IN-VITRO DRUG RELEASE FROM BIODEGRADABLE MATRICES: ANALYSIS, MODELING AND APPLICATION IN VASCULAR STENTS

LUCIANA LISA LAO

School of Materials Science and Engineering

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Abstract

Our laboratory has been developing a completely biodegradable coronary stent which is made of bi-layers of biodegradable polyesters. This thesis presents the investigations carried out to exploit the drug delivery potential of such a polymeric stent. The main objective of this work was to understand and control the release of anti-proliferative drugs from biodegradable polymer films. Such understanding enabled us to explore further the effect of the controlled release of anti-proliferative drug on smooth muscle cell viability.

In the bilayer films, an anti-proliferative drug such as paclitaxel is loaded on to the “top” layer that contacts the blood vessel endothelium, while an anti-thrombotic drug such as heparin may be incorporated into the “bottom” of lumen-facing side. Paclitaxel release from P(DL)LGA layer consists of three distinct stages: (i) extremely slow initial release, (ii) accelerated degradative, relaxation-induced release, and (iii) diffusional release. The effect of additives that accelerate release of paclitaxel from P(DL)LGA was studied using in vitro methods. Addition of 10 wt% PEG into P(DL)LGA reduced the induction period by half while replacing P(DL)LGA with PCL resulted in an initial burst followed by a rapid diffusion. Blending P(DL)LGA and PCL resulted in a unique, intermediate release profile that reflects the complementary effect of the two.
Abstract

On the other hand, heparin release from PLLA layer suffered from massive burst release followed by a slow diffusion. At the end of an 80-day release study, 30% of the drug loading remained trapped in PLLA. This problem was rectified by adding 10 wt% high molecular weight PEO. PLLA/PEO 90/10 (w/w) managed to reduce the initial burst and subsequently displayed a zero-order release rate until all drugs were released.

To better understand the experimental observations, two-step and three-step models were developed to predict bi-phasic and tri-phasic drug release from (unblended) bulk-degrading polymers. These models present deeper understanding of the underlying mechanisms and important parameters that control overall drug release process. On top of the usual burst and diffusion processes, the three-step model took into account a new concept of relaxation controlled release. In addition, a novel "heuristic" model was proposed for drug release from blends of low (or non-) miscible degradable polymers.

Finally, usefulness of controlled release of paclitaxel for stent applications was investigated. Cell counting and proliferation assays were employed to analyze the efficacy of each type of paclitaxel release kinetics in preventing human coronary artery smooth muscle cells proliferation. In this study, the moderate release kinetics appeared to be the best choice to prevent cell proliferation with consequently less adverse effect on re-endothelialization.
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CHAPTER 1
INTRODUCTION

1.1 Background

A stent is a tubular device, normally made of stainless steel used to open up any blocked ducts in human body. Since its first introduction in the mid 1980s [1], coronary stent has evolved from the first generation of bare metallic stents [1-3] to the second generation of drug-eluting, coated metallic stents [4-13]. Experience in recent years has shown that the metallic stent poses some problems, including restenosis (reblockage), corrosion and interference with magnetic diagnostic equipment [14-16].

Drug therapy has been identified as one of the most effective techniques in the latest battle against restenosis. Local delivery of anti-proliferative drugs such as sirolimus or paclitaxel has been enabled by the drug loaded polymer coating on the metallic stent. However, there are concerns about the coating stability over time, inflammation triggered by this layer of polymer coating and its low capacity of drug delivery [17-19]. In addition, another type of drug, i.e anti-thrombotic agent, has to be administered orally to the patients.
Lately, the development of temporary, biodegradable polymeric stent has been actively researched to be the best candidate to overcome the limitations of the first two generations of stents [20-24]. A bi-layer polymeric stent has been developed in our laboratory and is made of two layers of biodegradable polymers (PLLA and P(DL)LGA) sandwiched together [25-27]. Besides the benefits of temporary residence in the body, this completely polymer-based stent offers greater potential of local drug delivery. The demand to deliver both anti-proliferative and anti-thrombotic drugs concurrently can be met by this unique bi-layer stent as each layer carries each type of drug. This potential can only be exploited if the rate of the drug release can be programmed according to the specific treatment requirements.

1.2 Problem Statement

In our laboratory, a prototype of bi-layer degradable coronary stent has been developed from PLLA and P(DL)LGA. A clear advantage of this bi-layer, polymer-based stent is its great potential to deliver both anti-proliferative and anti-thrombotic drugs concurrently right on the target site – something which cannot be achieved by any kinds of coronary stents up to date. The challenge, however, lies on controlling and adjusting the release rate of each drug to suit the therapy requirements.
Introduction

In order to gain good control of drug release rates, it is important to understand the underlying release mechanisms and important parameters governing the release kinetics. Several release models in literature have attempted to predict drug release from biodegradable matrices [28-32]. However, most of them considered diffusion as the sole release mechanism and hence were applicable only to mono-phasic and at most, bi-phasic release profiles. *No models have yet been able to address the tri-phasic release profile which is commonly obtained from biodegradable systems.*

Coronary stent advancement has revolved around the local administration of anti-proliferative drugs (sirolimus/paclitaxel) through drug-eluting stents. This move was supported by abundant reports which show both drugs' potency to inhibit smooth muscle cell proliferation in-vitro. The incorporation of drugs into the stents has also returned good improvement to prevent restenosis [9-13]. *However, there is a gap in the literature as only very few, if any, papers have investigated whether fast or slow release kinetics resulted in the best inhibitory effect.*

1.3 Objectives

The objectives of this thesis can be divided into three major parts. The first part is to exploit the multiple drug delivery potential of the bi-layer polymeric films/stents. The top layer is loaded with an anti-proliferative agent, i.e paclitaxel, while the bottom layer is
loaded with an anti-thrombotic agent, i.e heparin. Hence it is first necessary to investigate and manipulate the in-vitro release behaviour of each drug from its respective mono-layer film as well as bi-layer configurations. If the release kinetics is found to be unsatisfactory, several approaches shall be taken to tailor and adjust the release rates to suit the coronary stent applications.

The second part is to correlate the experimental in-vitro release results with theoretical drug release models. As most of the available models have limitations, it is necessary to develop a novel model that is able to predict a more complex release profile from a biodegradable system. This new model development aims to elucidate the important parameters and governing release mechanisms that may change during the different stages of release. This is especially true given the fact that biodegradable matrix is, driven by degradation, dynamically changing at all times.

The third part is to investigate the effect of drug release kinetics on inhibiting coronary artery smooth muscle cells proliferation. At least three different paclitaxel release kinetics, including fast, moderate and slow release, shall be examined. Careful observation of smooth muscle cells viability and proliferation in response to the varying paclitaxel release kinetics shall be carried out. This study shall provide an early suggestion on the most efficacious release rate to inhibit cell proliferation and restenosis – the Achilles heel of coronary stents.
1.4 Scope

In this work, only the bi-layer film configuration of PLLA bottom layer and P(DL)LGA 53/47 top layer will be studied. Two types of drugs were loaded: (1) paclitaxel as the anti-proliferative agent and (2) heparin as the anti-thrombotic agent. Paclitaxel is loaded to only the top layer while heparin is loaded to only the bottom layer.

A preliminary study with a model drug (metoclopramide salt) will be conducted to equip the author with the basic in-vitro release techniques. Paclitaxel release study shall start from mono-layer P(DL)LGA 53/47 film and then followed by bi-layer P(DL)LGA/PLLA films and stents. The same procedure applies to heparin release study with PLLA as the drug carrier. Adjusting paclitaxel and heparin release rates will be attempted by the following methods: introducing several polymer additives and plasticizers such as PEO and PEG, choosing a more permeable polymer as the base matrix and if necessary, blending two different polymers. To verify the hypothesized release mechanisms, a separate set of degradation study will be conducted. It includes measurement of water absorption, weight loss and molecular weight decay as well as observation of surface deterioration by electron microscope.

Novel models developed in this thesis are meant to address drug release from erodible systems, specifically bulk-degrading polymers such as lactide / glycolide polymers. As polymer films act as the drug carriers in this project, the mathematical equations will be
solved solely for a planar dimension. In addition, the proposed heuristic blend model is intended to tailor and predict drug release from a blend of immiscible polymers.

Only human coronary artery smooth muscle cells were tested in the in-vitro cell study as they make up the highest fraction of components of the neo-intimal layer responsible for vessel re-narrowing. The study duration is restricted to a maximum of 17 days as cells would have reached confluence by then. Cell count assay which directly indicates the number of healthy cells in a sample and cell proliferation assay which measures the number of cells that are actively dividing in a culture are selected to present an early suggestion on which release kinetics works best in preventing restenosis.

1.5 Overview

This thesis report has been organized into seven chapters. The motivation that drives this research project, scope of the work and novel contributions upon completing the project have been highlighted in this opening chapter. To put our work in perspective, chapter 2 presents a summary of previously reported works, including past achievements as well as yet-to-be addressed critical issues in this field. It becomes the foundation of our work and sets the directions of this project. Chapter 3 outlines the methodology employed to carry out all the experiments in order to achieve the objectives of this research work. Subsequently, chapters 4, 5 and 6 form the “results and discussions” section of this report.
Chapter 4 discusses the in-vitro release profiles of paclitaxel and heparin from P(DL)LGA and PLLA, respectively, as well as the attempts to tailor the release profiles to the desirable outcomes. Of course, in-depth analyses of the release mechanisms accompanied all the in-vitro release results. In chapter 5, release models to predict drug release kinetics from unblended and blended degradable polymers are proposed. These models introduce new concepts and offer better insights on the whole drug release process. Chapter 6 reports the effect of paclitaxel release kinetics on smooth muscle cells proliferation. It is going to provide an early suggestion of the most suitable release profile to prevent restenosis. Finally, chapter 7 draws the final conclusions of all the investigative works in this thesis and recommends some works for future research.
CHAPTER 2
LITERATURE REVIEW

2.1 Coronary Stents: History, Advances and Challenges

2.1.1 Introduction to coronary stents

Coronary artery disease has been the leading cause of death in the past decades. It is largely caused by atherosclerosis, i.e., narrowing of inner diameter of arteries as a result of deposition of lipid/fatty layers and plaque. In the late 1970's Dr. Andreas Gruentzig [33, 34] introduced a non-invasive surgery to dilate the area of arterial blockage with the help of a catheter that has an inflatable small sausage-shaped balloon at its tip, a procedure termed percutaneous transluminal cardiovascular angioplasty (PTCA). Although PTCA showed some degree of success, several shortcomings were encountered.

Firstly, blood vessel tends to “spring back” to a certain degree after balloon dilation (elastic recoil) and causes the channel to become smaller. Secondly, the opening created is not smooth and even. The irregular shape and rough surface cause some turbulence to blood flow and negative remodeling. Thirdly, balloon dilation causes injury to the outermost lining of the vessel wall. The healing process in response to this injury involves
platelet aggregation, smooth muscle cell proliferation and excessive extra-cellular matrix production, usually termed neo-intimal hyperplasia. All three activities narrow the vessel's inner diameter and increase the risk of (complete) arterial re-blockage (restenosis) [35, 36].

To overcome the problems mentioned above, stents were introduced and quickly become the most popular choice for treatment of coronary artery disease. A stent is a slender, hollow tube which acts as a support that maintains or increases the lumen of a vessel. Stent implantation represents a major step forward in cardiovascular intervention as early trials showed significant reduction in restenosis (vessel's reclosure/reblockage) rates as compared to balloon angioplasty alone [37-39]. Stent evolution and advances can be categorized into several generations, as discussed in the next section.

2.1.2 Evolution of coronary stents

(a) Metallic stents

The first generation of stent was made of metals, mostly stainless steel, tantalum and nitinol. In the mid 1980s, Sigwart et al. first introduced a stainless steel stent into human coronary arteries [1]. Soon after, several new designs were developed and different metallic materials were tested. Animals and clinical trials of these metallic stents
Literature Review

exhibited significant improvement in minimizing complications and preventing stenosis after PTCA. For example, the Palmaz-Schatz stent implantation showed an absolute survival and event-free survival of 88% and 56% respectively after three years of metallic stent implantations [2]. Another longer-term study reported 86% survival rate, out of a total of 426 patients, eight years after the procedure [3].

With its excellent mechanical properties, metallic stents solve the problem of acute occlusion, immediate elastic recoil and negative remodeling. Nevertheless, several limitations and drawbacks associated with metallic stents are still present.

Firstly, stenting actually results in increased neo-intimal hyperplasia and causes late lumen loss up to as much as 0.8 to 1.0 mm, leading to a 56%-75% cross-sectional area loss [14-16].

Secondly, introduction of toxic corrosion products is inevitable due to the inherent metallic nature of the stent. These toxic products may cause abnormal tissue response and cause more serious systemic effects if allowed to flow with blood to the whole body.

Thirdly, the permanent residence of the metallic stent in patient’s body may cause long-term complications and inconvenience. For example, it limits the patient’s usage of certain diagnostic equipment due to dangerous ferromagnetic interaction.
Lastly, most surfaces of metals and alloys are thrombogenic. Their surface energies are largely higher than the 20-30 dynes/cm required for thrombo-resistance. As such, a large amount of thrombosis mediators and platelets adhere to stents.

(b) Drug eluting stents

Disappointing systemic drug therapies to prevent restenosis following cardiovascular intervention lead to the development of several drug delivery systems: perivascular and intraluminal delivery systems [40]. The most popular method, though, is through drug-eluting stents [4, 5].

The drug was incorporated to the metallic stent by applying a layer of drug-containing polymeric coating to the surface of the stent struts. The most common stent-coating technique is a wet application of a thin layer of drug-polymer solutions. Another method is to create a polymer sheath in which the drug is embedded. The sheath is then wrapped around the unexpanded stent. With stent deployment, the polymer sheath is trapped between the stent and vessel wall [14]. There are mainly two types of drugs incorporated into the drug-eluting stents [41]: (i) anti-proliferative drugs such as sirolimus and paclitaxel, and (ii) anti-thrombotic drugs such as heparin. More detailed explanations about these drugs’ mechanisms of actions can be found in the next section.
This second generation of stent has been reported to successfully reduce restenosis rate compared to uncoated stents [6-13]. Local delivery of heparin was proven effective to inhibit platelet-dependent thrombosis in amounts that are several orders of magnitude lower than the required systemic dose [6-8]. Minimal amount of neointimal proliferation was reported for stents that release 7-hexanoyltaxol (QP2), a paclitaxel analogue eight months after procedure [9]. ELUTES 6-month clinical trial reveals significant reduction of restenosis, from 20.6% in uncoated stents to 3.2% in polymer-coated paclitaxel eluting stents [10]. Similarly, after 9 months, polymer-based, paclitaxel-eluting stent (TAXUSTM, Boston Scientific) reduced restenosis from 26.6% to 7.9% [11]. Encouraging results were also obtained from sirolimus-eluting stents [12]. After 6 months, no change in minimal lumen diameter and no in-stent restenosis were recorded for sirolimus eluting BX VelocityTM stents [13].

Despite their notable success, some problems associated with this drug eluting coated stents are the stability of polymeric coating material over time and the large increase in costs compared to bare metallic stent [17, 18]. Besides, it has also been reported that the polymers used in stents' coatings triggered some inflammatory reactions [19]. Given only a few microns thick coating, the drug loading capacity is very limited. Further, only a single drug, usually antiproliferative agent, was administered while in actual fact, both anti-proliferative and anti-thrombotic agents are required for post-stenting treatment.

Therefore, the ideal stent should meet the following requirements [42]:
The stent should be biodegradable as restenosis and major cardiac events occur within 6 months following angioplasty. Hence, considering the short-term need and potential long-term complications of metal stents, a biodegradable stent would be preferred.

The stent must possess sufficient mechanical strength to avoid collapse yet be able to maintain longitudinal flexibility to follow vessel’s contour.

The stent should degrade to nontoxic products within reasonable time period of 12 to 24 months.

The stent should be able to deliver drugs locally that inhibit restenosis.

The stent must not be overly thrombogenic.

(c) Biodegradable polymeric stents

Biodegradable polymeric stents have been identified as the best candidate to fulfill the criteria of ideal stent design mentioned above. This degradable stent remains in situ for a designated period of time to render mechanical support that prevents acute elastic recoil. Afterwards it degrades gradually to non-toxic substances and allows the vessel wall to preserve its normal functions [20].

A number of researchers have tried to develop a completely biodegradable stent. The first bioabsorbable stent made of poly-L-lactide (PLLA) was developed by Duke University. This stent was able to withstand crush pressures of 800 to 1000 mmHg and maintain hoop
strength in a saline environment for over 30 days. It is higher than the most widely used metallic stent (Palmaz-Schatz) which can withstand pressure of 300 to 500 mmHg. Animal trial using this stent shows that it is biocompatible, induced little thrombotic response and generated minimal neointimal response [21, 22].

Another degradable stent was developed at Kyoto University using polyglycolic acid [23]. On the other hand, Tamai and co-workers [24] were the first to report the initial and 6-month results of implantation of a biodegradable poly (L-lactide), PLLA, stent in humans. This stent combines the features of a thermal self-expandable and a balloon expandable stent. Initially, the stent auto expands in response to the heat transmitted by a balloon inflated with a 70°C contrast-water mixture. Subsequently expansion is obtained with balloon inflation at pressure of 6 to 14 atm.

Although Tamai et al provides clinical and angiographic follow-up data that indicate this type of biodegradable stent may not be associated with more pronounced intimal hyperplasia than stainless steel stent, there is a possible concern related to the heat necessary to provide the rapid expansion of the stent. Even mild short-term temperature elevation for a few seconds can cause cell necrosis of the arterial wall. In addition, platelet adhesion to the vessel wall seems to be increased by a temperature of 55°C, which raises the risk of thrombosis.
Venkatraman and co-workers from Nanyang Technological University, Singapore have also developed an alternative biodegradable stent made of bilayer films of PLLA and P(DL)LGA. The collapse strength of this stent has been measured and was found to be satisfactory [25, 26]. In addition, this fully degradable polymeric stent can self-expand at body temperatures (similar to 37 degrees C), using the concept of elastic memory. It was obtained by temperature conditioning and careful modification of the thickness and composition of each polymer layer. [27]. The stent’s bilayer configuration offers a great potential to deliver two or more kinds of drugs locally. Built upon this work, this PhD project aims to study the multiple release of anti-proliferative and anti-thrombotic drugs from this bilayer stent.

2.1.3 Restenosis and its prevention

(a) Restenosis

Restenosis after percutaneous coronary balloon angioplasty and/or stenting remains the most significant problem. Several mechanisms of the occurrence of restenosis have been proposed. Basically restenosis occurs as a result of three complementary factors.

- Immediate elastic recoil and negative arterial remodeling

Artery (blood vessel) wall possesses some degree of elasticity. Therefore, right after the artery is dilated through a balloon expansion in angioplasty procedure,
vessel wall has a strong tendency to return back to its original condition. This elastic recovery or recoil contributes to immediate post-angioplasty lumen area loss. Over a period of time after angioplasty, arterial remodeling usually occurs. Arterial remodeling refers to changes in total vessel area or changes in area circumscribed by the internal elastic lamina. Arterial remodeling may consists of compensatory enlargement with an increase in total vessel cross-sectional area, or constriction with a decrease in total vessel area [36, 43]. Figure 2.1 shows a schematic cross section of a coronary artery (a) and constrictive arterial remodeling, or also called negative remodeling (b). It is obvious that arterial constriction results in restenosis, shown by significant reduction in the lumen area.

![Figure 2.1 Schematic drawing of a coronary artery cross-section: (a) before and (b) after negative arterial modeling](image)

**Figure 2.1** Schematic drawing of a coronary artery cross-section: (a) before and (b) after negative arterial modeling
• Excessive neo-intimal formation

During coronary angioplasty and stenting procedures, the balloon and implanted stents injure the endothelium lining of the vessel wall. The high pressure exerted by the balloon to dilate the vessels ruptures the intimal layer and causes tears or dissections within the artery, exposing the medial and adventitial layers. The wound healing process at the injury site causes an excessive formation of neo-intimal layers, sometimes termed neo-intimal hyperplasia [35, 44-46]. Upon endothelial denudation, a layer of platelets and fibrin deposit on the surface. Activated platelets on the surface attach to the circulating leukocytes, a process which results in leukocytes recruitment / attachment to the surface. Growth factors are subsequently released from platelets, leukocytes and smooth muscle cells (SMCs). These growth factors stimulate migration of SMCs from the media into the neo-intima and then followed by SMCs growth and proliferation. After several weeks, the resultant neo-intima thickens and consists largely of SMCs, extracellular matrix and macrophages. Over longer time period (weeks to months), the predominantly cellular neo-intimal layer changes to a more extracellular matrix-rich plaque. Collagen and proteoglycans constitute the major components of the mature restenotic plaque, covered by a layer of repaired endothelium.

• Thrombus formation and deposition

Endothelial lining of the vessel is a non-thrombogenic, blood-contacting layer of the arterial wall. The endothelial injury and/or dysfunction following angioplasty
or stenting procedure result in a loss of anti-thrombotic factors such as nitric oxide (NO). Hence, it allows platelet adhesion and aggregation on the surface, leading to thrombus formation, usually on the site of intimal dissection. It has also been found that a number of components comprising the forming thrombus may contribute to SMC proliferation, thus aggravating the undesired restenosis [47-49]. In addition, intrinsic thrombogenicity of the materials used for stent implants, such as metals and alloys, also contributes to the redeposition of platelet, fats and plagues.

(b) Prevention of in-stent restenosis

The introduction of stents has successfully eliminated the problems of elastic recoil and negative remodeling. However, in-stent restenosis caused by neo-intimal hyperplasia and thrombosis are still present up to this date. The latest efforts to combat in-stent restenosis focus on the local and/or systemic administration of anti-proliferative drugs (to prevent neo-intimal thickening) and anti-thrombotic drugs (to prevent thrombosis). The two most popular anti-proliferative drugs are sirolimus and paclitaxel.

- **Sirolimus**

Sirolimus (or sometimes called rapamycin) is a natural fermentation product produced by *Streptomyces hygroscopicus* and was initially discovered as an
antifungal agent in the mid 1970s. Sirolimus is a hydrophobic drug that has low stability in aqueous solution. It is a very potent regulator of cell cycle activation. Its cellular actions are mediated by binding to its intracellular receptor, the FK506 binding protein (FKBP12). The complex between sirolimus and FKBP12 inhibits the actions of FRAP, an important protein that regulates the availability of energy for protein synthesis and capacity to enter the cell cycle ultimately. As a result, there are increased levels of cyclin-dependent kinase inhibitors resulting in inhibition of cell cycle protein phosphorylation and subsequent cell cycle arrest at G1/S transition [41, 50-53].

A characteristic feature of sirolimus is its ability to inhibit growth factors signaling for both immune and nonimmune cells, including fibroblasts, endothelial cells, hepatocytes, and smooth muscle cells. In long term systemic treatment, sirolimus may induce adverse effects of hypercholesterolemia and hypertriglyceridemia. Other side effects reported include myocardial and retinal infarctions, gastrointestinal toxicity, mucosal necrosis, etc [41, 54-56].

• Paclitaxel

Paclitaxel is one of the best anti-neoplastic drugs isolated from *Taxus brevifolia*. It has a novel chemical structure and a unique mechanism of action. Although paclitaxel does not act by receptor-mediated mechanism, it is a highly lipophilic drug which enables it to penetrate cell membrane easily and hence promotes rapid cellular uptake [57, 58]. It was found that paclitaxel is a potent inhibitor of all
three phases of restenosis process: smooth muscle cell proliferation, migration and extracellular matrix formation [59].

In a cell cycle, paclitaxel disrupts M-phase by enhancing extraordinarily stable microtubules in the absence of (normally required) GTP. Microtubules are polymers of α- and β-tubulin dimers. Polymerization and de-polymerization of microtubules provide the pulling force necessary for mitotic spindle to separate two chromatids of a dividing cell into two daughter cells. Microtubule depolymerization is inhibited by paclitaxel, thus, inhibiting cell proliferation. Microtubules are also part of the cytoskeleton within cell’s cytoplasm. Cytoskeleton enables some cell motion and migration through microtubule growing (polymerization) and shrinking (depolymerization). Therefore, altering the structure and characteristics of microtubules also inhibits cell migration. Since paclitaxel’s mode of action involves a cytoskeleton-targeting therapy, its effect is long-lasting [41, 58-62]. Other than being used to prevent restenosis, paclitaxel has found wide applications to treat various cancers, especially breast and ovarian cancers [63, 64].

For anti-thrombotic agents, heparin is one of the most popular choices to date. It is a highly-sulfated glycosaminoglycan composed of chains of alternating residues of D-glucosamine and a uronic acid. The key structural unit of heparin is a unique pentasaccharide sequence that consists of three D-glucosamine and two uronic acid residues. This unique sequence accounts for a high-affinity binding/complex with
antithrombin III (ATIII). The formation of antithrombin – heparin complex accelerates the inactivation of the coagulation enzymes thrombin (factor IIa), factor Xa and factor IXa. Of these three enzymes, thrombin is the most sensitive to inhibition by heparin/ATIII complex. Accelerated inactivation of the active forms of proteases / enzymes prevents the subsequent conversion of fibrinogen to fibrin that is crucial for clot formation [65-67]. Although heparin is relatively non-toxic, systemic heparin administration (overdose) has been associated with serious and excessive bleeding complications [68].

Drug therapy of above-mentioned agents has been practiced clinically via implantation of various drug-eluting stents (DES). These commercially available stents carry different drugs and possess different release mechanisms/profiles [6-13]. However, despite DES’ varying degree of success, an in-depth study that investigates which release kinetics performs best in preventing restenosis is lacking in the literature [69]. Therefore, it would be of great interest to compare and screen various release kinetics and propose the most efficacious one.

2.2 Biodegradable Polymers

Biodegradable polymers have received significant interests in the past decades for the administration of pharmaceuticals and numerous biomedical applications. These
polymers, which can be metabolized in the body after preset duration, become especially attractive when only temporary interventions are required. Besides being degradable, a candidate biopolymer has to meet several pre-requisites. The polymer must be biocompatible and does not invoke excessive inflammatory or toxic responses. It has to possess suitable mechanical, physical and biological properties to match the intended biofunctionality. Lastly, it has to be easily processed into final products, has acceptable storage life and can be sterilized [70].

Therefore, out of the several types of degradable polymers available, a family of polyesters is one of the more popular choices. Several medical devices made of these degradable polyesters have been approved by Food and Drug Administration (FDA) for clinical uses.

2.2.1 Lactide and glycolide polymers

(a) Synthesis

Lactide/glycolide polymers are homo- and copolymers of lactic acid and glycolic acid, classified as aliphatic polyesters or poly (α-hydroxy acids). Based on their molecular weight, they can be categorized into two. The low molecular weight polymers (Mw ~ a few thousand daltons) are usually named poly(lactic acid), poly(glycolic acid) and
poly(lactid-co-glycolic acid) whereas the high molecular weight polymers (Mw>10,000 daltons) are called poly(lactide), poly(glycolide) and poly (lactide-co-glycolide). The difference among the two groups arises from different synthesis techniques.

The low molecular weight lactic/glycolic acid polymers can be prepared by direct condensation of lactic acid and/or glycolic acid (see Figure 2.2). A high temperature of around 130°C -190°C is required and sometimes a catalyst such as antimony oxide is added.

![Figure 2.2 Synthesis of (a) poly(lactic acid) and (b) poly(glycolic acid) by condensation polymerization](image)

**Figure 2.2** Synthesis of (a) poly(lactic acid) and (b) poly(glycolic acid) by condensation polymerization
The high molecular weight lactic/glycolic acid polymers are synthesized by ring-opening polymerization of the cyclic diester of lactic acid (lactide) and/or glycolic acid (glycolide), shown in Figure 2.3. No water removal or dehydration method is needed and different physical properties of cyclized monomers and linear polymers produced allow ready purification [70-74].

Figure 2.3  Synthesis of (a) poly(lactide) and (b) poly(glycolide) by ring-opening polymerization
(b) Properties

Lactic acid has one asymmetric carbon and can exist as two optical isomers, D-lactic acid and L-lactic acid. The polymers derived solely from D-lactide or L-lactide are semicrystalline, while those derived from a mixture of D- and L-lactide are amorphous. Poly-L-lactide (PLLA) is much more frequently used than poly-D-lactide (PDLA) because its biodegradation product, L-lactid acid, is naturally occurring and easily metabolized by the body. PLLA is highly crystalline with a melting point of 180°C. Due to its high initial strength and good strength retention, it is preferred in the production of surgical implants for internal fixation. On the other hand, poly-D,L-lactide (PDLLA) is irregular and completely amorphous. Its strength and modulus are lower but its elongation is higher. Its application includes medical products with lower requirements for strength and in the field of drug delivery. The degree of crystallinity affects the swelling behaviour of a polymer and its capability to undergo hydrolysis. As such, PLLA, being semicrystalline, degrades more slowly than amorphous PDLLA.

Polyglycolide is an important raw material for manufacturing surgical sutures. It has a highly regular chain conformation and hence exists as a semicrystalline polymer with melting point of 230°C. Its high degree of crystallinity translates into a very high tensile strength and modulus of elasticity. It is insoluble in most organic solvents. As lactic acid, with its extra methyl group, is more hydrophobic than glycolic acid, poly (lactide) absorbs less water and hence degrades more slowly than poly(glycolide).
Copolymers of L-lactide and glycolide are amorphous in the range of 25% to 70% glycolide, whereas copolymers of D,L-lactide and glycolide are amorphous in the range of 0% to 70% glycolide. The ratio of lactide to glycolide has an effect on the mechanical strength and strength retention during implantation. The copolymer composition also dictates the rate of biodegradation.

All homo- and copolymers of lactide/glycolide polymers have glass transition temperatures Tg above physiological temperature (37°C). Their Tg increases with the increase of lactide content in the polymer and molecular weight, ranging from 40°C up to 65°C [70-74].

2.2.2 Poly(ε-caprolactone)

(a) Synthesis

Poly(ε-caprolactone), PCL, has been synthesized by ring-opening polymerization of ε-caprolactone using different types of initiators, including anionic, cationic and coordination catalysts. The anionic method of polymerization is most useful for the synthesis of low molecular weight hydroxy-terminated oligomers and polymers. The cationic catalysts yield polymers with a molecular weight in the range of 15,000 to
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50,000. Coordination catalysts, such as stannous octoate, are used to obtain high molecular weight PCL [74].

![Synthesis of poly(ε-caprolactone) by ring opening polymerization](image)

**Figure 2.4 Synthesis of poly(ε-caprolactone) by ring opening polymerization**

(b) Properties

Poly(ε-caprolactone) is a highly crystalline polymer with melting point of about 60°C. Its degree of crystallinity decreases with the increase in the molecular weight; polymer of 5000 daltons is 80% crystalline while the 60,000 daltons polymer is 45% crystalline. Its highly crystalline structure hinders hydrolysis and slows down the degradation rate up to two years [75]. PCL, however, has an extremely low glass transition temperature (Tg \( \approx -60°C \)), rendering it at the rubbery state in most physiological conditions. Copolymerization with lactide has been shown to increase the Tg. PCL is soluble in chlorinated and aromatic hydrocarbons and cyclohexanone, but insoluble in aliphatic hydrocarbons, diethyl ether and alcohols [70, 74].
2.2.3 Biodegradation

Biodegradable polymers can be categorized into two groups on the basis of degradation mechanisms: bulk-degrading polymers and surface-degrading polymers. Bulk degradation is a homogenous process in which degradation occurs at a uniform rate throughout the polymer matrix. In contrast, surface degradation is a heterogeneous process in which degradation is confined to a thin surface layer of polymer.

All lactide/glycolide polymers and poly(ε-caprolactone) belong to the family of polyesters which degrade by bulk hydrolysis of the ester bonds. Bulk degradation can be described as four consecutive steps [71, 73].

- First, a polymer absorbs water and undergoes little swelling. The water penetrates into the amorphous region and disrupts secondary and tertiary structures stabilized by van der Waal's forces and hydrogen bonds.
- Second, cleavage of the covalent / ester bonds in the polymer backbone by hydrolysis begins. More and more carboxylic end groups are generated which may autocatalyze the hydrolysis. Molecular weight begins to decrease and loss of mechanical strength is observed.
- Third, massive cleavage of the backbone covalent bonds continues. At some critical value of molecular weight, significant weight loss begins to occur. Loss of physical and mechanical integrity is observed.
• Fourth, the polymer loses huge mass due to solubilization of oligomers into the surrounding medium. The polymer breaks down to many small fragments, which will be further hydrolyzed into free acids.

Biodegradation rate of lactide/glycolide polymers and poly(e-caprolactone) can be easily tailored by adjusting one of the following factors [71].

• Polymer composition
  The methyl group makes the lactide moieties in polymers more hydrophobic and provides steric hindrance to attack by water molecules. Thus glycolide polymers degrade faster than lactide polymers. Degradation rate of lactide/glycolide copolymers can be easily adjusted by varying the ratio of the two moieties. In general, copolymers with lower lactide content degrade more rapidly. Degradation rate of PCL is significantly increased by copolymerization with lactide and glycolide.

• Molecular weight
  Biodegradability increases as molecular weight decreases as low molecular weight polymers are more hydrophilic given their higher percentage of carboxylic and hydroxyl end groups. The presence of more carboxylic end groups may autocatalyze the biodegradation.

• Crystallinity
  The penetration of water into polymers greatly determines the rate of biodegradation. Water will readily penetrate amorphous regions, but the
semicrystalline nature of a polymer will limit water accessibility. It has also been proposed that degradation of semicrystalline polymers proceeds in two phases: in the first phase the amorphous regions are hydrolyzed, and then the crystalline regions in the second [76]. Thus higher degree of crystallinity slows down degradation rate

- Glass transition temperature

  The glass transition temperature reflects polymer chain mobility, which determines the ease of water diffusion and chemical attack. Therefore, the higher the Tg is, the slower the degradation rate is.

- Other parameters

  Physical dimension (surface-to-volume ratio), additives, impurities and the nature of drugs/agents incorporated in the polymer matrix may alter the degradation rate too.

2.3 Drug Delivery Systems

2.3.1 Types of drug delivery systems

The usefulness of polymers in drug delivery systems is well established. Currently available polymers for controlled release can be classified into four major categories [77, 78].
(a) Diffusion controlled systems

Diffusion controlled systems involve two types: reservoir and matrix. A reservoir consists of a drug core in powdered or liquid form. The core is surrounded by a layer of nonbiodegradable polymeric material through which the drug slowly diffuses. The properties of the drug and the polymer govern the diffusion rate of the drug and its release rate. One potential problem is the danger of "drug dumping" if the reservoir membrane accidentally ruptures. In the matrix type, the drug is uniformly distributed throughout the polymer matrix and is released at a uniform as drug particles dislodge from the polymer network [77-81].

(b) Solvent activated systems

Solvent activated systems also consist of two types: osmotically controlled systems and swelling controlled systems. In osmotic systems, external fluid moves across a semipermeable membrane to a region inside the device, where the drug is in high concentration. The inward movement of fluid forces the dissolved drug out of the device through a small orifice. On the other hand, the swelling system consists of hydrophilic crosslinked macromolecules that are able to take up large quantity of water which in turn dissolves the drugs and carries them away [77, 82-84].
(c) Chemically controlled systems

Chemically controlled systems also have two types: pendant-chain system and bioerodible or biodegradable system. For pendant-chain system, the drug molecule is chemically linked to the backbone of the polymer. In the body, this bond is broken by chemical hydrolysis or enzymatic cleavage with concomitant release of the drug. The drug may be linked directly to the polymer or via a "spacer group". For bioerodible system, the controlled release of drug involves polymers that gradually decompose. The drug is dispersed uniformly throughout the polymer and is slowly released as the polymer disintegrates [77, 85-88].

(d) Magnetically controlled systems

Magnetically responsive drug carrier systems have been developed for use in cancer chemotherapy. This system, composed of albumin and magnetic microspheres, is capable of area-specific localization and thus can be directed to the specific target [77].

2.3.2 Erodible drug delivery systems

Use of bioerodible polymers as drug delivery carriers has increased more significantly than other types of polymers because of the following two major advantages:

- Degradable polymeric carriers do not need to be removed from the body after its drug content is exhausted. Therefore, unlike nondegradable systems, no second surgery is required to retrieve the implanted delivery device.
Degradable polymeric carriers have the flexibility to deliver both hydrophilic and hydrophobic drugs. In many other systems, only water soluble drugs can be delivered as restricted by their mechanisms of release.

At least three basic approaches have been developed for drug release from erodible systems [77, 78, 88]:

1. Drug release by matrix solubilization

   Materials in this category include enteric coatings, generally classified as polyacids. In their unionized form they are water insoluble, but upon ionization of their carboxylic acid groups, they become water soluble. Erosion of the polymer surface/coating leads to concomitant release of physically entrapped drugs.

2. Erodible diffusional systems

   Erodible diffusional systems combine the attributes of a rate-controlling membrane, which provides a constant drug release rate from a reservoir, with erodibility, which eliminates the surgical removal of drug-depleted devices. Therefore, diffusion controlled the release of physically entrapped drug with bioabsorption of the polymer delayed until after drug depletion.

3. Monolithic systems

   In monolithic systems the drug is uniformly distributed in a polymer matrix and is released to the surrounding environment as the polymer bioerodes. In describing drug release from such systems, it is necessary to consider both polymer erosion and drug diffusion. If mobility of the drug in the matrix is such that rapid
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diffusional release is possible, its release kinetics will be first order, i.e. similar to nondegradable matrix system and amount of drug released is proportional to the square root of time. Zero order release (amount released is proportional to time) requires that the erosion process be confined to the surface of the solid device and that the drug be highly immobilized in the matrix. If drug diffusion and polymer erosion have comparable rates, combined diffusion and degradation controlled release is observed. This behaviour is usually found in the polymers that undergo bulk degradation.

2.4 Drug Release Kinetics and Models

2.4.1 Fick’s laws of diffusion

Diffusion is the spontaneous net movement of particles from an area of high concentration to an area of low concentration in a given volume of fluid, down the concentration gradient. In 1855, Adolf Fick introduced one of the earliest analyses of this mass transport phenomenon. His work was well recognized through the two fundamental equations, called Fick’s laws of diffusion [89].
• Fick’s First Law

Fick’s first law is used to describe steady-state diffusion, i.e., when the concentration within the diffusion volume does not change with respect to time. Concentration is dependent only on position. In one (spatial) dimension / planar geometry, this is written as

\[ J = -D \frac{\partial C}{\partial x} \]  
(Eq. 2.1)

\( J \) is the diffusion flux, i.e., amount of drug particles that passes through a unit area per unit time (\( \mu g \ cm^{-2} \cdot s^{-1} \)). \( C \) is the position-dependent drug concentration in the matrix (\( \mu g/ml \)). \( D \) is the drug diffusion coefficient (\( cm^2/s \)) and \( x \) is the position normal to the central plane of the membrane / film (cm). The minus sign shows that diffusion takes place down the concentration gradient.

• Fick’s Second Law

Fick’s second law is used to describe non-steady or continually changing state diffusion, i.e., when the concentration within the diffusion volume changes with respect to time as well as position. In one (spatial) dimension / planar geometry, this is written as

\[ \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \]  
(Eq. 2.2)

All the parameters carry the same meanings as in equation (2.1), except that \( C \) is the time- and position-dependent drug concentration in the matrix (\( \mu g/ml \)) and \( t \) is time (s). The main difference among the two equations lies on the fact that
concentration is only a function of position in the first law while concentration is a function of both position and time in the second law.

Both equations have formed the foundation of various theoretical and empirical drug release models developed in the past years. The following sections will highlight only the important models that bear direct relevancy with this research work.

2.4.2 Models of drug release from non-erodible, monolithic systems

Higuchi model has been widely accepted for drug release from a non-degradable monolithic system whereby drug particles are dispersed uniformly throughout the matrix [90-92]. It is assumed that steady-state / pseudo-steady-state diffusion exists such that Fick’s first law can be applied. The Fick’s first law, equation 2.1, can be rewritten as follows.

\[ R_t = -SD \frac{\partial C}{\partial x} \]  
(Eq. 2.3)

\( R_t \) is the rate of diffusion (g/s); \( S \) is the cross-sectional diffusion area (cm\(^2\)); \( D \) is the diffusion coefficient in the matrix (cm\(^2\)/s); \( C \) is the concentration of drug in polymer (g/cm\(^3\)) and \( x \) is the distance measured from solvent-matrix interface (cm).

The boundary conditions are:

\[ C = C_bK \quad \text{at} \ x = 0 \]  
(Eq. 2.4)

\[ C = C_s \quad \text{at} \ x = X(t) \]  
(Eq. 2.5)
C_b is drug concentration in the release medium and C_s is the saturation concentration in the matrix. K is the matrix-to-medium partition coefficient.

![Homogeneously dispersed drug](image)

**Figure 2.5 Schematic drawing of monolithic drug release system (Higuchi model)**

It is also assumed that the concentration profile in the diffusion region is linear at any time. Therefore, upon integrating equation 2.3

\[ R_t = SD \left( \frac{C_s - C_b K}{X(t)} \right) \]  

(Eq. 2.6)

To determine X(t), the following mass balance equation in the diffusion region is applied:

\[ R_t = \frac{dM_t}{dt} = \frac{d}{dt} \left\{ \left[ C_0^{-1/2} (C_s + C_b K) \right] SX(t) \right\} \]  

(Eq. 2.7)

After substituting R_t in equation 2.7 with equation 2.6 and then followed by a series of integrations, the final equation is written as:
In the sink condition, $C_b$ is maintained at very low concentration, close to zero. Also, in most cases, the matrix is usually loaded much higher than its saturation ($C_0 \gg C_s$). Therefore, equation 2.8 can be simplified to:

$$M_t = S[2DC_sC_0 t]^{1/2} \quad (Eq. 2.9)$$

This solution is a good approximation for monolithic system with $C_0 \gg C_s$ under pseudo-steady state condition. Exact solutions to this diffusion problem was developed by Paul and McSpaden [93] which improved the accuracy up to 11.3%. Further, Lee developed another model for monolithic system that can be applied at all ($C_0/C_s$) ratios [94].

2.4.3 Models of drug release from erodible, monolithic systems

Numerous drug release models of surface-eroding and bulk-eroding degradable systems have been reported in the literature. A review of the more important and useful mathematical models for both cases has been summarized by Siepmann and Gopferich [28]. In general, it is easier to model drug release from surface-eroding systems because the drug is released concurrent with the layer-by-layer erosion from the outermost surface of the matrix.
As the polyesters used in this work belong to bulk-erosing systems, the focus will be emphasized on the mathematical models developed for such systems. The majority of the reported models [29-31] have based their models on the widely-accepted Higuchi model for non-degrading systems. The constant diffusion coefficient in Higuchi model was modified to take into account the time-dependent matrix degradation.

For example, Charlier et al [29] postulate that the diffusion coefficient \( D_t \) depends on the polymer molecular weight \( (M_{w,t}) \) and varies in inverse ratio to it. This model was developed for drug release from a degradable matrix with initial loading above saturation. It works by the assumption that the system degrades by first order kinetics, the diffusion coefficient is inversely proportional to the polymer molecular weight and similar drug transport conditions as with the Higuchi model.

At a given time \( t \), diffusion field is limited at the outer part of the matrix system, as shown in Figure 2.6. At the solubility interface, the amount of solubilized drug versus the front solubility recession can be approximated. Solubilized drug is equal to amount of drug released at steady state. Therefore it is written as:

\[
\frac{dM}{dt} = C_0 S \frac{dh}{dt} \tag{Eq. 2.10}
\]

\( dM \) is the amount of drug released, \( S \) is the exposed area, \( C_0 \) is the initial drug concentration and \( dh \) is the recession of the front of solubility during period \( dt \).
Figure 2.6  Schematic diagram of drug concentration at time t (Couarraze model)

Polymer degradation follows the first order kinetics as follows

\[ \frac{dM_w}{dt} = -kM_w \rightarrow M_{w,t} = M_{w,0} \exp(-kt) \]  \hspace{1cm} (Eq. 2.11)

\( M_{w,t} \) is the polymer molecular weight at time \( t \), \( M_{w,0} \) is the initial polymer molecular weight and \( k \) is the degradation rate constant.

Assuming polymer’s molecular weight and diffusion coefficient are inversely proportional,

\[ \frac{D}{D_0} = \frac{M_{w,0}}{M_{w,t}} \rightarrow D = D_0 \exp(kt) \]  \hspace{1cm} (Eq. 2.12)

The drug transport (flux) is given by Fick’s first law:

\[ \frac{dM}{dt} = SDCs/h \]  \hspace{1cm} (Eq. 2.13)

Comparison between equation 2.10 and 2.13 leads to

\[ C_0 \cdot h \, dh = DCs \, dt \]  \hspace{1cm} (Eq. 2.14)
After integration and rearrangement, the amount of drug released can be expressed as

\[ M_t = A \left\{ \exp(kt) - 1 \right\}/k \] \[ M_t = A \left\{ \frac{\exp(kt) - 1}{k} \right\}^{1/2} \] \[ M_t = A \left\{ \frac{\exp(kt) - 1}{k} \right\}^{1/2} \] with \[ A = S \left[ \frac{2C_0C_sD_o}{112} \right]^{1/2} \] (Eq. 2.15)

Faisant et al [30, 31] also took the similar approach of modifying Higuchi model to suit erodible systems. However, they proposed a different expression for time-dependent diffusion coefficient \( D_t \). The dependence of \( D_t \) on \( M_{w,t} \) was described as \[ D_t = D_0 + (c/M_{w,t}) \] where \( c \) is a constant.

In the above treatments, both groups of researchers [29-31] have considered a single-step model of drug release, dominated by diffusion alone (although with time-dependent diffusion coefficient). These models demonstrated good agreement with their experimental data. However, it is worth mentioning that such success is obtained only for mono-phasic, single-step, drug release patterns. Therefore, their approach is applicable only if diffusion possesses the slowest rate and becomes the only governing mechanism throughout the period of release. These models will not work for bi- or tri-phasic drug release profiles.

A different model was suggested by Srikar and co-workers [95] to describe the release behaviour of hydrophilic fluorescence dye from electrospun polymer nanofibers, made of various combinations of poly (e-caprolactone), PCL and poly (methylmethacrylate), (PMMA). The in-vitro release study reveals an incomplete single stage release. Unlike the conventional diffusion process, the release mechanism was shown to be dependant on
drug desorption, assisted by the nanopores of the nanofibers. Subsequently, a model was developed – an exponential equation with a characteristic time $\tau$, whose value was controlled by polymer density and the intermolecular forces binding dye molecules to the polymer surface in the presence of water. The nanoporosity of the fibers ultimately determined the saturation point of this desorption process. As no substantial degradation took place within the time-frame of the study, total release equaled the amount of agents released via desorption.

A similar concept of drug desorption was also expressed in the model proposed by Batycky et al [32]. A bi-phasic release model was proposed for macromolecular (glycoprotein) release from polylactide-co-glycolide 50/50 microspheres. It took into account the presence of initial burst due to drug desorption from the surface of microspheres and surfaces of existing mesopores. Continuous diffusional release was postulated to take place after sufficient amount of pores developed and interconnected. An estimation of the delay (induction time or latent period) was attempted from a qualitative visual observation of surface pore coalescence with time. Good agreement between the model and experimental data was demonstrated. However, this model is still unable to predict a tri-phasic release pattern.

2.5 Summary
In the quest of better cardiology intervention techniques to restore blood flow in blocked arteries, simple balloon dilation (angioplasty) was enhanced with stent implantation. Over the years, continuous improvement has seen a gradual shift in stent deployment from bare metallic stents to drug-eluting, coated metallic stents (DES). Restenosis (reblockage), however, remains the biggest challenge up to date. Concurrent administration of two kinds of drugs (anti-proliferative and anti-thrombotic agents) has been identified to be the best solution to prevent restenosis. DES, however, only manages to deliver anti-proliferative drug, either paclitaxel or sirolimus. The development of degradable, polymeric stents is the latest breakthrough that can deliver both drugs in parallel. The challenge, however, lies on controlling and tailoring the release rate of each drug. Further literature survey reveals that despite abundant reports on varying degrees of success on DES’ animal and clinical trials, none of them thoroughly investigated which release kinetics worked best in preventing restenosis. Clearly, this is an important aspect of drug delivery via stent that has been overlooked in the literature.

Therefore, in this thesis, the release behaviour of paclitaxel and heparin and modification of their release rates from a bi-layer degradable stent would be studied. Next, novel release models that offer better insights on the release mechanisms would be presented. Lastly, comparison on the impact of various (fast, moderate and slow) paclitaxel release kinetics on arterial smooth muscle proliferation would be discussed in the light of the ideal release profile required to overcome restenosis.
CHAPTER 3
MATERIALS AND METHODS

3.1 Materials

3.1.1 Polymers

The polymers used in this project are basically aliphatic polyesters. Table 3.1 lists the important characteristics and properties of the polymers used in this research project.

Table 3.1  Characteristics and properties of polymers used

<table>
<thead>
<tr>
<th>Properties</th>
<th>Polymers</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLLA</td>
<td>P(DL)LGA 53/47</td>
</tr>
<tr>
<td>Melting Temperature, Tm (°C)</td>
<td>175</td>
</tr>
<tr>
<td>Glass Transition Temperature, Tg (°C)</td>
<td>59</td>
</tr>
<tr>
<td>Intrinsic Viscosity, IV (dl/g)</td>
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</tr>
<tr>
<td>Weight-average Mol. Weight, Mw (g/mol)</td>
<td>1.7 x 10^6</td>
</tr>
<tr>
<td>Number-average Mol. Weight, Mn (g/mol)</td>
<td>9.0 x 10^5</td>
</tr>
<tr>
<td>Polydispersity index, (Mw/Mn)</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* As determined by Modulated Differential Scanning Calorimetry
* As specified in certificate of analysis from manufacturer
^ As determined by Size Exclusion Chromatography
Biomedical grade of homopolymer poly (L-lactide), PLLA and copolymer poly (D,L-lactide-co-glycolide), P(DL)LGA 53/47, i.e 53 mole% D,L-lactide and 47 mole% glycolide, were purchased from Purac Far East, Singapore while poly (ε-caprolactone), PCL, was purchased from Aldrich, USA. These polymers formed the base films/matrices of all in-vitro drug release studies conducted in the subsequent experiments.

In an attempt to alter drug release kinetics, some polymer plasticizers/additives were added to the polymer matrices. Poly (ethylene glycol), PEG, with number average molecular weight of $1 \times 10^4$ g/mol was purchased from Fluka, USA while poly (ethylene oxide), PEO, with viscosity average molecular weight of $6 \times 10^5$ g/mol was purchased from Aldrich, USA. All the materials were used as received.

3.1.2 Drugs

Paclitaxel was purchased from Hande, China and was received as solid white powder. Its molecular weight is 853.9 g/mol. It is light-sensitive and insoluble in water. Figure 3.1 shows the chemical structure of paclitaxel.
Materials and Methods

Figure 3.1 Chemical structure of paclitaxel

Heparin sodium salt, derived from porcine intestinal mucosa, was purchased from Fluka, USA and was received in powder form. Its molecular weight is in the range of 4000 to 6000 g/mol and has good solubility in water (up to 50 mg/mL). Figure 3.2 shows the typical chemical structure of heparin.

Figure 3.2 Chemical structure of heparin
Materials and Methods

Metoclopramide monohydrochloride monohydrate, in short meto salt, was purchased from Sigma-Aldrich, USA and was received in powder form. Its molecular weight is 354.3. It is very soluble in water, freely soluble in alcohol, but sparingly insoluble in chloroform. Figure 3.3 shows the chemical structure of meto salt.

![Chemical structure of metoclopramide monohydrochloride monohydrate](image)

Figure 3.3 Chemical structure of metoclopramide monohydrochloride monohydrate

3.1.3 Solvents and reagents

HPLC grade dichloromethane, tetrahydrofuran, chloroform and acetonitrile for film preparation, HPLC tests and molecular weight analysis were obtained from Merck, Germany. Phosphate buffer saline (PBS) pH 7.4 for drug release study was purchased from Ohme Scientific, Singapore. Dimethyl sulfoxide (DMSO) to enhance paclitaxel's
solubility in release medium was purchased from Aldrich, USA. Toluidine blue dye for heparin quantification was purchased from Fluka, USA while n-hexane to extract the heparin-dye complex was purchased from Merck, Germany.

3.1.4 Cell line and culture medium

Human coronary artery smooth muscle cell line (hCASMC, passage 3) and cell culture medium SmGM®-2 Bullet kit were purchased from Clonetics, USA. The culture medium contains 5 μg/mL insulin, 0.5 ng/mL human epidermal growth factor, 2 ng/mL human fibroblast growth factor, 50 μg/mL gentamicin, 50 ng/mL amphotericin-B, 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

3.1.5 Cell assay kits

Cell count reagent, [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], in short WST-8, was purchased from Nacalai Tesque, Japan. Cell proliferation colorimetric ELISA, 5-bromo-2’-deoxyuridine (BrdU) kit was purchased from Roche, USA.
3.2 Methods

3.2.1 Film preparation

All films were prepared through the method of solution casting. Polymer pellets (PLLA, P(DL)LGA or PCL) were dissolved in dichloromethane and stirred continuously until homogenous polymer solutions were obtained. Depending on the film formulation, some plasticizers (PEG or PEO) might be added to the polymer solution too. For drug containing films, predetermined amount of drug (paclitaxel, heparin or meto salt) was added to solution and stirred to dissolve and/or distribute all drug particles uniformly.

The polymer solution was then cast on a glass panel using an automatic film applicator that utilizes a casting knife with adjustable height and speed. For bi-layer films, the bottom layer was cast first; after it hardened and the solvent evaporated, another layer was cast on top. Subsequently the films were dried in a vacuum oven at 55°C for 1 week to evaporate all residual solvent. Weight loss analysis with Thermogravimetric Analyzer, TGA (TA Instruments Q500) revealed 0.3~1 wt% and 1~1.5 wt% residual solvent in single and double layer films respectively.

The thickness of the dried film was measured using a micrometer. Only those films with thickness in the range of 75 ~ 80 μm and 155 ~ 160 μm, for single and double layer films
respectively, were accepted and used for subsequent studies. In the case of double layer films, cryogenic cross-sectioning was performed and then analyzed under scanning electron microscope. Two distinct polymer layers were seen and the individual thickness of each layer was measured. The results were compared to the micrometer's measurement to ensure accuracy.

3.2.2 In-vitro drug release study

Three rectangular strips with dimension of 14mm x 14mm were cut from each film. Each strip was then individually immersed in a vial containing phosphate buffer saline, PBS pH 7.4. Only for paclitaxel release study, 10% (v/v) dimethyl sulfoxide (DMSO) was added to PBS to enhance paclitaxel's solubility and prevent drug saturation. It has been confirmed that no plasticization effect (reduction in glass transition temperature, Tg) was observed for the films immersed in PBS/DMSO 90/10 as opposed to pure PBS (data not shown).

All vials were kept in incubator at 37°C throughout the release study. At every predetermined time point, a portion of the release medium was drawn from each vial and replaced with fresh medium of equal or larger amount to maintain sink condition. The amount of drug released was quantified by analyzing the drawn aliquot.
Materials and Methods

- Paclitaxel quantification
  For paclitaxel quantification, the aliquot was injected into reverse phase High Performance Liquid Chromatography (HPLC, Agilent 1100, Santa Clara, USA) using a ZORBAX Eclipse XDB-C18 column of pore size 5 µm. The mobile phase was acetonitrile / water 50/50 (v/v), the flow rate was 1 mL/min and the detector’s wavelength was set at 227 nm.

- Heparin quantification
  In the case of heparin release, an indirect measurement was carried out with the help of toluidine blue dye. 2 mL of the drawn aliquot was reacted with 2.5 mL 0.0025% toluidine blue (25 mg toluidine blue in 1000 mL 0.01 N HCl containing 0.2% NaCl). After thorough mixing, 5 mL hexane was added to remove the heparin-dye complex. The remaining, unreacted, toluidine blue was quantified with UV-Visible Spectrophotometer (Shimadzu UV-250) at wavelength of 631 nm with deionized water as the reference [96].

- Metoclopramide monohydrochloride quantification
  For meto salt quantification, the aliquot was directly scanned with UV-Visible Spectrophotometer at wavelength of 309 nm with deionized water as the reference.
3.2.3 In-vitro degradation study

The films were cut into rectangular strips with dimension of 10mm x 10mm. Each strip was weighed, individually immersed in a vial containing release medium and kept in incubator at 37°C. At every predetermined time point, the sample was taken out and weighed after removing adsorbed water on the surface of the film. The sample was weighed again after thorough drying in vacuum oven at 37°C.

- Water absorption and weight loss measurement

Water absorption and weight loss were calculated as follows.

\[
\text{Water absorption (\%)} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100\% \tag{Eq. 3.1}
\]

\[
\text{Weight loss (\%)} = \frac{W_{0} - W_{\text{dry}}}{W_{0}} \times 100\% \tag{Eq. 3.2}
\]

where \(W_{0}\) is the initial weight of the film prior to immersion; \(W_{\text{wet}}\) is the wet weight of the film measured immediately upon retrieval from the release medium; \(W_{\text{dry}}\) is the final weight of the film after thorough drying to remove absorbed water.

- Molecular weight analysis

Gel Permeation Chromatography (GPC, Agilent series 1100, Santa Clara, USA) was used to monitor the change in the sample’s molecular weight as a result of
Materials and Methods

degradation. For P(DL)LGA, each sample was dissolved in 1 mL dichloromethane (DCM). After 24 hours, 4 mL tetrahydrofuran (THF) was added and mixed homogenously. For PLLA and PCL, each sample was dissolved in 5 mL chloroform for 24 hours. The resulting dilute polymer solution was injected into GPC that is fixed with PLgel 5 µm column at 35°C and refractive index (RI) detector. The flow rate was 1 mL/min and the mobile phase was either THF/DCM 80/20 (v/v) or chloroform. The former was used for P(DL)LGA while the latter was used for PLLA or PCL. The calibration was done using a series of standard polystyrene of known molecular weight.

- Surface topography observation

Scanning Electron Microscope (JEOL, model 5410) was used to observe the surface of the films for evidence of degradation and formation of micropores. The samples were first coated with gold using a gold sputtering coater for 40 seconds. After which the samples were analyzed under SEM at operating voltage of 10 kV.

3.2.4 Culture of human coronary artery smooth muscle cells

Human coronary artery smooth muscle cells (hCASMCs) were grown in SMC basal medium supplemented with 5 µg/mL insulin, 0.5 ng/mL human epidermal growth factor, 2 ng/mL human fibroblast growth factor, 50 µg/mL gentamicin, 50 ng/mL amphotericin-B, 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. All the culture flasks
were kept at 37°C in a humidified 5% CO₂ incubator. Smooth muscle cells were identified on the basis of their typical structure under light microscopy. Cells in passage 6 were used in the cell count and proliferation assays studies.

### 3.2.5 Cell assays’ experimental setup

Cultured hCASMCs were seeded into 48-well plates with a seeding density of $2 \times 10^4$ cells/well. 400 µL complete medium was added into each well to let the cells attach to the wells. After 24 hour incubation at 37°C / 5% CO₂, the medium was changed to SMC basal medium with 0.5% FBS and 1% antibiotics but without the growth factors to produce cell quiescence. After another 24 hours, the medium was changed back to 700 µL/well complete culture medium.

Polymer samples were sterilized by soaking them in filtered 70% ethanol/water for 20 minutes and then washed with sterile PBS pH 7.4. The sterilized polymer samples, with or without paclitaxel, were individually placed into the 48-well plates with cells attached at the bottom. The samples were completely submerged yet they did not rest on the cells. Figure 3.4 demonstrates the setup of each well.

A set of triplicate wells were prepared for each film configuration and a set of wells without any films serve as a reference. Cell culture medium was refreshed every 2 days.
Materials and Methods

Figure 3.4  Experimental setup of cell assays

3.2.6 Cell count assay

At every predetermined time point, the films were removed from every well. The medium was refreshed with 400 μL complete culture medium and 40 μL WST-8 reagent was added. The culture plate was then incubated at 37°C / 5% CO₂ for 2 hours. WST-8 is a sensitive colorimetric assay that allows for the determination of the number of viable cells. Ready-to-use, unreacted, WST-8 reagent is pink in color. Upon interactions with viable cells, WST-8 is reduced by the activity of cells’ dehydrogenases to give a yellow coloured product (formazan), which is soluble in the culture medium. The amount of the formazan dye generated is directly proportional to the number of living cells. Multi-well plate reader (Tecan, Infinite ® 200) was used to measure WST-8 absorbance at 450 nm and reference set at 630 nm.
**Materials and Methods**

**3.2.7 Proliferation assay**

To study cell proliferation behaviour, 5-bromo-2'-deoxyuridine (BrdU), ELISA colorimetric kit was used. Cell proliferation was determined by measuring the amount of BrdU incorporation during DNA synthesis, i.e the S phase of a cell cycle. BrdU is as a non-radioactive alternative to the commonly used [3H]-thymidine. After BrdU incorporation, cells were fixed and denatured. BrdU was detected by anti-BrdU antibody conjugated with peroxidase (anti-BrdU-POD), which formed linkage with tetramethyl-benzidine (TMB) substrate.

The assay was carried out in accordance to the manufacturer’s protocol. After WST-8 assay test, the cells were washed carefully with PBS pH 7.4 thrice. Subsequently, 360 µL complete culture medium was pipetted to each well and 40 µL BrdU labeling solution was added. After the plate was reincubated for 2 hours at 37°C / 5% CO₂, the medium was removed, the cells were fixed and the DNA was denatured in one step using 800 µL FixDenat for 30 minutes at 25°C. To bind the incorporated BrdU, 400 µL anti-BrdU-POD was added and the plate was incubated at 25°C for 90 minutes. After which, the extra anti-BrdU-POD was removed by washing buffer. Subsequently, substrate TMB solution was added and incubated at 25°C for 20 minutes. The product of TMB reaction with POD was detected by the developed green color and quantified using a multi-well plate reader (Tecan, Infinite ® 200) at 370 nm and reference wavelength 492 nm.
3.2.8 Statistical analysis

WST-8 and BrdU assays results from triplicate wells were averaged. At every time point, hCASMCs response to variable drug release rates was analyzed using single-factor ANOVA. Whenever ANOVA was positive, the student t test was performed between every pair of release formulations. The statistical significance of all tests was defined at p<0.05 level.
CHAPTER 4

IN-VITRO DRUG RELEASE FROM BIODEGRADABLE MATRICES

4.1 Introduction

As highlighted in Chapter 2, biodegradable stents are the latest breakthrough in the battle against restenosis. A bi-layer, biodegradable polymeric stent with the potential of delivering two or more types of drugs at the target site, has been developed in our laboratories. Figure 4.1 shows the structure of a bi-layer film which can be processed into a helical coronary stent. This is the film configuration adopted in this project.

![Figure 4.1 Schematic drawing of a bi-layer film / stent](image)

Figure 4.1  Schematic drawing of a bi-layer film / stent
Bi-layer systems made of P(DL)LGA 53/47 and PLLA were chosen based on previous studies in our group. Thorough investigation on the mechanical properties of various biodegradable polymers shows that the best combination of collapse strength, stent expandability and period of residence was obtained from the combination of these two polymers. More in-depth discussions on the bi-layer stents’ mechanical properties and polymers selection can be found in previous publications of our group [25-27].

The top layer of this bi-layer system is made of fast-degrading polymer (P(DL)LGA 53/47) which will deliver paclitaxel to the arterial wall. The bottom layer is made of a slow-degrading polymer (PLLA) which will release heparin that prevents thrombus redeposition. At the same time, this PLLA layer functions as a mechanical support to the stent and prevents the artery from collapsing.

This chapter begins with a preliminary study on the release of a model drug, metoclopramide monohydrochloride monohydrate (meto salt) from P(DL)LGA film. The study then focuses on the drug release behaviours of paclitaxel and heparin from each layer. Attempts to tailor the release rates by matrix modification and addition of plasticizers, are discussed extensively next. Results on the degradation study are also presented to verify and support the release mechanisms proposed.
4.2 Metoclopramide Monohydrochloride Monohydrate Release

Preliminary work on a model drug helped the author to familiarize with the drug release experimental techniques before moving on to the drugs of interest (paclitaxel and heparin). Metoclopramide monohydrochloride monohydrate, in short meto salt, was chosen as the model drug due to its excellent solubility in release medium and ease of analysis. Table 4.1 summarizes the films prepared for meto salt release study.

Table 4.1 List of samples prepared for meto salt in-vitro release study

<table>
<thead>
<tr>
<th>No</th>
<th>Sample Configuration</th>
<th>Polymer</th>
<th>Drug loading (%)</th>
<th>Thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Monolayer film</td>
<td>P(DL)LGA 53/47</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td>80</td>
<td></td>
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<td>3</td>
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<td>90</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>80</td>
<td></td>
</tr>
</tbody>
</table>

The list of samples prepared as in Table 4.1 was designed to investigate two factors that may control drug release profiles: drug loading and film thickness.

Figure 4.2 shows the in-vitro release profiles of metoclopramide salt from various monolayer P(DL)LGA 53/47 films.
Drug Release from Biodegradable Matrices

Figure 4.2 In-vitro metoclopramide salt release profiles from monolayer P(DL)LGA 53/47 films of different thicknesses and drug loadings

*Note:* Each data point is an average of triplicate samples while error bar represents ± standard deviation
In general, the release profiles could be distinguished into two main phases:

1. an initial burst in the first two days followed by insignificant additional release (induction period) up to day 12, and
2. an accelerated second phase release up to saturation at day 30.

The initial burst was caused by rapid dissolution of metoclopramide salt particles, found mostly on the surface of the films. When the films were placed in contact with the release medium, these loosely bound drug particles were released immediately. From Figure 4.2, a comparison of different thicknesses of the film shows that thinner films suffered greater burst than thicker ones. The reason lies in the thinner film’s greater surface-to-thickness ratio so that on a percentage basis, a higher burst is seen.

In addition, increasing meto salt loading from 1% to 5% caused an approximately five-fold increase in the initial burst. Drug solubility in the polymer matrix plays a key role in determining the magnitude of burst release. Meto salt, being a hydrophilic drug, would have low solubility in the hydrophobic P(DL) LGA 53/47 film. Therefore, the increase of meto salt loading in the film only translated to more severe agglomeration of undissolved drug particles and ultimately led to greater burst.

The burst phenomenon is best illustrated by the SEM pictures in Figure 4.3. Prior to immersion, agglomerations of drug particles are evident on the surface of P(DL) LGA 53/47 film loaded with 5% metoclopramide salt (Figure 4.3-c). These drug particles were
quickly dissolved after 1 day immersion in release medium and left many micropores behind (Figure 4.3-d).

Figure 4.3 SEM pictures of 80 μm thick P(DL)LGA films loaded with metoclopramide salt: (a) 1% at day 0, (b) 1% at day 1, (c) 5% at day 0 and (d) 5% at day 1
Drug Release from Biodegradable Matrices

Following the initial burst, metoclopramide release rate slowed down and very little additional release was recorded up to day 12. It was caused by insufficient free volume (in the relatively glassy P(DL)LGA) to support further drug release. Only a small amount of water penetrated the matrix and triggered the hydrolysis process of the ester bonds, leading to matrix degradation. This period is termed the ‘latent period’ and is discussed in greater length in Chapter 5.

At the end of the latent period, the second phase release kicked in and was marked by a significant increase in the cumulative release. This acceleration was a result of polymer matrix degradation which created more water-filled pores as shown in Figure 4.4. Combination of Figures 4.3 and 4.4 illustrates the chronological development of micropores in the P(DL)LGA films. Evidently, more micropores were generated as the immersion time extended and degradation continued.

As drug diffusion through water filled pores is easier than through polymer matrix, these pores obviously became the preferred escape path and hence accelerated the release. Drug release continued until all drug particles were exhausted from the polymer film. Finally, meto salt release from P(DL)LGA 53/47 film reached saturation and release was completed within 30 days.
Figure 4.4 SEM pictures of 80 μm thick P(DL)LGA films loaded with metoclopramide salt: (a) 1% at day 12, (b) 1% at day 17, (c) 5% at day 12 and (d) 5% at day 17
4.3 Paclitaxel Release

4.3.1 Paclitaxel release from single and bi-layer films and stents

According to the bi-layer film/stent configuration shown in Figure 4.1, a series of film combinations were prepared. Basically, the top layer, made of P(DL)LGA 53/47, was loaded with paclitaxel. Table 4.2 lists all films prepared with different layer thicknesses and paclitaxel loadings.

Table 4.2 List of paclitaxel containing films made of P(DL)LGA 53/47 and PLLA with various thickness and drug loadings

<table>
<thead>
<tr>
<th>No</th>
<th>Sample Configuration</th>
<th>Top layer</th>
<th>Bottom layer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Polymer</td>
<td>Drug loading (%)</td>
<td>Thickness (μm)</td>
</tr>
<tr>
<td>1</td>
<td>Monolayer film</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td>2</td>
<td>P(DL)LGA 53/47</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>3</td>
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<tr>
<td>5</td>
<td>Bi-layer film</td>
<td>1</td>
<td>30</td>
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<tr>
<td>6</td>
<td>Bi-layer film</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>7</td>
<td>Bi-layer stent</td>
<td>1</td>
<td>80</td>
</tr>
</tbody>
</table>
Drug Release from Biodegradable Matrices

The first four samples were made of mono-layer P(DL)LGA 53/47 films, whereby paclitaxel was allowed to diffuse out from both sides of the films. Thickness and amount of initial drug loading were varied to assess their effect on paclitaxel release profiles. Samples 5 and 6 were made of bi-layer films, whereby each of them had an additional blank PLLA layer attached to the paclitaxel-containing P(DL)LGA 53/47 layer. This configuration led to drug elution only from one side of the film because PLLA, a slowly degrading polymer, served as a barrier layer. Hence, it allows investigation on the effect of release directionality to the overall paclitaxel release profiles. Finally, sample 7 is the actual bi-layer stent prototype made of paclitaxel-loaded P(DL)LGA 53/47 top layer and a blank PLLA bottom layer.

(a) In vitro release of paclitaxel from monolayer P(DL)LGA 53/47 films

Figure 4.5 shows paclitaxel release profiles from single layer P(DL)LGA 53/47 film with varying thickness and initial drug loading. It is obvious that all configurations show very similar release behavior. It can be concluded that drug loading and film thickness within the range of interest did not significantly change the profile of paclitaxel release, although the amounts released at any time point of course differed. However, as only the practically useful dimensions and loadings for coronary stent application were investigated in this work, caution should be exercised when such conclusion is extended to vastly different dimensions and loadings from those shown in Figure 4.5.
Drug Release from Biodegradable Matrices

Figure 4.5  In-vitro paclitaxel release profiles from monolayer P(DL)LGA 53/47 films of different thicknesses and drug loadings

*Note: Each data point is an average of triplicate samples while error bar represents ± standard deviation*
Drug Release from Biodegradable Matrices

Paclitaxel release profile can be divided into three stages [97]: (i) extremely slow initial release up to day 25, (ii) accelerated degradation-induced release from day 25 to day 48, and (iii) diffusion controlled release up to saturation at day 80.

- Stage 1 (day 0 ~ day 25)
  
  In the first stage, only a small amount of paclitaxel was released from P(DL)LGA 53/47 film regardless of the loading and thickness. Paclitaxel is an extremely hydrophobic drug with very limited aqueous solubility, ranging from 0.25 µg/mL to 0.7 µg/mL [98-100]. Its hydrophobicity allows it to dissolve and distribute uniformly within the hydrophobic P(DL)LGA 53/47 polymer matrix as confirmed by the MDSC test result in Figure 4.6.

![Figure 4.6 MDSC curves of pure paclitaxel drug powder and P(DL)LGA 53/47 film loaded with 3% paclitaxel](image)

Figure 4.6  MDSC curves of pure paclitaxel drug powder and P(DL)LGA 53/47 film loaded with 3% paclitaxel
Pure paclitaxel exists as white powder crystals with melting peaks at 220–230°C. However, upon loading into the P(DL)LGA 53/47 films, these characteristic melting peaks disappeared. This result indicates that paclitaxel no longer existed as drug crystals; instead, it was dissolved in the matrix. An SEM photo also shows no drug agglomeration and no drug-polymer phase separation even at 3% loading (data not shown). Thus, within the loading range studied here, paclitaxel is expected to be substantially in the dissolved state.

The slow initial release in the first stage could be attributed to the very low diffusivity of paclitaxel through the solid, glassy P(DL)LGA film as well as the low partitioning into an aqueous medium. During this stage, there was little water ingress into the relatively hydrophobic polymer as evidenced by a separate water absorption study. Figure 4.7 reveals insignificant water absorption up to 23 days following immersion in PBS pH 7.4.

With time, P(DL)LGA 53/47 films underwent random chain scissions due to hydrolysis, via a process usually referred to as bulk degradation. The consequent continuous decrease in molecular weight of the film is shown in Figure 4.8.
Figure 4.7 Water absorption by P(DL)LGA 53/47 films loaded with paclitaxel

Figure 4.8 Molecular weight decay of P(DL)LGA 53/47 films loaded with paclitaxel
Small differences were observed when film thickness was changed: the thicker films seemed to degrade somewhat faster than the thinner ones up to day 17. It was most likely to be caused by a mild autocatalytic degradation in thicker films as oligomers produced via hydrolytic scission were trapped in the thicker films. This effect disappeared with prolonged degradation, as after 20 days, all the films converged towards the same molecular weight levels.

Therefore the first stage release (up to day 25) can be considered as an induction period whereby only a small amount of water penetrated the film. This small amount of water was insufficient to cause either significant overall erosion of the polymer (only little weight loss was detected) or substantial plasticization of the polymer but initiated the degradation of polymer film in preparation for accelerated second stage release.

- Stage 2 (day 25 – day 48)

This stage is marked with the onset of a remarkably faster release of paclitaxel. In this stage, the water absorption was accelerated, shown by a steady increase in water absorption after day 23 in Figure 4.7. Besides, degradation had been substantial enough to cause leaching of a significant amount of oligomers, as indicated by commencement of weight loss from day 28 onwards in Figure 4.9.
Figure 4.9 Weight loss of P(DL)LGA 53/47 films loaded with paclitaxel

The SEM photo of the sample in Figure 4.10 shows that after 28 days degradation, micropores were formed on an initially smooth P(DL)LGA film surface. All the degradation data reconfirm the transformation of the film from an initially solid, glassy polymer to a porous, plasticized matrix. This combination of changes (interconnected pore formation and plasticization) led to the observed faster release.
A quick comparison across all films configurations in Figure 4.5 shows no difference in the second stage release rate with respect to drug loading and thickness. This finding was expected as Figures 4.7, 4.8 and 4.9 show no significant difference in water absorption, molecular weight decay and weight loss of the films.

In short, the second stage release was accelerated by P(DL)LGA 53/47 degradation which transformed the matrix from glassy state into somewhat porous one. The release rate was controlled by how fast the shorter polymer chains rearrange in favor of the creation of more free volume and micropores.
Stage 3 (day 48 ~ day 80)

As degradation continued, more and bigger pores were formed by leaching-out of degraded polymer products. At this stage, these pores were pervasive in the matrix and their interconnectivity was established. Therefore, drug release was controlled by diffusion through the water filled pores. Finally, due to drug exhaustion, the release rate slowed down when release was about to reach the saturation at day 80.

It is worth mentioning that the absence of burst and unique existence of “induction” period in the first stage was also observed by other researchers during their investigations on paclitaxel release from poly(anhydride-co-imides) microspheres [101], P(DL)LGA 50/50 nanoparticles [102] and P(DL)LGA 50/50 film [103]. As long as the drug is present below its dissolution limit in the polymer, this so-called induction phase of slow release will be exhibited.

(b) In vitro release of paclitaxel from bi-layer P(DL)LGA 53/47 and PLLA films and stents

Figure 4.11 shows the comparison of paclitaxel release from P(DL)LGA monolayer and bi-layer P(DL)LGA/PLLA films. For the bi-layer films, paclitaxel was loaded only to the top P(DL)LGA layer, while the PLLA bottom layer was left blank. The exact configurations of the bi-layer films can be found in Table 4.2, samples (5) and (6).
Drug Release from Biodegradable Matrices

Figure 4.11 In vitro paclitaxel release from bilayer P(DL)LGA/PLLA films loaded with 1% paclitaxel with top layer thickness of (a) 30 μm and (b) 80 μm

Note: Each data point is an average of triplicate samples while error bar represents ± standard deviation.
The general trend of paclitaxel release profile is similar for both monolayer and bilayer cases and the three release stages mentioned above can be easily identified. This indifference is supported by the similar degradation behaviour of P(DL)LGA layer with and without the presence of bottom PLLA layer (see Figure 4.12). For the double-layer film, tetrahydrofuran was used to selectively dissolve only the P(DL)LGA top layer and its molecular weight was analysed with GPC.

![Molecular weight decay of P(DL)LGA 53/47 with and without blank PLLA bottom layer](image)

**Figure 4.12** Molecular weight decay of P(DL)LGA 53/47 with and without blank PLLA bottom layer
Although Figure 4.11 shows that the general release trends are similar for monolayer and bi-layer films, the presence of blank PLLA bottom layer seems to reduce the second stage release rate and approximately 20–25% of the drug was not released at all. This was caused by partitioning of drug from the P(DL)LGA 53/47 top layer into the blank PLLA layer.

To prove this hypothesis, the sample was removed from the vial at the end of the release study. After 100 days of immersion, the stability of interface between the two layers was compromised. The P(DL)LGA top layer swelled considerably and degraded much faster than the PLLA bottom layer. Therefore, delamination occurred and the P(DL)LGA layer detached from the PLLA layer. The PLLA layer was isolated and dissolved to extract “trapped” paclitaxel and was quantified using HPLC. About 25% and 20% of the initial drug loaded to the P(DL)LGA top layer were recovered from the 30 μm and 80 μm thick films, respectively.

The portion of drug that partitioned into PLLA layer could not diffuse out within the period of the release study. This observation is reasonable as a separate paclitaxel release study from single layer PLLA film loaded with 1% and 3% paclitaxel showed negligible (near zero) release even after the film was immersed and monitored for 120 days (data not shown). Compared to amorphous P(DL)LGA 53/47, semicrystalline PLLA degrades very slowly and hence hinders drug release, especially of hydrophobic drugs like paclitaxel.
Figure 4.13 compares the paclitaxel release profiles from a bilayer film and stent. The monolayer release profile was added as a reference. Almost identical release profiles were observed for both bilayer film and stent, identified with the distinct three stages of release. Therefore, it can be concluded that the stent-making process, that transforms a bilayer film into a helical stent via a proprietary heat treatment process, did not alter the drug release profiles. In other words, the in-vitro drug release studies from stents can be adequately represented with studies from planar films.

![Paclitaxel release from a bilayer film and stent](image)

*Note: Each data point is an average of triplicate samples while error bar represents ± standard deviation*

**Figure 4.13** In vitro paclitaxel release profiles from bilayer P(DL)LAGA / PLLA film and stent loaded with 1% paclitaxel
4.3.2 Modification of paclitaxel release profiles

It is very obvious that paclitaxel release from P(DL)LGA 53/47 matrix suffered from a long induction period. Therefore, several approaches were taken to accelerate the release rate and shorten the induction period.

- Adding 10 wt% poly(ethylene glycol), PEG to the P(DL)LGA matrix. Lack of water absorption by hydrophobic P(DL)LGA matrix in the early days was one of the main reasons which hindered release and caused the long induction period. Therefore, addition of hydrophilic PEG (Mn = 10,000 g/mol) would attract more water to the matrix as early as possible. Besides, more water leads to faster degradation which in turn aids the drug release process.

- Switching the base polymer to poly (ε-caprolactone), PCL. PCL has been known to posses extremely low glass transition temperature, Tg ≈ 60°C. Therefore, at physiological condition (37°C, humid), it is in rubbery state and thus would allow faster drug diffusion than the glassy P(DL)LGA 53/47.

- Blending P(DL)LGA 53/47 with PCL Blending two or more materials is usually approached as a way to combine the benefits of each component. Therefore, the rationale behind this approach was to obtain an intermediate release between the fast diffusion of PCL and sustained release of P(DL)LGA.
Drug Release from Biodegradable Matrices

Figure 4.14 shows the in-vitro release results of the approaches mentioned above performed using 80 μm thick films. Paclitaxel release rate was accelerated to different extents by modifying the matrix composition. Addition of PEG significantly shortened the induction period while PCL exhibited a fast release with some burst and P(DL)LA/PCL blend displayed distinct combined effects of release behaviours from both components [104]. The release mechanism of each formulation is discussed next.

![Adjustable Paclitaxel Release Profiles](image)

*Note: Each data point is an average of triplicate samples while error bar represents ± standard deviation*

Figure 4.14 Adjustable in vitro paclitaxel release profiles via modification of matrix compositions
Drug Release from Biodegradable Matrices

(a) P(DL)LGA/PEG 90/10 (w/w)

P(DL)LGA-PEG film displayed a three-stage release profile, similar to that of P(DL)LGA film, but at a much faster pace. The initial slow release in the first stage was shortened from 25 days (in pure P(DL)LGA matrix) to 12 days. As mentioned earlier, the limiting factor that hindered release in the first stage was the limited water penetration into the hydrophobic P(DL)LGA. Addition of 10 wt % hydrophilic PEG led to a remarkable improvement instantly (even at day 1), as evidenced by a water absorption test in Figure 4.15.

![Figure 4.15 Water absorption by P(DL)LGA 53/47 films with and without PEG](image)

Figure 4.15 Water absorption by P(DL)LGA 53/47 films with and without PEG
As a result, paclitaxel release was increased considerably; after 12 days, a cumulative 6% drug was released as opposed to only 1% in pure P(DL)LGA matrix. The enhanced water absorption also translates into accelerated hydrolysis of P(DL)LGA chains leading to earlier onset of the second stage. And indeed, separate analysis of molecular weight decay of P(DL)LGA matrix in Figure 4.16 demonstrated accelerated degradation with the addition of PEG.

Figure 4.16 Molecular weight decay of P(DL)LGA 53/47 films with and without PEG
Ultimately, the coupled effect of enhanced water ingress and faster degradation in P(DL)LGA-PEG film caused the drug release to reach completion in 45 days, down from 80 days.

(b) PCL

Unlike P(DL)LGA and P(DL)LGA/PEG films, PCL film displayed a common single stage release pattern. Paclitaxel release started with an approximately 24% initial burst. It was then controlled by diffusion until drug exhaustion at day 30. PCL is a slow degrading polymer as shown by negligible molecular weight decay in Figure 4.17.

![Molecular Weight of PCL film](image.png)

**Figure 4.17** Molecular weight decay of PCL film
During the 30-day period of release, PCL's molecular weight did not change much. As such, degradation did not influence the drug release process for PCL in the time frame studied. Instead, paclitaxel was released by simple diffusion through the rubbery matrix owing to PCL's low glass transition temperature (Tg ≈ -60°C). At test condition of 37°C and surrounded by saline, drug molecules could move easily through the free volume across rubbery PCL chains and led to diffusion-controlled release profile.

(c) P(DL)LGA/PCL blend

As seen from Figure 4.14, release from P(DL)LGA has a long induction period of zero to little release while release from PCL suffers from high burst and is relatively short-lived (only 30 days to completion). A blend of the two was postulated to give an intermediate release profile for paclitaxel in applications involving drug-eluting stents. Indeed, P(DL)LGA/PCL 65/35 blend film exhibited a unique release pattern whereby P(DL)LGA and PCL mutually complemented each other and resulted in a combined/additive release profile (see Figure 4.14)

PCL helped to contribute to drug release in the early days and hence eliminated the long induction period, characteristic of P(DL)LGA alone. On the other hand, P(DL)LGA helped to sustain drug release to longer time, up to 70 days, as opposed to only 30 days in PCL alone. Mechanisms of release from P(DL)LGA/PCL blends are discussed in greater depth during the model development for release from blends in chapter 5.
4.4 Heparin Release

4.4.1 Heparin release from single-layer films

Investigation of heparin release from the bilayer polymeric stent was started with a study of heparin release from monolayer PLLA films. PLLA is the bottom layer of the bilayer stent configuration (see Figure 4.1) that will be in contact with blood in the vessel lumen.

Note: Each data point is an average of triplicate samples while error bar represents ± standard deviation

Figure 4.18 In-vitro heparin release profiles from monolayer PLLA films of different drug loadings
Drug Release from Biodegradable Matrices

Figure 4.18 shows heparin release profiles obtained from two PLLA films of 80 µm thickness. The first film was loaded with 1% heparin while the other was loaded with 5% heparin.

Heparin release from PLLA films were marked with massive initial burst of up to 45% of the total drug loading. PLLA is a hydrophobic and semicrystalline polymer which has low capability to dissolve hydrophilic drugs including heparin. Due to low solubility of heparin in PLLA, most of the loaded heparin agglomerated in the matrix and a large portion of the drug particles precipitated on the surface of the matrix. These loosely bound drug particles on the surface were immediately dissolved when the matrix was immersed in the release medium, leading to initial burst release.

The severe burst release recorded in our study agrees with previous reports in the literature. Tan and co-workers [105] performed similar investigation of heparin release from PLLA matrix and reported about 40% of the drug were released by burst in the first 3 days. Other researchers working on heparin release from different biodegradable polymers also observed the similar phenomenon in their studies. Edelman et al [106] noticed 10 to 15% burst from P(DL)LGA 50/50 and 70/30 microspheres, Luong-Van et al [107] reported 20 to 30% burst from electrospun PCL fiber while Yang et al [108] encountered 15 and 25% burst from microspheres made of P(DL)LGA 50/50 and P(DL)LGA 75/25 respectively.
After the burst, a very slow diffusion contributed to a gradual increase in the cumulative release. In fact, after 80 days of release, only approximately 70% heparin was released. The remaining 30% remained “trapped” in the PLLA film – extracted from the films at the end of the release study. This slow release kinetics was mainly caused by PLLA’s rather high Tg and extremely low degradation rate. A separate degradation study revealed negligible molecular weight decay, water absorption and weight loss (data are shown in the next section). On top of that, the Tg of PLLA is approximately 60°C; as such, it existed as a rigid, glassy polymer network at the physiological environment (37°C) in this study. Chain motion was restricted and only very limited free volume was available for drug diffusion.

4.4.2 Modification of heparin release profiles

Figure 4.18 indicated a rather poor control of heparin release from PLLA matrix. The amount of burst was huge and 30% of drug was wasted and remained in the matrix. Therefore, modification of heparin release profiles aimed to reduce the initial burst and prevent drug wastage. Edelman et al [106] has attempted to reduce the burst by sequestering the P(DL)LGA 70/30 microspheres (loaded with heparin) in alginate gels. This method was able to reduce burst without changing the later phases of the release. On the other hand, Tan et al [105] added 5 wt% plasticizer (PEG) to the PLLA matrix in order to suppress the burst effect.
Drug Release from Biodegradable Matrices

In our work, however, a high molecular weight polyethylene oxide (PEO) was added to the matrix, at either 10 wt% or 25 wt%. PEO, being hydrophilic, was expected to encourage water penetration into an otherwise hydrophobic matrix. In addition, it would also interact favorably with heparin particles and control its release rate from the PLLA matrix. Figure 4.19 shows the in-vitro release results from the PLLA matrix supplemented with 0, 10 and 25 wt% PEO.

![Heparin Release from monolayer PLLA with and without PEO](image)

*Note: Each data point is an average of triplicate samples while error bar represents ± standard deviation*

Figure 4.19  In-vitro heparin release profiles from monolayer PLLA films supplemented with PEO.
Addition of high Mw PEO significantly altered the heparin release behaviour from PLLA matrix. Introduction of 10 wt% PEO managed to reduce the initial burst significantly, from 42% to 26%. Following which, a zero-order release rate was obtained until day 60. Due to drug exhaustion, release subsequently slowed down and reached completion at day 80. At the end of the release study, almost all drug particles were released and hence no drug was wasted as seen in the case of pure PLLA. However, increasing PEO content to 25% led to higher burst and drug dumping up to 78% in the first few days.

![Water absorption of PLLA films with and without PEO](image)

**Figure 4.20** Water absorption by PLLA films loaded with 5% heparin, with and without addition of PEO.
As can be seen from Figure 4.20, PLLA film alone did not absorb much water. This condition was improved by the addition of PEO. PLLA/PEO 90/10 and PLLA/PEO 75/25 were able to immediately absorb 25% and 95% water respectively. For PLLA/PEO 90/10, this phenomenon definitely helped heparin dissolution and release from the matrix after burst, as opposed to minimal release from pure PLLA film. For PLLA/PEO 75/25, the massive water absorption led to a very rapid heparin release and accounted for 60% burst and 78% cumulative release within the first 4 days.

The zero order heparin release which follows after burst (Figure 4.19) was related to the rate at which PEO leached or dissolved away from polymer matrix. As mentioned earlier, hydrophilic heparin prefers to associate with hydrophilic PEO than with hydrophobic PLLA. As such, heparin release rate relied heavily on the rate of PEO dissolution. To prove this hypothesis, a separate weight loss study was conducted (see Figure 4.21).
Figure 4.21  Weight loss of PLLA films loaded with 5% heparin, with and without addition of PEO.

In Figure 4.21, PLLA/PEO 90/10 displayed a gradual, near zero-order mass loss for about 70 days. This finding agrees well with the zero-order release rate within the same period. PLLA/PEO 75/25, on the other hand, immediately suffered from a huge mass loss and then increased steadily, in accordance to its huge initial burst and fast release afterwards.

Further investigation of heparin release was extended to the bilayer film configuration. PLLA/PEO 90/10 was selected as the bottom layer because it offers controlled (zero-order) yet sustainable release profile.
Figure 4.22  In-vitro heparin release profiles from PLLA/PEO 90/10 films with and without blank P(DL)LAGA top layer.

Figure 4.22 shows the heparin release from PLLA/PEO 90/10 bottom layer topped with a blank P(DL)LAGA layer. The presence of blank P(DL)LAGA top layer did not alter the heparin release behavior significantly. This blank P(DL)LAGA layer slightly reduced the burst and somewhat slowed the release rate, as opposed to release from monolayer PLLA. This rather mild slowing down of release was expected as P(DL)LAGA is a much faster degrading polymer compared to PLLA and thus could not act as an effective barrier layer to drug release. As such, it can be concluded that P(DL)LAGA top layer did not hinder heparin release from PLLA/PEO 90/10 bottom layer.
CHAPTER 5

MODELING OF DRUG RELEASE FROM DEGRADABLE POLYMERS AND THEIR BLENDS

5.1 Introduction

In chapter 2, an overview of drug release models has been presented. Most of these models successfully represented single-step release kinetics, dominated by (time dependent) diffusion alone. To address two-step release kinetics, another dimension, namely burst release, was added to the drug release model proposed by Batycky et al [32]. Nevertheless, the applications of all of these models are limited to mono-phasic and bi-phasic drug release patterns only.

Various in-vitro release profiles have been exhibited in chapter 4; among which are distinct tri-phasic release behaviour and a unique drug release profile from polymer blends. The previously reported models were unable to illustrate the tri-phasic release and none of them addressed drug release from blends of degradable polymers. In an attempt to gain better insights on release mechanisms that governed the release kinetics in chapter 4, a new model was developed for tri-phasic release. In addition, a novel model that predicts release kinetics from blends of degradable polymers is proposed in this chapter.
5.2 Description of Drug Release Process and Kinetics

Drug release rate is defined as the rate at which drug is removed from a polymer matrix to a surrounding aqueous (buffer) phase or physiological solution. Basically, there are three steps which occur chronologically and lead to drug release into the medium:

1. solvent (water) penetration into the matrix, usually accompanied by rapid burst;
2. a degradation-dependent "relaxation of the network" that creates more free volume to promote drug dissolution and
3. drug removal to the surrounding medium, usually by diffusion process.

During the period of drug release from the biodegradable polymer, the rate of each step generally increases as degradation (hydrolysis) proceeds. At any time, the step with the lowest rate will become the rate limiting step and will ultimately control the overall drug release rate. The exact release profile, however, varies and depends on factors such as the nature of drugs (hydrophilic/hydrophobic), polymer degradation rate, water permeability and drug-polymer matrix interaction. Sometimes one or more steps may proceed extremely fast and hence have no chance to control release rate and overall profile. In such cases, these steps will be omitted from release models as only the rate limiting steps determine release rates and are reflected in the models.

Of the 3 steps mentioned above, most drugs affect step number 2. Dissolution of the drug is governed by the drug's water solubility and partition coefficient in water. Hydrophilic
drugs usually have good water solubility and favor water to hydrophobic polymer matrices whereas the opposite is true for hydrophobic drugs. Therefore, step 2 usually occurs very fast for hydrophilic drugs and hence it is not the limiting step. In the case of an extremely hydrophobic drug like paclitaxel, step 2 is slow especially when the amount of water is insufficient to meet the solubility requirement of the drug. Thus, drug dissolution may control the release rate during the time frame when polymer degradation has not created sufficient number of water filled pores and “open” swollen network.

Numerous drug release studies from nondegradable and degradable systems have reported step 3 (diffusion) as the sole controlling factor. This approach is correct as diffusion probably possesses the slowest rate and hence becomes the “all-time” rate limiting step in those cases. In this work however, the importance of step 1 and 2, especially in the case of hydrophobic drug, will be highlighted during the development of a tri-phasic model. This three-step model is one of the important building blocks of the novel drug release model from blend systems proposed in the next stage.

5.3 Modeling of Drug Release from Degradable Polymers

Many factors affect the rate of polymer degradation which in turn affects drug delivery kinetics, including water permeability and water solubility, degree of crystallinity, glass transition temperature and physical dimension (size and surface-to-volume ratio). Another
important factor that can affect drug release rate is the type of drug incorporated to the polymer carriers. Significantly different release profiles were obtained from in-vitro release studies of hydrophilic and hydrophobic drugs from P(DL)LGA 53/47 films (compare Figures 4.2 and 4.5 in Chapter 4). In order to explain the different drug release mechanisms, two release models were proposed – one for each drug [109]. An attempt to compare the models with experimental results is presented as well.

5.3.1 Modeling of hydrophilic drug release

Metoclopramide monohydrochloride is very hydrophilic and freely soluble in water with aqueous solubility of larger than 50 mg/ml [110]. Drug dissolution occurs readily and thus step 2 is an insignificant factor to the overall drug release rate. Step 1 and 3 are dominant at different times depending on the extent of polymer degradation. Step 1 describes initial burst limited by water absorption in the early stage while step 3 represents drug diffusion in the later stage. Therefore, the total drug release was described as the sum of the fraction of drug release due to an initial burst, $\phi_b$, plus the fraction of drug release through diffusion, $\phi_d$ [109]. Mathematically, $\phi_b + \phi_d = 1$.

The fraction of drug release through initial burst, $\phi_b$, occurs within the first few days of release study. It is a result of immediate desorption of drug particles located at or near the surface of a film following immersion in the release medium. The kinetics of initial burst
follows an exponential relationship, as pointed out by Batycky et al [32], and is dictated by the rate of drug desorption, termed burst constant, $k_b$.

\[
\left( \frac{M_t}{M_\infty} \right)_{\text{burst}} = 1 - \exp(-k_b t)
\]  

(Eq. 5.1)

where $M_t$ is the amount of drug release at time $t$ (µg), $M_\infty$ is the amount of drug release at infinity (µg) and $t$ is time (s). Equation 5.1 will be used in our model to determine the fraction of drug release via burst, $\phi_b$.

To derive the equation for the fraction of drug release through diffusion, Fick's second law of diffusion under non-steady state condition for film/planar geometry was utilized.

\[
\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2}
\]  

(Eq. 5.2)

$C$ is time and position-dependent drug concentration in the matrix (µg/ml), $D$ is the drug diffusion coefficient (cm$^2$/s), $t$ is time (s) and $x$ is the position normal to the central plane of the film (cm).

The following initial and boundary conditions were applied to solve equation (5.2).

\[
C = C_0 \quad -l < x < l, \ t = 0
\]  

(Eq. 5.3)

\[
C = 0 \quad \text{at } x = -l
\]  

(Eq. 5.4)

\[
C = 0 \quad \text{at } x = l
\]  

(Eq. 5.5)

$C_0$ is the initial drug concentration in the matrix (µg/ml) while $l$ is the half-thickness of the film (mm). Equation 5.3 indicates uniform drug distribution in the film prior to diffusion.
Equations 5.4 and 5.5 indicate that the surface concentration is equal to zero at any time.

The solution to this diffusion problem is as follows [111].

\[
\left\{ \frac{M_t}{M_\infty} \right\}_{\text{diff}} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left\{ -\frac{D(2n+1)^2 \pi^2 t}{4l^2} \right\} \quad (\text{Eq. 5.6})
\]

Equation 5.6 will be used in our model to determine the fraction of drug release via diffusion, \(\phi_d\).

Based on the analysis above, both modes of drug release were combined in our proposed model. The total fraction of hydrophilic drug release, \((M_t/M_\infty)_{\text{total}}\), was described as the sum of drug release due to an initial burst (first term on the right hand-side) plus the diffusion-controlled release (second term on the right hand-side).

\[
\left\{ \frac{M_t}{M_\infty} \right\}_{\text{total}} = \phi_b \left\{ 1 - \exp(-k_b t) \right\} + \phi_d \left\{ 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp\left\{ -\frac{D(2n+1)^2 \pi^2 (t-t_b)}{4l^2} \right\} \right\} \quad (\text{Eq. 5.7})
\]

In equation 5.7, the second term on the right hand-side was slightly modified from equation 5.6 through the introduction of a new parameter, i.e \(t_b\). This parameter represents the end of burst release and at the same time, the commencement of diffusion-controlled release. All other parameters carry the same meanings as have been discussed earlier.

MATLAB was then used to fit equation 5.7 to our experimental data. The fitting procedure involves translating the mathematical expression (equation 5.7) into a
MATLAB function. The values of certain parameters of the function were known while some of them were set free. These free parameters were optimized until the best fitted values were obtained by the least-square method. At the end, the programme returned the values of these unknown (free) parameters and the goodness of fit ($R^2$ value). All these parameters are summarized in Table 5.1.

**Table 5.1 Parameters of metoclopramide salt release from P(DL)LGA 53/47 films**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Unit</th>
<th>Drug loading and film thickness</th>
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<td>Known parameters</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>$\phi_b$</td>
<td>fraction of burst release</td>
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<tr>
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<tr>
<td>Parameters determined by the model</td>
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<tr>
<td>$D$</td>
<td>diffusion coefficient</td>
<td>cm$^2$/s</td>
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<tr>
<td>$k_b$</td>
<td>burst constant</td>
<td>day$^{-1}$</td>
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<tr>
<td>$t_b$</td>
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<td>Goodness of fit</td>
<td>$R^2$ correlation coefficient</td>
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<td></td>
<td></td>
<td></td>
<td>0.992</td>
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</table>

It is important to note that the $R^2$ value reports the overall goodness of fit of all stages in the model as compared to the experimental data. In all cases, good correlation ($R^2 = 0.99$) was obtained, as shown in Figures 5.1 and 5.2.
Figure 5.1 Model and experimental data matching of metoclopramide release from P(DL)LGA 53/47 films (30 μm thick) with (a) 1% and (b) 5% drug loading
Figure 5.2 Model and experimental data matching of metoclopramide release from 
P(DL)LGA 53/47 films (80 μm thick) with (a) 1% and (b) 5% drug loading
As can be seen from Figures 5.1 and 5.2, after burst in the first day, an induction period was observed before significantly accelerated release occurred. This is equivalent to what has been reported in the literature as a “dual sorption” process. Here, the equivalent term is “dual release” or “two-level release”. During the induction period, water penetration (step 1) controlled the drug release rate. Limited water penetration resulted only in a small amount of additional release. At the end of this period, given by a symbol $t_b$, sufficient water absorption was obtained; thus, drug diffusional release (step 3) took over and controlled the rate until saturation.

The values of the model's parameters, listed in Table 5.1, agree well with the experimental data. The burst period, $t_b$, is similar for all films and was found to be approximately 11 or 12 days. These values are very close to those obtained from the in-vitro release experiments. The burst constant, $k_b$, (i.e., rate of drug desorption) is slightly higher for films with higher initial loading and reflects the presence of greater agglomeration of undissolved drug particles as loading is increased.

Drug diffusion coefficients of thicker films seem to be somewhat higher than those of thinner films, partly due to faster degradation of thicker films as shown in Figure 5.3.
The degradation of P(DL)LGA 53/47 films can be described by first order kinetics as follows:

\[
\frac{dM_w}{dt} = -kM_w \quad \text{(Eq. 5.8)}
\]

\[
M_{w,t} = M_{w,0} \exp(-kt) \quad \text{(Eq. 5.9)}
\]

Here, \( M_{w,0} \) and \( M_{w,t} \) are the weight average molecular weights at time zero and \( t \), respectively, while \( k \) is the degradation constant. Table 5.2 tabulates the degradation constants of all meto salt films determined by equation 5.9.
Table 5.2  First order degradation constants of metoclopramide loaded P(DL)LGA 53/47 films

<table>
<thead>
<tr>
<th>Films</th>
<th>k (day⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% meto, 30 µm</td>
<td>0.085</td>
<td>0.98</td>
</tr>
<tr>
<td>5% meto, 30 µm</td>
<td>0.075</td>
<td>0.96</td>
</tr>
<tr>
<td>1% meto, 80 µm</td>
<td>0.099</td>
<td>0.95</td>
</tr>
<tr>
<td>5% meto, 80 µm</td>
<td>0.093</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Generally, thicker films have higher values of degradation constants, k. Thinner films degraded somewhat more slowly because the resultant low molecular weight oligomers (from chain scission process) can diffuse much faster out of thinner films. If these oligomers were to remain "trapped" in the polymer matrix, a likely scenario for the thicker films, their carboxylic (acid) end groups would accelerate hydrolysis and hence lead to autocatalysis [112-114].

5.3.2 Modeling of hydrophobic drug release

A completely different scenario was experienced by hydrophobic drug release, in particular paclitaxel release. Paclitaxel is extremely hydrophobic and practically insoluble in water. Drug dissolution takes place with much difficulty especially in the early days when only very limited free volume was available for paclitaxel transport pathway. It has
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to rely on degradation to break long chains and polymer relaxation to create a more “open” network. Therefore, during certain periods, degradation-dependent relaxation of the polymer chains (step 2), plays a critical role to create more free volume for drug dissolution and to promote further release.

In short, unlike hydrophilic drug model, the model for hydrophobic drug has to take into consideration all three steps as they became the rate limiting steps at different times throughout the period of release. The total fraction of drug release is thus a summation of burst release (first term on the right hand-side), relaxation-induced drug dissolution release (second term on the right hand-side) and diffusion-controlled release (third term on the right hand-side) [109].

\[
\begin{aligned}
\left\{ \frac{M_t}{M_\infty} \right\}_{total} &= \phi_b \{1-\exp(-k_{bt}t)\} + \phi_r \{\exp[k_r(t-t_b)]-1\} \\
+ \phi_d \left\{ 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left[ -\frac{D(2n+1)^2 \pi^2 (t-t_r)}{4l^2} \right] \right\}
\end{aligned}
\]  
(Eq. 5.10)

Equation 5.10 was derived by expanding equation 5.7 and adding a term which represents relaxation-induced drug dissolution release, i.e the second term on the right-hand side. All the parameters in equation 5.10 bear the same meanings as described earlier for equation 5.7. In addition, \(\phi_r\) is the coefficient of drug dissolution release due to polymer relaxation,
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$k_r$ is the degradative relaxation constant and $t_r$ is the end of relaxation-induced, drug-dissolution controlled release.

Polymer samples are known to exhibit relaxation when they are exposed to a thermodynamically compatible liquid. For example, a hydrophilic glassy polymer may swell in the presence of water to give a swollen gel. This process is characterized by a relaxation time, $\lambda$, which is usually depended on the molecular weight of the polymer. In fact, due to the polydispersity of the molecular weight, the relaxation is expressed as a spectrum of relaxation times or as an average relaxation time, $\lambda$. Astarita and Sarti [115] and Joshi and Astarita [116] showed that transport phenomena and water uptake during relaxational change may be expressed with an exponential expression with relaxation constant $k=\lambda^{-1}$ if relaxation is the prevailing term. It will be shown next that this idea can also be applied to biodegradable system.

In his pioneering work, Lee [94] showed that in controlled release systems, phase erosion and state erosion are physically similar systems. Based on his idea, the relaxational phenomena can be observed not only in state erosion, i.e., swelling (glassy to rubbery transition), but also in phase erosion, i.e., chemically degrading systems.

Thus, it is appropriate to express the additional relaxation factor which controls drug release from a degradable system by an exponential term as shown in equation 5.10. Clearly the relaxation constant, $k_r$, is dictated by how fast molecular chains relax as the
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polymer degrades, and allow additional water penetration. Obviously the chain length or the molecular weight of polymer plays an important role as higher molecular weight chains require longer relaxation time. The relaxation process of degrading polymers can be described by Figure 5.4 (adapted from the basic ideas of disentanglements expressed by de Gennes [117]).

![Figure 5.4 Relaxation of P(DL)LGA 53/47 chains induced by swelling and degradation](image)

<table>
<thead>
<tr>
<th>Time</th>
<th>Mol weight</th>
<th># entanglement</th>
</tr>
</thead>
<tbody>
<tr>
<td>$t_0$</td>
<td>$M_{w,0}$</td>
<td>$N_0$</td>
</tr>
<tr>
<td>$t_1$</td>
<td>$M_{w,1} &lt; M_{w,0}$</td>
<td>$N_1 &lt; N_0$</td>
</tr>
<tr>
<td>$t_2$</td>
<td>$M_{w,2} &lt; M_{w,1} &lt; M_{w,0}$</td>
<td>$N_2 &lt; N_1 &lt; N_0$</td>
</tr>
<tr>
<td>$t$</td>
<td>$M_{w,1} &lt; M_{w,0}$</td>
<td>$N_1 &lt; N_0$</td>
</tr>
</tbody>
</table>

Initially the polymer chains are very long, $M_{w,0}$, and there are $N_0$ entanglement points. Due to hydrolysis of ester bonds, chains become shorter, i.e $M_{w,1} < M_{w,0}$, and the number
of entanglement decreases, $N_t < N_0$. As degradation proceeds, some short chains (oligomers) “dissolve out” of the polymer matrix and the degree of entanglement keeps decreasing. As a result, the remaining polymer chains have to rearrange and thus create a more “open” network. In short, relaxation of the polymer matrix takes place continuously in response to swelling (water penetration) and the dynamically changing chain length, $M_w$, due to degradation.

It is worth mentioning that as the local amount of water around a drug particle is not high (considering paclitaxel’s extremely low aqueous solubility), the drug dissolution process occurs with great difficulty. In addition, other researchers have demonstrated that paclitaxel has much stronger affinity for hydrophobic substance which acts as an additional hindrance to its dissolution and release rate. Paclitaxel’s partition coefficient for [octanol]/[water] is as high as 10,000 [118] and for [lipid]/[water] is 9,700 [98]. Hence, paclitaxel remains in the hydrophobic PDL)LAGA 53/47 matrix and this fact highlights the importance of relaxation processes. Along with the chain relaxation process, the creation of a looser network and the breaking down of chain entanglements “free” the trapped drug molecules; thus boosting water penetration and promoting drug dissolution of the otherwise hydrophobic paclitaxel.

The chain degradative relaxation constant, $k_r$, depends heavily on the rate of production of shorter polymer chains, i.e. on the polymer’s degradation rate. Thus, as a first approximation, the value of $k_r$ was taken to be of the order of the polymer’s degradation
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current. The degradation of P(DL)LGA 53/47 films that contain paclitaxel is followed by the films' molecular weight reduction as illustrated in Figure 4.8 (previous chapter). Equation 5.9 was used to fit the data and calculate the first order degradation constant of paclitaxel films; the results are tabulated in Table 5.3.

Table 5.3 First order degradation constants of paclitaxel loaded P(DL)LGA 53/47 films

<table>
<thead>
<tr>
<th>Films</th>
<th>k (day⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLGA 0.03mm, 1% paclitaxel</td>
<td>0.070</td>
<td>0.98</td>
</tr>
<tr>
<td>PLGA 0.03mm, 3% paclitaxel</td>
<td>0.072</td>
<td>0.96</td>
</tr>
<tr>
<td>PLGA 0.08mm, 1% paclitaxel</td>
<td>0.093</td>
<td>0.99</td>
</tr>
<tr>
<td>PLGA 0.08mm, 3% paclitaxel</td>
<td>0.093</td>
<td>0.98</td>
</tr>
</tbody>
</table>

An attempt to approximate k values in Table 5.3 with k₀ and use this value in equation 5.10 gave good match with the experimental paclitaxel release data. Figures 5.5 and 5.6 show the comparison between the model and the in-vitro release data. Correlation coefficients, R², of at least 0.99 were obtained in all cases of P(DL)LGA films with different thicknesses and paclitaxel loadings.
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Figure 5.5  Model and experimental data matching of paclitaxel release from P(DL)LGA 53/47 films (30 μm thick) with (a) 1% and (b) 3% drug loading

Note: Each data point is an average of three repeated experiments (n=3)
Modeling of Drug Release

Note: Each data point is an average of three repeated experiments (n=3)

Figure 5.6 Model and experimental data matching of paclitaxel release from P(DL)LGA 53/47 films (80 μm thick) with (a) 1% and (b) 3% drug loading
Again, MATLAB programming software was used to determine some of the model parameters of paclitaxel release from P(DL)LGA 53/47 films. The results are summarized in Table 5.4.

### Table 5.4 Parameters of paclitaxel release from P(DL)LGA 53/47 films

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Unit</th>
<th>Drug loading and film thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1% 0.03 mm</td>
</tr>
<tr>
<td>Known parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \phi_b )</td>
<td>fraction of burst release</td>
<td>-</td>
<td>0.028</td>
</tr>
<tr>
<td>( l )</td>
<td>half-thickness</td>
<td>Mm</td>
<td>0.015</td>
</tr>
<tr>
<td>( k_r )</td>
<td>degr relaxation constant</td>
<td>day(^{-1})</td>
<td>0.070</td>
</tr>
<tr>
<td>Parameters determined by the model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( D )</td>
<td>diffusion coefficient</td>
<td>cm(^2)/s</td>
<td>1.65x10(^{-12})</td>
</tr>
<tr>
<td>( k_b )</td>
<td>burst constant</td>
<td>day(^{-1})</td>
<td>0.083</td>
</tr>
<tr>
<td>( t_b )</td>
<td>end of burst release</td>
<td>days</td>
<td>20.99</td>
</tr>
<tr>
<td>( t_r )</td>
<td>end of relaxation release</td>
<td>days</td>
<td>47.14</td>
</tr>
<tr>
<td>( \phi_r )</td>
<td>relaxation release coeff</td>
<td>-</td>
<td>0.074</td>
</tr>
<tr>
<td>Goodness of fit</td>
<td></td>
<td></td>
<td>R(^2)</td>
</tr>
</tbody>
</table>

\( *: k_r \) is approximated to be equal to \( k \) (1st order degradation constant); please refer to Table 5.3

Regardless of the initial loading and thickness of the samples, almost no initial burst was observed for paclitaxel release from P(DL)LGA 53/47 films, \( \phi_b = 0.02 \sim 0.03 \). This finding means there were no loose drug particles present on the surface of the films. Instead, a long induction-burst period was observed, \( t_b \approx 20 \) days, in agreement with
negligible / near zero water absorption by paclitaxel-loaded P(DL)LGA films until about 23 days of immersion (see Figure 4.7). Despite the negligible burst, the model, equation 5.10, still takes into account the possibility of burst phenomenon as it might happen if the initial drug loading is increased significantly.

The second stage of drug release, controlled by the drug dissolution (relaxation) process, was marked by the creation and growth of micropores as a result of dissolution of short oligomers and rearrangement of remaining polymer chains. As degradation continued, water became easily available to the drug. Thus, dissolution occurred fast enough and no longer controlled the release rate. At this point, drug diffusion through water filled pores became the rate limiting step and controlled the release rate until saturation. The diffusion coefficient values obtained from our model are in agreement with those measured by other researchers for similar biodegradable matrices [119, 120].

5.4 Modeling of Drug Release from Degradable Polymer Blends

Although degradation rate can be tailored to adjust drug release rate, it is often necessary to blend two or more polymers to achieve desirable release kinetics and/or for other purposes. However, the previously reported models work only for drug release from single (unblended) polymer systems. Thus, in this section, a new model is developed to predict drug release from a blend of degradable polymers, particularly paclitaxel release
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from P(DL)GGA/PCL blends. Comparisons between model predictions and experimentally measured data are also presented.

5.4.1 Modeling of drug release from PCL film

PCL belongs to the class of slowly degrading polymers; it takes more than 2 years to be completely degraded [75]. Our own degradation study also noted very mild molecular weight decay (see Figure 4.17 in the previous chapter). In addition, PCL film’s surfaces observed under SEM did not show any notable sign of degradation after 28 days of immersion in release medium (Figure 5.7). As such, degradation does not influence the drug release process for PCL in the time frame studied.

However, PCL has very low glass transition temperature ($T_g \approx -60^\circ C$). Hence, at test condition of $37^\circ C$ and surrounded by saline, PCL chains were in a highly flexible rubbery state. At the temperature of testing ($T_{test} >> T_g$), there is sufficient free volume in the PCL matrix. This fact implies that step 2 (relaxation to create more free volume) was not a rate limiting step.
Figure 5.7  SEM pictures of PCL films (a) prior to immersion and (b) after 28 days of immersion in release medium
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Therefore, after initial burst and water penetration to the matrix (step 1), drug release proceeded to diffusion (step 3). In short, the total fraction of drug release from PCL, \( \left( \frac{M_t}{M_{oo}} \right)_{\text{PCL}} \), can be described as the sum of drug release due to an initial burst plus the diffusion-controlled release portion [121]. Similar two-step release scenario has been discussed earlier in section 5.3.1, prior to the derivation of equation 5.7. As such, the same equation can be applied and has been re-written with "PCL subscript" to differentiate it with P(DL)LGA release in the blend systems later on.

\[
\left\{ \frac{M_t}{M_{oo}} \right\}_{\text{PCL}} = \phi_{b,\text{PCL}} \left( 1 - \exp(-k_{b,\text{PCL}}t) \right) + \phi_{d,\text{PCL}} \left\{ 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left( - \frac{D_{\text{PCL}}(2n+1)^2 \pi^2 (t-t_{b,\text{PCL}})}{4t^2} \right) \right\}
\]

(Eq. 5.11)

Physical meanings of all other parameters have been discussed earlier and are tabulated in Table 5.5 for a quick reference. MATLAB, a programming software, was used to fit equation 5.11 to the experimental data and returned good agreement (\( R^2 = 0.999 \)). Table 5.5 lists the values of all parameters determined by the model while Figure 5.8 shows the plot of the model in comparison with the experimental release profile.

From Figure 5.8, it is obvious that PCL exhibited an initial burst of up to 24% in the first 2 days. This observation tallies with the model parameters which yielded, in Table 5.5, the fraction of drug release, \( \phi_{b,\text{PCL}} \), of 0.22, the burst constant, \( k_{b,\text{PCL}} \), of 25 day\(^{-1} \) and the end of burst release, \( t_{b,\text{PCL}} \), at day 2.48.
Diffusion is the main governing mechanism that controlled paclitaxel release from PCL. The model was used to determine paclitaxel diffusion coefficient through PCL matrix, reported for the first time in literature, and was found to be $8.10 \times 10^{-11}$ cm$^2$/s. Paclitaxel diffusional release from PCL proceeded relatively fast until release reached completion at around day 30. This somewhat easy diffusion agrees well with literature finding which indicates that PCL is permeable to lipophilic drugs such as steroids [122]. As such, paclitaxel, being lipophilic too, did not face much difficulty to diffuse through PCL.
Table 5.5  Models parameters of paclitaxel release from PCL, PLGA and PLGA/PCL blend films

<table>
<thead>
<tr>
<th>Parameters determined by the model</th>
<th>Description</th>
<th>Unit</th>
<th>Pure PCL</th>
<th>Pure PLGA</th>
<th>PLGA/PCL 50/50</th>
<th>PLGA/PCL 55/45</th>
</tr>
</thead>
<tbody>
<tr>
<td>fPCL,PLGA</td>
<td>fraction of drug released from PLGA phase</td>
<td>-</td>
<td>0</td>
<td>0.169</td>
<td>0.199</td>
<td></td>
</tr>
<tr>
<td>fPCL,PLGA</td>
<td>fraction of drug released from PCL phase</td>
<td>-</td>
<td>0.22</td>
<td>0.45</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td>DpCL</td>
<td>drug diffusion coefficient in PCL phase</td>
<td>cm²/s</td>
<td>8.10x10⁻¹²</td>
<td>1.99x10⁻¹¹</td>
<td>1.21x10⁻¹²</td>
<td></td>
</tr>
<tr>
<td>kPCL</td>
<td>burst constant of PCL phase</td>
<td>day⁻¹</td>
<td>25.00</td>
<td>27.8</td>
<td>24.40</td>
<td></td>
</tr>
<tr>
<td>tPCL</td>
<td>end of burst release from PCL phase</td>
<td>days</td>
<td>2.48</td>
<td>1.08</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td>DpPLGA</td>
<td>drug diffusion coefficient in PLGA phase</td>
<td>cm²/s</td>
<td>6.29x10⁻¹²</td>
<td>8.03x10⁻¹²</td>
<td>8.10x10⁻¹²</td>
<td></td>
</tr>
<tr>
<td>kPPLGA</td>
<td>burst constant of PLGA phase</td>
<td>day⁻¹</td>
<td>0.10</td>
<td>0.26</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>tPPLGA</td>
<td>end of burst release from PLGA phase</td>
<td>days</td>
<td>20.16</td>
<td>19.43</td>
<td>18.77</td>
<td></td>
</tr>
<tr>
<td>tPPLGA</td>
<td>end of relaxation rel from PLGA phase</td>
<td>days</td>
<td>46.66</td>
<td>46.02</td>
<td>46.00</td>
<td></td>
</tr>
<tr>
<td>rPLGA</td>
<td>relaxation release coeff of PLGA phase</td>
<td>-</td>
<td>0.04</td>
<td>0.058</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td>Goodness of fit</td>
<td>R²</td>
<td>correlation factor</td>
<td>-</td>
<td>0.999</td>
<td>0.995</td>
<td>0.998</td>
</tr>
<tr>
<td>Calculation of partition coefficient</td>
<td>K</td>
<td>Partition coefficient of drug, [PCL]/[PLGA]</td>
<td>-</td>
<td>-</td>
<td>4.917</td>
<td>4.889</td>
</tr>
</tbody>
</table>

Finally, it is worth mentioning that it is possible to illustrate drug release from PCL with a diffusion equation alone, i.e using only the second term on the right-hand side of eq. 5.11. An equally good match was obtained ($R^2=0.994$) and the diffusion coefficient, $D_{PCL}$, was found to be equal to $8.45x10^{-12}$ cm²/s. This value is very close to the one tabulated in Table 5.5. Nevertheless, to develop the drug release model for blended polymer systems in section 5.4.3, eq. 5.11 will be used as it is more complete and readily accommodates burst when it is required.
5.4.2 Modeling of drug release from P(DL)LGA film

Paclitaxel release from P(DL)LGA 53/47 films have been discussed in section 5.3.2. To differentiate P(DL)LGA release from PCL release in the development of blend model, equation 5.10 is re-written with "PLGA" subscript as equation 5.12. Its model parameters are summarized in Table 5.5 along with the values obtained from the 80 μm thick film loaded with 1% paclitaxel.

\[
\left\{ \frac{M_t}{M_{\infty}} \right\} = \phi_{b,PLGA}\{1-\exp(-k_b,PLGA t)\} + \phi_{r,PLGA}\{\exp[k_r,PLGA(t-t_{b,PLGA})]-1\} + \\
\phi_{s,PLGA}\left[1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2}\exp\left(-\frac{D_{PLGA}(2n+1)^2\pi^2(t-t_{b,PLGA})}{4\ell^2}\right)\right]
\]

(Eq. 5.12)

5.4.3 Modeling of drug release from blends of P(DL)LGA/PCL_y films

Given low (or non-) miscibility of PCL and P(DL)LGA, it is reasonable to consider the blend system as consisting of PCL rich and PLGA rich phases. In fact, Figure 5.9 shows the SEM pictures of P(DL)LGA/PCL blend films at the start of release study and 4 weeks later. It is obvious that two separate phases were formed and as expected, the PLGA-rich phase degraded much faster than PCL-rich phase after immersion in release medium.
Figure 5.9  SEM pictures of P(DL)LGA/PCL 60/40 blend films prior to immersion (a) and after 28 days of immersion in release medium (b and c)
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Therefore, a "heuristic" approach has been considered whereby it is postulated that drug partitions into either phase and remains in that particular phase until it is released. Further, the release from each phase follows the same mechanism of its respective unblended state. The overall fraction of drug release is a summation of drug released from PCL phase and PLGA phase [121].

Based on the assumptions above, the following model was developed for paclitaxel release from blend of PLGA/PCL films.

\[
\left\{ \frac{M_t}{M_{\text{blend}}} \right\} = f_{pCL} \left\{ \frac{M_t}{M_{\text{PCL}}} \right\} + f_{PLGA} \left\{ \frac{M_t}{M_{\text{PLGA}}} \right\} \quad \text{(Eq. 5.13)}
\]

Here, \( f_{pCL} \) and \( f_{PLGA} \) are the fractions of drug that partition into and are released from PCL and PLGA phases respectively. The sum of the two fractions is equal to 1 (\( f_{PLGA} + f_{pCL} = 1 \)). Substituting equations (5.11) and (5.12) into equation (5.13) gave us the following extended equation for paclitaxel release from blend of PLGA and PCL.

\[
\left\{ \frac{M_t}{M_{\text{blend}}} \right\} = f_{pCL} \left\{ \frac{M_t}{M_{\text{PCL}}} \right\} + f_{PLGA} \left\{ \frac{M_t}{M_{\text{PLGA}}} \right\} 
\begin{align*}
&\quad \phi_{pCL} \{1 - \exp(-k_{pCL}t)\} + \phi_{pCL} \left\{ \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left[ -D_{pCL}(2n+1)^2 \pi^2 (t-t_{b,pCL}) \right] \right\} \\
&\quad + f_{PLGA} \left\{ \phi_{PLGA} \{1 - \exp(-k_{PLGA}t)\} + \phi_{PLGA} \left\{ \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} \exp \left[ -D_{PLGA}(2n+1)^2 \pi^2 (t-t_{b,PLGA}) \right] \right\} \right\} \\
&\quad \text{(Eq. 5.14)}
\end{align*}
\]
Again, MATLAB was used to match the proposed model, equation 5.14, with in vitro release profiles obtained from two blend systems: PLGA/PCL 50/50 and PLGA/PCL 55/45. Excellent correlation factors were obtained for both compositions ($R^2$ values > 0.99). Figure 5.10 shows the agreement of model and experimental data while Table 5.5 lists all the parameters used and determined by the model in equation 5.14.

![Paclitaxel release from PLGA/PCL 50/50](image)

*Note: Each data point is an average of three repeated experiments (n=3)*

**Figure 5.10** Model and experimental data matching of paclitaxel release from PLGA/PCL 50/50 blend film
Figure 5.10 clearly shows a combined/additive effect of release mechanisms from both PCL and PLGA. PCL helped to contribute to drug release in the early days and hence eliminated the long induction period, characteristic of PLGA alone. On the other hand, PLGA helped to sustain drug release to longer time, up to 70 days, as opposed to only 30 days in PCL alone.

Further, Table 5.5 shows that the values of model parameters that control drug release from PCL phase of the blend are very similar to those given by the unblended PCL film. Burst constant $k_{b,PCL}$, end of burst release $t_{b,PCL}$, and diffusion coefficient $D_{PCL}$ of blends were consistent with those of PCL alone. Similarly, the values of model parameters that control drug release from PLGA phase of the blends did not change much from the values of the unblended PLGA film. These include all parameters (see Table 5.5) that dictate three release steps from PLGA: burst, relaxation-induced drug dissolution and diffusion. This consistency shows that there is no major shift/change in the release mechanism from each polymer phase before and after blending – as postulated prior to model development.

The only notable difference is a little increase in fraction of burst of the unblended PCL from $\phi_{b, PCL} = 0.22$ to $\phi_{b, PCL} = 0.39$ for fraction of burst of PCL phase in the blends. It was caused by unequal drug partition in PLGA and PCL phases of blend which led to higher effective drug loading (>1%) in PCL phase of blend than drug loading in the unblended PCL (1%). Drug partition also holds a very crucial role in determining the fractions of drug release from each phase, $f_{PLGA}$ and $f_{PCL}$.
Partition coefficient is an important parameter that specifically affects drug release profile from a blend system. It is the ratio of concentrations of the drug in the two phases of a mixture of immiscible matrices. Partition coefficient shows the drug distribution and its preference for either of the two phases in the blend system.

Partition coefficient can be derived from \( f_{\text{PCL}} \) as follows.

\[
f_{\text{PCL}} = \frac{\text{amount of drug in PCL}}{\text{amount of drug in PCL} + \text{amount of drug in PLGA}} \quad (\text{Eq. 5.15})
\]

Given that the specific gravities of PCL and PLGA are quite similar (\( \rho_{\text{PCL}} = 1.15 \text{ g/ml} \), and \( \rho_{\text{PLGA}} = 1.25 \text{ g/ml} \)),

\[
f_{\text{PCL}} = \frac{W_{\text{PCL}} \left[ \text{PCL} \right]}{W_{\text{PCL}} \left[ \text{PCL} \right] + W_{\text{PLGA}} \left[ \text{PLGA} \right]} \quad (\text{Eq. 5.16})
\]

\( W_{\text{PCL}} \) and \( W_{\text{PLGA}} \) are the weight fractions of PCL and PLGA phases respectively whereas \( \left[ \text{PCL} \right] \) and \( \left[ \text{PLGA} \right] \) are the concentrations of drug in PCL and PLGA phases respectively.

By definition, partition coefficient \( K = \left[ \text{PCL} \right]/\left[ \text{PLGA} \right] \), thus

\[
f_{\text{PCL}} = \frac{W_{\text{PCL}} K}{W_{\text{PCL}} K + W_{\text{PLGA}}} \quad (\text{Eq. 5.17})
\]
From Table 5.5, the blend model in equation 5.14 gives $f_{PCL}$ (PLGA/PCL 50/50) of 0.831. Using equation 5.17, the partition coefficient $K$ was calculated to be 4.92. When the same modeling and calculation was done for another blend composition (PLGA/PCL 55/45), the partition coefficient $K$ obtained was 4.89, very close to the first one. This finding was expected as partition coefficient is an intrinsic parameter of a constant value regardless of the ratio of the blend components. As such, this constant $K$-value and excellent correlation coefficients confirm that our "heuristic-based" model is suitable for this blend system.

To further investigate the applicability of this blend model, the same partition coefficient $K$ value was plugged back to equation 5.14 and used to predict the release profiles of other blend ratios. At the same time, several other blend films were prepared and their in-vitro release profiles were recorded. Comparisons between the model and actual experimental data returned good matches for PLGA/PCL 60/40 and PLGA/PCL 65/35 blend films ($R^2 \geq 0.99$), as demonstrated in Figure 5.11.
Modeling of Drug Release

Figure 5.11  Model predictions and experimental data of paclitaxel release from (a) PLGA/PCL 60/40 and (b) PLGA/PCL 65/35 blend films

Note: Each data point is an average of three repeated experiments (n=3)
However, the experimental data deviated from the model for PLGA/PCL 75/25 blend film as seen in Figure 5.12. The blend model works by the assumption that the total release is a sum of drug release contributions from two phases. The drug from each phase is released through interconnected paths of its own phase across the film. When the weight fraction of one component is reduced considerably, it is expected that the minor component will assume the forms of isolated islets within the major phase. The interconnectivity of the minor phase is thus lost. Therefore, drug release from the minor phase, in this case PCL phase, would be disrupted. Thus in Figure 5.12, it can be seen that the actual drug release was substantially less than that predicted by our model.

Figure 5.12 Model prediction and experimental data of paclitaxel release from PLGA/PCL 75/25 blend film

Note: Each data point is an average of three repeated experiments (n=3)
A similar theory, called percolation theory [123], agrees with our finding and has reported that the critical fraction to obtain undisrupted continuous path in any blend systems is approximately 20%-25%. Therefore, it can be concluded that the model deviation from actual data is caused mainly by the inability of minor component (PCL) to form established interconnected paths.
CHAPTER 6

IMPACT OF VARIOUS RELEASE KINETICS ON THE INHIBITION OF SMOOTH MUSCLE CELLS GROWTH

6.1 Introduction

Chapter 2 presents the history and evolution of coronary stents in the field of interventional cardiology. Restenosis, however, remains the top challenge to date. Despite the recent success of drug eluting stents, there is very little literature on the role of (paclitaxel) release kinetics on smooth muscle cells proliferation – the main culprit of restenosis [69].

Only one stent design has allowed for variation of paclitaxel release rate: the strut-filled design developed by Conor MedSystems Inc [124]. In a clinical trial involving 6 different paclitaxel formulations [125], whose overall paclitaxel release duration was varied from 10 to 30 days, it was found that the slower-releasing formulation had a bigger effect on reducing neo-intimal hyperplasia. Thus there was a hint that release kinetics played a role in preventing hyperplasia, but the range of release rates studied was narrow.

In this work, a broader range of release rates and doses of paclitaxel was studied from degradable polymer systems. The tailorable release kinetics has been described
extensively in section 4.3. This study focuses on investigating which release profile, i.e. fast or moderate or slow release, works best in preventing smooth muscle cell growth. Hopefully, the results can shed some light and provide an early indication on the most suitable release kinetics to combat restenosis via drug therapy.

6.2 Overview of Various Paclitaxel Release Profiles

Three distinct paclitaxel release profiles were chosen from section 4.3.2 and tested on human coronary smooth muscle cells in-vitro. Figure 6.1 summarizes the selected release profiles.

All the films in Figure 6.1 carried the same amount of paclitaxel (same doses) but varied in the rate of paclitaxel elution. PLGA exhibited an extremely slow release rate but sustained the release up to 80 days. PLGA/PEG had a moderate release rate and required about 45 days to reach completion. Lastly, PCL showed the fastest release rate by releasing its entire dose within 30 days. Detailed explanations on the release mechanism of each film can be found in section 4.3.2.

The efficacies of the release kinetics of these three films (fast, moderate and slow) on inhibiting smooth muscle cells proliferation were investigated via in-vitro cell count and proliferation assays.
Figure 6.1 Various paclitaxel release profiles selected for smooth muscle cells proliferation assays

All samples were sterilized with ethanol/water 70/30 (v/v) at 25°C for 15 minutes prior to incubation with smooth muscle cells. This relatively mild sterilization condition did not alter the release profiles of the films. Less than 1% drug loss was observed for slowest releasing film (PLGA) while only about 1.5% drug was lost from moderate releasing film (PLGA-PEG). More substantial drug loss of about 13% was suffered by the fastest releasing film PCL. All data were measured as an average of three repeated samples. Nevertheless, as suggested by our separate residual release study, no significant changes occurred to the proposed release patterns as shown in Figure 6.1.
6.3 Cell Count and Proliferation Assays

The cell cycle, or cell-division cycle, is the series of events that take place in a eukaryotic cell leading to its replication. Figure 6.2 shows the schematic diagram of a cell cycle.

![Schematic diagram of a cell cycle](image)

G0 refers to the quiescence state of “post-mitotic” cells. Cells in G0 stage are non-proliferative and do not participate in cell division / cell cycle. When stimulated by some triggering events, they re-enter the cell cycle at G1 stage and thus restart the cell proliferation. G1 is the phase that is marked by synthesis of various enzymes required in the S phase, mainly those needed for DNA replication. S-phase starts when DNA synthesis commences and completes when all chromosomes have been replicated. During
Cellular Response on Various Release Kinetics

this phase, the amount of DNA in the cell is effectively doubled. The cell then enters the G2 phase which involves synthesis of proteins for the production of microtubules, required during the process of mitosis. Finally, in the M (mitotic) phase, the cell undergoes nuclear and cytoplasmic division and splits itself into two distinct cells, often called “daughter cells”.

Paclitaxel inhibits cell proliferation by enhancing the stability of the microtubules, i.e polymers of α- and β-tubulin dimers. In actual fact, polymerization and de-polymerization of microtubules provide the pulling force necessary for mitotic spindle to separate two chromatids of a dividing cell into two daughter cells. Therefore, paclitaxel inhibits cell proliferation by disrupting the G2/M phase of the cell cycle.

To study the inhibitory effect of different paclitaxel release kinetics on smooth muscle cells proliferation, a number of samples were prepared (see Table 6.1). In total, seven combinations were prepared in triplicates for every time point. The first three samples were made of P(DL)LGA 53/47, PLGA/PEG 90/10 and PCL. They were loaded with paclitaxel and displayed the slow, medium and fast release profiles as mentioned in the previous section. Samples 4 to 6 were blank/unloaded films, prepared from exactly the same polymers as samples 1 to 3, to make up the controls. Lastly, a set of triplicate wells without any films serve as a reference. Two different dosages were investigated, i.e sets A and B with 4 and 0.4 μg paclitaxel per sample respectively.
Table 6.1  List of polymer films and combinations investigated with proliferation assays

<table>
<thead>
<tr>
<th>No.</th>
<th>Abbreviation</th>
<th>Polymer Matrix</th>
<th>Drug dosage (µg)</th>
<th>Set A</th>
<th>Set B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PLGA-PTX</td>
<td>P(DL)LG A 53/47</td>
<td>4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>PLGAPEG-PTX</td>
<td>P(DL)LG A + 10wt% PEG</td>
<td>4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>PCL-PTX</td>
<td>PCL</td>
<td>4</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PLGA-ctrl</td>
<td>P(DL)LG A 53/47</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>PLGAPEG-ctrl</td>
<td>P(DL)LG A + 10wt% PEG</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>PCL-ctrl</td>
<td>PCL</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Reference</td>
<td>No polymer film</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

6.3.1 Inhibition of SMCs growth by paclitaxel-eluting films loaded with higher dose

Figure 6.3 shows the cell count results by WST-8 assay of cells subjected to films grouped under set A, i.e with higher paclitaxel dose. WST absorbance value is directly proportional to the number of viable cells, which means higher WST value translates to larger amount of viable cells.

Cell proliferation was marked by BrdU incorporation during DNA synthesis in the S-phase of a cell cycle. The results are shown in Figure 6.4, whereby higher BrdU absorbance reflects greater DNA synthesis activities and cell proliferation.
Cellular Response on Various Release Kinetics

**Figure 6.3**  WST-8 assay of paclitaxel-eluting films loaded with 4 µg dose

**Figure 6.4**  BrdU assay of paclitaxel-eluting films loaded with 4 µg dose
WST-8 assay in Figure 6.3 demonstrated excellent and prolonged cell growth inhibition by PLGA-PTX, PLGAPEG-PTX and PCL-PTX films at all time points. No statistically significant difference (p-value>0.05, Table 6.2) in WST-8 absorbance was observed among these three paclitaxel eluting films. This finding is emphasized by equally minimum DNA synthesis activities (p-value>0.05, Table 6.2) by PLGA-PTX, PLGAPEG-PTX and PCL-PTX films as shown in Figure 6.4.

Table 6.2 Statistical analysis test results (ANOVA’s p-values) on WST-8 and BrdU assays subjected to set A films

On the other hand, cell proliferation was not inhibited in wells treated with the PLGA-ctrl, PLGAPEG-ctrl, and PCL-ctrl films. No significantly different cellular response among the three control films was observed on both WST-8 and BrdU incorporation assays (p-values>0.05, see Table 6.2). However, in comparison with the reference wells, i.e without any films, the cell growth in control wells was slightly delayed.
For set A films, the ratio of paclitaxel dose (4 µg) in each drug-eluting film over cell seeding density (20,000 cells) was designed to be about four to seven times lower than that of TAXUS™ stent [126-128]. The TAXUS™ stent contains 85~140 µg drug per 15-mm long stent, contacting an estimated 1.41x10^5 cells (treatment area of 1.41 cm^2).

Figures 6.3 and 6.4 clearly demonstrate that PLGA-PTX, PLGAPEG-PTX and PCL-PTX films exhibit equal excellent inhibition to hCASMCs proliferation even though the amount of paclitaxel release varied from as low as 0.45% (0.03 µM) to as high as 24% (1.64 µM) on the first day. This result indicates that a small amount of paclitaxel (at concentration as low as 0.03 µM) is enough to stop hCASMCs proliferation cycle. This result is in agreement with some literature reports that the minimum effective concentrations needed to achieve 90% and 50% inhibition in human aortic SMCs are 0.01 µM and 0.005 µM respectively [58] and 0.005 µM to get 60% inhibition in breast normal cells [129].

This finding also implies that high loading (>100 µg) as in TAXUS™ stent may be redundant to prevent hCASMCs proliferation. In actual fact, it has been reported that only 10% to 25% of the total dose in TAXUS™ stents was released and contributed to cell proliferation inhibition [126-128]. The remaining 75% to 90% remained trapped in the stent and could be wasted. Another work reported remarkable suppression of restenosis in New Zealand rabbits after administering paclitaxel via double balloon catheter [130]. Though the dose used was about 85 µg, only 2 to 3% was successfully delivered to the
vessel wall. Nevertheless, this low amount of paclitaxel proved to be effective to prevent restenosis. In addition, in a 4-month clinical trial, the Conor MedSystems study referred to previously [125] showed efficacy with a 10 µg dose of paclitaxel, delivered over 30 days. Our findings (Figures 6.3 and 6.4), combined with these reports, reiterate paclitaxel’s potency and suggest that smaller dose could be chosen to obtain similar efficacy and avoid wastage.

6.3.2 Inhibition of SMCs growth by paclitaxel-eluting films loaded with lower dose

Too high paclitaxel concentrations (\text{Conc}_{\text{ext}} \gg \text{Conc}_{\text{effective}}) in the previous study masked the effect of paclitaxel release kinetics on hCASMCs proliferation. Therefore, another set of experiments were carried out using set B films, whose paclitaxel dose was ten times lower than that of set A films (see Table 6.1).

Figures 6.5 and 6.6 show hCASMCs viability and proliferation in response to set B films (i.e with lower paclitaxel dose), monitored by WST-8 and BrdU assays respectively. Despite their lower dose, PLGA-PTX, PLGAPEG-PTX and PCL-PTX films still exhibited inhibition to cell growth and proliferation when compared to the controls. However, from day 7 onwards, the cell count assay in Figure 6.5 revealed different extent of inhibition depending on film’s release rate (p-value<0.05, Table 6.3). In line with this observation, higher DNA synthesis activities were noted on days 7 to 11 in wells containing slowest releasing PLGA-PTX film (see Figure 6.6).
Cellular Response on Various Release Kinetics

Figure 6.5  WST-8 assay of paclitaxel-eluting films loaded with 0.4 μg dose

Figure 6.6  BrdU assay of paclitaxel-eluting films loaded with 0.4 μg dose
Table 6.3 Statistical analysis test results (ANOVA's p-values) on proliferation assays subjected to set B films

<table>
<thead>
<tr>
<th>Time points (days)</th>
<th>Paclitaxel-eluting films (PLGA-PTX, PLGAPEG-PTX and PCL-PTX)</th>
<th>Control films (PLGA-ctrl, PLGAPEG-ctrl and PCL-ctrl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WST-8 assay</td>
<td>BrdU assay</td>
</tr>
<tr>
<td>2</td>
<td>0.578</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>0.516</td>
<td>0.427</td>
</tr>
<tr>
<td>7</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>9</td>
<td>0.009</td>
<td>0.002</td>
</tr>
<tr>
<td>11</td>
<td>0.009</td>
<td>0.014</td>
</tr>
<tr>
<td>14</td>
<td>&lt;0.001</td>
<td>0.092</td>
</tr>
<tr>
<td>17</td>
<td>&lt;0.001</td>
<td>0.29</td>
</tr>
</tbody>
</table>

When paclitaxel dose was reduced to 0.4 μg, as in set B films, we started to observe some effect of release kinetics on hCASMCs proliferation from day 7 onwards (see Figure 6.5). WST-8 assay shows that PCL-PTX film had the strongest inhibition effect, followed by PLGAPEG-PTX film and lastly PLGA-PTX film. PLGA-PTX film suffered a temporary loss of inhibitory effect from day 4 to day 7 and hence allowed the cell count to increase. The inhibition was restored afterwards and cell count maintained at the same level as day 7, except for a small but statistically insignificant increment at day 11 (p-value>0.05, t-test between days 9 and 11). The corresponding BrdU assay (Figure 6.6) complements the WST-8 results as it showed slightly more active DNA synthesis in PLGA-PTX wells from day 7 to 11.
PLGAPEG-PTX film had complete inhibitory action throughout the 17-day study period as shown by constant cell count and low DNA synthesis activities (Figures 6.5 and 6.6). PCL-PTX film had similar inhibitory action as that of PLGAPEG-PTX film up to day 11. Significant reduction in cell count of PCL-PTX wells was observed at day 14 and dropped further at day 17. This reduction in cell count could be due to cell death (necrosis) as a result of prolonged exposure to high concentration of paclitaxel – a phenomenon that has been previously investigated. Yeung et al. reported that paclitaxel inhibits cell proliferation by inducing apoptosis at low concentration (0.005 ~ 0.05 μM) and necrosis at high concentration, especially at concentration > 5 μM [129].

To sum up the discussion above, the merits and shortcomings of each release kinetics [104] will be briefly highlighted here. Fast release kinetics (represented by PCL-PTX) with its strong 20% burst effect assures the earliest full inhibition of hCASMCs proliferation immediately after the stenting procedure. The inhibitory effect can be sustained for 17 days (as tested) or even longer. However, as paclitaxel is very potent, it risks releasing too high a drug concentration which causes hCASMCs death (necrosis). It should also be noted that too high local paclitaxel concentration (leading to local toxicity) may result in undesirable hindrance to endothelial cell growth. Endothelial cells growth and proliferation are very crucial in recovering the damaged anti-thrombotic lining of artery (re-endothelialization) and overall healing process. In fact it has been reported that drug-eluting stents have caused delayed healing to a certain extent and faced a late thrombotic risk due to incomplete re-endothelialization [131].
The moderate release kinetics (represented by PLGAPEG-PTX) has demonstrated excellent inhibition of hCASMCs proliferation while mitigating the necrosis danger by working at lower (released) drug concentrations. Throughout the 17-day period of study, hCASMCs proliferation was suppressed at all times. As such, this release profile appears to be the best choice within this period of study. Moreover, drug release is sustained for up to 45 days which may be sufficient to prevent restenosis, as the paclitaxel effect has been reported to be long-lasting [41]. However, with this study alone, we cannot rule out the possibility that 45 days is insufficient. Besides, the steep accelerated second stage release may induce late-stage necrosis risk (day 20–30).

The slow release kinetics (represented by PLGA-PTX) displayed a fair inhibition to hCASMCs proliferation. Insufficient drug release and marginally low paclitaxel concentration in the early days caused temporary loss of inhibitory effect and allowed some degree of cell proliferation. Hence, in this 17-day study, slow kinetics seems to be a less attractive choice. In the long term, however, this slow release kinetics may be a good candidate as it is able to provide continuous supply of drug for up to 3 months. Besides, its second stage release that shows gentler slope over wider timeframe can potentially reduce late toxicity risk.

On the basis of this study, however, it appears to us that the moderate release profile (represented by PLGAPEG-PTX) is the best choice provided the duration can be pushed
to about 60 days. Of course this conclusion needs to be proven with longer-term (>6 months) study in animal and/or clinical trials. The clinical trial of Conor MedSystems stents mentioned previously [125] certainly hints that this hypothesis may have validity, as it was found that (at 4 months) the lowest in-stent late loss was obtained from the formulation with longest-duration of release regardless of the initial dose.
CHAPTER 7
CONCLUSIONS AND FUTURE WORK

7.1 Conclusions

7.1.1 Manipulation of drug release from degradable matrices (films/stents)

(a) Paclitaxel release

Paclitaxel release from P(DL)LGA 53/47 film exhibited a three-stage release pattern: (i) extremely slow initial release up to day 25, (ii) accelerated degradative, relaxation-induced release from day 25 to day 48, and (iii) diffusion controlled release up to saturation at day 80. Drug loadings (1\% and 3\%) as well as thickness (0.03 mm and 0.08 mm) generally did not affect the shapes of the paclitaxel release profiles. No burst effects were observed even at 3\% drug loading; the drug appeared soluble at this loading.

The three stages of the release are related to changes in the matrix, as confirmed by weight loss, water uptake and molecular weight measurements. The first stage release can be considered as an induction period as there was only very little drug release due to near zero water penetration. In the second stage, P(DL)LGA 53/47 degradation transformed the initially glassy matrix into somewhat porous. The release rate was controlled by how fast
the polymer chains relax to create more free volume. In the last stage, diffusion through water-filled pores became the key mechanism.

In the study of paclitaxel release from bilayer films and stents, it was found that addition of PLLA bottom layer did not significantly alter the release pattern and the three-stage release was maintained. However, about 20–25% paclitaxel from the top P(DL)LGA layer partitioned into the PLLA bottom layer and remained in PLLA layer even after 90 days of immersion in release medium. As drug release profiles from a bilayer film and a bilayer stent are almost identical, it can be concluded that the stent-making process has no impact on drug release. In other words, the in-vitro drug release studies from stents can be adequately represented with studies from planar films.

Paclitaxel release profile was successfully tailored by modifying the matrix composition. Introduction of 10 wt% PEG in P(DL)LGA film increased water absorption and accelerated the polymer degradation. As a result, the induction period was halved and the release completed within 45 days. When the base polymer of the matrix was changed to PCL, a common single stage release pattern was obtained. Paclitaxel release started with a huge initial burst and then controlled by diffusion until drug exhaustion at day 30. Blending P(DL)LGA and PCL resulted in a unique, intermediate release profile that sees the complementary / additive effect of PCL’s fast diffusion and P(DL)LGA’s sustained release.
(b) Heparin release

Heparin release from PLLA films were marked with massive initial burst of up to 45% of the total drug loading. After which, contributions from a very slow diffusion led to only 70% cumulative release at the end of 80-day release study. The remaining 30% remained “trapped” in the PLLA film.

This poor control of heparin release from PLLA film was greatly improved by the addition of 10 wt% high Mw PEO. PEO is a hydrophilic polymer and hence will interact more favorably with heparin than hydrophobic PLLA. As a result, PLLA/PEO 90/10 managed to reduce the initial burst significantly, from 42% to 26%. Subsequently, it displayed a zero-order release rate until day 60. Due to drug exhaustion, release subsequently slowed down and reached completion at day 80. At the end of the release study, almost all drug particles were released and hence no drug was wasted as seen in the case of pure PLLA.

Further investigation of heparin release was extended to the bilayer film configuration with PLLA/PEO 90/10 as the bottom layer and blank P(DL)LGA as the top layer. As P(DL)LGA is a much faster degrading polymer compared to PLLA, it did not cause much hindrance to heparin release. Apart from slightly lower burst, the release pattern was largely maintained, including the characteristic zero-order release rate.
7.1.2 Elucidation of release mechanisms and modeling of drug release from degradable polymers/blends

Two models were proposed for drug release from poly(DL-lactide-co-glycolide) 53/47 films: a two-step model and a three-step model. The two-step model consists of burst release and diffusion controlled release. This model is most suitable for release of hydrophilic drugs such as metoclopramide salt from a hydrophobic, glassy matrix. Further, it is important to note that (meto) salt release from biodegradable polymers has a feature of a relatively quick onset of diffusion, prior to substantial degradation. This was possible as burst release of the salt particles created water-filled pores that served as a favorable diffusion path. Nevertheless, degradation contributed in creating micro-pores that enhanced the pores connectivity and supported drug diffusion in the later days. This seemingly less significant role of degradation in the overall salt release process contrasts with the case of hydrophobic drug release.

The three-step model was deveoped for release of hydrophobic drugs such as paclitaxel from a hydrophobic, glassy matrix. The release model consists of three stages of release: burst release, relaxation induced drug dissolution controlled release and finally diffusion controlled release. Polymer matrix relaxation phenomenon, induced by matrix degradation, plays an important role in controlling the rate of drug dissolution especially when the drug has strong affinity for the polymer.
Lastly, a novel “heuristic” model was proposed for drug release from blend of low (or non-) miscible polymers, represented by P(DL)LGA and PCL. The overall fraction of drug release is a summation of drug released from PCL-rich phase and PLGA-rich phase. The crucial parameter (partition coefficient K) that specifically controls drug distribution and release from such blend system was also determined. The applicability of this blend model has been tested and proven effective on a range of P(DL)LGA/PCL ratios. However, a deviation was observed when minor component’s fraction became so low that its interconnected phase, acting as drug release avenue, was disrupted.

### 7.1.3 Application and usefulness of controlled-release in vascular stents

Chapter 6 clearly demonstrates the usefulness of controlled-release of paclitaxel in coronary stents application. Three different paclitaxel release kinetics, obtained from prior in-vitro release studies, were tested on their efficacies in inhibiting smooth muscle cells proliferation. Fast, moderate and slow release profiles were given by PCL-PTX, PLGAPEG-PTX and PLGA-PTX films respectively.

When the dose administered is such that paclitaxel concentration is well above the minimum effective concentration, the effect of release kinetics on inhibiting cell proliferation was masked and not obvious. Further investigation with lower dose revealed different cells’ responses to different release kinetics.
Conclusions and Future Work

Fast releasing film gave perfect inhibition immediately but could pose the danger of local toxicity. Slow releasing film suffered a brief loss of inhibitory action in early days but may be beneficial in the later days given its sustainable release of up to 3 months. This is especially promising if the dose is adjusted upwards to cover the deficiency in early days. Based on this study, however, moderate releasing film appeared to be the best choice to obtain full inhibitory effect at reduced risk.

7.1.4 Summary

In the first part of the project, at least four different paclitaxel release profiles were obtained through simple modification of matrix compositions. On the other hand, heparin release was carefully adjusted such that a zero-order rate was achieved. These findings have clearly demonstrated that paclitaxel and heparin release rates can be controlled and programmed. In the second part, novel models have been proposed to address bi-phasic and tri-phasic release profiles from biodegradable systems. A heuristic model was also developed for drug release from blends of degradable polymers. All the models demonstrated good agreement with the in-vitro release data. In the third part, the merits and shortcomings of fast, moderate and slow release kinetics on inhibiting smooth muscle cells proliferation was outlined. It was suggested that for treatment of restenosis in vascular stents application, moderate release kinetics was most efficacious and posed lower toxicity risks. In short, it can be concluded that the results and conclusions have clearly met all the objectives outlined in the opening chapter.

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7.2 Future Work

The results and knowledge presented in this thesis will help set directions for the future work that can be conducted. To further substantiate the research work that has been conducted in this project, the following studies could be done in the future.

(1) The effect of paclitaxel release kinetics on smooth muscle cells migration

Restenosis is mainly caused by excessive neo-intimal formation, which is largely made up of smooth muscle cells. These SMCs originate from the media and migrate to the neo-intima where they continue to grow and multiply. Therefore, apart from inhibiting SMCs proliferation, preventing SMCs migration from the media into the neo-intima is equally important. Some cell motion is enabled by the cytoskeleton through microtubule polymerization and depolymerization. As paclitaxel inhibits depolymerization of microtubules, it can inhibit cell migration too. Therefore, it would be of great interest to determine the optimum paclitaxel release kinetics to prevent or minimize SMCs migration.
(2) The effect of paclitaxel release kinetics on endothelial cells growth and attachment

The response of smooth muscle cells to various paclitaxel releasing films were conducted in this study. The results re-affirm literature finding that paclitaxel is indeed very potent. However, too high concentration of paclitaxel may unfavorably inhibit endothelial cells growth and attachment, leading to delayed re-endothelialization. In severe cases, it may cause late restenosis and other complications. Thus, it is very important to carefully adjust paclitaxel doses, investigate and select the best release kinetics that inhibits smooth muscle cells proliferation without adversely compromises endothelialization process.

(3) The effect of heparin release kinetics on platelet attachment

For all blood contacting devices including coronary stents, it is imperative to take measures to prevent thrombosis/blood cloting. That is why in our work, heparin (anti-thrombotic drug) was released from the blood-contacting PLLA layer of the bi-layer stent. Thus, it is very beneficial to find out the effect of heparin release kinetics on preventing platelet attachment and thrombosis and suggest the most efficacious one.

(4) In-vivo study of the multi-drug eluting biodegradable coronary stent
Conclusions and Future Work

The results of in-vitro multiple drug release study were reported in this thesis. Although the experimental procedures of the in-vitro study have been designed to mimic the in-vivo situation by maintaining sink condition, using body temperature 37°C and phosphate buffer saline at body pH 7.4, etc, an in-vivo release study is required to verify the results.

Furthermore, in this work, the efficacies of different paclitaxel release kinetics on smooth muscle cells proliferation were tested in-vitro for 17 days. The results obtained have to be verified with a longer-term study in animal and/or clinical trials. This in-vivo study will provide a more thorough investigation on the long-term efficacies in preventing restenosis after several months. Besides, inflammatory and immunogenic reactions (if any) as well as toxicity of degradation products can be observed.

(5) Mechanical tests of bi-layer degradable stents made of “alternative” top layers

The in-vitro release study was started with P(DL)LGA 53/47 top layer as the paclitaxel-eluting layer. However, its release kinetics was found to be too slow. In line with the attempt to modify and/or accelerate paclitaxel release rate, several other formulations, including addition of PEG and blending with PCL, were developed. Therefore, it is important to investigate the suitability of these formulations for stent applications. Further tests such as collapse pressure and stent expansion should be conducted.
Conclusions and Future Work

(6) Refinement of the proposed drug release models

The models proposed in this thesis were developed by recognizing the most important and determining factors such as drug desorption, diffusion, degradative relaxation and partition coefficient. Nevertheless, drug release from biodegradable systems is a complex problem that involves many other factors. Therefore, it will be advantageous to improve and refine the models by incorporating other key parameters such as polymer initial molecular weight, crystallinity, etc.

(7) Extension of the drug release models for macromolecules (protein) release and systems of different shapes and double-layer configurations

The mathematical models proposed in this work cater to the release of small drug molecules from bulk-degrading polymer films (planar systems). It would be of great interest to extend the applicability of the models to macromolecules release and other systems such as microspheres, nanospheres, cylinders, capsules, etc by modifying the dimensional parameters accordingly.

In addition, improving the model such that it can be used to predict release from a bi-layer polymer matrix will be a significant contribution. A careful study should be conducted on the interface between the two layers, drug partitioning between the top and bottom layer, rate of delamination, cross-diffusion across different layers, etc.
References


References


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References


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