table 4.10 PLLA properties synthesized at different times at 220 °C

Polymerization time (hours)	M _w (Da)	M _{wD}	Appearance	Yield (%)
0.5	33, 000	1.8	Reddish brown	73.7
2	43, 000	1.4	Brown	52.3
4	38, 000	1.7	Dark brown	45.7
8	34, 000	1.4	Black	1.0

Figure 4.9 M_w of PLLA synthesized at different times at 220 °C

The molecular weight increased significantly from 33,000 Da, 0.5 hour to 43,000 Da at 2 hours. However, the molecular weight decreased with polymerization time longer than 2 hours. At 220 °C, the yield was strongly dependent on the

reaction time. The yield reduced from 74 % (0.5 hours) to 1 % after 8 hours of reaction time. The low yield obtained at 8 hours was mainly due to severe lactide formation, and thermal/ thermal oxidative degradation which turned the polymer into carbon, CO and CO₂.

Effect of reaction temperatures

Figure 4.10 M_w of PLLA synthesized at different reaction times at 180 °C and 220 °C respectively

A series of PLLA molecular weight synthesized at two different polymerization temperatures, 180 °C and 220 °C, are shown in **Figure 4.10**. The maximum M_w obtained was at 180 °C with a M_w of 54,000 Da. The lower M_w obtained at 220 °C could be due to the higher reaction temperature, not only enhances polymerization but also caused various side reactions that led to depolymerization. The gradients of the two curves were calculated as shown in **Figure 4.10** to serve as a rough guideline on the polymerization rate. The gradient obtained for 220 °C was 6667 Da/hr and 3632 Da/hr for 180 °C. This showed that the polymerization rate at 220 °C was about double the rate at 180 °C. Hence, the reaction duration required to reach the maximum M_w was much shorter than at 180 °C which took 18 hours. However, the 18 hours synthesis window provided better control in obtaining a specific molecular weight.

The polymer yield at 220 °C dropped drastically to 1 % after 8 hours of reaction, whereas the yield at 180 °C only reduced slightly to 40 % after 20 hours of reaction. This suggested that with more energy at 220 °C the rate of lactide formation and thermal/ thermal oxidative degradation were enhanced. The discoloration was also more significant at 220 °C than at 180 °C. Hence, a lower reaction temperature was required to minimize various side reactions.

The effect of reaction times and temperatures on thermal properties
The effect of reaction time on PLLA thermal properties at 180 °C and 220 °C are listed in the **Table 4.11** and **Table 4.12** respectively.

Table 4.11 Thermal properties of PLLA synthesized at different durations at 180 °C

Polymerization time (hours)	T _d (°C)	T _g (°C)	T _c (°C)	T _m (°C)	ΔH _m (J/g)	Crystallizability (%)
8	200	40.1	79.4	148.7	38.1	41
12	198	47.3	115.8	131.2	1.7	1.8
18	195	49.1	NA	NA	0	0
20	185	46.5	NA	NA	0	0

Table 4.12 Thermal properties of PLLA synthesized at different durations at 220 °C

Polymerization time (hours)	T _d (°C)	T _g (°C)	T _c (°C)	T _m (°C)	ΔH _m (J/g)	Crystallizability (%)
0.5	208	50.2	100.4	141.0 (T _{m1}) 147.9 (T _{m2})	27.9	30.0
2	205	48.9	115.5	140.2	16.2	17.4
4	204	49.3	118.2	136.9	6.5	7.0
8	145	46.8	NA	NA	0	0

Decomposition temperature (T_d) of both PLLA synthesized at 180 °C and 220 °C decreased with an increase in reaction time. The decrease in thermal stability

was due to various side reactions, which caused the formation of impurities. For the same polymerization time of 8 hours, the T_d for the 220 °C PLLA was significantly lower than the T_d for the 180 °C PLLA. However, with the reduction of polymerization time to 4 hours, the T_d for the 220 °C PLLA was slightly higher than that of 180 °C PLLA. This showed that the thermal stability of the polymer can be improved by having shorter reaction time and/ or lower reaction temperature.

The T_g of the PLLA synthesized at 180 °C presented a similar trend with the change of M_w (Refer to **Figure 4.8**). The T_g decreased slightly with reaction time at reaction temperature 220 °C. In general, all the T_g varied in the range of 40 °C to 50 °C, and were neither affected significantly by the reaction temperature nor the reaction time.

The effects of reaction time and temperature on the PLLA crystallization behaviours were studied by determining the T_c , T_m and crystallizability of the polymers. The T_c of PLLA synthesized at both temperatures increased with reaction duration, showing that the polymer was becoming harder to crystallize. With further increase in the reaction duration, the crystallization peaks disappeared. The polymers remained amorphous and thus no melting peak could be observed. The T_m of the polymers synthesized at both temperatures decreased with reaction duration. This showed that the polymer obtained at longer reaction time formed less perfect crystals, due to racemization and degradation. The maximum crystallizability (8 hours, 41 %) at 180 °C was higher than the maximum crystallizability (0.5 hours, 30 %) at 220 °C. This showed that racemization was less significant at lower temperature. However, the reaction temperatures did not show any significant effects on T_m and T_c .

4.5. Chemical Structure Characterization of PLLA

4.5.1. FTIR

All the PLLA synthesized had similar FTIR spectra irrespective of the different catalyst systems and reaction conditions used. A typical FTIR spectrum of PLLA is shown in **Figure 4.11**. The spectrum of the PLLA showed a significant transmittance peak at 1756 cm^{-1} due to the C=O stretch of the carbonyl group. The broad band around 3600 cm^{-1} was due to the free -OH of the water vapour absorbed by KBr and the -OH end group of the polymer. The details of each peak are listed in **Table 4.13** [23, 89, 93].

Figure 4.11 FTIR spectra of PLLA

Table 4.13 The wavelength and corresponding chemical bonds in PLLA FTIR spectra

Wavelength (cm ⁻¹)	Bonding of PLLA
3653	Free OH
3503	Stretch of -OH in hydroxyl end group
2996	Asymmetric -CH ₃ stretch
2942	Symmetric -CH ₃ stretch
2878	-CH stretch
2331~2353	CO ₂ presents in air
1756	C=O stretch in ester linkage
1456	Asymmetric -CH ₃ bending
1384	Symmetric -CH ₃ bending
1362	-CH bend
1267	C-O stretch in carboxylic end group
1184, 1045	-C-O stretch in ester linkage
862	-O-C-C in phase stretch which the -O- connected to H

4.5.2. ¹H NMR

4.5.2.1. Polymer structure

A typical ¹H NMR spectrum of PLLA is shown in **Figure 4.12**. The quartet at 5.1 ppm was contributed by the methine groups of L-lactate repeat units. The doublet at 1.5 ppm was attributed to the methyl groups of L-lactate repeat units. The methine and methyl regions were enlarged as shown in **Figure 4.13a and 4.13b** respectively. In the enlarged methine region, two small peaks could be observed at 4.99 ppm and 4.3 ppm. The peak at 4.99 ppm was attributed to the methine groups in -COOH end group L-lactate units, while the peak at 4.3 ppm was that of -OH end group L-lactate units. From the enlarged methyl region as shown in **Figure 4.13b**, the -COOH end groups L-lactate units were located at 1.43 ppm, and the -OH end group was located at 1.37 ppm [23, 89].

Figure 4.12 ^1H NMR spectrum of PLLA produced with binary-catalyst system

Figure 4.13 Enlarged (a) methine and (b) methyl region of ^1H NMR spectrum

4.5.2.2. Oligomer structure

The ^1H NMR spectrum of lactic acid oligomer (OLLA) was obtained to verify the structure of the synthesized oligomer. The study of the oligomer structure was necessary in order to understand the mechanism of the synthesis process. In

addition, the knowledge of the synthesized oligomer structure could be used as a comparison to the structure of oligomeric by-products from hydrolytic degradation.

In the present work, DMSO- d_6 was used as the solvent instead of $CDCl_3$. According to Espartero et al [94], a better spectrum resolution could be obtained using DMSO- d_6 as the solvent. As many peaks in oligomer spectra were either very close to each other or overlapping, increasing the spectra resolution enabled each peak to be recognized more accurately.

Figure 4.14 shows a typical 1H NMR diagram of the OLLA. The methine groups of OLLA located at the region from 5.5 to 4.0 ppm. The signals of methyl groups were at 1.2 to 1.5 ppm. The peaks located at 3.34 ppm and 2.5 ppm were attributed to the solvents, dichloromethane and DMSO respectively [94]. The structural details were further studied by enlarging the methine and methyl regions in the spectrum.

Figure 4.14 1H NMR spectrum of oligomer

The enlarged methine region is shown in **Figure 4.15**. Each peak corresponding to the constitutive unit of the oligomers was labelled [94]. The labelling of the

different constitutive units of the oligomers is shown in **Figure 4.16**. The most significant quartet at 5.2 ppm in **Figure 4.15** was attributed to the L-lactate repeat units (8) and (5) in the oligomer chains, as shown in **Figure 4.16**. The quartets located at 5.0 ppm and 4.93 ppm were attributed to the –COOH end group units (4) and (2) respectively. Unit (4) was the unit from oligomers whereas unit (2) was the unit from dimers. The quartet located at 4.21 ppm was contributed by the –OH end group units (3) and (7). The quartet at the most upfield (4.13 ppm) was assigned to the monomeric units (1). The enlarged methyl region spectrum is shown in **Figure 4.17**. The most intense doublet at 1.47 ppm was the signal of the oligomer (8) units (**Figure 4.16**). The two small doublets presented at 1.42 ppm and 1.28 ppm were attributed to the –COOH end group units (4) and –OH end group unit (7) of the oligomer (**Figure 4.16**). The smallest doublet was the signal of monomeric lactic acid methyl group (1).

Figure 4.15 Enlarged methine region of the ^1H NMR spectrum of the oligomer

Figure 4.16 Labelling of different constitutive units of the oligomers

Figure 4.17 Enlarged methyl region of the ^1H NMR spectrum of the oligomer

In both the methine and methyl signals, the chemical shifts at the downfield region were caused by the repeat units at the center of the polymer chains (methine 5.2 ppm, methyl 1.47 ppm). The chemical shifts at the up field region were contributed by the monomeric units (methine 4.13 ppm, methyl 1.24 ppm).

4.6. Hydrolytic Degradation of PLLA Synthesized

4.6.1. Change in molecular weight and mass

In-vitro hydrolysis of PLLA synthesized in the range of medium M_w (30,000 Da) was investigated to serve as a guideline for applications as multi-functional scaffold in-situ pore formation and drug incorporation. PLLA in film form were degraded in PBS at pH 7.4 at 37 °C for up to 135 days. The number average molecular weight (M_n) of the degraded PLLA as a function of hydrolytic degradation time is listed in **Table 4.14**.

Table 4.14 Molecular weight of PLLA films at different degradation time

Days	M_w (Da)	M_n (Da)	M_{wD}
0	29100	18400	1.58
3	28910	18062	1.60
6	28813	17975	1.60
9	27879	17235	1.62
13	26916	17161	1.57
16	27817	17491	1.59
19	26921	17166	1.57
26	26666	16410	1.62
31	26233	15984	1.64
36	25453	15549	1.64
41	24921	15324	1.63
47	24425	14908	1.64
61	23572	14480	1.63
68	23855	13803	1.73
84	20557	12121	1.70
118	21295	13357	1.59
135	19609	11485	1.71

The M_n of the degraded PLLA films decreased with degradation time. The decrease in M_n was detected as early as 3 days after immersion. After 135 days,

the M_n decreased by approximately 38 %. The reduction in M_n with degradation time was due to hydrolytic chain scission of PLLA ester bonds. **Figure 4.18 (a) and (b)** present plots of $1/M_n$ and $\ln M_n$ versus time, modelling the uncatalysed and autocatalysed degradation models respectively. The plots were fitted with linear trend lines. The R^2 correlation coefficients and the rate constants were displayed in **Table 4.15**.

Figure 4.18 (a) Uncatalysed and (b) autocatalysed models for PLLA films

Table 4.15 Correlation coefficients and rate constants of uncatalysed and autocatalysed models

Model	R ²	K
Uncatalysed	0.9821	3 x 10 ⁻⁷
Autocatalysed	0.9798	0.0041

Figure 4.18 (a) and (b) shows a strong statistical linear correlation up to 68 days, with R² closed to 1. The changes of M_n deviated slightly from the straight line after 68 days. Both uncatalysed and autocatalysed models were proposed by assuming that the extent of degradation was not large and no significant mass loss occurred during the time frame. The mass loss observed in the present study was 9 % up to 68 days. The linear correlation of the plots suggested that both models held true even with the presence of minimum mass loss.

Both the R² correlation coefficients obtained for uncatalysed model and autocatalysed model were around 0.98, up to 68 days of degradation. This suggested that no clear distinction could be derived between these two models during the 68 days. This finding complemented the report of Anderson [95]. His report concluded that the hydrolytic degradation of semicrystalline polyesters might not proceed exclusively by non-catalytic or autocatalytic mechanisms and speculating that both may contribute to the rate of chain scission. In contrast, Li et al [76] and Weir et al [96] reported that the semicrystalline PLLA underwent significant autocatalysed degradation. The difference in findings could be due to the sample size. The samples prepared for degradation study here were in the form of thin film with 45 μm thickness. The samples used by Li et al and Weir et al were in the form of compressed moulded circulated plates; and compressed moulded tensile bar of 2 mm and 0.8 mm thickness, respectively. This showed that the smaller size samples did not go through autocatalysed degradation unlike samples of larger size which exhibited significant autocatalysed degradation. Similar result was reported by Grizzi et al [97] where small-sized

devices such as thin films and microspheres degraded homogenously because no internal autocatalysis occurred.

The change of M_{wD} (M_w/M_n) with different hydrolytic degradation time is plotted in **Figure 4.19**. The results showed that M_{wD} increased gradually with degradation time from 1.58 to 1.71 in a period of 135 days. The increment of M_{wD} indicates the occurrence of random hydrolytic chain scission and the formation of oligomer fragments in the polymer matrix.

Figure 4.19 Molecular weight distribution of PLLA films at different degradation time

The change in mass of the dried films at different degradation times is shown in **Figure 4.20**. The commencement of mass loss was detected after 3 days of immersion and the mass loss followed a parabolic curve. A mass loss of 9.3 % was detected after 68 days of degradation. From 68 days onwards to 135 days, the mass loss observed was only 0.8 %.

Figure 4.20 Mass loss of PLLA films at different degradation time

It is interesting to note that the mass loss of the medium molecular weight PLLA occurred without any time delay. On the other hand, in the degradation of high molecular weight PLLA, a delay in mass loss of several weeks was always observed [76, 95, 96, 98, 99]. It was reported by Weir et al [96] that mass loss started when the M_n reduced to about 20,000 Da, and the water uptake was at around 1 %. In our experiments, the initial M_n of PLLA film was 18,400 Da, which was lower than the M_n suggested by Weir et al [96]. PLLA with its low molecular weight consisted of high concentration of oligomers even before degradation. These oligomer fragments were either soluble and could dissolve immediately upon immersion; or became soluble after minimum hydrolytic chain scission. Hence, our synthesized PLLA experienced an instant commencement of mass loss upon immersion in the PBS at 37 °C.

The water uptake of the PLLA films with degradation time is shown in **Figure 4.21**. The water uptake after 3 days was about 0.92 %. It then increased to approximately 13 % after 135 days. The water uptake of our PLLA films was much higher than the water uptake of high molecular weight PLLA. High molecular weight PLLA takes several weeks to attain 1 % water uptake due to its

hydrophobic nature [96]. PLLA, with its low molecular weight, have high end groups concentration (carboxylic and hydroxyl end groups) and consequently high hydrophilicity. The increase in hydrophilicity enhanced the water absorption into the PLLA matrix.

Figure 4.21 Water uptake of PLLA films at different degradation time

The high water uptake of PLLA films was believed to accelerate mass loss, which was observed in samples even after 3 days of degradation. According to Weir et al [96], the onset of mass loss appeared to coincide with the 1 % water uptake. The 0.92 % water uptake obtained after 3 days of degradation might enable the PLLA films to swell sufficiently for the soluble oligomer to diffuse out of the polymer matrix.

4.6.2. Morphology and degradation

MDSC measurements were performed to study the changes in DOC , T_m , T_g and T_c of the films with different degradation times. As illustrated in **Figure 4.22**, the DOC obtained from the MDSC first scan of PLLA film increased gradually with degradation time. The increase in DOC could be explained by two different mechanisms.

Figure 4.22 Degree of crystallinity of PLLA films at different degradation time

First, the increase of DOC was assigned to different degradation rates in the amorphous and crystalline regions. DOC is essentially the proportion of crystalline regions to amorphous regions in the polymer. The packing density of amorphous regions is lower than crystalline region. Water can penetrate into the amorphous regions more easily and increase the degradation rate of these regions. According to Li et al [76] and Chu et al [98], the hydrolysis degradation of the semicrystalline polymer can be divided into two stages. In the first stage, hydrolytic degradation occurs mainly in the amorphous regions due to higher water absorption. During this stage, the DOC of the polymer increases with degradation time. This increase is due to the dissolution of the amorphous regions. However, the actual amount of the crystals remains unchanged. The second stage begins after most of the amorphous regions have dissolved. During this stage, the crystalline regions are slowly destroyed through hydrolytic chain scission reaction. Therefore, the DOC of the second stage decreases with degradation time. The degradation at the second stage is slower than that of first stage. The increase of DOC as shown in **Figure 4.22** suggested that the

degradation of semicrystalline PLLA was still in the first stage. This supports the mass loss profile observed in **Figure 4.20**, where the loss in mass was due to the degradation and dissolution of the amorphous regions. The mass loss in the amorphous regions accounted for the increase in DOC. The increase in DOC could also be attributed to “degradation-induced crystallization” [76, 96]. The long polymer chains in the amorphous regions formed shorter chains through hydrolytic chain scission. These shorter chains with higher mobility were able to crystallize easily, thus increasing the DOC.

The T_g decreased from 54 °C to 49 °C and the crystallization temperature (T_c) decreased from 94 °C to 88 °C (**Figure 4.23**) with degradation time of up to 135 days. The reduction in T_g indicated the increase in main chain segmental mobility. This was related to the reduction in polymer chain length in the amorphous regions through hydrolytic chain scission. The decrease in T_c with degradation time suggested an increase in crystallizability of the polymers chains in the amorphous regions. The reduction in polymer chain length through hydrolytic chain scission enhanced the ease of the polymer chains in the amorphous regions to form crystal. The reduction of T_g and T_c suggested that degradation significantly occurred in the amorphous regions during the time of investigation.

Figure 4.23 Glass transition temperature (T_g) and crystallization temperature (T_c) of PLLA films at different degradation time

Double melting peaks were observed from the MDSC thermograms. The temperatures of the double melting peaks were assigned as low melting temperature, T_{mL} , and high melting temperature, T_{mH} . The melting temperatures from the first MDSC scan decreased with degradation time (**Figure 4.24**). The T_{mL} decreased from 138°C to 132 °C after 135 days, and the T_{mH} decreased from 146°C to 142 °C. The decrease of the melting temperatures could be due to the reduction in the crystals size and the uniformity of the crystals regions. Chu et al [98] suggested that because of the existence of the crystals defects, a few portions of the crystalline regions could also be destroyed simultaneously, but more slowly, during the degradation of the amorphous regions. Another possibility for the reduction in the melting temperature with degradation time was the decrease in the polymer molecular weight due to hydrolytic chain scission.

Figure 4.24 Melting temperatures (T_{mH} and T_{mL}) of PLLA films at different degradation time

Chapter 5. Multi-functional Scaffold Fabrication

5.1. Printing of Scaffold

5.1.1. Binder selection

Organic powder was generally printed with the corresponding solvent ('ink') in 3DP. This solvent must be able to dissolve the material readily during the printing process. This limited the material-solvent selection. For example, PGA with low solubility in most of the solvents was not favourable to be processed by 3DP. PLLA was generally printed with chloroform. However, chloroform is toxic and hard to be completely removed from the scaffold [33]. In addition, this solvent was not suitable for Z-Corp Z400 3D printer. Hence, an alternative was sought.

In this study, an indirect binding approach was explored, whereby the PLLA powder (M_w 130,000 Da) was mixed with a solid phase binder. The criterion for the solid phase binder is that it should readily dissolve in the solvent deposited by 3DP, so that the dissolved binder would bind the discrete PLLA particles together upon solidification. The PLLA particles would remain discrete as the polymer was not soluble in the solvent used in 3DP. In this case, water was chosen as the solvent for its non-toxicity and the possibility to incorporate various water-soluble drugs or bio-agents. Hence, the solid phase binder should be highly water soluble, biocompatible, and swell-proof when in contact with water. Furthermore, it has to be chemically and physically inert at elevated temperature and in body fluid. It must also have good binding ability with PLLA powder. The candidates for the solid phase binder included Pluronic, PVA, cellulose, polyethylene glycol, sodium alginic, and Arabic Gum. Arabic Gum (AG), was chosen based on the solubility and thermal properties studies.

By using indirect binding approach, the selection for scaffold material is unlimited as long as the material could be bound by AG. In addition, the properties of the 3DP scaffold could be tailored by mixing and printing different materials of

different solubility. This finding is a major improvement over the current 3DP with chloroform as solvent in terms of powder material selection.

5.1.2. Printing parameter optimization

Three parameters were investigated in this study, which included the PLLA particle size and two printer parameters: shell/ core saturation values and layer thickness. The series of printer parameters studied are listed in **Table 5.1**. The shell/ core saturation values controlled the amount of solvent deposited on the outer layer and inner portion of the scaffold respectively. The layer thickness was the thickness of each powder layer spread out during printing. The values for the binder/ volume ratio and saturation level were reported. However, these values were dependent on the input values for shell/ core saturation and layer thickness. Hence, the binder/ volume ratio could not be varied independently.

Table 5.1 Printer parameters optimization

Binder/Volume Ratio (%)		Saturation Value		Saturation Level (%)	Layer Thickness (mm)	Distortion (mm)
Shell	Core	Shell	Core			
20.0	6.0	1.00	0.30	190	0.088	4.0
27.2	12.0	1.36	0.60	258	0.088	7.0
14.0	6.0	1.00	0.43	133	0.125	4.0
19.0	8.4	1.36	0.60	180	0.125	1.0
10.0	6.0	1.00	0.60	95	0.175	5.0
13.6	6.0	1.36	0.60	129	0.175	4.0
15.7	6.0	1.80	0.69	149	0.200	1.5
7.8	6.0	1.00	0.77	74	0.225	1.5
8.6	6.0	1.10	0.77	81	0.225	2.0
9.3	6.0	1.20	0.77	88	0.225	3.0
10.1	6.0	1.30	0.77	96	0.225	2.5
10.6	4.7	1.36	0.60	100	0.225	5.0
10.9	6.0	1.40	0.77	103	0.225	2.5
11.7	6.0	1.50	0.77	111	0.225	2.0
12.4	6.0	1.60	0.77	118	0.225	2.0
13.2	6.0	1.70	0.77	125	0.225	1.5
14.0	0.0	1.80	0.00	133	0.225	1.0
14.0	1.6	1.80	0.20	133	0.225	0.8
14.0	3.1	1.80	0.40	133	0.225	1.0
14.0	4.7	1.80	0.60	133	0.225	1.1
14.0	6.0	1.80	0.77	133	0.225	0.5
14.0	6.2	1.80	0.80	133	0.225	0.8
14.0	7.8	1.80	1.00	133	0.225	1.0
14.0	9.3	1.80	1.20	133	0.225	0.5
14.0	10.9	1.80	1.40	133	0.225	0.2
14.0	12.4	1.80	1.60	133	0.225	0.0
14.0	14.0	1.80	1.80	133	0.225	0.3
14.0	15.6	1.80	2.00	133	0.225	1.0
14.8	6.0	1.90	0.77	140	0.225	1.8
15.6	6.0	2.00	0.77	148	0.225	1.5

The printer parameters were optimized in terms of the severity of planar distortion and tolerance of the scaffold. Planar distortion is often encountered especially for thin and small printed parts. The distortion was due to the stair-stepping effect, in which the top layer of the scaffold was displaced from the bottom layer by a distance, caused by the raster-scanning action of the print head. The hydrophobic nature and light weight of the PLLA particles made them easy to disperse and float on the water droplets deposited, which contributed to the planar distortion. The final set of optimized parameters chosen, is as highlighted in **Table 5.1**, produced no distortion and stair-stepping effect.

In addition, the orientation and location of the printed scaffold in the build chamber were found to affect the onset of the stair-stepping effect. To avoid the effect, the object should be oriented such that its length was parallel to the Y-axis of the 3D printer.

PLLA particles of two different sizes, 212-500 μm and 106-212 μm were investigated. The parts printed with particles of size 212-500 μm had worse planar distortion and were too fragile that they crumpled upon handling. Scaffolds printed with PLLA particles of size 106-212 μm were found to have good tolerance and with less distortion. It was therefore believed that by decreasing the particle size, the tolerance and strength of the printed part improved, and the planar distortion was reduced. However the particle size had to be kept at a fixed range in order to obtain the desired pore size, since particle size and pore size are directly correlated. The secondary electron images (SEI) of the as-printed scaffold are shown in **Figure 5.1**.

Figure 5.1 SEI of as-printed scaffold with particles of size 106-212 μm

5.2. Post-printing Processes

One of the general drawbacks of 3DP scaffold was its poor mechanical properties. In this study, the as-printed scaffolds were too fragile to withstand any mechanical test. The inferior mechanical properties were the result of the weak AG binding strength to the particles, and the high porosity of the scaffolds. Therefore post-printing process was required to enhance the mechanical properties, and to prevent the disintegration of the scaffold upon dissolution of AG in the water. Infiltration and annealing, as well as a combination of both were investigated.

5.2.1. Infiltration

Infiltration increased the mechanical strength of the scaffold by filling in some of the pores and increasing the strut thickness of the structure, thereby reinforcing the scaffold. The infiltrant must penetrate the entire scaffold so that the effects of infiltration were uniform throughout the scaffold. The infiltration step should increase the mechanical strength of the scaffolds while maintaining the scaffold's porosity so that cells could grow and proliferate on it. Due to the above requirements, the infiltrant should be liquid enough to fully permeate the scaffold, yet contained the right amount of dissolved polymer so that the strengthening effect was attained.

PLLA and PCL in different concentrations were used as the infiltrant. DCM and THF at a 15: 85 volume ratio was used as the solvent. This ratio was chosen because it contained enough DCM to dissolve the PLLA and/ or PCL, but not enough to dissolve the scaffold itself. In addition to the above, high DCM content caused fast evaporation of solvent which in turn built up stress and warpage.

In order to ensure uniform penetration of infiltrant through the entire scaffold, different infiltration methods were experimented upon as mentioned in **section 3.5.2.1**. In the first method, the scaffolds were soaked in the infiltrant for up to 12 hours without ultrasonic bath. In the second method, the as-printed parts were soaked in the infiltrant and placed in an ultrasonic bath for 10 minutes before the scaffold disintegrated by ultrasonic bombardment. The infiltrant was not able to penetrate into the middle of the scaffold in both the methods. The third method was considered effective but limited to certain infiltrant concentrations. Infiltrant with polymer concentration above 20 wt% was too viscous for the vacuum pump to force through the scaffold. The last method, whereby the plunger was strong enough to force the infiltrant through the scaffold with concentration up to 35 wt% and thus thorough penetration was obtained. The last method of infiltration was then employed for the following investigation.

5.2.2. Annealing

Annealing was another method which served to increase the strength of a porous material by increasing the adhesion between particles in the scaffold. During annealing, the material was held at an elevated temperature to facilitate polymer diffusion. This enabled the formation of bridges between individual PLLA particles, so that the scaffold did not collapse even when the AG binder was removed. The strength of the scaffold was determined by the strength of adhesion of the contact surfaces between the individual particles.

The effects of annealing environment, duration and temperature on the inter-particle binding were studied and evaluated with the ultrasonic bath test.

Scaffolds were annealed in an oven for both vacuum and nitrogen environments, for 1 to 15 hours at temperatures ranging from 100 to 190 °C. This temperature range was taken as below the T_d and above the T_g of the polymer. The thermal properties of the polymer are listed in **Table 5.2**.

Table 5.2 Thermal properties of PLLA

T_d (°C)	T_g (°C)	T_m (°C)	T_c (°C) ^a
200	55-68	170	132

^a Obtained from MDSC second scan. No significant crystallization peak was observed in the first scan

The annealing temperature range of 150 to 190 °C produced the desired effects. The scaffolds did not disintegrate during the ultrasonic bath test. In order to minimize the extent of discoloration, the temperature of 160 °C was chosen. A nitrogen environment was preferred over a vacuum environment due to its improved heat conduction, especially to the middle section of the scaffolds. Good heat conduction was required because PLLA was inherently non-conductive. By providing good heat conduction, the same annealing outcome could be attained with the annealing temperature set at 20 °C lower for the same annealing duration. The SEI of annealed scaffold at different magnifications are shown in **Figure 5.2**. Binding due to diffusion between two particles could be observed in **Figure 5.2 (b)**.

Figure 5.2 Annealed scaffold at different magnification (a) X200, (b) X1,000

5.2.3. Mechanical properties of scaffolds with different post-printing processes

The effect of the infiltration and annealing conditions were further investigated. The post-printing processes were optimized in terms of infiltrant materials, infiltrant concentrations and the sequence of two processes combined.

5.2.3.1. Types of infiltrant

Two types of infiltrants, PCL and PLLA with the same concentration of 10 wt% were used in this study. The mechanical properties and the SEI of the scaffolds are shown in **Figure 5.3** and **Figure 5.4** respectively.

Figure 5.3 Mechanical properties of scaffolds with different types of infiltrants (PCL and PLLA)

Figure 5.4 (a) As-printed scaffold and scaffolds infiltrated with (b) PLLA and (c) PCL

Comparing the 10 wt% PCL with 10 wt% PLLA infiltrated scaffolds, PCL increased the UTS and the corresponding strain of the scaffolds respectively by 64 % (0.58 MPa), and 96 % (2.08 % strain) more than PLLA. Energy at maximum load is the energy absorbed by the sample when undergoing deformation and is calculated from the integrated area under the stress-strain curve from 0 MPa up to UTS. A material with low yield strength may absorb more energy than another material with higher yield strength. Hence, this energy is a good indicator of toughness. However, true toughness should be investigated via impact testing. PCL infiltrated scaffold exhibited higher energy at maximum load by a significant increase of 328 % (5.38 mJ). A drop in modulus by 50 % was observed in the scaffolds infiltrated with PCL as compared to that with PLLA.

The higher strain, energy and lower modulus obtained by PCL infiltration was expected since PCL in rubbery stage (T_g -65~ -60 °C) was more ductile and less

stiff as compared to PLLA in glassy stage [35]. Overall, PCL infiltrated scaffold demonstrated better mechanical properties as compared to PLLA infiltrated scaffold. In addition, attainment of higher UTS would make the scaffold more useful. Hence, PCL infiltration which gave higher UTS was chosen for further investigation.

5.2.3.2. Sequence of post-printing processes

Four groups of scaffolds were treated with different post-printing processes. Two groups of scaffolds were treated with single post-printing process with either infiltration (I only) or annealing (A only). Another two groups of scaffolds were treated with both post-printing processes. One of the groups was treated with infiltration followed by annealing (I+A), whereas the scaffolds in the other group was first annealed, followed by infiltration (A+I). The infiltrant used was PCL at 20 wt% and annealing was conducted at 160 °C for 15 hours.

Figure 5.5 Mechanical properties of scaffolds with different post-printing process sequences

Scaffolds that were annealed only (A only) were brittle and failed at the grips, thus no reliable results were obtained. As for the samples annealed before or after infiltration, they showed a significant effect on the UTS and the modulus of the scaffold. Similar UTS and modulus obtained in the I+A samples and I only samples suggested that the strength of I+A samples were mainly contributed by infiltration. However, the scaffold treated with A+I exhibited the highest UTS and modulus. Annealing before infiltration had significant effect in improving the scaffold mechanical strength. The average values of the strain and energy obtained by A+I was lower but comparable to those of I only. Overall, A+I scaffolds demonstrated better mechanical properties. In addition, attainment of higher UTS would make the scaffold more useful. Hence, A+I that gave higher UTS was chosen for further investigation.

5.2.3.3. Infiltrant concentration

Three different PCL infiltrant concentrations, 20 wt%, 35 wt% and 50 wt% were investigated under the optimized post-printing processes sequence (I+A) as discussed in the previous section. At 50 wt% concentration, PCL could not be completely dissolved in the DCM and THF (15:85) solvent mixture and was discarded. The mechanical properties of scaffolds annealed and infiltrated with 20 wt% and 35 wt% PCL are shown in **Figure 5.6**.

Figure 5.6 Mechanical properties of scaffolds with different infiltrant concentration

Significant improvement in the mechanical properties with more than 100 % increment was obtained by increasing the infiltrant concentration. Higher concentration infiltrant formed a thicker PCL layer to bind the PLLA particles and obtained thicker scaffold struts. In addition, more macro-pores were filled and this caused the reduction of porosity and pore size, thereby an increase in scaffold strength.

5.3. Incorporation of Lower M_w PLLA

The synthesized PLLA powder of M_w 15,000 Da (PLLA_{low}) was mixed together with the PLLA powder of M_w 130,000 Da (PLLA_{high}), at two different weight ratios of 70: 30 and 50: 50 (PLLA_{high}/ PLLA_{low}). The mixing ratio of the total amount of PLLA to AG was kept at 80: 20 (PLLA/ AG). The scaffolds were printed according to the optimized printing parameters as mentioned in **section 5.1.2**.

5.3.1. Annealing conditions

The thermal properties of both PLLA obtained from MDSC and TGA are listed in **Table 5.3**. It was noted that the thermal properties of the synthesized PLLA_{low} were significantly lower than that of PLLA_{high} due to the smaller of M_w [100] and probably the stereo-irregularity (DOR 24 %). Hence the previous annealing condition of 160 °C for 15 hours for PLLA_{high} would not be suitable for this dual M_w scaffold. In this case, the ideal annealing temperature and duration should fulfil two requirements: (1) able to melt PLLA_{low} particles in order to enhance inter-particles binding, with minimal thermal degradation; (2) able to promote the diffusion of PLLA_{high} particles, while preventing the melt and collapse of the scaffold structure. The upper limit of annealing temperature should either be below T_d of PLLA_{low} or T_m of PLLA_{high}, whichever was lower. The lower temperature limit should be above T_m of PLLA_{low}, in order to melt the PLLA_{low} and formed inter-particle binding. In this case, the two annealing temperatures of 150 °C and 160 °C were studied with durations of 7 hours and 15 hours for each temperature.

Table 5.3 Thermal properties of PLLA_{high} and PLLA_{low}

	T _d (°C)	T _g (°C)	T _m (°C)	T _c (°C) ^a
PLLA _{high}	200	55-68	170	132 ^a
PLLA _{low}	165	25-47	140	81

^a Obtained from MDSC second scan. No significant crystallization peak was observed in the first scan.

Scaffolds with mixing ratio of PLLA_{high}/ PLLA_{low} of 70: 30 were first annealed under these different annealing conditions and then infiltrated with 20 wt% of PCL. The mechanical results obtained are shown in **Figure 5.7**.

Figure 5.7 Mechanical properties of scaffolds (PLLA_{high}/ PLLA_{low}) with different annealing conditions

The mechanical results of the scaffolds annealed 150 °C improved significantly with annealing time, but the scaffolds annealed at 160 °C deteriorated mechanically with annealing time. The scaffold annealed at 150 °C (15 hours) had the best mechanical properties in the study. Hence, this annealing condition was chosen for PLLA_{high} /PLLA_{low} of 50: 50.

5.3.2. Mixing ratio

The effect of different mixing ratio of PLLA_{high}/ PLLA_{low} on the scaffold mechanical properties was investigated. Scaffolds with two mixing ratios 70: 30 and 50: 50 PLLA_{high}/ PLLA_{low} were both annealed at 150 °C for 15 hours and infiltrated with PCL 20 wt% respectively. The mechanical properties of the scaffolds are shown in **Figure 5.8**. Scaffold made by PLLA_{high} only (100: 0) were included for comparison. The post-printing processes and sequence for the 100: 0 scaffolds were the same as above except the annealing temperature was at 160 °C, which was the optimal temperature for the 100: 0 scaffolds.

Figure 5.8 Mechanical properties of scaffolds (PLLA_{high}/ PLLA_{low}) with different mixing ratios

The 70: 30 scaffolds exhibited the best mechanical properties among the samples. The incorporation of PLLA_{low} contributed significantly to the increment of the scaffold's mechanical properties. The UTS and modulus increased by 315 % and 90 % for the 70: 30; and by 100 % and 44 % for the 50: 50. The strain increment was 147 % for 70: 30 and 45 % for 50: 50.

5.3.3. Infiltration concentration

The mechanical properties of the 70: 30 scaffolds infiltrated with different PCL concentrations are shown in **Figure 5.9**. All the scaffolds were treated with the same post-printing processes and sequence except that the annealing temperature for 100: 0 scaffolds were conducted at 160 °C.

Figure 5.9 Mechanical properties of scaffolds with different infiltrant concentration

The mechanical strength of the 70: 30 scaffolds increased with PCL concentration from 20 to 35 wt%. The highest UTS, and modulus attained were the 70: 30 scaffolds infiltrated with 35 wt% of PCL, which were 19 MPa and 421 MPa respectively. The average strain and the energy for these scaffolds were also higher than the other scaffolds.

5.4. Binding of PLLA Particles and Infiltration Efficacy (IE)

The mechanical strength of the 3DP scaffolds could be attributed to inter-particles binding and infiltration. Inter-particles connections were formed by three methods: AG, annealing induced diffusion between the contact points of PLLA_{high}

particles, and annealing induced melting of PLLA_{low} particles. In general, the inter-particles binding formed were relatively weak and only played a role in maintaining the scaffold structure during processing; rather than contributing to the mechanical strength applicable for practical engineering usage. Infiltration of the scaffold was found to be the key factor in improving the scaffold mechanical strength. The infiltrant enhanced the strut thickness and thus the whole structure. The IE was measured (refer to **section 3.5.2.1**) and a close dependence of strength on this parameter was observed. By increasing the infiltration efficacy, the UTS and modulus increased correspondingly.

IE was decided by the ability of the infiltrant to penetrate and be retained in the scaffold. The major factors affecting IE included infiltrant material, annealing condition, mixing ratio of PLLA_{high}/PLLA_{low} and infiltrant concentration.

5.4.1. Types of infiltrant

The IE of scaffolds infiltrated by PCL 20 wt% and PLLA 20 wt% under I+A sequence were investigated. The IE of the former was 56.7 % $\pm$ 3.2 %, whereas a layer of coating was formed on the surface of the latter scaffolds and no full penetration was obtained. PCL, with lower viscosity than PLLA [22, 101], could penetrate through the scaffold more easily and more thoroughly. It could fill up smaller pores and gaps between the particles. As a result, higher UTS (2.4 MPa) and modulus (98.5 MPa) were obtained by PCL infiltrated scaffold. Scaffolds with PLLA coating were not strong enough and mostly failed at the grips during tensile test.

5.4.2. Post-printing processes

The direct effect of annealing to mechanical properties was not significant. However, annealing played an important role in determining the IE, thus the mechanical properties. The IE of scaffolds with different post-printing process sequences are shown in **Figure 5.10(a)** with the corresponding UTS and modulus **Figure 5.10(b)**. Another set of IE and the strength of the scaffolds with

different annealing conditions are shown in **Figure 5.11**. The UTS and modulus of both sets of scaffolds had the similar trend as the IE. Higher IE gave higher UTS and modulus. This indicated that IE was a critical factor in determining the mechanical strength of the scaffold.

Figure 5.10(a) IE of scaffolds with different post-printing processes sequence and (b) the corresponding UTS and modulus

The IE of A+I was higher than I+A. It was found to be more efficient to do the infiltration after annealing. Annealing usually increases the scaffold density, which is accompanied by the decrease in porosity and pore size [102- 104]. In this case, the density of the as-printed scaffold increased from 1.255 g/ cm³ to 1.276 g/ cm³ after annealing at 160 °C for 15 hours. The increase in density accompanied with the decrease in pore size might be more effective in retaining the low viscosity PCL infiltrant in the scaffold, thereby increasing the IE. Scaffolds treated with I+A and I only had similar IE. This was because the densities for infiltration of these two sets of scaffolds were the same. The annealing of I+A was conducted only after the infiltration and thus had no effect on the IE. Furthermore, similar mechanical properties obtained from these two treatment sequences excluded the contribution of inter-particles binding caused by the later annealing. The annealing induced diffusion between the PLLA particles in contact could be impeded due to the presence of PCL infiltrant wrapping around the particles.

Figure 5.11(a) IE of scaffolds (70: 30) with different annealing conditions and (b) the corresponding UTS and modulus

The difference in IE for annealing conditions at 150 °C for 7 and 15 hours, and at 160 °C for 7 hours could be due to the difference in scaffold density (**Figure 5.11**). The cross sections area of the scaffolds was measured to determine the effect of annealing on sample shrinkage, which served as a guide for density changes. The cross sections area of the scaffolds shrunk from 10.86 mm² (150 °C, 7 hours) to 10.36 mm² (150 °C, 15 hours) with the increase of annealing duration. The cross sections of the scaffolds further shrunk to 10.29 mm² at 160 °C, 7 hours and 9.76 mm² at 160 °C, 15 hours. More shrinkage was observed at the scaffolds with higher annealing temperature or longer annealing duration. This in turn increased the IE. The low IE observed in the scaffolds annealed at 160 °C for 15 hours could be due to excessive diffusion and melting of PLLA particles causing formation of close pores and overly compact structure that impeded the penetration of infiltrant. This showed that the effect of infiltrant was more significant than inter-particles binding on the mechanical strength.

5.4.3. Mixing ratio

Figure 5.12(a) IE of scaffolds with different mixing ratios and (b) the corresponding UTS and modulus

A strong correlation between IE and scaffold strength was observed in **Figure 5.12**. The changes in IE with different PLLA_{high}/ PLLA_{low} mixing ratio could again be attributed to the density of the scaffold. The cross section area of the scaffolds reduced from 13.27 mm² (100:0) to 10.36 mm² (150 °C, 15 hours) and 9.33 mm² (50: 50). The shrinkage showed that the structure was more compact with the increase of PLLA_{low} amount. The 30 wt% of PLLA_{low} in 70: 30 scaffolds melted during annealing and formed a more compact structure than 100: 0 scaffolds without any PLLA_{low}. This compact structure was able to retain the PCL infiltrant more effectively, and was believed to have minimal penetration problem. However, with the increase of PLLA_{low} to 50 wt%, the melting of this low PLLA could have blocked some open pores. This excessive compact structure could impede the infiltrant penetration and reduced the IE.

5.4.4. Infiltration concentration

Figure 5.13 and **5.14** show two sets of scaffolds (100: 0 and 70: 30) infiltrated with two different infiltrant concentrations 20 wt% and 35 wt% PCL. The UTS and modulus for both sets of scaffolds increased significantly with IE, and the IE was in turn affected by the infiltrant concentration. Increasing the infiltrant

concentration reduced the viscosity of the infiltrant, and as a result more infiltrant was retained in the scaffold.

Figure 5.13(a) IE of scaffolds (100:0) with different infiltrant concentrations and (b) the corresponding UTS and modulus

Figure 5.14(a) IE of scaffolds (70:30) with different infiltrant concentrations and (b) the corresponding UTS and modulus

5.5. Scaffold Architecture

The architectures of two groups of scaffolds (70: 30, infiltrated with PCL 20 wt% and 35 wt%) were studied. The scaffold architecture was characterized in terms of density, open porosity, open pore volume/ mass (V_p/ M), surface area/ mass (SA/ M) and pore diameter (**Table 5.4**). The SEI of the outer surface and the cross section tensile fracture of the scaffolds are shown in **Figure 5.15**.

Table 5.4 Architecture of scaffold with optimized mechanical properties

Scaffold (70: 30)	Density ^a (g/cm ³)	Open porosity ^a (%)	V _p / M ^a (cm ³ /g)	SA/ M ^b (m ² /g)	Pore Diameter ^a (μm)	
					macro	micro
PCL 20 wt%	1.1865 (+3.4/ -2.4 %)	47.74 (+7.5/ -10.5 %)	0.7746 (+17.3/ -19.2 %)	1.3743	155 (+146/ -88)	6 (+3.3/ -5.8)
PCL 35 wt%	1.1782 (+5.7/ -5.9 %)	40.08 (+4.9/ -8.4 %)	0.5698 (+ 8.1/ -15.9 %)	1.3614	82 (+62/ -33)	4 (+2.4/ -5.4)

^a measured by density bottle.

^b measured by BET

The size of the macro-pore for PCL 20 wt% scaffolds was 155 μm in average. The PCL 35 wt% scaffolds had significantly less macro-pores, and the average pore size was smaller. **Figure 5.15(c)** and **(d)** show the micro-pores architecture of scaffold surface at higher magnification (X1,000). The size of the micro-pore calculated for PCL 20 wt% ranged from 2.7 to 11.8 μm, while the PCL 35 wt% micro-pore size measured from 6.4 to 9.4 μm. The formation of micro-pores could be due to the evaporation of solvents from the infiltrant. In **Figure 5.15(e)** and **(f)**, the lighter coloured material was PCL infiltrant, and the solid in darker colour was PLLA particles. The PLLA particles were observed to be enfolded and bound by the PCL infiltrant. The fibre form of PCL infiltrant was due to the deformation caused by tensile test.

Figure 5.15 SEI of scaffolds (70:30) with different infiltrant concentrations (a) PCL 20 wt%, surface structure (b) PCL 35 wt% surface structure (c) PCL 20 wt% micro-pores (X1,000) (d) PCL 35 wt% micro-pores (X1,000) (e) PCL 20 wt% cross section and (f) PCL 35 wt% cross section.

As discussed in the previous section, higher IE was obtained with increase in infiltration concentration. The increase in infiltrant amount retained in the scaffold

enhanced the strut thickness and the strength of the structure. However, this also reduced the porosity and pore size. With the increase in concentration 20 to 35 wt%, the porosity reduced by 8 %. The pore volume/ mass also reduced from 0.78 to 0.57 cm³/g, and the average pore diameter reduced from 155 to 82 μ m.

High surface area allows significant cell-surface interactions such as cellular attachment [1, 116]. Reduction in porosity decreases the surface area/ mass (SA/ M), but decrease in pore size increases the SA/ M. Though scaffolds PCL 35 wt% had lower porosity than scaffolds PCL 20 wt%, the SA/ M obtained for these two sets of scaffolds were similar. This could be due to smaller pore sizes obtained for scaffold PCL 35 wt%.

5.6. Multi-functional Scaffold with Incorporation of PLLA_{low}

The incorporation of PLLA_{low} into the scaffold offered a few underlying advantages and potential applications. Three major aspects of the PLLA_{low} functions were discussed below.

5.6.1. Mechanical strength enhancer

The incorporation of PLLA_{low} into PLLA_{high} scaffolds (i.e. PLLA_{high}/ PLLA_{low} 70: 30 and 50:50) exhibited better mechanical properties as compared to the scaffolds without the incorporation of PLLA_{low} (100: 0). Due to the lower melting temperature of PLLA_{low}, the polymer melted at the annealing temperatures to form inter-particles bonding, which increased IE and the strength of the scaffolds. In the meantime, the PLLA_{high} with its melting temperature higher than the annealing temperatures did not melt and was able to maintain the scaffold structure.

The UTS, and strain at UTS for the 70: 30 scaffolds were higher than the 3DP polymeric scaffolds made by Giordano et al [33]. However, the tests were carried out at a strain rate of 0.25 mm/min while Giordano et al tested their samples at a

strain rate of 1 mm/min. Hence, the UTS and modulus of these samples were expected to be higher at 1 mm/min [105, 106].

5.6.2. In-situ pores formation

To produce a scaffold with suitable mechanical strength was one of the essential requirements, especially at the beginning stage of strategy I implantation, as suggested by Dietmar et al [3]. At this stage, the scaffold had to sustain any in vivo stresses and loading without the support of neotissue [2, 3]. A scaffold with high porosity, interconnectivity and correct pore size was another critical factor in tissue engineering. This was particularly vital at the later stage of implantation when the cells start to migrate deep into the scaffold [4]. Unfortunately, the mechanical strength of a scaffold is compromised with increase in porosity and pore size. Therefore a balance between these two parameters is needed in order to optimize the performance of the scaffold. It is a challenge to make scaffolds of high strength for using the traditional approach to introduce porosity.

This problem can be solved through the approach of in-situ pores formation with this multi-functional scaffold. The difference in degradation rate of different M_w of PLLA could be exploited in this case. PLLA_{high} takes about a year to degrade before mass loss occurred [76, 96, 107]. On the other hand, PLLA_{low}, according to the result reported in **section 4.6**, had an instantaneous mass loss. Hence, the hydrolytic degradation and dissolution of PLLA_{low} particles (106 μm -212 μm) incorporated in the PLLA_{high} scaffold matrix would introduce pores in-situ. The pore dimensions could be defined by the size of the PLLA_{low} particles used, and the porosity could be controlled by the amount of PLLA_{low} incorporated. According to Venkatram et al, two-phase scaffolds (PLGA in polyanhydride matrix; and p(CPP:SA) 20: 80 in p(CPP:SA) 80: 20 matrix) were constructed by compressive moulding and pores were successfully introduced in-situ through the hydrolysis of minor phase to even the interior of the scaffold [108].

One of the advantages of using 3DP with AG was the versatility in materials selection. The polymers used need not necessary to be soluble in the “ink” deposited by 3DP. In this case, multiple polymers with different degradation properties could be mixed and printed together in order to manipulate the timing of pores formation and the corresponding mechanical strength. By proper selection of the polymers, the scaffold could be designed to be with high mechanical strength and low porosity at the initial stage, followed by a sequential transition to lower strength and higher porosity as neotissue resumes. The rate of transition could be manipulated according to the requirement of specific tissue. In addition, with the infiltration of a slow degradation rate PCL [42], the possibility of the scaffold collapse due to loss of other polymers (as porogen) could be minimized.

5.6.3. Controlled release of biofactors delivery for TE

The needs to deliver biofactors for TE and the methods to deliver were stated in chapter 2. The use of conventional porous scaffold for mechanical support in tissue engineering in conjugation with biofactors delivery is likely to be one of the best ways going forward. Various biofactor(s) incorporation methods were explored in combination with scaffold fabrication techniques [7, 105- 108]. However, the current developed multi-functional scaffolds are mostly limited to single biofactor delivery, with a decreasing release rate with respect to time. More advanced controlled delivery is required in order to generate functional tissue. The major challenges include the delivery of several biofactors at different time frames with different concentrations. In addition, constant release rates are preferred especially for biofactors that possess narrow range of therapeutic indices.

The AG indirect binding approach enables the printing of polymers with different degradation properties, as the carriers of various biofactors, in a scaffold. As the polymer carriers degrade at different rates, controlled release of multiple biofactors at different periods of implantation can be obtained [104]. The 3DP

method, that can deposit the ink at specific locations, allows for the possibility of fabricating scaffold with controlled release approximating zero order release kinetics [75]. Hence, 3DP can be exploited and applied in combination with AG indirect binding to achieve multiple biofactors zero order kinetics controlled release.

Chapter 6. Conclusion

6.1. Synthesis of PLLA

6.1.1. Catalyst systems

It was proven here that the binary-catalyst system (SnCl_2 / TSA) is the most effective polycondensation catalyst to produce relatively high molecular weight PLLA. Compared to other single polycondensation catalysts, this catalyst system also minimized the discoloration effect and produced PLLA with acceptable crystallizability of 30.8 %. The relatively good properties obtained from the PLLA synthesized were due to the combination effect of the two catalysts in the binary-catalyst system. The metal ion catalyst SnCl_2 was efficient in promoting dehydration reaction and increasing the molecular weight of the PLLA, while TSA was effective in minimizing side reactions including discoloration and racemization. The third catalyst used Sn(II) Oct was not an effective polycondensation catalyst in terms of increasing the molecular weight and reducing various side reactions, such as discoloration and racemization reaction.

The catalyst(s) used to produce PLLA had significant effect on the degree of racemization. This consequently affected the crystallizability of the polymer. It was found that the crystallizability was inversely correlated with DOR. PLLA synthesized by TSA had the least DOR of 9.1 %, and so the highest crystallizability of 55 %. PLLA produced by the binary-catalyst had a DOR of 24 % and a crystallizability of 31 %. PLLA produced by Sn(II) Oct with 38 % of degree of racemization was amorphous and could not crystallize during the second heating scan of MDSC.

6.1.2. Process Optimization

In the oligomerization step, the optimum oligomer M_n that produced the highest polymer molecular weight was around 600 Da. The process yield increased while the polymer molecular weight decreased when the oligomer M_n was raised above 600 Da.

Three reaction parameters of polymerization step were optimized in this study. These included the ramping sequence, the reaction temperature and duration. The most effective ramping sequence in the polymerization step to produce high molecular weight polymer was RS4. It was found that the ramping sequence with simultaneous increase in temperature and decrease in pressure enhanced both the yield and the molecular weight. Reaction pressure less than 10 torr enhanced dehydration but also caused various side reactions, which deteriorated the polymer. Hence, the pressure 10 torr was used throughout the study.

Two different polymerization temperatures, 180 °C and 220 °C were studied with different reaction durations. The highest M_w obtained at 180 °C for 18 hours was 54,000 Da and 43,000 Da at 220 °C for 2 hours. The polymerization rate at 220 °C was much higher than that at 180 °C. However, the high reaction temperature not only enhanced the polymerization but also caused various side reactions that led to depolymerization. Thermal stability, thermal properties and crystallization behaviour can be improved by either having a shorter reaction duration and/ or lower reaction temperature. In summary, PLLA produced at 180 °C was able to attain a higher molecular weight, yield, better thermal stability and crystallization behaviour, as compared to the PLLA synthesized at 220 °C. In addition, the 18 hours of synthesis window at 180 °C provided more controllable duration for obtaining a specific molecular weight.

In this study, PLLA with M_w more than 10,000 Da, good crystallization behaviour and stable thermal properties was obtained through polycondensation synthesis. With the 54,000 Da M_w attained after 18 hours of reaction, it showed the potential of melt polycondensation to synthesize high molecular weight PLA, with the use of binary-catalysts system.

6.2. Scaffold Printing

The indirect binding approach proposed in this study involved the use of AG and water to bind the PLLA particles. Hence eliminating chloroform as a binder. This approach also enabled a wider selection of materials for scaffold fabrication. The properties of the 3DP scaffold could be tailored by mixing and printing different materials, even with different solubility. This finding is a major improvement over the current 3DP with chloroform as a solvent in terms of polymeric powder selection.

6.3. Post-printing Processes and Incorporation of Low Mw PLLA

As the mechanical properties of scaffold obtained by 3DP with AG were low, post printing treatment(s) were required to enhance the mechanical properties. In addition, the scaffolds made up of a mixture of PLLA_{low} and PLLA_{high} had better mechanical properties than the scaffolds made up of PLLA_{high} only. The best mechanical properties were obtained with the annealing of scaffold at 150 °C for 15 hours, followed by an infiltration of PCL 35 wt%. The PLLA_{high}/ PLLA_{low} mixing ratio used for this scaffold was 70: 30.

A new infiltration set-up ensured uniform penetration, where a plunger was used to force the infiltrant through the scaffold. This method was successfully applied even to infiltrant of concentration of up to 35 wt%. The IE was found to have a close and direct dependence on the scaffold strength and modulus. IE determined the ability of the infiltrant to penetrate and retain in the scaffold. Better infiltrant penetration and/ or retention of the scaffold enhanced IE and thus the mechanical properties. The major factors affecting IE included infiltrant material, annealing condition, mixing ratio of PLLA_{high}/ PLLA_{low} and infiltrant concentration.

Annealing and the incorporation of PLLA_{low} resulted in inter-particles binding and a more compact structure. In general, the inter-particles binding was relatively

weak, but was needed in maintaining the scaffold structure during processing. However, with a more compact structure formed through annealing and the melting of PLLA_{low}, more infiltrant was retained in the scaffold and thus enhanced the IE and mechanical properties.

6.4. Hydrolytic Degradation of Synthesized PLLA

The polymer M_w decreased with degradation time, where hydrolytic chain scission of the PLLA ester bonds was observed right from the beginning of the study. The degradation of PLLA films was studied with autocatalysed and uncatalysed models. No clear distinction could be derived between these two models. This suggested that the hydrolytic degradation was contributed by both autocatalysed and uncatalysed mechanisms simultaneously. Both the models hold true even with mass loss up to 9 %. There was no clear differentiation between surface and interior degradation of the samples. The increment of M_{wD} with degradation time indicated hydrolytic random chain scission and the formation of oligomer fragments in the polymer matrix. The increase of DOC and the reduction of T_g and T_c with degradation time indicated that the degradation mainly occurred in the amorphous regions within the time frame investigated, 135 days. The reduction of T_m was mainly due to the reduction of the polymer molecular weight through hydrolytic chain scission.

It was found that the mass loss profile of the PLLA synthesized by melt polycondensation was different from the mass loss profile of high molecular weight PLLA produced by ROP. The synthesized PLLA displayed a parabolic mass loss due to the initial low molecular weight and high water uptake. The instant mass loss observed upon immersion of sample in PBS showed that the low M_w PLLA is a suitable candidate for porogen application and as a fast controlled release drug carrier. The rate of pores formation could be tailored to suit the growth rate of different tissues, and attain a gradually transition of scaffold mechanical properties. With the proposed indirect binding approach, this

low M_w PLLA can also be substituted by other polymers with different degradation properties to meet various requirements.

6.5. Mechanical Properties and Morphology of the Scaffold

The mechanical properties of the scaffolds (70: 30, A+I) infiltrated with PCL 20 wt% and 35 wt% respectively were investigated. The scaffold infiltrated with PCL 20 wt% had 14.6 MPa, modulus 334.6 MPa and strain at UTS 10.78 %. The scaffold infiltrated with PCL 35 wt% had higher UTS 18.9 MPa, modulus 420.6 MPa, and strain at UTS 11.89 % compared with the scaffolds infiltrated with PCL 20 wt%. The mechanical properties obtained were rather high as compared to other 3DP polymeric scaffolds.

The mechanical properties obtained from the scaffolds infiltrated with PCL 20 wt% and PCL 35 wt% fell in the mechanical range of soft connective tissues, such as anterior cruciate knee ligament (ACL) and ankle medial collateral ligament [109]. However, most of the tensile test of the soft connective tissue was conducted at high testing strain rate such as 100- 1000 mm/ min with pre-test loading [110- 113], while strain rate of 0.25 mm/min was used in the scaffolds. In view of the low testing strain rate (0.25 mm/ min), the UTS and modulus were expected to increase with higher strain rate applied; whereas the strain at UTS would reduce [105, 106]. Since, the testing conditions of the soft connective tissues reported were different from the testing condition applied in this study, more accurate mechanical properties should be obtained through more realistic testing methods such as creep test. Hence, the mechanical properties of the scaffold obtained here serves as guide to its application.

The average size of the macro-pore for PCL 20 wt% infiltrated scaffolds was 155 μm with an open porosity of 48 %. Scaffolds with PCL 35 wt% had significantly less macro-pores, and the average pore size was smaller, 82 μm , with 40 %

open porosity. The pore size and porosity was expected to increase further during in-situ pore formation with the degradation of the PLLA_{low}.

Chapter 7. Future Work

7.1. In Vitro Study of Hydrolytic Degradation and In-situ Pores Formation

In order to evaluate the in-situ pores formation, the degradation profile of the scaffolds having the optimum properties could be studied. This study would investigate the changes in the scaffold molecular weight, mass loss, water uptake and thermal properties during the hydrolytic degradation process. In addition to the above, the changes in porosity, pore size, density and surface area of the scaffolds throughout the hydrolytic degradation process would assess the performance of the PLLA_{low} which is incorporated as a porogen for in-situ pores formation.

7.2. In Vitro Study of Biocompatibility

In order to assess the scaffold biocompatibility, in vitro cell culture could be carried out where cell attachment, growth, and long term matrix elaboration are investigated. The primary ACL fibroblasts derived from either explants or digestive cultures could be used to study the cell response to the scaffold geometry. The delivery of proteins/ gene such as growth factors or adhesion proteins to enhance cellular proliferation should also be investigated. The changes of the scaffolds mechanical properties with time due to the effect of cell growth, hydrolytic degradation and pores formed in-situ should also be taken into consideration.

7.3. Study of Biofactors Controlled Delivery

In order to attain multi-drugs release profiles at different stages of implantation, two methods to incorporate these biofactors could be studied. In the first method, biofactors are incorporated in matrix materials PLLA_{high}, PLLA_{low}, and PCL infiltrant respectively, while the polymers are in solution stage. In the case of PLLA_{high} and PLLA_{low}, the drug-containing polymers solutions will first be dried

and then be grounded into desired particle sizes for 3DP. A fast release profile is expected on the biofactor incorporated into PLLA_{low} particles, whereas biofactor included into PLLA_{high} particles will give a slower release. Mixing the drug into the PCL infiltrant will provide a much slower controlled release.

The second approach is to mix the biofactors with the water-based ink and deposited spatially in designed pattern during 3DP process. The aim is to produce a control release kinetics that will approximate zero order. The release rate and release time can be controlled by either specifying the position of the biofactors within the scaffold, or by controlling the local composition and microstructure with the 3DP process. The changes of local composition and microstructure are to be achieved by modifying the binder composition, printing speed or binder deposition concentration. The mechanism of resorption can also be controlled by manipulating the composition and microstructure with the scaffold during construction [75].

In the second approach, the biofactor release is expected to be largely associated with the surface of the polymer particles and subject to a more rapid release as compared to the first approach. In contrast, the first approach is predicted to result in a more even distribution of biofactor throughout the respective polymer carrier, with release regulated by the degradation of the polymer.

7.4. Scaffold Mechanical Properties Study for Specific Application

The mechanical properties obtained from the scaffolds infiltrated with PCL 20 wt% and PCL 35 wt% fell in the mechanical range of soft connective tissues, such as anterior cruciate knee ligament (ACL) and ankle medial collateral ligament [109]. However, most of the tensile test of the soft connective tissue was conducted at high testing strain rate such as 100- 1000 mm/ min with pre-test loading [110- 113], while strain rate of 0.25 mm/min was used in the

scaffolds. In view of the low testing strain rate (0.25 mm/ min), the UTS and modulus were expected to increase with higher strain rate applied; whereas the strain at UTS would reduce [105, 106]. Since, the testing conditions of the soft connective tissues reported were different from the testing condition applied in this study, more accurate mechanical properties should be obtained through more realistic testing methods such as creep test. Hence, the mechanical properties of the scaffold obtained here serves as guide to its application.

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