The degradation and drug-eluting properties of biodegradable polymers and their potential use as coatings for coronary stents.

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Abstract

The introduction in 2002 of the first drug-eluting stents (DES) revolutionized interventional cardiology and their use led to a dramatic reduction in the rates of in-stent restenosis (ISR) and the need for subsequent revascularization procedures as compared with bare metal stents and balloon angioplasty. Stents provide mechanical support to maintain an open lumen of the damaged vessel and enable local drug delivery to the area of vascular injury, thereby averting the need to deliver the high doses of drug required if they were to be delivered systemically. However the long term residency of the polymeric coating formulations currently used and the fact that most of the anti-restenotic agents remain in the polymer has raised some concerns regarding late stent thrombosis, delayed arterial healing (which may be an unintended effect of the anti-restenotic agents used on the stents) and hypersensitivity reactions to the polymers.

Biodegradable polymers with shorter residency times may help reduce the long term undesirable effects and facilitate the release of active agents over a more controlled and desirable profile. In this study the degradation and paclitaxel elution profile of two biodegradable polymers, a tyrosine containing polyarylate termed TyRx P22-10 and poly($_{D,L}$-lactide-*co*-glycolide) (PLGA) coated onto coronary stents was determined in an acellular *in vivo* model using gel permeation chromatography (GPC), mass loss, scanning electron (SEM) and light microscopy (LM) as the analytical tools.

The presence of paclitaxel had no significant effect on the degradation rate of TyRx and 50:50 PLGA. TyRx degraded rapidly over a period of 120 days from an original molecular weight (MWt) of approximately 59,000 to around 9,000. By 150 days the MWt of the polymer had degraded to around 5,000 after which degradation proceeded more slowly. Degradation of PLGA was faster than TyRx and was dependant on the GA:LA (glycolide:lactide) ratio with higher GA content leading to faster degradation rates. PLGA coatings degraded to a MWt of around 12,000 in 30, 45 and 90 days in 50:50, 75:25 and 85:15 PLGA respectively after which the rate of degradation decreased markedly. At a critical MWt (approximately 5,000 and 12,000 for TyRx and PLGA respectively) the polymer chains become soluble in the aqueous media and polymer erosion occurs, leading to bulk mass loss from the polymer matrix. As polymer mass loss progressed visual deterioration in the stent coatings became evident with the creation of pits and pores creating a more porous structure within the stent coating eventually leading to the exposure of the bare metal of the stent as the bulk of the polymer coating eroded. Complete solubilisation of the TyRx coating had not occurred by the end of the study period and was in excess of 280 days. Complete solubilisation of 50:50 and 75:25 PLGA had occurred by 45 and 105 days respectively, while traces of the 85:15 PLGA coating could still be detected after 120 days

The elution of the paclitaxel was linked to the degradation state of the polymer and occurred over two distinct phases: a diffusion controlled stage (lag phase) in which the drug slowly diffused out of the polymer matrix, this coincided with hydrolytic degradation of the polymer and was followed by an erosion controlled stage of more rapid release associated with mass loss of the polymer and the appearance of a more porous structure of the stent coating which was visible using SEM. The onset of polymer erosion was slow in the TyRx coatings due to the low water solubility of the degraded monomers resulting in a lag phase of around 250 days before there was any significant amount of paclitaxel release. The onset of polymer erosion was faster in the PLGA coatings resulting in a shorter lag phase in paclitaxel elution.

Paclitaxel release was fastest from a 200μg 50:50 PLGA coating with a minimal lag phase. The paclitaxel elution profile was similar for all the 400μg PLGA coatings but occurred over different time scales dependant on the GA content of the polymer. Elution of paclitaxel was fastest in the 50:50 PLGA coating and slowest in the 85:15 PLGA coating. There was a lag phase in paclitaxel release of 15, 30 and 60 days in the 50:50, 75:25 and 85:15 PLGA respectively and complete elution of the drug had occurred by 45, 75 and 120 days in the 50:50, 75:25 and 85:15 PLGA coatings respectively.

The drug release profile of PLGA devices has been modified by blending PLGA with low molecular weight, hydrophilic, water soluble copolymers or blending different PLGA copolymers. The degradation and drug release profile of PLGA was further studied *in vitro* by blending a hydrophilic low molecular weight pluronic or 50:50 PLGA into 85:15 PLGA films coated onto PTFE discs. In this study blending hydrophilic pluronics had no effect on the degradation rate or release of paclitaxel from 50:50 and 85:15 PLGA films. But the onset of polymer erosion and the duration of the lag phase in paclitaxel release from 85:15 PLGA films was reduced by blending 50:50 PLGA into the films. Blending 50:50 PLGA with a low molecular weight of around 6,000 resulted in increased rates of mass loss and further reductions in the length of the lag phase in paclitaxel release and could be considered a viable method to modify 85:15 PLGA for use as a coronary stent coating.

Clearly there is significant potential to develop endovascular stents to augment the physical physiological role of these devices to prevent ISR using coatings which either provide significant benefits directly by promoting the natural healing of the endothelium and/or indirectly by delivering molecules that can control the surrounding tissues in which they are dwelling.

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Glossary

BMS	Bare metal stent
Da	Dalton
DES	Drug-eluting stent
DTE	Desaminotyrosyl –L-tyrosine ethyl ester
DTH	Desaminotyrosyl –L-tyrosine hexyl ester
DTR	Desaminotyrosyl –L-tyrosine ester
DTO	Desaminotyrosyl –L-tyrosine octyl ester
FGF	Fibroblast growth factor
FR	Fast release
GA	Glycolic acid
GPC	Gel permeation chromatography
ISR	In-stent restenosis
kDa	Kilodalton
LA	Lactic acid
LM	Light microscopy
LMWt	Low molecular weight
LST	Late stent thrombosis
MACE	Major adverse cardiac events
MI	Myocardial infarction
M_n	Weight number molecular weight
M_p	Peak molecular weight
M_w	Weight average molecular weight
MR	Medium release
MWt	Molecular weight
PBS	Phosphate buffered saline
PCI	Percutaneous coronary intervention
PDI	Polydispersity index
PDGF	Platelet derived growth factor
PEG	Polyethylene glycol
PEO	Poly(ethylene oxide)
PLGA	Poly($_{D,L}$-lactide-*co*-glycolide)
PLLA	Poly($_{L}$-lactic acid)
PMMA	Polymethyl methacrylate
PPO	Poly(propylene oxide)
SEM	Scanning electron microscopy
SMC	Smooth muscle cells
SR	Slow release
ST	Stent thrombosis
TLR	Target lesion revascularization
T_g	Glass transition temperature
THF	Tetrahydrofuran
TyRx	Tyrosine polyarylate P22-10
VSMC	Vascular smooth muscle cells
w/w	Weight to weight ratio

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Introduction

1.1 Introduction and purpose of the study.

Coronary stents are small expandable wire mesh devices shaped in a tube that are used to unblock diseased arteries in the heart and peripheral vessels [1, 2]. Stenting is now the most common form of interventional treatment for symptomatic coronary artery disease [1]. But the effectiveness of the procedure may be compromised due to cellular in-growth (in-stent restenosis -ISR) into the lumen of the vessel through and around the stent struts causing re-occlusion of the artery following the injury caused by placement of the stent [1, 3]. Drug-eluting stents (DES), in which an anti-restenotic agent or drug is released from a polymeric coating on the stent, provides a mechanism of site specific drug delivery to the site of injury [4] and have been demonstrated to have significant advantages as compared to bare metal stents (BMS) in reducing ISR and loss of luminal capacity [5-7]. However, recent reports of late stent thrombosis (LST) and delayed arterial healing in patients receiving DES has raised concerns about the safety of DES and the non-degradable polymer coatings that are currently used and the fact that most of the anti-restenotic agents remain sequestered in the durable polymer coating in some stents [8-14].

The dosage and the release kinetics of the drugs needs to be optimized to avoid the potential risks associated with DES [15]. The action of the drugs used in DES not only inhibit neointima proliferation but also delay the process of re-endothelialisation and arterial healing of the stented section which may be a cause of late stent thrombosis (LST) [16]. The optimal dose and elution profile for paclitaxel DES has not yet been identified but it would be such that sufficient drug is delivered to prevent proliferation of smooth muscle cells but without affecting the re-endotheliazation process [16].

Drug delivery from coronary stents has become an important area of research for scientists, cardiologists and industry to address the issues with currently available DES [16, 17]. Biocompatible, biodegradable polymers have a number of advantages for drug delivery from medical devices [18] and complete and uneventful degradation of biodegradable polymers with few toxic effects from their degradation products and

which facilitate controlled delivery of the drug may present a way to improve the performance of DES. Complete dissolution of the polymer may help reduce any hypersensitivity effects associated with the polymer coating and avoid any possible long term consequences due to disruption and deterioration to the integrity of durable polymer coatings, while complete elution of the contained drug may reduce any concerns regarding the long term impact that may be associated with its long term presence in the stent [12, 19].

In this research project the degradation and paclitaxel elution properties of two biocompatible, biodegradable polymers, namely a tyrosine derived polyarylates called TyRx™ and poly($_{D,L}$-lactide-*co*-glycolide) (PLGA) was determined to assess their potential as polymer coatings for paclitaxel DES. Adequate drug release properties as well as blood and tissue biocompatibility are amongst the requirements of a polymer coating for DES [20]. Tyrosine polyarylates have been shown to have good biocompatibility [21] and show a diffusion controlled release mechanism of low molecular weight drugs [22] and have been used for the release of water soluble peptides [23] and anticoagulants [24] cited by [25]. PLGA are aliphatic biodegradable polymers that have been used in a number of applications for sustained drug-release [26] and have excellent biocompatibility and are considered safe [27] cited by [28] and [29, 30] cited by [26]. Due to their biocompatibility, biodegradability and drug delivery properties, TyRx and PLGA were considered as suitable candidates to test the hypothesis that controlled drug delivery and complete polymer dissolution can be achieved using coronary stents coated with biodegradable polymers.

1.2 Coronary stents: development, advantages and limitations.

1.2.1 Development of drug-eluting stents

The term 'stent' was originally used by Charles R Stent to describe a curved device which was used as a scaffold for oral skin grafts [2]. Stents have also been described as devices or moulds that are used to hold a skin graft in place or to provide support for tubular structures that are being anastomosed (Dorlands Medical Dictionary cited by [2]). While coronary stents are probably the best known and most common form of this device there are many other applications of such devices and they have been

used in other natural body conduits, such as central and peripheral arteries and veins, bile ducts, oesophagus, colon, trachea or large bronchi, ureters, and urethra [2].

In late 1970 Andreas Gruentzig and A. Senning described a catheter based method for treatment of obstructive coronary atherosclerosis which they termed percutaneous transluminal coronary angioplasty (PCTA) [31] cited by [32]. Problems with such procedures occurred due to vascular recoil of the artery and in particular blockage of the vessel due to proliferation of smooth muscle cells (restenosis) and accumulation of intercellular matrix [20]. In 1987 Sigwart *et al* [33] used bare metal stents in the arteries of patients who had coronary or coronary–bypass graft surgery and were having complications due to restenosis or abrupt closure [32]. The bare metal stents (BMS) were superior in giving mechanical support to the damaged vessel, acting as a scaffold to keep the vessel open and reduced the incidence of vessel recoil [4]. The rate of restenosis was reduced with the introduction of BMS as compared to balloon angioplasty (PCI) [20, 33] and two randomised trials demonstrated that use of bare metal stents reduced the incidence of restenosis as compared to balloon angioplasty (32-42% for balloon angioplasty as compared with 22-32% for BMS [34, 35] cited by [32]). But ISR was a major problem in 20-30% of BMS devices leading to dramatic loss of luminal capacity [20].

1.2.2 Restenosis and in-stent restenosis (ISR).

Restenosis has been a major problem associated with vascular procedures such as balloon angioplasty and stenting [36]. Restenosis is the arterial healing response after injury to the vessel wall during transluminal coronary revascularization and results in loss of lumen diameter of the repaired vessel [36]. Injury occurs during balloon angioplasty as the balloon is inflated (sometimes several times) to compress the plaque and so unblock the artery. Similarly, injury occurs during stenting. Prior to use, the stent is in its collapsed form attached onto the outside of a balloon catheter. Placement of the stent involves inserting the catheter into an artery usually via the wrist or groin, which is then advanced to the site of the diseased artery [3]. Once in place at the site of vessel occlusion the balloon is inflated, expanding the stent which compresses the plaque thereby opening up the artery. The balloon is then deflated and the

catheter withdrawn leaving the stent in- situ acting as a scaffold and support to maintain an open lumen of the artery [3].

Stretching of the vessel caused by the high pressures exerted when the balloon is inflated during placement of the stent causes mechanical injury causing rupture of the elastic lamina, intimal and/or medial tears, endothelial denudation and crushing of the occluding plaque [37-40]. Following this trauma there is a maladaptive response by the coronary artery leading to a sequence of events consisting of thrombosis, inflammation, cellular proliferation, and extracellular matrix production which result in a loss of lumen capacity post-operatively over approximately 6 months [41, 42]. Loss of lumen diameter following balloon angioplasty consists of three phases: early loss due to elastic recoil, late loss due to negative remodelling and neointimal hyperplasia [36, 42]. Endovascular stents act as a mechanical scaffold and virtually eliminate vessel recoil and reduce lumen loss due to remodelling [42, 43]. There are however differences between the inflammatory response associated with balloon angioplasty and that induced by the injury caused during stent implantation [44] and the presence of the stent may amplify the proliferative component of restenosis [36, 42].

ISR is solely caused by neointimal hyperplasia. Mechanical injury caused by stent placement and the foreign body response to the presence of the stent, incite acute and chronic inflammation in the vessel wall [45]. The processes leading to ISR have been described previously [36-40, 44, 45].

Within minutes of the injury to the endothelium of the vessel, platelet rich thrombus deposition begins at the site of injury and this is rapidly and simultaneously followed by activation and aggregation of platelets which along with fibrin, forms clots at the site of injury on and around the stent struts [38, 40]. Thrombus formation occurs within 1-3 days after stenting injury but platelet deposition and thrombus formation may persist for 2-4 weeks after stent placement [45]. Activated platelets express adhesion molecules such as P-selectin and glycoprotein (GP) Ibα which attach to circulating leukocytes via platelet receptors such as P-selectin glycoprotein ligand. The loosely attached leukocytes then begin to migrate along the injured surface, facilitated by a gradient of chemoattractant cytokines called chemokines which are released by the smooth muscle cells (SMC) and resident macrophages. Cytokines

stimulate the leukocytes to become more tightly bound to the platelets via leukocyte integrin adhesion molecules, such as Mac-1, by attaching directly to platelet receptors such as GP Ibα and via cross linking with fibrinogen to the GP IIb/IIIa receptors [38, 39].

Prior to injury the SMC are quiescent, but mechanical injury and the subsequent release of growth factors induce a change from a contractile to a synthetic phenotype [36, 39] and a phase of cellular proliferation commences. Following activation of platelets and thrombus formation, vasoactive agents and mitogens such as thrombin, thromboxane A2 and platelet derived growth factor (PDGF) are released from SMC, platelets and inflammatory cells. [46, 47] cited by [37]. This induces the proliferation and migration of SMC's from the media and into the neointima, resulting in a neointima consisting of SMC's, extracellular matrix and macrophages. Formation of this neointima occurs over several weeks after injury, but over longer periods of time there is increased production of extracellular matrix with fewer cellular elements [38, 39]. Thrombin induces proliferation in SMC and the secretion of platelet derived growth factor (PDGF) and fibroblast growth factor (FGF). Thrombin may lead to intimal hyperplasia via induction of PDGF and FGF production by SMC. Accordingly, thrombin-dependent VSMC proliferation and migration may contribute to restenosis after stent deployment [45]. Serotonin is another growth factor released during platelet activation and thrombus formation. Clot bound thrombin may potentiate the mitogenic effect of serotonin and thereby prolong the period of VSMC proliferation [45].

Inflammatory cells have subsequently been found to have a critical role in ISR and the presence of the stent induces a more prolonged and intense inflammatory response [38]. Acute inflammation occurs within 24 hours of stent placement. Infiltration of the vessel wall by neutrophils is followed by adhesion and infiltration of monocytes. Farb *et al* [46] found that the severity of the inflammatory response was associated with the contact between the stent struts and the vessel wall, with increased numbers of acute inflammatory cells produced when the stent struts were adjacent to the injured media or lipid core as opposed to the struts pressing on the fibrous plaque. Infiltration of chronic inflammatory cells e.g. leukocytes, histiocytes and giant cells occurs around the stent struts and produce cytokines such as inter-

leukin-1 and tumour necrosis factor (TNF) which further stimulate the proliferation and migration of VSMC [45].

1.2.3 Drug-eluting stents (DES).

DES were designed to provide the mechanical support offered by the BMS but with the capacity to deliver anti-restenotic drugs from a polymer coating (drug delivery system) to combat some of the problems associated with BMS by preventing ISR [20]. DES essentially consist of the stent platform, the drug or active agent and a coating (normally a polymer) which controls the release of the active agent [12]. Proliferation and migration of Vascular Smooth Muscle Cells (VSMC) and extracellular matrix deposition can be significantly reduced by passivating the metal surfaces of the stent using polymer coatings [48] and adding slowly released anti-restenotic drugs to the polymer coatings can further reduce in-stent restenosis [36]. Delivery of the anti-restenotic agent via the DES facilitates drug release at the site of vessel injury at concentrations sufficient to prevent cell proliferation and migration but not at high enough concentrations to cause toxicity in surrounding tissues and organs [48] thereby avoiding the use of high concentrations of the drugs (which may have cytotoxic effects) that would be required were they administered systemically [20].

Drug-eluting stents (DES), have been demonstrated to have significant advantages as compared to Bare Metal Stents (BMS) in reducing neointimal hyperplasia, ISR and loss of luminal capacity [5-7] and the reduction in ISR seen with the use of DES as compared to BMS has in turn reduced the need for subsequent revascularization procedures [49, 50]. Over 1.5 million patients worldwide are now treated using metallic coronary stents per year. Polymer coated stents containing drugs such as paclitaxel or sirolimus are increasingly used in preference to bare metal stents (BMS) [11] and about 85% of the percutaneous coronary interventions (PCI) in the US are now done using drug eluting stents (DES) [8, 11, 13]. In the USA in 2006 the market for coronary stents was worth some $5 billion and DES accounted for about 90% of the revenue [7].

1.2.4 Efficacy of DES

Both the Cypher and the Taxus DES have been shown to significantly reduce rates of ISR and angiographic events as compared with BMS [51]. Eisenberg and Konnyu [6] reviewed randomised controlled trials comparing paclitaxel or sirolimus (or its derivatives) DES's to BMS and DES's to other DES's published in the PubMed database and found a definite therapeutic advantage associated with sirolimus and polymeric paclitaxel coated stents in comparison to BMS. A number of combinations of agents, polymer and stent platforms have been developed for clinical use [20, 52] but only two DES have been shown to have both a beneficial effect and a suitable safety profile in large–scale randomised clinical trials, namely the Cypher™ stent consisting of a durable polymer coating and the drug sirolimus (rapamycin) and the Taxus™ paclitaxel eluting stent which is coated with a non-biodegradable durable polymer [52]. The Taxus™ Express$^{2™}$ stent is coated with a styrene isobutylenestyrene triblock copolymer (SIBS) called Translute™ and contains 1μg/mm^2 paclitaxel [53] which is equivalent to 108μg on a 16mm Express Stent [12]. Two formulations of the Express stent have been used in clinical practice (and Taxus IV-VI clinical trials): a slow release formulation (SR) and a medium (MR) release formulation. The SR and MR stents contain 8.8% w/w and 25% w/w paclitaxel respectively but with the same dose density of 1μg/mm^2 paclitaxel [4]. The NIRx™ – paclitaxel/SIBS-coated coronary stent (Boston Scientific, USA) was used in the Taxus I to III trials in SR and MR formulations (1.0 μg/mm^2 loaded drug/stent surface area; total dose 85 μg per stent) [52, 54].

The safety and efficacy of the Taxus DES was evaluated in randomised double blind multicenter investigations comparing 6 studies (Taxus I-VI) in comparison with BMS (nb except for Taxus III which was an open-label investigation) and the results have been reviewed elsewhere [4, 6, 52, 54]. The safety of the Taxus DES was demonstrated in the Taxus I trial which compared MACE (major adverse cardiac events) rates between the Taxus (SR formulation) stent and BMS and found that at 12 months the MACE rate was 3% and 10% in the DES and BMS groups respectively with no patients requiring target lesion revascularization (TLR) in the Taxus arm of the trial. The Taxus II study demonstrated lower rates of target vessel revascularisation and restenosis in patients receiving either SR or MR formulations of paclitaxel

as compared with controls (patients receiving BMS). At 12 months the MACE rate was 10.9%, 9.9% and 21.7% for the SR, MR formulations and BMS respectively with TLR of 4.7%, 3.8% and 14.4% respectively. Additionally there was no significant difference between the SR and MR formulations in efficacy or safety in this trial. The Taxus II study involved patients with standard risk, de novo coronary lesions. The Taxus IV trial, which was larger than Taxus II, was designed to evaluate the Taxus SR stent in a broad spectrum of patients and lesion subsets to more closely reflect 'real life' situations than had been used in previous trials. Patients enrolled in previous trials had had focal lesions in larger vessels, but in Taxus IV the patients had either symptomatic artery disease, objective evidence of ischemia and the target lesions were solitary de novo lesions 10-28mm in length occurring in vessels with a diameter of 2.5-3.75mm. This trial confirmed the results from previous trials and demonstrated lower rates of restenosis, TLR and MACE for the Taxus SR stent as compared with BMS with the benefit being maintained at two years. In Taxus V and VI the Taxus SR and MR stent formulation were evaluated in higher risk patient populations, defined as those requiring 2.25 – 4.00mm stents or vessels with longer lesions requiring overlapping stents. In these trials, the TLR rate was significantly lower in the Taxus SR and MR groups as compared with the BMS control group at 9 months. Furthermore there were less MACE events, lower rates of restenosis in patients receiving the Taxus stents as compared with BMS and there was no significant difference in rates of death, myocardial infarction and stent thrombosis (which was low in all groups).

There have been seven randomised controlled trials designed to evaluate the efficacy and safety of the sirolimus DES (Cypher stent) [6]. The early trials demonstrated the safety and efficacy of sirolimus DES in reducing TLR, significantly reducing rates of restenosis with similar rates of death and myocardial infarction as patients receiving BMS in a population of lower risk patients. The improvements in restenosis rates and TLR were seen in trials involving more complex lesions and in patients with diabetes. The random controlled trials have consistently demonstrated the sirolimus DES capacity to reduce or prevent ISR and to significantly reduce the need for subsequent TLR procedures for up to one year, while having a safety profile comparable to that of BMS [6].

1.2.5 Late stent thrombosis and delayed endothelial healing

Despite the success of stenting some concerns remain and recent reports concerning late events including increased stent thrombosis, myocardial infarction and death have called into question the long term safety of DES and may be a consequence of delayed arterial healing associated with DES [7, 13, 49, 50]. The most likely causes for this affect appear to be either the presence of the durable polymer causing an inflammatory response, and/or an unintended effect of the anti-restenotic agents used in DES [7, 15, 49, 55]. Although there are no reported toxic side-effects related to the use of paclitaxel after 6 or 12 months, concern has been raised about the long-term biological effect of the non-erodable polymer used on the Taxus stent as well as the persistence of significant quantities of drug still present in the polymer at 30 days; 92.5% for the slow release formulation or 78.1% for the moderate release [12] and only 10% and 25% respectively of the paclitaxel is released in the slow-release and moderate-release formulations of the polymer [54, 56].

The possibility that late complications leading to excess stent thrombosis in DES was raised in 2003-2004 [54], an infrequent but nevertheless catastrophic complication that may be manifest as myocardial infarction (MI) or sudden death [57]. McFadden *et al* [11] reported 4 cases (2 paclitaxel and 2 sirolimus DES) of stent thrombosis (ST) occurring 2-15 months after stenting and these cases were considered unusual since late ST was rare with BMS [58]. The term 'late ST' generally refers to ST occurring at least 1 month following implantation, whereas events occurring more than 12 months following implantation are referred to as 'very late ST' [59]. Subacute ST (1 to 30 days after implantation) rates have been found to be <1.0% using BMS [58] and randomised trials using large datasets have shown similar rates of ST occurring up to 30 days after stenting between BMS and DES (for review see [57, 58]).

Studies using data from registries, randomised trials and meta-analyses which have investigated the relative risk of stent thrombosis from BMS and DES over varying follow-up periods have yielded conflicting results [57]. Follow-up of up to one year from randomised trials did not show any significant increased risk of late thrombosis due to DES as compared with BMS in which patients were also taking combined therapy of clopidrogel and aspirin to prevent clotting [60] cited by [9] and [61]. Mo-

reno *et al* [61] found no difference in stent thrombosis between either paclitaxel and sirolimus DES or between DES and BMS provided that appropriate anti-platelet therapy is applied. But the authors found that stent length increased the rate of stent thrombosis and longer stents were of the DES type. Mauri *et al* [62] found no significant difference for Sirolimus DES compared with BMS. Spaulding *et al* [63] found no significant difference in death rate, stent thrombosis or myocardial infarction between Sirolimus and Paclitaxel DES as compared with BMS but in patients with diabetes a significant advantage was found in patients receiving BMS.

But incidences of late stent thrombosis have been reported [11, 64, 65]. Stone *et al* [50] found that after 1 year stent thrombosis in patients receiving either Sirolimus DES or Paclitaxel DES was significantly higher as compared to patients receiving BMS but that over four years follow up there was no significant difference in the cumulative rates of death or myocardial infarction between DES and BMS. Pooled analysis of the RAVEL, SIRIUS, C-SIRIUS, and E-SIRIUS trials showed that between 1 and 4 years after implantation there were five cases of late thrombosis with sirolimus DES as opposed to no cases in the BMS group. While pooled data from the Taxus II, IV, V and VI revealed eight cases of late thrombosis from the DES group as opposed to one case from the BMS group [66] cited by [67], although the survival free time from stent thrombosis at three years was not significantly different for the DES versus the BMS [59].

The BASKET-LATE trial, in which patients receiving either a DES or BMS and had stopped anti-platelet therapy 6 months after stenting, showed that the incidence of late cardiac death or non-fatal myocardial infarction was greater for DES compared with BMS (4.9% and 1.3% respectively). Given that the DES still showed an advantage in reducing ISR, late clinical events, possibly related to late stent thrombosis, were cited as likely causes for the effect [68]. Bavry *et al* [69] performed a meta-analyses on 14 contemporary clinical trials comparing paclitaxel and sirolimus DES with BMS and found 5 events per 1000 of late stent thrombosis in patients receiving DES with 2.8 events in the BMS group. Based on the type of DES there was 3.5 and 4.9 events per 1000 patients for sirolimus DES and BMS respectively and 6.3 and 1.1 events per 1000 patients for paclitaxel DES and BMS respectively. Daeman *et al* [67] found early and late stent thrombosis occurred with both types of DES, but late

stent thrombosis was more prevalent in patients receiving the paclitaxel DES. Data from the Large Multicenter Registry-Stent Group using 'real world patient populations' showed no significant difference in death, myocardial infarction and target vessel revascularization between sirolimus and paclitaxel DES at 9 months [70]. Using this data Simonton *et al* [70] concluded that clinical restenosis and major adverse cardiac events are infrequent and similar for patients receiving either sirolimus or paclitaxel DES. Stettler *et al* [71] reviewed controlled trials from databases such as Medline, EmBase, CENTRAL and other published studies up to March 2007 using data from patients with symptoms or signs of myocardial ischemia due to coronary artery disease and compared Taxus and Cypher DES and BMS. They concluded that DES and BMS are associated with similar rates of cardiac mortality. Sirolimus DES was associated with a lower rate of myocardial infarction as compared with paclitaxel DES or BMS. There was little evidence of an overall increase in ST using DES as compared with BMS but paclitaxel DES was associated with an increase in LST as compared with sirolimus DES and BMS. Both types of DES showed a marked reduction in target revascularisation rates as compared with BMS.

While there is no significant evidence to suggest that there is greater risk of ST from DES as compared with BMS [58], the incidence of very late stent thrombosis (through years 1 to 4 post implantation) appears to be around 0.2-0.6% higher for DES as compared with BMS [57-59, 67, 69]. Whether this risk continues after year 4 is as yet unknown [59].

The cause of late stent thrombosis is likely to be multifactorial [58, 72-74] involving:

- the stent deployment procedure (sub optimal stent deployment and problems with blood flow through the stent).
- stent design (e.g. strut thickness)
- the patient (discontinuing antiplatelet therapy, resistance to antiplatelet treatment, intrinsic thrombogenicity, type of lesions).
- polymer coating (causing hypersensitivity, inflammation and being thrombogenic).
- anti-restenotic drugs causing delayed healing and possibly increasing the risk of late stent apposition.

In terms of the polymer and the action of the anti-restenotic drugs the DES can affect LST in three main ways

- hypersensitivity to the stent coating
- thrombogenicity caused by the action of the anti-restenotic drugs
- the effect of the anti-restenotic drugs on endothelial healing

1.2.5.1 Polymer coat hypersensitivity effects

An inflammatory response to stent coatings and drug releasing polymers has been shown in animal models and the biomedical polymers currently used on stents incite inflammation to a variable degree that is proportional to the mass of polymer on the coating [75]. The durable polymers currently used on the Taxus and Cypher stents have been associated with chronic eosinophilic infiltration of the arterial wall indicating hypersensitivity reactions in animal studies [76] and in a small number of cases involving humans [74]. Hypersensitivity to metals such as molybdenum, chromium and nickel have been reported but the reaction is different to that associated with DES and the effect has led to restenosis rather thrombosis. Cases of hypersensitivity associated with BMS resulted in infiltration of macrophages, T-lymphocytes with few B-lymphocytes and is not associated with an eosinophil rich infiltrate [13].

In pigs, hypersensitivity reactions start to occur about 28 days after Cypher stent placement, with a gradual increase in the presence of granulatomous reactions, with eosinophilic infiltrate over the following 5 months. Since the hypersensitivity reaction peaks following complete elution of sirolimus from the Cypher stent, the effect is likely related to the polymer coating on the stent [74]. Given that sirolimus has been shown to suppress eosinophil infiltration in an animal model of bronchial hypersensitivity [13] it may be that granulation is retarded initially due to the presence of the sirolimus in the stent. Biodegradable and non-biodegradable polymers when coated onto stents have been shown to induce inflammatory reactions in porcine coronary artery [77] but this study has been criticised as no BMS control stents were used, the stent samples were not sterilized and the degradation of the polymer was not characterized [7].

Virmani *et al* [13] reported a case of severe localized hypersensitivity reaction in response to the polymer coating resulting in myocardial infarction in a patient who had received a Cypher stent. CD45-positive lymphocytes, macrophages, plasma cells and eosinophils were found to have infiltrated into the intima, media and the adventia of the arterial wall at the stented zone. Thick layers of fibrin thrombus were found separating the stent struts from the underlying plaque and there was a focal giant cell reaction surrounding fragments of the polymer coating which had become detached from the stent struts. Virmani *et al* [14] attributed that the presence of a nonreabsorbable polymer alone may have induced the chronic inflammation leading to the persistent fibrin deposition, varying degrees of inflammation and delayed endothelial healing in patients at 12 months after receiving a stent containing a paclitaxel derivative with a polyacrylate sleeve. But giant cells, which are usually seen in reactions to polymers, were not observed in this study.

In 2003 the Food and Drug Administration (FDA) reported a number of cases of hypersensitivity reactions in humans after stent placement but later concluded these were due to concomitantly prescribed medications such as clopidogrel [55]. Nebecker *et al* [55] looked events from FDA's adverse-device-event database, the published literature, and from the Research on Adverse Drug/Device Events And Reports (RADAR) project. Out of 5,783 reports identified for the DES in the FDA database there were 262 hypersensitivity events. Although hypersensitivity to clopidogrel therapy has been reported, only 2 of these cases were caused by clopidogrel and 10 cases were probably caused by the DES. Out of all the sources examined 17 cases of hypersensitivity to the DES were confirmed, with 14 cases being reported for Cypher stent and 3 for the Taxus stent. The Cypher and Taxus DES are coated with different carrier polymers but whether this accounts for the differences in the hypersensitivity between the DES is uncertain. Translute™ (used in the Taxus paclitaxel DES) is a triblock copolymer, poly(styrene-b-isobutylene-b-styrene) [4] while the sirolimus containing Cypher stent is coated with a permanent polymer composed of polyethylene-co-vinyl acetate and poly-*n*-butyl methacrylate [53]. Given that LST due to polymer induced inflammation has only been proven in a minority of cases, it may be that this effect is restricted to those patients who posses a proinflammatory phenotype [74].

1.2.5.2 Prothrombic effect of the drugs

The drugs loaded onto a DES may themselves exert a prothrombogenic effect. At the concentrations found in the arterial cell wall after stent deployment both paclitaxel and sirolimus can increase tissue factor expression. This may contribute to creating a prothrombotic environment [74] since tissue factor expression is a principal activator of the coagulation cascade that activates factors IX and X [78]. Sirolimus binds to FK-binding protein 12 which inhibits the mammalian target of sirolimus (mTOR). mTOR is a downstream target of the phosphatidylinositol-3-kinase pathway which in turn has an inhibitory effect on the regulation of tissue factor in endothelial cells and monocytes. As a result of the inhibition of mTOR there is an increase in thrombin- and tumour necrosis factor (TNF)-α induced endothelial tissue factor expression and activity [74]. Paclitaxel can enhance tissue factor expression and activity in endothelial cells by activating c-Jun NH_2-terminal kinase which is a mediator of endothelial and monocytic tissue factor induction [79, 80]. Additionally, plasminogen activator inhibitor type 1 which is a potent inhibitor of fibrinolysis and a mediator of acute thrombosis is selectively enhanced by paclitaxel and sirolimus [73].

1.2.5.3 Delayed healing

Both paclitaxel and sirolimus are delivered directly into the arterial wall from the stents and due to their lipophilic properties they are readily absorbed and retained by the cells of the vessel [74] where due to their anti-proliferative properties SMC proliferation and migration is inhibited thereby preventing formation of a neointima [79]. But due to the non-specific targeting of anti-restenotic agents such as paclitaxel and sirolimus, the intended anti-restenotic activity of the drugs may have the unintended effect of delaying arterial healing and the process of re-endothelialisation [15, 74, 76]. A coverage of endothelial cells over stent struts and the injured arterial wall is essential for the maintenance of long-term luminal patency as these cells provide essential structural functions [73] and an intact endothelium separates thrombogenic elements in the artery wall, any underlying plaques and the stent struts from the blood stream and secretes antithrombotic and vasodilatory substances [57] such as histamine . The delay in arterial healing with persistent fibrin deposition, inflammation, impaired re-endothelialisation and exposed stent struts are factors which can

increase the likelihood of late stent thrombosis [76] and extends the window during which stents are prone to thrombosis [81].

Following denudation due to stent placement, re-endothelialisation of the damaged artery wall and stent struts occurs by endothelial cell proliferation and migration from intact areas of arterial segments into the adjacent damaged areas of the stented segment in BMS [73, 74]. *In-vitro* studies have shown that paclitaxel and sirolimus suppress endothelial cells and consequently impair the normal healing process of the injured arterial wall [74, 76, 82]. Bone marrow–derived endothelial progenitor cells may also be involved in re-endothelialisation. Sirolimus has been shown to inhibit proliferation, migration and differentiation of human endothelial progenitor cells *in-vitro* [74, 76] while paclitaxel has been shown to inhibit endothelial cells at nanomolar concentrations [76]. Thus the anti-restenotic drugs on the Taxus and Cypher stents may further impede endothelialisation by affecting the number, homing and differentiation of endothelial progenitor cells [74, 76, 80].

Joner *et al* [73] compared re-endothelialisation in a number of DES and BMS in a rabbit iliac artery model. They found significantly reduced endothelial coverage over stent struts in paclitaxel and sirolimus DES as compared with BMS at 14 days (but not at 28 days). Furthermore platelet-endothelial adhesion molecule (PECAM)-1 at cell-to-cell contacts points and thrombomodulin (TM) was reduced in the DES as compared with a BMS. PECAM-1, an endothelial antigen, is critical for endothelial homeostasis and is a transmembrane glycoprotein found in areas of cell-to-cell contact between neighbouring endothelial cells. During periods of cell growth and migration, junctions between cells are poor and PECAM-1 expression is reduced. Endothelial regrowth as assessed by PECAM-1 expression was reduced in DES as compared with BMS at 14 and 28 days indicating inhibition of endothelial cell migration and proliferation, endothelial injury and perhaps evidence of increased rate of cell turnover. Increased vascular endothelial growth factor (VEGF) levels are associated with regenerating endothelial cells and levels peak early after arterial injury and then diminish as healing progresses. Joner *et al* [73] found highest levels of VEGF in DES as compared with BMS at 14 and 28 days which was associated with poor endothelial healing.

TM is a regulator of platelets and coagulation and loss of TM function can cause thrombosis in the arterial and venous circulatory system [83] cited by [73]. Joner *et al* [73] found reduced expression of TM in DES as compared with BMS (which in turn had reduced TM as compared with non-stented segments at 14 and 28 days, indicating a change in endothelial homeostasis to a more prothrombotic state in DES. This change becomes important as the combination of a dysfunctional endothelium combined with increased vessel wall thrombogenicity due to poor endothelial coverage of the injured artery wall and the stent struts may increase the possibility of LST.

Other studies using animal models have shown incomplete healing, poorer rates of endothelialisation and fibrin deposition for DES as compared with BMS [10, 84]. Paclitaxel DES when placed in rabbit iliac arteries were associated with delayed intimal healing with increased local arterial inflammation and fibrin deposition as compared with BMS for up to 180 days by Drachman *et al* [84] using a poly(lactide-*co*-$\sum$-caprolactone) copolymer coated stent containing 200μg paclitaxel. Farb *et al* [10] using a similar model but using stents containing lower doses of paclitaxel (up to 40μg per stent) contained in a chondroitin sulphate/gelatine coating also found delayed arterial healing and local arterial inflammation at 28 days but this effect had disappeared by 90 days. In this study chondroitin sulphate/gelatine coated stents without paclitaxel had lower incidence of intimal haemorrhage and reduced inflammation as compared with the DES, and so the effect was probably due to the action of paclitaxel.

While the process of arterial repair following stent placement occurs at a faster rate in pigs and rabbits than in humans after stent placement the sequence of biological events during the healing process are very similar [73, 76]. In patients receiving BMS endothelialisation is either complete or near complete at 3 to 4 months [81]. Joner *et al* [82] found that in human patients both Cypher and Taxus DES stents had significantly higher fibrin scores and decreased endothelial healing as compared with BMS for a similar duration of implantation. The study also revealed higher fibrin scores and decreased endothelialisation from DES in which late thrombosis had occurred as compared with patent DES. Poor endothelial junction formation with microthrombi of focal platelet aggregation was seen at 16 months in a patient that had received a sirolimus DES [74]. Delayed arterial healing has been blamed for endo-

thelial dysfunction [85] and poorer vasodilation [86] in patients receiving Sirolimus DES. Virmani *et al* [14] in 12 month follow-up studies found evidence of persistent fibrin deposition with varying degrees of inflammation in patients receiving a paclitaxel derivative in a DES, pathological changes that represent delayed healing which are usually observed up to only 3 months in human coronary arteries with stainless steel balloon-expandable stents. Awata *et al* [87] used angioscopy to compare endothelial repair in patients receiving either BMS or sirolimus DES. The study showed that while there was complete neointimal coverage in patients receiving BMS by 3 to 6 months, coverage was poorer in patients receiving DES up to 2 years after implantation and the low grade coverage of the stent struts left the underlying plaques exposed to thrombogenic elements in the blood stream.

1.3 Elution of antirestenotic drugs from polymer stent coatings

1.3.1 Polymers as stent coatings and drug release vehicles

A number of polymers are known to have the potential to act as a drug delivery coating for coronary stents (for review see [88]). The polymer coating on DES acts both as a carrier vehicle and as a means to control the release of the anti-restenotic agent [17].

The polymeric coating needs to fulfil a number of criteria [17, 89] including;

- have good vascular compatibility and have no adverse reactions above those seen with a BMS
- be able to maintain good mechanical integrity during handling, clinical deployment and while in situ
- be able to withstand processing, sterilization and storage
- have suitable drug release properties
- compatibility with the anti-restenotic drug

Polymer coatings are tested for biocompatibility, but polymer delamination and fragments of polymer that have flaked from the stent coating have been found to incite foreign body hypersensitivity reactions [13, 37, 55] (see section 1.5). Further-

more, mechanical integrity of the polymer while in situ is important since release of delaminated polymer fragments have the potential to form microemboli which could be large enough to cause arterial blockage and myocardial infarction [37].

Release of the drug is controlled either by chemical or physical mechanisms [16]. Chemical mechanisms of drug release involve grafting the active drug to the polymer carrier via covalent bonds which are then cleaved by chemical or enzymatic action thereby releasing the active drug [88].

Physical means of controlling drug release include:

- diffusion of drug molecules through a polymer matrix or layer
- dissolution or degradation of the polymer matrix controlling release rate
- osmotic pressure
- ion exchange (for ionised drugs)

Diffusion-controlled drug release and dissolution/degradation-controlled drug release are the most common forms of drug release mechanisms from currently available DES [16].

Permanent or non-erodible polymers are utilised for diffusion control release and these polymers tend to be water insoluble. In this system the drug is dispersed within the polymer coating and the rate at which the drug is released is dependent on the rate at which it can diffuse through the polymer coating. Within the polymer some of the drug particles will be solubilised and dissolve into and saturate the polymer matrix. These molecules can then diffuse through the polymer into the surrounding medium. As saturation of the polymer is lost more of the particulate fraction of the drug can solubilise and dissolve into the polymer matrix and diffuse though the polymer into the release media. As the drug is released pores may be left in the polymer matrix which can fill with the aqueous media from the exterior facilitating further drug release through the pores [88]. Drug molecules closest to the polymer surface and exterior elute the fastest while the molecules further within in the body of the polymer matrix have further to travel to the external environment and hence are released the slowest [16, 37].

The current Taxus stent is an example of diffusion controlled release. The stent is coated with a permanent polymer (poly(styrene-*b*-isobutylene-*b*-styrene) (SIBS) triblock copolymer) called Translute™. By varying the ratio of drug to carrier polymer different rates of drug release are achieved. The three formulations that are available have the same amount of paclitaxel but differ in the amount of polymer coating, with drug-polymer ratios of 35:65, 25:75 and 8.8:91.2 respectively for the fast, medium and slow release formulations [16, 89]. The solubility of paclitaxel is typically 2-5% in hydrocarbon-based polymers [7]. This concentration is exceeded in all the Taxus formulations and the paclitaxel molecules exists as discrete and dispersed particles within the polymer matrix [89]. The elution profile of paclitaxel from SIBS polymer is in the form of an initial burst phase during the first two days after implantation due to the dissolution of paclitaxel available in particulate form at the surface of the polymer followed by a longer lasting phase of slower sustained release [89].

In the Cypher stent poly(ethylene-*co*-vinyl acetate) (PEVA) and poly(n-butyl methylacrylate) (PMBA) are mixed with a ratio of 67% polymer to 33% drug (sirolimus) and this is added to the stent surface. A topcoat of PMBA (with no added drug) is then added which acts as a rate controlling membrane. During storage, drug diffuses into the topcoat and upon stent placement this faction of drug is rapidly released giving an early 'burst' of drug release. The PBMA topcoat minimizes the early burst phase and then controls the kinetics of further drug release as drug molecules have to diffuse from the basecoat of PEVA and PBMA, through the topcoat of PBMA across a diffusion gradient to the external environment (in this case the artery wall) [16].

Other examples of diffusion controlled release include the reservoir system (e.g. the Conor stent) in which wells and reservoirs are formed on the stent, filled with the drug and covered with a thin polymer membrane which controls drug release via diffusion. Rate of drug release is controlled by the concentration gradient between the polymer matrix and the outside of the barrier coating [88].

Biodegradable polymers are examples of dissolution/degradation-controlled systems and release of the drug from the polymer matrix is via diffusion through the polymer

coating and the rate at which this occurs will depend upon the extent of degradation and dissolution of the polymer [88, 90].

The factors governing the elution of drugs from biodegradable polymers has been described by Siepmann and Göpferich [90]. For polymers, biodegradation consists of a number of stages. Initially the polymer becomes hydrated as the aqueous medium enters the polymer bulk, drug dissolution occurs (the extent of which is dependent upon the hydrophilicity/hydrophobicity of the drug) and the drug will begin to diffuse through the polymer matrix. The molecular weight of a polymer and the length of the polymer chains affect the diffusion of the drug through the polymer as small chains offer less resistance to diffusion than do longer chains. The rate of diffusion of the drug at this initial stage is usually slower as the molecular weight of the polymer is higher and the polymer chains longer. There then follows a period of polymer degradation (either via hydrolysis and/or enzymatic reactions) reducing the molecular weight of the polymer chains thereby facilitating faster drug diffusion [90]. Polymer erosion is the process of mass loss from the polymer bulk and occurs when the molecular weight of the polymer chains decrease and at a critical molecular weight the resulting oligomers can solubilise and diffuse out of the polymer matrix. As erosion proceeds there is a decrease in polymer strength and integrity and creation of a more porous structure in the polymer bulk [91, 92]. Polymer erosion increases drug release by carrying along drug molecules with the eroded product and additionally, erosion increases the diffusional space by expanding the pore volume in the polymer matrix thereby accelerating drug release by diffusion [93].

Degradable polymers undergo either surface erosion or bulk erosion and the method by which erosion occurs is dependant on factors such as the rate polymer degradation and the rate at which the aqueous media infuses into the polymer bulk [28, 90]. Surface erosion occurs when the degradation rate of the polymer is faster than water infusion into the polymer bulk resulting in hydrolysis occurring mainly on the outermost surface of the polymer bulk. But, if the rate at which the aqueous media infuses into the polymer is greater than the rate at which the polymer degrades the polymer will undergo bulk erosion. In bulk eroding polymers the entire polymer system rapidly becomes hydrated and degradation occurs throughout the polymer mass [28, 90, 91]. Polymers based on very reactive functional groups tend to degrade fast and are

more likely to be surface eroding polymers, whereas slower degrading polymers tend to be bulk eroding [90]

A number of factors affect the rate of polymer degradation. The speed of hydrolysis is controlled by the type of bonds present in the polymer, the polymer structure and environmental factors [91]. Structural factors affecting the speed of hydrolysis include composition (which determines the hydrophilicity of the matrix), crystallinity, glass transition temperature (T_g), molecular weight and the type of bonds present in the polymer [18, 88, 94]. Environmental factors include pH and the temperature of the release medium [91].

Hydrolytic degradation of a polymer depends on the access of water to the biodegradable bonds in the polymer chains. The hydrophilicity (or hydrophobicity) of a polymer is determined by its composition and is a measure of how readily it is wetted by water [94]. The rate at which water penetrates the polymer matrix affects the degradation rate with hydrophilic polymers tending to degrade faster than hydrophobic polymers [88, 91, 94]. For example, studies have shown that increasing the glycolic acid content of poly(lactide-*co*-glycolide) copolymers of similar molecular weight increases the hydrophilicity of the polymer and consequently the degradation rate of the polymer [95].

The degree of crystallinity of a polymer has implications for both the degradation rate of the polymer and its mechanical properties. Crystallinity of a polymer refers to the degree of structural order within the matrix. Chemically similar chains or chain regions tend to form polymer crystals and the ability of polymer chains to crystallise is dependent on the degree of regularity of their chemical structure [94]. Increasing crystallinity tends to increase the mechanical strength of the polymer but decreases the rate of degradation [88, 94]. Polymer chains within crystalline regions are tightly and regularly ordered whereas the amorphous regions are characterised by lower chain density with greater degree of randomness and free motion of the chains [94]. The higher free volume and greater chain mobility of the amorphous regions of the polymer allows easier access of water (and other agents such as enzymes and free radicals) to hydrolytically vulnerable linkages in the polymer chains resulting in faster degradation as compared with crystalline regions [94].

The glass transition temperature (T_g) is the temperature at which the polymer exhibits a transition between two solid phases [94]. Below the T_g, the polymers are in the glassy state, the chains are much less mobile and the free volume available for diffusion of small molecules like water and drugs within the polymeric matrix is significantly decreased. Above the T_g, polymers are in the rubbery state and this is associated with high mobility of the polymer chains which facilitates easier drug diffusion through the polymer [94, 96]. The T_g of the polymer changes during degradation and consequently the elution of the drug can be orders of magnitude lower in the glassy state as compared with the rubbery state.

Environmental factors such as temperature and pH can have a dramatic effect on the degradation rate of a polymer [18, 94]. A number of studies have shown that increased temperature can increase degradation rate and drug elution rates of polymers. Hakkarainen *et al* [97] showed that degradation and mass loss was faster at 60°C than at 37°C in poly(lactide-*co*-glycolide) (PLGA) and poly($_L$-lactide) (PLLA) polymers. Elevated polymer degradation and drug release with increasing temperature has also been reported in studies by Aso *et al* [98] and Zolnik *et al* [99]. For clinical applications of biomaterials, temperature is of relevance with respect to the T_g of the polymer since polymers with T_g less than 37°C will degrade faster and release their drug load faster than polymers with higher T_g.

Both strongly alkaline and acidic media can accelerate polymer degradation. Studies have shown accelerated degradation of PLLA and PLGA in solutions with pH 5 and pH>9 [18]. For most clinical applications the degradation at pH 7.4 is most relevant and is the pH used for *in vitro* testing of DES. However activated macrophages can create an environment of pH 5 around implanted materials and accelerated degradation has been observed in some PLGA implants at pH 5 at the later stages of degradation [94]. This may have implications for degradation of polymer coatings on DES during the inflammatory response following stent placement in the artery.

In summary, the rate of polymer degradation and drug elution depends on different properties [88]:

- the higher the crystallinity of the polymer the slower the degradation and drug release rate.

- The type of degradable bonds present in the polymer backbone affects the degradation rate. For example in PLGA copolymers the glycolic acid units are more susceptible to hydrolysis than the lactic acid units. Faster degrading polymers have faster drug release rates.
- Hydrophilic polymers degrade faster than hydrophobic polymers
- Polymers with high molecular weight have slower degradation and drug release rates than low molecular weight polymers

1.3.2 Elution and anti-restenotic action of Paclitaxel

Paclitaxel is a natural diterpenoid and can be extracted from the bark of the Pacific Yew, from species including *Taxus brevifola* and *Taxus media* [4, 52]. Paclitaxel works by binding to beta-tubulin thereby stabilising the microtubules and inhibiting the depolymerization of tubulin. Given that microtubules are found in both the cytoplasm and nuclei of cells this action has a number of effects on cellular processes such as cell division and migration, activation, maintenance of cytoskeletal framework and intracellular and transmembrane protein transport [4, 52]. Stabilizing the microtubules blocks the cells ability to break down the mitotic spindle during mitosis therefore inhibiting cell division [19, 39]. At high concentrations paclitaxel is cytotoxic and arrests the cell cycle in the G_2/M phase and promotes apoptosis. At low concentrations paclitaxel arrests the cell cycle in the G_0/G_1 and G_1/G_2 premitotic phase and there is no apparent cytotoxicity, necrosis or apoptosis induction [52]. Due to its high lypophylicity paclitaxel is rapidly taken up by cells and due to the alterations induced in the cytoskeleton its effects are long lasting [100].

Rapamycin inhibits SMC during G1 (growth phase) of the cell cycle thereby preventing production of RNA and protein synthesis. Cells are therefore prevented from entering S phase and replicating. Rapamycin targets (TOR) protein kinase element in the downstream signalling pathway controlling mRNA translation and cell growth [19, 39].

For DES to be effective the active drug must be released by the polymer coating and then enter the media of the arterial wall to its active sites on the SMC where it must be retained at concentrations that can inhibit SMC proliferation and migration [101,

102]. The artery itself comprises three main layers; and innermost layer consisting of a single layer of endothelial cells bounded by a layer of sub-endothelial connective tissue. The middle layer which is mainly composed of SMC concentrically arranged around the lumen is termed the tunica media. The tunica adventitia is the outermost layer and is mainly composed of longitudinally arranged fibroelastic connective tissue. Bands of elastic fibres are located in the outermost part of the intima and media forming the internal and external elastic laminae [103].

Tissue concentration, distribution and clearance of a drug delivered from a stent to the arterial wall are mainly dependant on transport forces such as diffusion and convection, and the physicochemical properties of the drug such as its molecular weight, charge and hydrophobicity. Within the arterial wall drug transport and distribution will be further modulated due to the structure of the artery and effects such as protein binding of the drug and facilitation of movement of the drug via carrier molecules [103].

A pathway of drug transport and clearance from DES was proposed by Yang and Burt [103] and is shown in Figure 1.1. The drug is released from the polymer coating and partitions into intima or the media of the artery and is then transported via passive diffusion and convection through and within the artery tissue. Drug molecules released into the intima will partition into the internal elastic lamina and then into the media. The drug migrates via diffusion through the media to the external elastic lamina where the drug partitions into the external elastic lamina and then into the adventia. Drug can be cleared from the artery via release through the endothelium and into the blood stream or via adventitial clearance through the vasa vasorum, via lymphatic drainage or through loss into connective tissues [103].

The structure of the artery, consisting of layers of smooth muscle cells between sheets of elastin affects drug distribution and transport as the drug may interact with elements within the ultrastructure [104]. Soluble, hydrophilic drugs readily penetrate and diffuse across tissues. Insoluble hydrophobic drugs interact with soluble proteins in the circulatory system and with both fixed and soluble proteins within the artery interstitium [105].

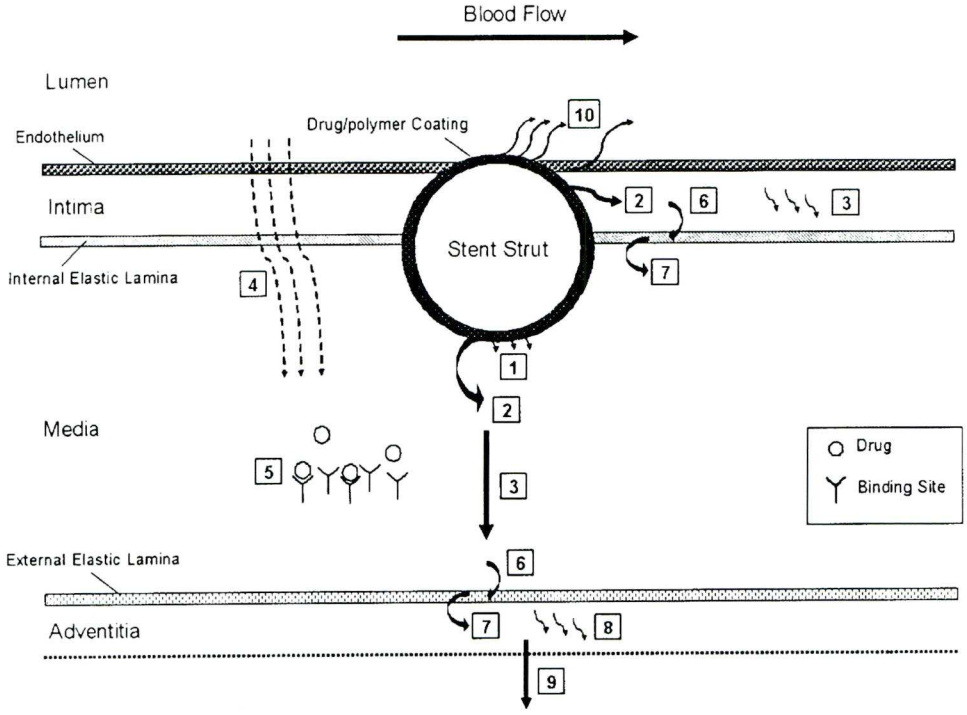

Figure 1.1 Proposed pathway of drug transport and clearance from drug-eluting stents across and within the artery. Taken from Yang and Burt [103]

1. The drug dissolves in the polymer matrix and diffuses through the matrix to be released
2. Drug partitions from the polymer matrix into the intima or the media
3. Drug diffuses down a concentration gradient through the intima or the media
4. Physiologic transmural hydrostatic pressure gradient results in convective transport of drug through the vessel wall tissues
5. Drug binding and interactions with proteins in the vessel wall.
6. Drug partitions from the intima into the internal elastic lamina, or drug partitions from the media to the external elastic lamina
7. The drug partitions from the elastic lamina into the media or the adventitia
8. The drug diffuses through the adventitia
9. Adventitial clearance through the vasa vasorum, lymphatic drainage, loss into connective tissues
10. Drug release into the blood stream or washout from superficial intimal tissues.

Paclitaxel is hydrophobic and insoluble in water but highly soluble in organic solvents and diffuses more slowly through the blood vessel wall than hydrophilic molecules such as dextran or heparin [105, 106] resulting in high arterial retention [101, 104]. Paclitaxel is highly lipophilic and is rapidly taken up by cells as it readily passes through the cell membrane [107]. It combines readily to arterial tissues and binds to fixed tissue elements within the tissue interstitium and also associates with or partitions into hydrophobic structures such as lipid and cellular membranes [105].

In addition to non-specific binding to cellular proteins, paclitaxel also binds specifically to its protein target, polymerized microtubules which possibly explains the uneven distribution of paclitaxel within the artery wall with most of the paclitaxel remaining in the subintimal space and partitioned significantly in the adventitia with higher concentrations of paclitaxel remaining beneath the stent struts long after release [106]. Paclitaxel binds strongly to albumin and albumin can penetrate the arterial wall, especially so when the endothelium is denudated e.g. after stent placement [104].

The interaction of paclitaxel with tissue elements within the artery wall impedes the transport of paclitaxel down its diffusion gradient across the artery as there is competition between forward diffusion of soluble drug and the repeated binding and release of the drug to and from arterial elements resulting in a lower diffusivity and leading to high retention of the drug within the artery [105]. Hence paclitaxel is delivered into the arterial wall, where it is retained for a period of time and is thus able to exert its anti-restenotic effect.

1.4 Paclitaxel release profiles – effect on in-stent restenosis and delayed arterial healing.

The dosage and elution profile of anti-restenotic agents needs to be sufficient to prevent restenosis but not at concentrations that would impair arterial healing and perhaps give rise to late cardiac events [15, 16].

In order for the drug to be efficacious the anti-restenotic agent needs to be released over a minimum period of time. Proliferation of SMC may begin one day after stent deployment and may persist for up to 2 weeks [108] cited by [16] while the genes potentially responsible for proliferation of the SMC are activated for up to 21 days [108] cited by [12]. Consequently delivery of anti-restenotic agents may need to be delivered for at least 3 weeks to prevent proliferation and migration of SMC [16] and possibly for 2-3 months after implantation [7].

For paclitaxel DES differing release profiles have a profound effect on efficacy and indices of injury including fibrin deposition. In the PICES trial on human patients a

Conor stent in which small wells were loaded with varying concentrations of paclitaxel and then covered with PLGA was studied. Release of a 10μg dose of paclitaxel within 10 days showed no significant reduction in neointimal hyperplasia as compared to a BMS while the same dose delivered over 30 days showed a 69% reduction in volume obstruction as compared to BMS [12]. From this study 30μg/30day and 10μg/30day stent loads were found to be effective at reducing ISR and in the EUROSTAR trial the 10μg/30 day load with abluminal unidirectional release of the drug was found to result in lower levels of late lumen loss in human subjects [109]. But in the CoStar trial in which a Conor stent with abluminal unidirectional release of a 10μg/30 day load was compared with a Taxus stent, the 10μg/30 day load was found to be inferior to the Taxus stent at reducing ISR and was no better at reducing ISR than BMS [109]. The reasons for the poorer performance in the CoStar trial as compared with PICES and EUROSTAR trials are unclear but may be due to a more complex patient population in the CoStar trial in which a higher density of clinical events would be expected as compared with the study populations in the previous trials [109].

Kamath *et al* [4] using a porcine artery model found more adverse affects from higher doses of paclitaxel in a fast release formulation and that these effects diminished in a dose-dependant manner as the dose was reduced. A 1μg/mm^2 (paclitaxel/ mm stent area) dose was found to have more vascular compatibility when released in a slow (SR) and medium (MR) rate release formulation than in the fast release formulation. In the TAXUS II study both the SR and MR formulations were equally efficacious at reducing ISR and major adverse cardiac events (MACE) at 12 months follow up as compared to BMS despite there being differences in the amount of paclitaxel eluted from the stents. Over the first ten days there is an 8 fold increase in the amount of paclitaxel delivered from the MR formulation as compared with the SR formulation [56, 89]. The total amount of drug loaded onto the SR and MR TAXUS stent is the same (108μg/16mm stent) [12, 89] with the release profile controlled by the ratio of drug to polymer (8.8% and 25% for SR and MR respectively). But approximately 92.5% and 78.1% of the paclitaxel in the SR and MR formulations respectively is retained on the stent after 30 days [12] which suggests that approximately 8.5 and 23.6μg of paclitaxel are delivered from the stent over the first 30 days. A substantial portion of the drug in both SR and MR release formulations re-

mains within the stent for a considerable length of time [7, 56] with perhaps up to 90% and 75% respectively of the paclitaxel remaining at 6 months [7, 52].

From this it would appear that restenosis can be prevented using relatively small doses of paclitaxel (10-20μg total drug) released over a minimum of 10 days and probably over 30 days. But it is unclear what contribution, if any, the paclitaxel remaining on the stent has on future ISR.

If there is a maximum period and dosage after which further paclitaxel release does not affect ISR then further elution of the drug may be counter productive by delaying arterial healing and re-endothelialisation of the vessel. *In vitro* studies have shown that paclitaxel inhibits migration and proliferation of smooth muscle cells at 10 to 100 fold lower concentrations than those required to inhibit endothelial cells proliferation [110] and developing a release system capable of delivering sufficient paclitaxel to prevent ISR whilst not delaying endothelial healing would appear as a desirable and achievable goal. The optimal drug release profile for DES is yet to be established and may be different for different drugs, stent platforms and agents [7].

1.5 Preclinical methods of evaluating DES.

New DES formulations require vigorous testing prior to approval by the Food and Drug Administration and equivalent bodies [111]. Drug release, polymer degradation, biocompatibility and mechanical properties of the DES need to be evaluated prior to the stent being further tested in clinical trials [112, 113] and selection of suitable models to asses these parameters are crucial.

Typically, novel DES are tested *in vitro* to define the degradation and drug elution profile of the polymer coating prior to further testing of the formulation *in vivo*. *In vitro* polymer degradation and drug elution should be examined at body temperature under infinite sink conditions and with agitation to prevent boundary layer effects [113]. Often *in vitro* testing involves incubating the stent (expanded and unexpanded) at 37°C in a physiological release media on a shaker or stirrer at 60-120rpm. The incubation media that has been used in *in vitro* models includes phos-

phate buffered saline, isotonic sodium chloride, porcine plasma and may contain additives such as surfactants and cosolvents [114].

Tissue chamber models, in which chambers containing polymeric materials are implanted subcutaneously in animals such as rats, mice and farm animals, have been used to determine the biocompatibility of polymers, the inflammatory response to implanted materials and the pharmacokinetics of a range of anti-inflammatory drugs [115-117].

The rat subcutaneous implant model is an established small animal model used to determine the impact of the *in vivo* environment on drug elution, molecular weight change and mass loss of biodegradable polymers (S Zhong pers comm. [115, 118]). In this model the test article or polymer is contained within polymethyl methacrylate (PMMA) chambers, which are sealed with a semi-permeable membrane and then implanted subcutaneously into the back of male Wistar rats. The procedure causes minimal stress to the animals and is therefore ethically acceptable [117]. The filter allows cellular exudate to diffuse into the chamber but excludes cellular elements. The model is seen as a reliable method to test the properties of the polymer prior to further testing in animal artery models (Boston Scientific in litt).

The model has a number of advantages over *in vitro* models including:

- The test article is exposed to complete interstitial exudates fluid environment enabling degradation of the polymer in an *in vivo* physiological medium at body temperature and pH
- The semi-permeable membrane facilitates diffusion of eluted drug and polymer degradation products to diffuse out of the chamber and degradation and drug elution is subjected to *in vivo* clearance kinetics
- Drug elution rates are not affected by boundary layer effects due to natural agitation of the chamber exudate caused by the animals movements
- The model is sufficiently discriminatory to detect weaknesses in implant configuration (changes in physical coating characteristics over time)
- The model is pathophysiologically relevant with respect to observations related to inflammation and neo-vascularization

- The model results in uniform and repeatable *in vivo* conditions based on in-bred animal strains
- The test article is exposed to hydrolases and oxidoreductases secreted by macrophages and fibroblasts. This is of relevance for polymers which are susceptible to enzymatic cleavage.

The device is retrievable for physical and chemical analysis. This is true also for *in vitro* models. But analysis of degradation and coating integrity is difficult in stents retrieved from animal arteries due to the presence of adhered tissue which needs to be removed prior to visual assessment by scanning electron microscopy and light microscopy. Additionally, the adhered tissue can give rise to peaks in chromatograms in studies using gel permeation chromatography which can impair assessment of the degradation rate of the polymer coating. Removal of the adhered tissue either physically or chemically (e.g. by use of enzymes) may damage the stent coating and give a false assessment of the degradation, drug elution and visual appearance of the stent coating.

In common with *in vitro* models, the tissue chamber model does not replicate all conditions of the vascular implant site and does not take into account the effect of factors such as flow dynamics, shear stress, continuous pulsatile effects and strut encapsulation by SMC.

Stent formulations with a satisfactory performance in the initial preclinical trials are further tested using animal models. A consensus statement which set out recommendations for best scientific practice in preclinical studies of DES recommended using the porcine coronary artery model and the rabbit iliac artery model to determine the safety and efficacy of DES [113].

1.6 Overview of the study.

In chapters 2 and 3 of this thesis the degradation and paclitaxel elution profile of two biodegradable polymers, namely TyRx and PLGA, are examined to assess their suitability as coatings for DES. Further studies to modulate polymer degradation and pa-

clitaxel elution by mixing different blends of PLGA and incorporating hydrophilic copolymers into PLGA polymers are covered in chapter 4.

Molecular weight change is a measure of the reduction in size of the polymer chains within the matrix and serves as a measure of polymer degradation [119] and was determined using gel permeation chromatography (GPC). As a result of polymer degradation polymer erosion occurs and this was measured by calculating the change in mass of the stents as degradation proceeded. Paclitaxel elution was monitored by measuring the amount of drug remaining on the stent after implantation using GPC. Degradation and erosion of the polymer can weaken the structure of the polymer matrix and cause fragments of the polymer to break away from the coating (delamination) and the visual deterioration of the stent coating was determined using light microscopy and scanning electron microscopy.

In chapters 2 and 3 polymer degradation and drug elution is determined using an *in vivo* rat tissue chamber model. All of the animal procedures were conducted under home office regulations with the appropriate licence in place. In chapter 4 the degradation and drug release properties of PLGA were modified by blending low molecular weight hydrophilic pluronics and PLGA copolymers with different lactide:glycolide (LA:GA) content and differing molecular weights. This study was performed *in vitro* using polymer coated Teflon (PTFE) discs incubated in PBS, pH 7.4 at 37°C.

In chapter 5 the data obtained is discussed in relation to the polymer degradation and drug elution profiles of paclitaxel DES and with clinical studies and assessed for its suitability for coronary DES.

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The Degradation and Drug Elution Profile of TyRx, a Tyrosine-Containing Polyarylate

2.1 Introduction.

Tyrosine-derived polyarylates are a group of thermally stable polymers with a range of mechanical properties from soft elastomeric materials such as poly(DTO sebacate) to tougher, strong materials such as poly(DTE succinate) [1]. The polymers were designed as part of a library of structurally related polymers and a good structure to property relationship has been established [2-3] cited by [1]. The desaminotyrosyl tyrosine ester (DTR) usually comprises about 80-90% of the polymer mass and is the key component for defining properties (by its solubility) such as vascular compatibility and degradation profile. Polyarylates are not cytotoxic and cells attach and proliferate on their surface depending on the polymers hydrophobicity. Degradation of tyrosine-derived polymers has been shown to produce less acidic degradants than poly (lactic-co-glycolic acid) and poly (lactic acid), this reduction may explain the good tissue compatibility of the tyrosine derived polymers [1]. A comparison of the degradation and tissue response to extruded pins made from a tyrosine-derived polyarylates (poly(DTE-adipate), a tyrosine derived polycarbonate and PLLA (poly (L-lactide)) in a subcutaneous rat model by Hooper *et al* [4] showed that the tyrosine-derived polyarylates elicited the mildest inflammatory response and that as degradation of the polymer proceeded tissue in-growth into the pins followed.

Early tyrosine containing polyarylates had slow degradation times of months to years, but for applications such as DES a faster rate of degradation is required. The properties of the polymer can be altered by varying the length of the diacids (such as succinic acid, adipic acid or sebatic acid) in the polymer backbone and/or the length of the alkyl ester pendant chains. The arylate bond within the backbone of the polymer structure introduces a site within the structure that is susceptible to hydrolysis [1]. Tyrosine-derived monomers which are produced during degradation are not readily water soluble and mass loss from these polymers occurs very slowly and only towards the end of the degradation process [1, 4].

Previous studies have shown that lengthening the pendant chain has little effect on degradation rate of thin films of poly(DTE adipate), poly(DTH adipate) and poly(DTO adipate). Changes in pendant chain length affect the glass transition temperature and the hydrophobicity of the polymer and these effects may counteract each other with respect to the degradation rate in these polymers [5]. Increasing the pendant chain length decreases the glass transition temperature (T_g) and increases the hydrophobicity of the polymers. The hydrophobicity of the polymers is increased with increasing pendant chain length leading to lower degradation rates. But at 37°C polymers with the shorter pendant chains were glassy, while those with longer chain length were rubbery. Polymers with lower T_g and in the rubbery state have faster degradation rates as compared with those in the glassy state.

T_g also has implications for the diffusion rate and controlled release of drugs; Poly(DTH adipate) and poly(DTO adipate) which are rubbery at 37°C eluted a model compound (p-nitroaniline) 10 times faster than poly(DTE adipate) which is glassy at 37°C [5], possibly due to the fact that in the rubbery state polymers have a higher free volume than polymers in the glassy state, therefore more readily allowing the diffusion of small free molecules [5-6]. Previous studies have shown a diffusion controlled release mechanism of low molecular weight drugs from the adipic acid series of tyrosine-based polyarylates [5]. Additionally, tyrosine-derived polyarylates have been used for the release of water soluble peptides [7] and for the release of anticoagulants from polymer coated carbon fibres [8] cited by [1].

Given the good mechanical strength, coating properties, biocompatibility (especially important considering the concern surrounding the possible role of any residual polymer in eliciting unfavourable late cardiac events in DES) and low toxicity of their degradation products tyrosine-derived polymers may have significant future potential for use as a polymer for a DES.

The aim of this study was to examine the degradation profile of TyRx P22-10 (Figure 2.1) in an in-vivo rat model to evaluate its potential as a coating and drug delivery polymer for use on coronary stents. TyRx P22-10 is a tyrosine-derived polyarylate with a succinate diacid monomer with an ethyl ester pendant group and 10% free acid in the pendant groups and a T_g of 84°C (S.Zhong. pers comm.).

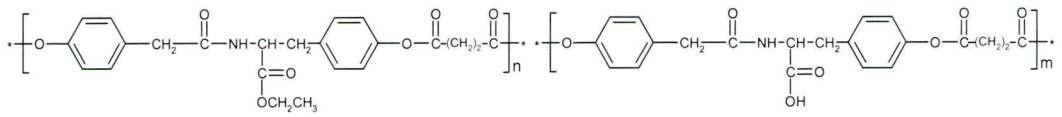

Figure 2.1 Structure of TyRx P22-10: A tyrosine-derived polyarylate containing succinate

This study tested the hypothesis that the introduction of a succinic acid monomer into the polymer backbone with 10% free acid in the pendant chain would increase the degradation rate of the polymer and elution of paclitaxel and be of a suitable duration for DES. The suitability of the polymer was assessed by determining the degradation profile of TyRx P22-10 polymer coated onto coronary stents with and without paclitaxel in a rat in-vivo model using Gel Permeation Chromatography (GPC) and using Light Microscopy (LM), Scanning Electron Microscopy (SEM) to assess the visual condition of the polymer coat as it degraded. In this model the stent is contained in a sealed in a PMMA chamber which provides an acellular environment and the degradation media is derived from the host as extracellular exudate which collects into the chamber. Acid degradation products were measured by monitoring the pH of the explanted chamber exudate.

2.2 Materials and methods

2.2.1 Materials

Tetrahydrofuran (THF): Romil-SpS Super Purity solvent, 99.9% destabilised

Methanol: Romil-SpS Super Purity solvent, 99.9%.

Paclitaxel from *Taxus yannanensis*: Sigma-Aldrich

TyRx coated Liberté™ WH 16mm stents obtained from Boston Scientific

Implantation chambers: prepared from medical grade 20mm lengths of 10mm diameter medical grade polymethyl methacrylate (Goodfellows UK).

Chamber seals: 22μm cellulose nitrate millipore filters.

2.2.2 Preparation of stents and implant devices

400μg of TyRx P22-10 with or without 2.5% paclitaxel was coated onto a Liberté 16mm WH coronary stent. The stents were placed in PMMA chambers which were

then sealed with a 0.22μm millipore filter using medical grade silicon glue (Nusil Silicone Technology, USA) (Figure 2.2) and were sterilised by autoclaving.

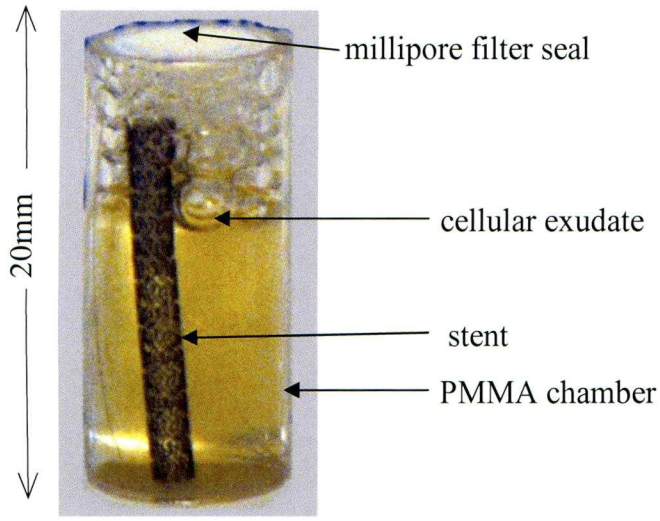

Figure 2.2 PMMA chamber containing a stent with cellular exudate

Implantation of the test chambers was performed as described by Baldwin and Hunt [9]. Four test chambers containing one stent each were implanted subcutaneously into the backs of male Wistar rats, two either side of the spine, locating them on top of the dorso lumbar muscles and also below the shoulders. At the appropriate time points the implants were removed for experimental analysis. The stents were washed by quickly dipping the stent twice into ELGA Purelab UHQ water. Excess moisture was quickly blotted off the stent and the sample was placed into a clean polystyrene vial, and dried under vacuum at room temperature. The samples were weighed every 24 hours until a stable weight was achieved and the samples were dry. The sample vials were then tightly capped and stored at 4°C in polythene sealed bags containing Silica desiccant. Four stents were recovered from each animal at each time point. All stents were examined using optical light microscopy. Three stents were used for GPC analysis and one for SEM.

2.2.3 Gel permeation chromatography:

For GPC analysis each stent was added to 1ml of solvent containing 9:1 v/v THF and Methanol in a capped glass vial and shaken gently for 3 hours to extract the polymer. GPC was performed using three 300 x7.5mm, 5μm Polymer Laboratories PLgel columns with pore sizes 500Å, 10^3Å and 10^5Å with a flow rate of 1ml per minute using THF as the mobile phase and a Polymer Labs ELS 1000 evaporative light scatter detector. Calibration standards were prepared using Easi-Cal PS-2 (Polymer Labs)

polystyrene standards, with a molecular weight range of 580 – 377400, dissolved in THF to give a 0.1% solution. Calibration standards were run in triplicate and a calibration was performed at the beginning, middle and end of every GPC run. Raw polymer was prepared by weighing an amount of polymer and adding THF/Methanol mixture to give a final concentration of 2mg/ml and gently mixing at 120rpm at room temperature for 3 hrs.

The injection volume was 250µl with a polymer concentration of approximately 400µg/ml solvent for the stent samples and 100µl for the raw polymer and Easi-Cal standards) with three measurements per sample. Quantitative analysis was performed using Caliber Software (Polymer Labs). Paclitaxel analysis was determined using peak area of the samples and quantified using a calibration curve prepared using standards containing 5, 10, 20, 50 and 100µg/ml paclitaxel dissolved in 9:1 THF/Methanol. 250µl of the standards were injected onto the GPC and the peak area was analysed in the same method as the stent samples.

2.2.4 pH measurements.

Cellular exudate recovered from the chambers was measured using a pH meter (Mettler Toledo) and the visual appearance of the exudate noted and recorded as clear (indicating an intact chamber seal), cloudy, red (indicating presence of red blood cells and presence of tissue in-growth(indicating severe loss of chamber seal integrity.

2.2.5 SEM and light microscopy

The explanted stents were examined by light microscopy by two methods; stereo dissection microscope and reflected light microscopy using differential interference contrast and standard reflectance. SEM was performed using a Leo 1550 Field Emission Scanning Electron Microscope (Zeiss). For SEM, one stent from each animal was mounted onto an aluminium stub using double sided adhesive carbon tape sputter coated with chromium using an EMITECH K575X coater.

2.2.6 Mass loss assessment.

The weight of polymer coated onto each stent was recorded prior to implantation. The mass of the polymer remaining on the stent after implantation was determined after the explanted stents had been dried using a Sartorius CP2P balance.

2.3 Results

2.3.1 GPC data.

2.3.1.1 TyRx molecular weight data

A calibration graph of log molecular weight versus retention time was generated and used to determine the following for each sample:

- Peak molecular weight (M_p) – the molecular weight of the highest peak and therefore the mode of the molecular weight distribution.
- Weight average molecular weight (M_w) – this takes into account the molecular weight of the chains in determining the contributions to the molecular weight average
- Number average molecular weight (M_n) – is the mean molecular weight of all the chains in the sample
- Polydispersity Dispersity Index (PDI) - is a measure of the distribution of molecular mass in a given polymer sample and is calculated by dividing the weight average molecular weight by the number average molecular weight
- Peak Height
- Peak Area.

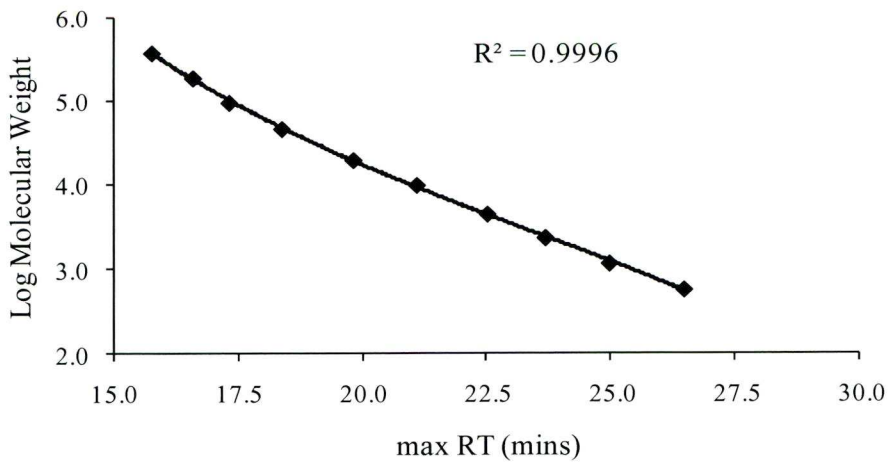

Figure 2.3 Typical GPC Calibration Graph for TyRx degradation Studies.
The X-axis is the retention time on the column for the maximum peak from the polymer standard. The Y-axis is the log of the molecular weight of the calibration standard. The plot is 3rd order polynomial.

A typical calibration is shown in Figure 2.3. An R^2 ≥0.999 was required for the calibration to be acceptable. The GPC data obtained is given in Table 2.1 and refers to the polymer remaining on the stent. Data for M_w, M_p, M_n and PDI is shown in Figures 2.4 to 2.7.

Table 2.1 GPC data: Degradation of TyRx with and without paclitaxel (Ptxl).
The data refers to the M_w, M_p, M_n and PDI of the polymer remaining on the stent.sd=standard deviation.

implant time (days)	polymer	Mean M_n	sd	Mean M_w	sd	Mean M_p	sd	PDI
14	TyRx+Pctxl	25558.44	2366.10	36357.50	1465.57	47919.08	3332.89	1.53
	TyRx	24615.86	187.36	36930.11	176.20	46373.56	1976.57	1.50
30	TyRx+Pctxl	19720.00	269.08	25350.89	133.91	25377.33	730.61	1.31
	TyRx	16767.78	216.15	23623.56	166.63	25048.56	946.83	1.41
60	TyRx+Pctxl	9391.00	360.09	12930.89	481.23	12631.94	643.93	1.37
	TyRx	8747.31	259.96	11594.53	290.92	12163.31	638.33	1.33
90	TyRx+Pctxl	7445.44	54.17	10285.61	81.74	9800.13	405.36	1.35
	TyRx	7174.26	150.90	9157.30	111.23	9405.63	474.72	1.27
120	TyRx+Pctxl	5790.44	78.95	7148.48	68.11	6680.22	327.03	1.23
	TyRx	5187.60	74.50	6425.02	67.54	6101.54	283.69	1.24
150	TyRx+Pctxl	4558.70	163.06	5537.52	211.36	5245.89	257.78	1.21
	TyRx	4268.78	33.85	5045.41	40.31	4751.22	94.00	1.18
180	TyRx+Pctxl	4118.11	41.83	4611.44	53.29	4032.81	196.15	1.12
	TyRx	3559.11	59.33	4224.52	46.18	3544.59	147.64	1.19
220	TyRx+Pctxl	3463.85	135.29	3927.67	112.95	3356.15	220.67	1.13
	TyRx	3553.15	64.45	3921.70	75.15	3192.04	277.48	1.10
250	TyRx+Pctxl	3804.37	54.49	4436.67	57.54	3662.04	94.27	1.17
	TyRx	3316.37	69.21	3820.88	77.00	2989.25	239.66	1.11
280	TyRx+Pctxl	3517.03	33.37	3921.33	38.43	3110.36	89.49	1.11
	TyRx	3161.04	21.36	3494.30	26.56	2815.59	19.42	1.10

The change in molecular weight in TyRx polymer in implanted stents is shown in Figure 2.4. Analysis by ANOVA showed that addition of paclitaxel had no effect on the M_w change in TyRx (p=0.082). The degradation of the polymer was over two phases consisting of an initial rapid phase to 60 days followed by a period of slower degradation. The original M_w of the polymer was 59,000 and by 14 days this had degraded by 40% to approximately 37,000 and by 60 days the M_w had further degraded to 12-13,000. By 150 days implantation the M_w had degraded to approximately 5,000 after which degradation proceeded more slowly to approximately 3,500 to 4,000 by 280 days, equivalent to 6-7% of the original M_w at the end of the study period.

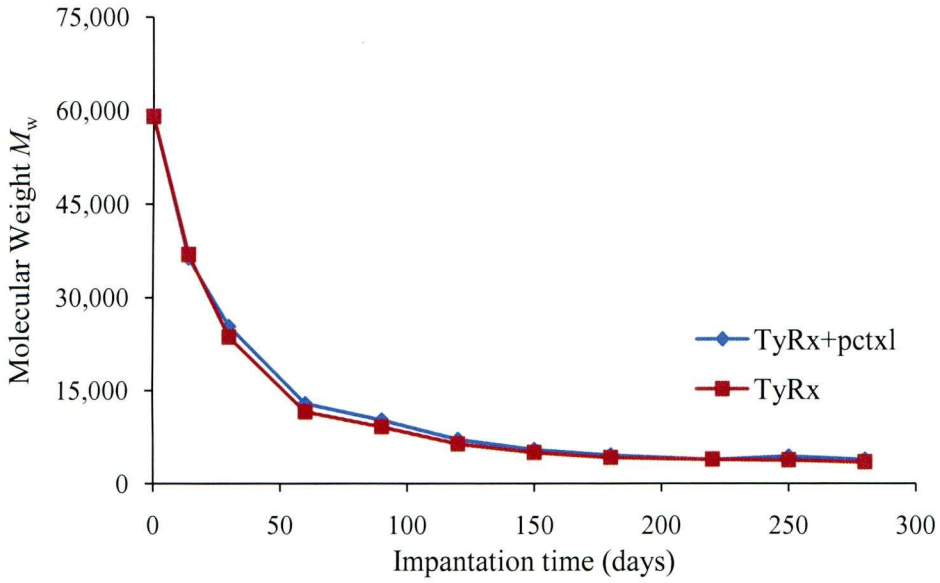

Figure 2.4. Molecular weight change of TyRx polymer during implantation
The graph shows the change in weight average molecular weight of the polymer (with and without paclitaxel) remaining on the stent. pctxl=paclitaxel.

There was no significant difference (p=0.356) in the polydispersity (PDI) during the trial due to the presence of paclitaxel in the polymer but PDI decreased significantly (p<0.001) with increasing implant time. The PDI of the polymer is shown in Figure 2.5. The PDI of the raw polymer was 1.572. At 14 days the PDI was 1.515 but by 180 days it had reduced to 1.207. The PDI continued to decrease after 180 days to 1.150 at 280 days but the decrease was not significant.

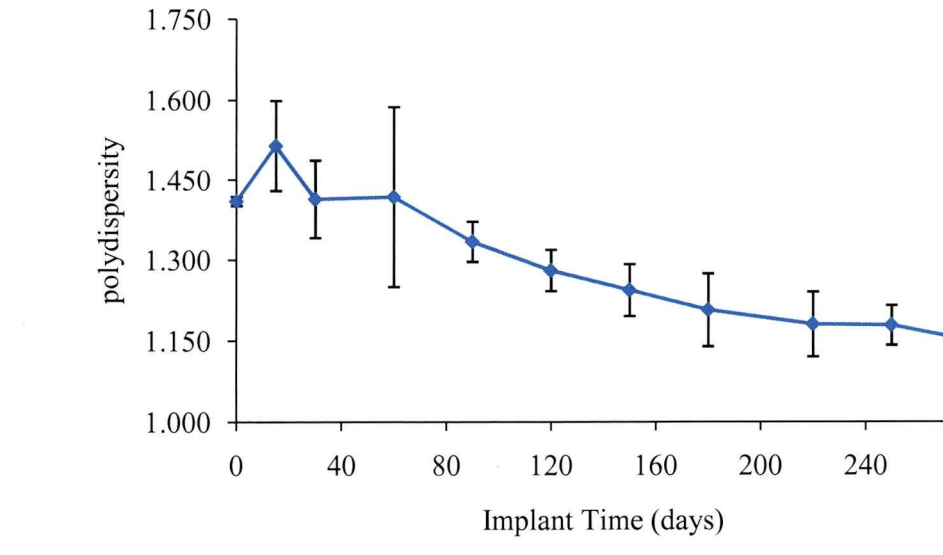

Figure 2.5 Polydispersity of TyRx polymer coated onto coronary stents (combined data from TyRx with and without paclitaxel) during implantation. Figure shows the PDI of polymer remaining on the stent. Error bars are the standard deviation.

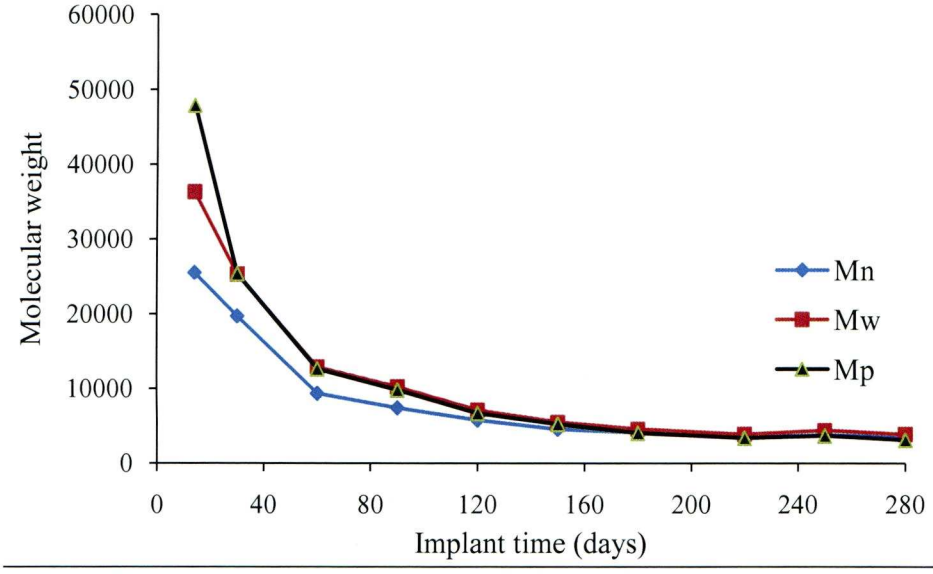

Figure 2.6 Changes in peak molecular weight (M_p), weight average molecular weight (M_w) and number average molecular weight (M_n) during degradation of TyRx polymer containing paclitaxel.

The changes in peak molecular weight (M_p), weight average molecular weight (M_w) and number average molecular weight (M_n) of TyRx polymer coated on stents with and without paclitaxel are shown in Figures 2.6 and 2.7. During the first 90 days the values for M_p, M_w and M_n are different indicating differences in the length of the polymer chains that comprise the polymer, but as degradation progresses M_p, M_n and M_w become similar indicating that the polymer chains become more uniform in size and weight.

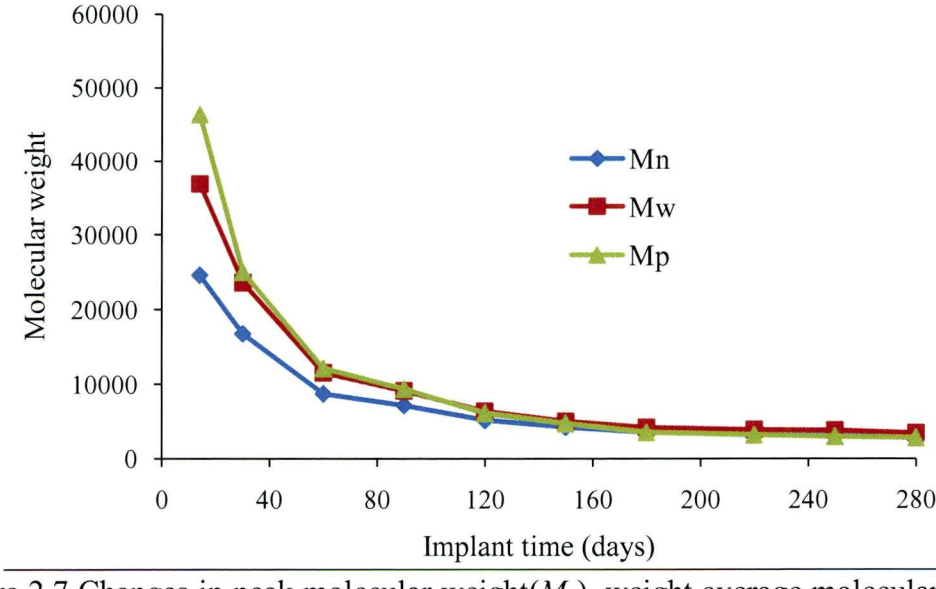

Figure 2.7 Changes in peak molecular weight(M_p), weight average molecular weight (M_w) and number average molecular weight (M_n) during degradation of TyRx polymer.

2.3.1.2 Paclitaxel assessment

Attempts were made to quantify the amount of paclitaxel remaining on the stent using peak area data from GPC analysis. A peak for paclitaxel can be seen on the GPC chromatograms but at the concentrations in the set of stents in this trial the peak is small on the ELS detector. Paclitaxel has a molecular weight of 853. But due to loss of integrity of the seal in a large number of the chambers cellular exudate and tissue in-growth occurred and peaks with molecular weights of around 600 to 1100 where observed on the chromatograms which often overlapped or completely obscured the paclitaxel peak (see Appendix, Figures A1-A4). Thus paclitaxel quantification was difficult and only possible in a small number of cases.

The calibration data for paclitaxel assay is shown in Figure 2.8 and the amount of paclitaxel remaining on the stent after implantation is shown Table 2.2 & Figure 2.9.

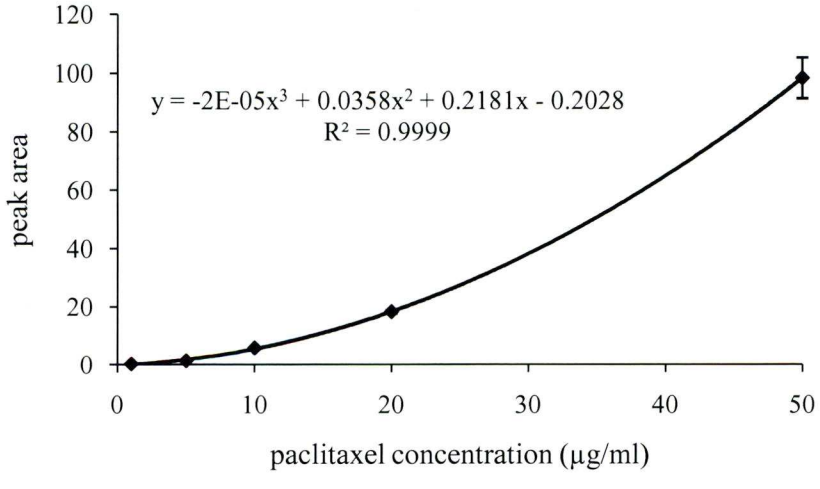

Figure 2.8 Paclitaxel Calibration. Peak area data derived from GPC chromatograms. Function is 3rd order polynomial. Error bars = standard deviation.

At the start of the trial the stents contained 10µg of paclitaxel. Recovery of paclitaxel in this assay was 109% for the controls. Analysis using ANOVA showed that over the trial period the concentration of paclitaxel decreased significantly ($p=0.003$).

Table 2.2 Paclitaxel concentration on TyRx coated stents.

Implant Time (days)	Paclitaxel Concentration (μg/stent)		number of stents	% Paclitaxel
	mean	St dev		
Controls	10.96	1.00	4	100
15	10.91	1.75	2	99.5
90	8.75	0.37	2	79.8
120	6.64	0.00	1	60.6
150	8.47	0.40	3	77.3
220	10.55	1.79	2	96.2
250	6.87	2.60	7	62.7
280	3.08	0.54	2	28.1

Controls data from un-implanted stents analysed as other stent samples. Percentage paclitaxel is the amount of drug remaining on the stent as a % of the controls, St dev=standard deviation

At 15 days there was negligible elution of paclitaxel. After 90 and 150 days implantation, 20% and 23% respectively of the paclitaxel had been eluted but analysis using ANOVA showed that the decrease in paclitaxel was not significant as compared the controls (90 days, p=0.185 and 150 days p=0.096). At 250 days approximately 38% of the paclitaxel had been eluted and this was significantly lower than the control values (p=0.003). After 280 days implantation 72% of the paclitaxel had been eluted and this was significantly lower than at 250 days (p=0.021) and all other time points.

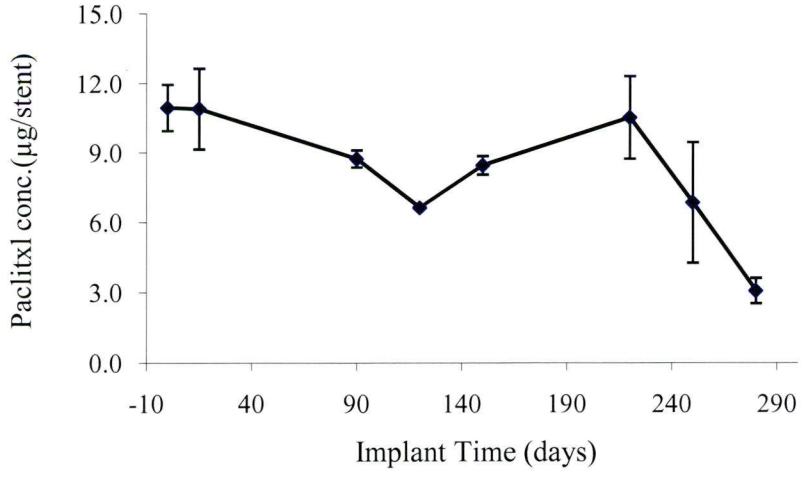

Figure 2.9 Elution of Paclitaxel from TyRx coated stents during implantation.
Graph shows the mean value of paclitaxel remaining on the stent. Error bars represent the standard deviation of the mean. The first data point is from control (unimplanted) stents.

2.3.2 pH data.

In total 216 chambers were sampled and the pH of the chamber exudate is shown in Figure 2.12. Approximately 35% of the stents had a clear exudate indicating that the chamber seal retained its integrity while 14% had a cloudy exudate. The remainder had and exudate that was either yellow through to red from the presence of red blood cell, some samples had tissue adhered to the stent indicating a loss of integrity of the chamber seal.

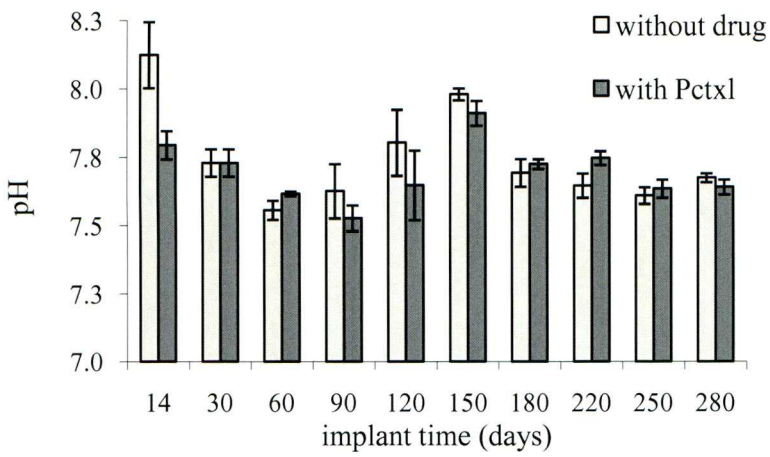

Figure 2.10 pH of cellular exudate collected in the PMMA chambers. The exudate from chambers containing stents coated with TyRx with or without paclitaxel. Error bars show +/- the standard deviation.

The mean pH of the exudate was 7.72 (SE=0.02) and 7.69 (SE=0.2) for the TyRx polymer group and TyRx plus paclitaxel groups respectively. Analysis by ANOVA showed that paclitaxel had no significant effect on exudates pH ($P>0.05$). At 14 days and 150 days time points the pH (7.96 and 7.95 respectively) was significantly higher than the other time points $p<0.001$ but no trends in pH over time were observed and the significance of the 14 day and 150 day data was unclear.

2.3.3 Light microscopy & SEM.

Data from Light Microscopy (LM) and SEM is shown in Figures 2.11 to 2.13. Similar changes in appearance were seen in both the TyRx and TyRx+ paclitaxel groups. With increasing time the appearance of the polymer coating changed from smooth and clear to opaque, white and chalky with areas of exposed bare metal where the polymer had degraded and solubilisation of polymer chains had occurred. The slow erosion of polymer from the stent is evident by the slow change in appearance of the stent coating over time with a trend of increasing areas of bare metal occurring steadily over time but with large areas of intact polymer even at the later stages of degradation at 280 days (Figure 2.11.d).

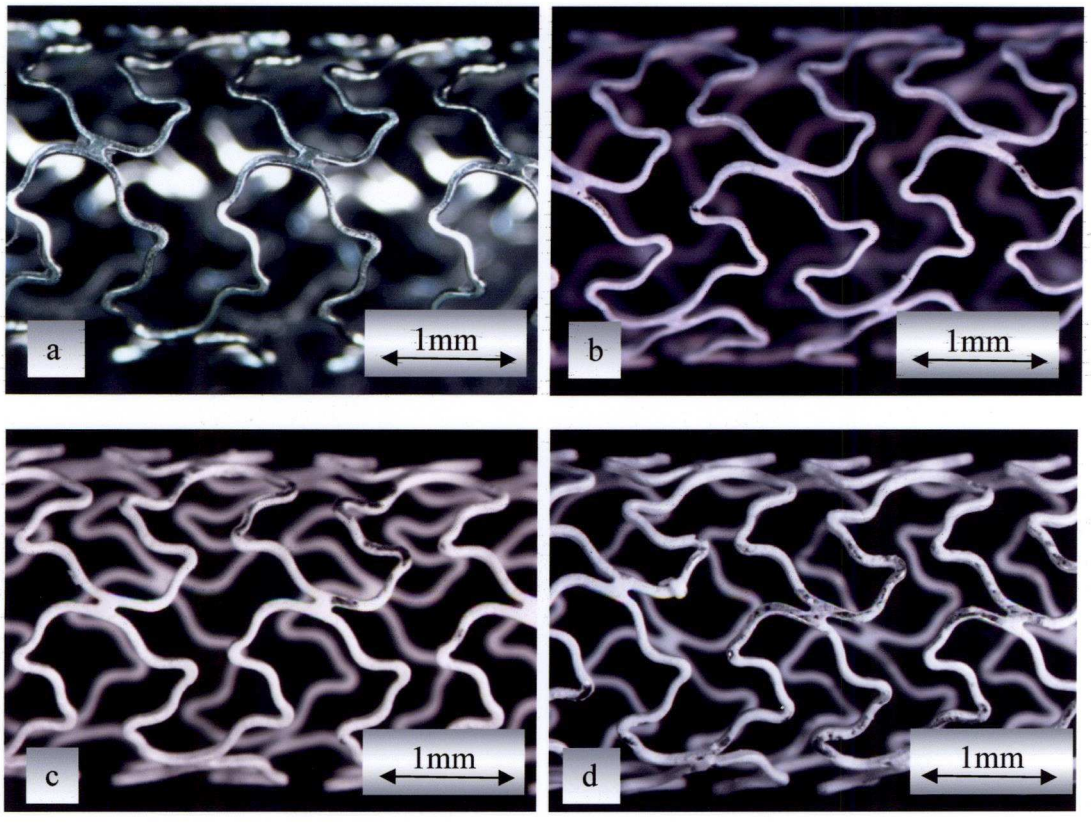

Figure 2.11 Light microscopy images of explanted stents.
Clockwise from top left: a:30 days, b:150 days,c: 250 days and d:280 days. Magnification x1.6

Blistering on the polymer was observed at 30 days and had become more extensive at 60 days. By 90 and 180 days areas of bare metal could be observed and the opacity of the polymer had increased and by 250 days large areas of the metal stent were exposed, where degraded polymer had either been dissolved or had become detached from the stent (Figure 2.12). However, despite the occurrence of areas of bare metal

and apparent deterioration of the polymer, large areas of the stent remained coated by degraded, but intact polymer.

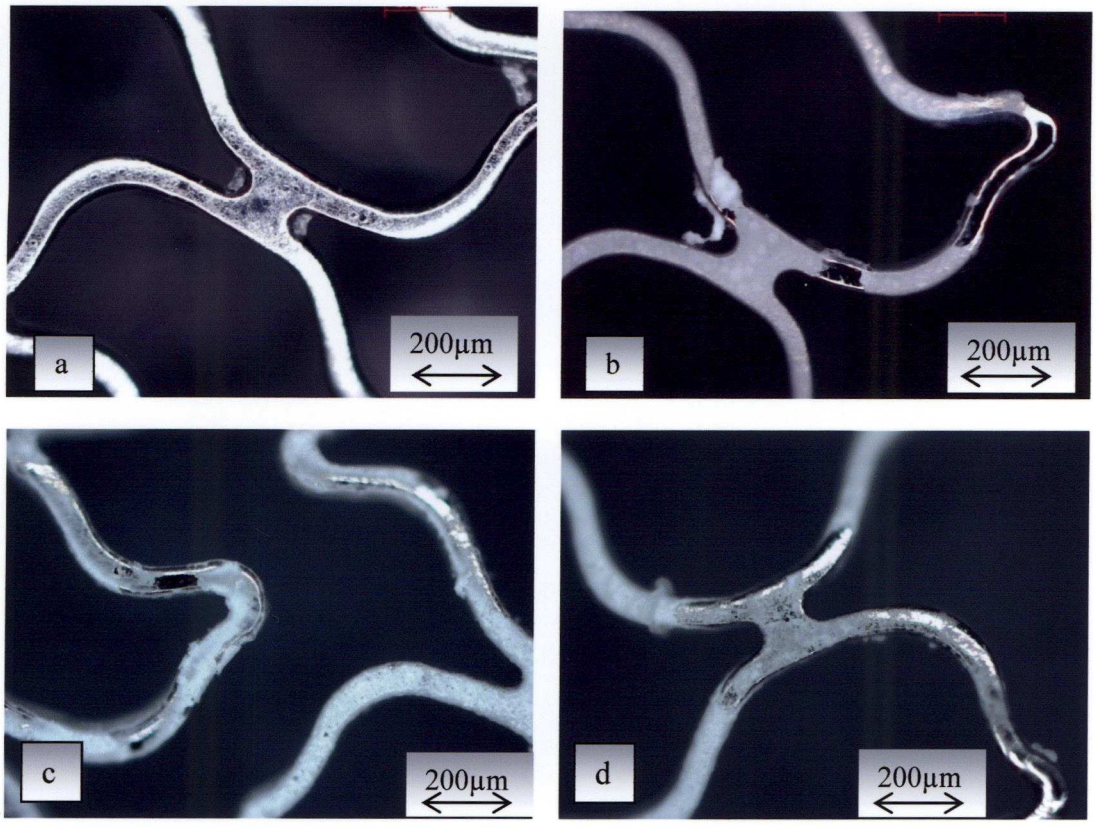

Figure 2.12 Light microscopy images of explanted stents showing deterioration of the polymer coating. Areas of bare metal (silvered areas) increase as implant time progresses. a: 60 day, b:90 day, c:180 day and d:250 days Implantation. Images taken at x5 magnification using filter 2.

Figure 2.13 shows SEM images of a 120 day implanted stent. The layer of polymer revealed a porous/honeycomb appearance with areas of bare metal visible with some small deposits of polymer still adhered to the metal. The porous honeycomb appearance of the polymer was indicative of hydrolytic degradation.

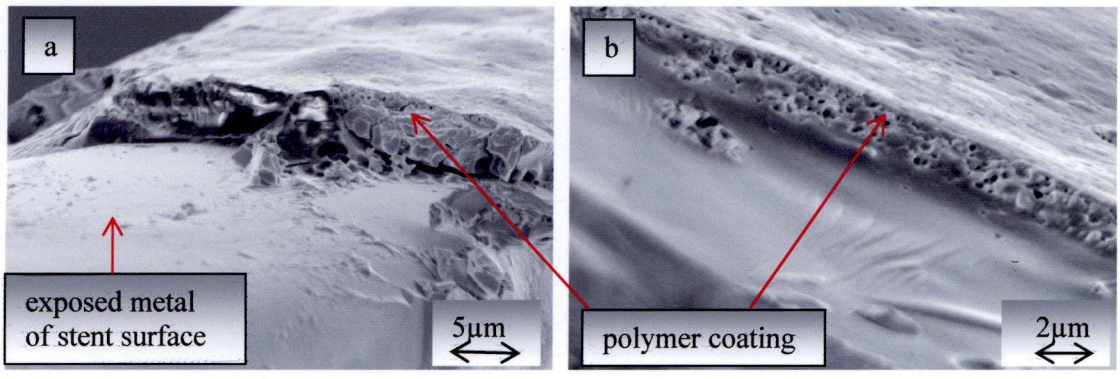

Figure 2.13 SEM Images of 120 day implant stents showing polymer coating and areas of extensive erosion revealing the bare metal of the stent. Images taken using In-Lens and EHT=5KV.

2.3.4 Mass loss data.

After explanation cellular exudate and host tissue was present on the stent, much of which was not removed using the given washing protocol. Thus it was impossible to obtain accurate post explanation mass data for the stents.

The peak height and peak area data from GPC is dependant on the amount of polymer in the samples and gives an indication of the amount of polymer remaining on the stent. Due to overlapping peaks on the chromatograms from either cellular exudate and/or host tissue components, peak area would not give reliable data in this trial (see Appendix 1). But peak height should be unaffected by the presence of other peaks and gives an indication in changes in polymer mass on the stent.

Peak height data from GPC analysis is shown in Figure 2.14. Analysis by ANOVA showed no significant difference in peak height during the study between the TyRx group and the TyRx + paclitaxel group ($p= 0.719$), but implant time significantly affected peak height ($p<0.001$).

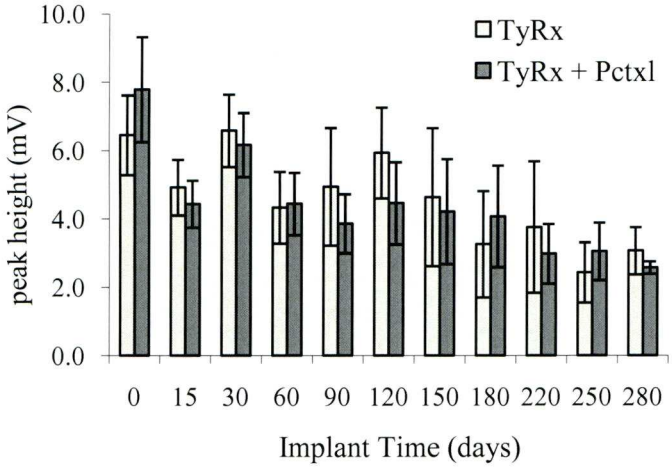

Figure 2.14 Effect of implantation time on peak height of TyRx polymer.
Peak Height data taken from GPC data. Control data from un-implanted stents is shown at 0 days.

Mean peak height data from the TyRx and TyRx + paclitaxel groups combined is shown in Figure 2.15. Overall the peak height decreases over time indicating that mass loss is occurring. But the data shows large variation about the mean values.

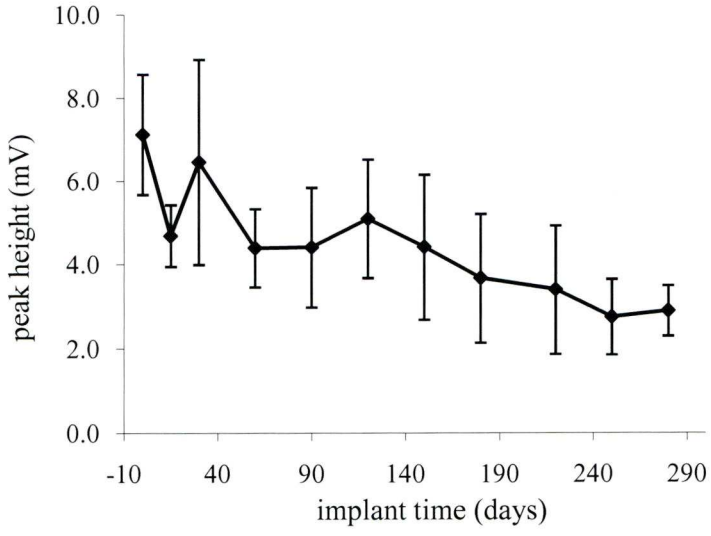

Figure 2.15 Peak height of TyRx during implantation.
Combined data from TyRx and TyRx + paclitaxel groups. Error Bars are the standard deviation. The first data point is from control (un-implanted) stents.

Analysis using ANOVA showed that by 150 days implantation the peak height had decreased significantly compared to that of the controls ($p<0.001$) indicating that mass loss had occurred. Significant ($p=0.015$) reduction in peak height continued after 150 days from 4.42mV (stdev=1.7) to 3.4mV (stdev=1.53) at 220 days. At 280 days peak height was 2.89mV (stdev=0.6) but this was not significantly different to peak height at 220 days.

2.4 Discussion.

The presence of 2.5% paclitaxel (w:w) had no effect on the degradation profile, exudate pH or mass loss of TyRx polymer. The original molecular weight of the polymer was 59,000 and by 60 days this had decreased by approximately 80% to around 12,000. The degradation rate then slowed and at the end of the 280 day study period the molecular weight was about 6% of the initial M_w. (approximately 4,000).

The degradation of TyRx P22-10 is affected by two opposing effects. Tyrosine containing polyarylates with short diacid components and short pendant chains (such as TyRx) have higher T_g and are glassy at 37°C, decreasing the degradation rate. But the shorter pendant chain decreases the hydrophobicity of the polymer which should result in higher degradation rates [5]. 10% carboxyl acid was included in the pendant chain to increase the degradation rate of the polymer. The degradation rate of TyRx P22-10 in this study was faster than that for thin films of poly(DTE adipate), poly(DTO adipate) and poly(DTH adipate) which degraded to 30-40% of their original molecular weights in 180 days when incubated *in vitro* in PBS at pH7.4 at 37°C [5] whereas in the present study TyRx P22-10 degraded to about 7% of the original molecular weight over 180 days. Initial degradation rate to around 220 days was faster for TyRx P22-10 than for for poly(DTE adipate) implants [4] but at around 280-290 days the polymers had degraded to a similar degree.

The peak molecular weight (M_p), weight average molecular weight (M_w) and the mean number average molecular weight (M_n) of the polymer were different in the early stages of the trial but by 60 days their values became similar as a result of degradation generating molecular chains of similar length and molecular weight. The decrease in polydispersity as implantation time increased also indicated a trend to-

wards greater uniformity in the length of the polymer chains as a result of hydrolytic degradation. This is in contrast to the changes in polydispersity seen during *in vivo* degradation of poly(DTE adipate) and poly(DTE carbonate), tyrosine derived polyarylates and polycarbonates respectively, which showed an increase in polydispersity as implant time increased due to degradation via random hydrolytic chain cleavage [4]. The reason and significance for this difference between TyRx and poly(DTE adipate) is unclear.

One feature of the degradation of tyrosine-derived polyarylates is a slow rate of mass loss, possibly due to the hydrophobic nature of the polymers which retards permeation of water into the polymer bulk and the insolubility of the degradation products in aqueous media [5] and occurs only at the very end of the degradation process [1]. Fiordeliso *et al* [5] found that the mass of polyarylate films when incubated in physiological buffer solution at 37°C was unchanged after 26 weeks while Hooper *et al* [4] found that mass of poly(DTE adipate) pins incubated in buffer and *in vivo* decreased by <5% over 295 days.

In vitro studies using TyRx have shown a mass loss of approximately 20% by 120 days but *in vivo* data from the same laboratory showed that TyRx P22-10 pellets and coated stents in PMMA chambers had negligible mass loss up to 90 days despite a reduction in the molecular weight to around 15% of the original (unpublished data). It is unclear if there were any problems due to cellular exudate or adhered tissue contaminating the pellets or stents which would increase the weight of the explanted stent and mask any polymer mass loss and explain the differences between the *in vivo* and *in vitro* data.

In this study the presence of cellular exudate and/or host tissue on some of the stents made measurements of mass loss using stent weight unreliable. But the peak heights of the polymers on the GPC chromatograms gave an indication of loss of mass over implant time. A clear trend could be seen of reduced polymer peak height with increasing implant time, suggesting that mass loss was occurring after about 60 days implantation and continued to 280 days. However, the relationship between amount of polymer and peak height from the ELS detector is not linear and small changes in concentration causes large increases in signal intensity and a 50% reduction in peak

height does not correspond to a 50% reduction in polymer mass. Further evidence of mass loss from the polymer could be seen by the visual deterioration of the stent coating using LM with areas of bare metal of the stent becoming visible after 60 days and more bare metal becoming exposed as the polymer chains slowly solubilised as implantation time increased. SEM revealed a porous microstructure indicative of hydrolytic degradation within the body of the polymer suggesting that bulk erosion was occurring. But despite the degradation much of the polymer remained intact on the stent at 280days.

Due to presence of overlapping peaks in the GPC chromatograms and the low response on the ELS for paclitaxel which resulted in small peaks making peak detection difficult on some occasions it was difficult to quantify paclitaxel concentration in some samples. But the data that was obtained showed a three phase release of the drug from the polymer coating. An initial slow phase of the release up to 15 days was followed by a more steady release of drug. Approximately 2μg (approx. 20% of initial loading) of paclitaxel had been released by 90 days and at 250 days approximately 6-7μg (approx. 38% of initial loading) had been eluted. Elution rate was then faster to 280 days by which time over 70% of the drug had been eluted.

A number of factors govern the elution of a drug from a polymer during degradation [6]. The mobility of drug molecules within the polymer matrix increases as the polymer molecular weight decreases resulting in faster drug release [10] since small chains offer less restriction for drug diffusion than long chains [6]. Erosion of the polymer mass enhances elution of a drug as drug molecules are carried along with the eroded polymer products out of the polymer matrix. Additionally creation of larger pore spaces and vacuoles increases the diffusional space within the polymer bulk accelerating the release of the drug by diffusion [11]. Glass transition temperature (T_g) also influences drug elution rate. Rubbery polymers have a higher free volume fraction than glassy polymers and this may facilitate faster diffusion through the polymer matrix [5]. The elution of *p*- nitroaniline was 10 fold faster from films of poly(DTH adipate) and poly(DTO adipate) which have a T_g of <37°C and were rubbery as compared with poly(DTE adipate) which was glassy [5].

Release of *p*- nitroaniline from films coated with the polyarylates poly(DTE adipate), poly(DTH adipate) and poly(DTO adipate) was via diffusion controlled release mechanism as shown by the release kinetics, i.e. linear correlation between release and the square root of the release time [5]. Figure 2.18 shows the release of paclitaxel from TyRx 22-10 plotted against the square root of the release time. The data suggests a linear relationship between cumulative paclitaxel release and the square root of time between 15 and 250 days implantation time, indicating diffusion controlled release of the drug during this period as has been seen in other studies [5]. The rate of diffusion increases after 250 days possibly as a result of more extensive mass loss and an increased porous structure of the polymer coating after this period.

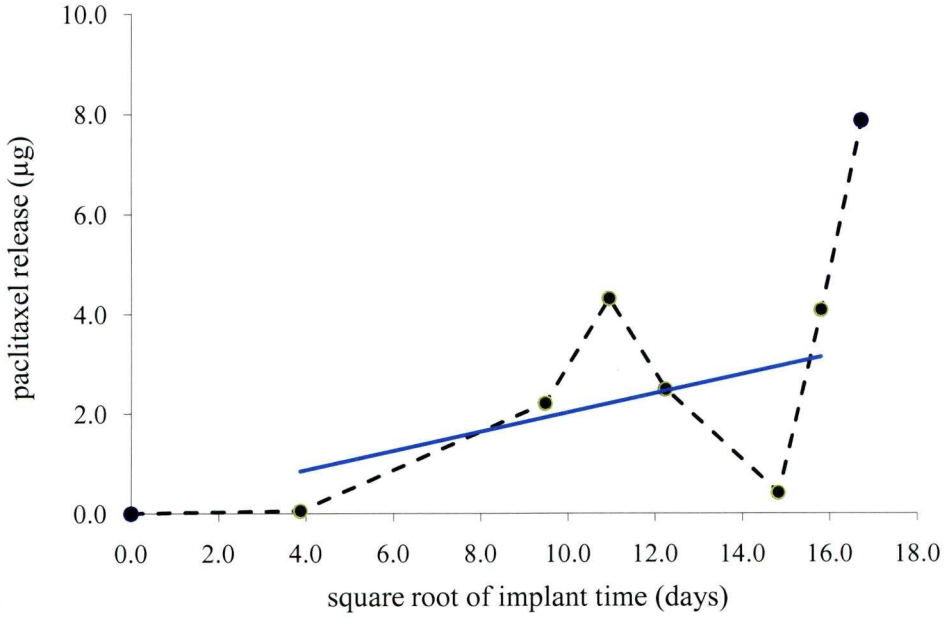

Figure 2.16 Release of paclitaxel from TyRx 22-10 plotted against the square root of the implant time. The black dashed line shows release of paclitaxel during the study period. The solid blue line is the trendline for the points from 15 to 250 days.

Figure 2.17 shows the relationship between molecular weight change in the polymer, paclitaxel release and change in peak height during implantation. The reasons for the initial slow release phase are unclear but may be due to a number of factors. At 15 days implant time the polymer had degraded by only about 35% of its initial molecular weight indicating larger polymer chains which hinders drug elution. The elution of paclitaxel appears to be diffusion controlled from 15 days to 220 days. During this

period the polymer molecular weight degrades from around 40,300 to approximately 4,200 but despite this degradation only around 4μg (approx. 38% of original amount) of paclitaxel was released.

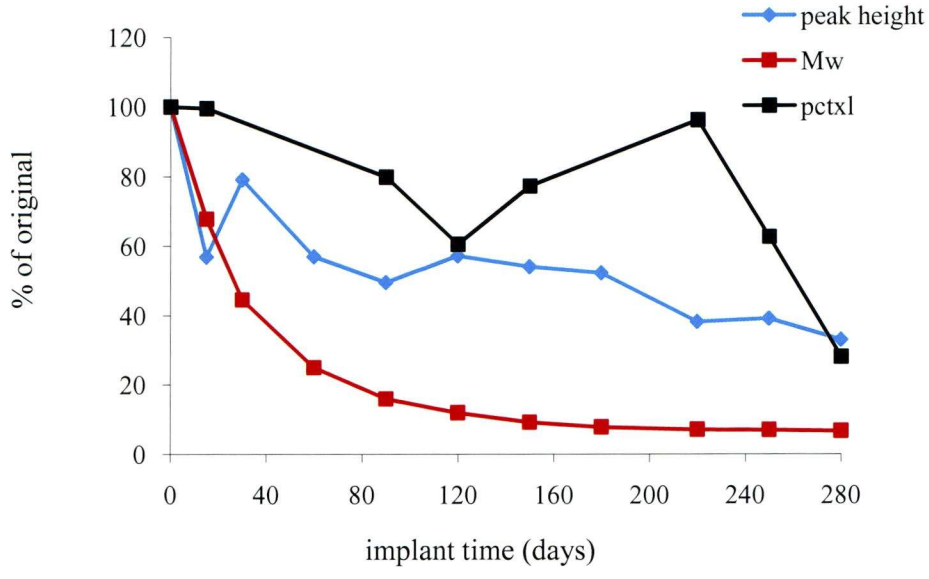

Figure 2.17 Molecular weight change, paclitaxel elution and peak height expressed as percentage of original value during implantation. M_w = polymer molecular weight, pctxl= paclitaxel elution. The peak height is height of the paclitaxel peak from GPC chromatograms

The slow rate of paclitaxel elution despite the extensive degradation of the polymer up to 220 days may be attributed a number of factors. The T_g of TyRx P22-10 is 84°C and conditions within the chamber would therefore be below the T_g and the polymer would be in a glassy state. This glassy state is associated with less mobile polymer chains and less free volume and slower elution rates [6]. The hydrophobic nature of polyarylates may retard ingress of aqueous media which may also slow diffusion of drugs through the polymer matrix. Other studies have indicated low water uptake by tyrosine containing polyarylates. Hooper *et al* [4] found less than 2% water uptake in poly(DTE adipate) implants. But Schachter and Kohn [7] found that thin films of poly(DTH adipate) adipate absorbed 10% of their initial weight after 2 hours when incubated in PBS buffer, pH7.4, at 37°C. Finally, the rate of mass loss tends to be minimal in tyrosine containing polyarylates and occurs only at the end of the degradation process [4]. The present data shows indirect evidence that mass loss was occurring slowly and steadily during implantation and paclitaxel elution appears

to mirror polymer erosion. As polymer erosion becomes extensive a more porous structure of the polymer coating is created allowing for easier and more rapid diffusion of a drug into the external environment. At 280 days there was visual evidence of extensive degradation of the polymer coating and presence of a porous structure within the polymer body (from SEM data, Figure 2.13) and by this time point 38% of the paclitaxel had been eluted.

The importance of the therapeutic window for drug delivery following stent placement was discussed in Chapter 1 and refers to the dosage and duration of paclitaxel required to be effective in preventing restenosis whilst not causing toxic effects or delaying arterial healing [12]. Studies by Serryus *et al* [13] and Kammath *et al* [14] indicate a minimum period of paclitaxel delivery of approximately 30 days and during this time span between 8.5μg (7.5% of total drug loading) and 23.6μg (11.9% total drug loading) respectively of paclitaxel would be delivered from the SR and MR Taxus™ coronary stent. In the present study approximately 2.2μg and 4.0μg of paclitaxel had been released at 90 days and 250 days respectively. Only by 280 days, when 7.9μg had been released, had an equivalent amount of paclitaxel been eluted as compared to that of the Taxus™ SR stent at 30 days. Serryus *et al* [13] found that drug loadings of 10μg/stent released over 30 days to be efficacious in reducing ISR and had low indices of injury to the vessel wall. Further studies would be required to test whether the release of paclitaxel in the present formulation of TyRx P22-10 would be effective in preventing or reducing ISR but based on the observations of Kammath [14] and Serryus *et al* [13] the present formulation of TyRx P22-10 does not appear to have a suitable elution profile of paclitaxel.

Cellular exudate recovered from the PMMA chambers had a mean pH of 7.72 (SE=0.013). Adding paclitaxel to TyRx had no significant effect on pH and overall there was no significant change in pH over time (although at time points 14 days and 150 days pH was significantly higher than at other time points). Succinic acid is a degradation product from TyRx P22-10, and the pH of the exudate remained slightly basic throughout the trial indicating that the buffering capacity of the exudate was not exceeded by the accumulation of breakdown products within the PMMA chamber.

Degradation of tyrosine-derived polymers has been shown to produce less acidic degradation products than poly (lactic-co-glycolic acid) and poly (lactic acid) and this reduction in acid degradation products may explain the good tissue compatibility of the tyrosine derived polymers [1]. A comparison of the degradation and tissue response to extruded pins made from a tyrosine-derived polyarylates (poly(DTE-adipate) a tyrosine derived polycarbonate and poly (lactic acid) in a subcutaneous rat model by Hooper *et al* [4] showed that the tyrosine-derived polyarylates elicited the mildest inflammatory response and that as degradation of the polymer proceeded tissue in-growth into the pins was evident. Given that the presence of polymer remaining on the currently used implanted stents has been suggested as a cause for an extended and enhanced inflammatory response a polymer with better biocompatibility may prove an attractive alternative coating material for arterial stents.

2.5 Conclusions

Degradation of TyRx P22-10 was advanced by 9 months but mass loss was minimal and dissolution was incomplete and the stent retained a largely intact coating of low molecular weight polymer. Paclitaxel elution was slow and occurred over the 280 day study period with approximately 3μg of the drug still retained in the polymer coating of the stent at the end of the study period and may not be released at a suitable dosage or duration to prevent ISR. There was no significant shift in interstitial fluid pH as a consequence of degradation and TyRx P22-10 is expected to have good tissue compatibility as has been seen in other tyrosine containing polyarylates.

Some of the requirements of a coating for a DES is that it must provide good structural integrity throughout degradation, consistent and controlled drug delivery at the desired concentration, be non-thrombogenic and generate as benign a response from the host as possible or no more adverse effects as that from a BMS [15]. The coating of TyRx P22-10 exhibited good structural integrity but the presence of undissolved polymer containing some paclitaxel over a time scale of 9 months reduces its suitability as a polymer for DES. Further work on the polymer to enhance degradation, mass loss and drug elution would be required to address these issues.

2.6 References.

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The Degradation and Paclitaxel Elution Profile of Poly($_{D,L}$-lactide-*co*-glycolide) (PLGA).

3.1 Introduction

Devices such as DES in which a drug is dissolved or dispersed within the polymer coating are termed monolithic devices. Release of drugs from such devices is via diffusion of the drug through the polymer coating, by erosion of the polymer or a combination of both [1]. Lactic acid homopolymers and copolymers of lactic acid and glycolic acid have been the subject of much interest in the medical and pharmaceutical field due to their biodegradability and toxicological safety; poly($_{D,L}$-lactide-*co*-glycolide) (PLGA) has been approved for human clinical use [2] .

PLGA polymers are aliphatic biodegradable, bulk eroding polymers that have been used in a number of applications for sustained drug-release [1]. PLGA has excellent biocompatibility and is considered safe [3] cited by [4] and [5-6] cited by [1]. In addition to being biodegradable they are biocompatible and bioabsorbable and their degradation products are non-toxic, nonimunogenic and noncarcinogenic. They are easily processed, have good mechanical properties and can be sterilized [7]. PLGA polymers are copolymers based on repeating units of glycolic acid (HO-CH_2-COOH) and lactic acid (HO-CH(CH_3)-COOH) [8] and the ratio of lactic acid (LA) to glycolic acid (GA) in the copolymer largely dictates the degradation of the device [2]. PLGA is named according to the percentage of lactic and glycolic units in the copolymer;- e.g. PLGA 75:25 comprises 75% lactic and 25% glycolic units [9]. A wide range of thermal, mechanical and biological properties can be obtained by varying the chemical and configurational structures in the polymer chains. PLGA degrades into the natural metabolites glycolic and lactic acid which can then be removed by the body via normal metabolic pathways [1, 8-11].

Biodegradation of PLGA occurs through 4 main steps [12]:

- Hydration; the aqueous media penetrates into the polymer matrix resulting in relaxation of the polymer and a reduction in the glass transition temperature (T_g)

- Initial degradation; hydrolysis commences in the hydrated regions of the polymer matrix cleaving the polymer backbone thereby reducing the polymer molecular weight (MWt). Degradation continues resulting in further decrease in MWt but without any appreciable loss in polymer mass.
- Further degradation; as the decrease in molecular weight continues there is an associated rapid loss in polymer bulk and oligomeric fragments and formation of soluble monomers.
- Solubilisation; continued polymer chain cleavage results in the formation of soluble monomers and molecules resulting in extensive polymer erosion and mass loss and ultimately complete solubilisation of the polymer.

Degradation of PLGA is via hydrolysis of the ester bonds during which polymer chains are cleaved into oligomers and finally monomers [2, 13]. The rate of degradation is affected by a number of factors including the polymer composition (ratio of LA to GA moieties), molecular weight, hydrophilicity, crystallinity, specimen size and the nature of the hydrolysing medium including pH, ionic strength and temperature of external medium, [12] & [14-16] cited by [11].

Degradable polymers are described as being either surface eroding or bulk eroding polymers. Surface erosion occurs when the rate of polymer degradation is much faster than water penetration into the polymer bulk [17] and hydrolysis is confined to the outer surface and the interior matrix of the polymer remains essentially unchanged [2]. PLGA is a bulk eroding polymer since the rate of water penetration into the polymer matrix is faster than the rate of polymer degradation. As a result, polymer degradation is not confined to the surface of the polymer but occurs throughout the whole of the polymer matrix [4, 17]. Degradation of the polymer chains results in creation of carboxylic end groups within the matrix of the polymer which can further increase ester hydrolysis via autocatalysis and increase the rate of degradation within the bulk of the polymer as compared with the surface [11, 18].

Increasing the GA content of PLGA copolymers is associated with faster degradation rates. Wu and Wang [12] studied PLGA with similar molecular weights but with different GA:LA ratios and found that the biodegradation rate constant increased with

increasing GA content. The composition of the PLGA determines the hydrophilicity and wettability of the polymer and increasing the GA content increases the hydrophilicity and hence the hydration of the polymer [12]. The pendant methyl group on the lactic acid moieties sterically hinders the attack of water molecules and increases the hydrophobicity of the polymer [12]. Hydrolytic scission of the ester bonds occurs more readily on the linkage between GA and LA or GA [18] and the inclusion of GA units in the polymer introduces vulnerable points on the macromolecular chains [9] since the GA units are hydrolysed much faster than lactic units due to their higher hydrophilicity [2].

It is generally accepted that higher MWt PLGA polymers degrade slower than polymers with smaller MWt's [2]. But Wu and Wang [12] found the opposite - that higher MWt polymers degrade faster than those of similar composition but with lower MWt's. They speculated that the increased length of the polymer chains increased the chances of hydrolytic attack by water molecules. Crystallinity is affected by MWt and the effect of crystallinity on degradation is unclear with some reports suggesting faster degradation as crystallinity increases while others report the opposite [2]. Increasing the proportion of $_{L}$-LA units in the copolymer increases the crystallinity of the polymer and results in lower degradation rate constants [19]. Li *et al* [9] found that the preferential degradation of the GA units enriched the remaining polymer fragments with respect to $_{L}$-LA units resulting in an increase in polymer crystallinity. In general polymer degradation and drug elution are accelerated by greater hydrophilicity and less crystallinity which can be achieved by increasing the GA content of the copolymer. Slower drug release and degradation are associated with hydrophobic, crystalline PLLA polymers [2]

Polymer device size has been shown to affect the degradation rate of PLGA. Dunne *et al* [13] and Klose *et al* [20] showed that larger microspheres have a faster degradation rate than smaller ones while Grizzi *et al* [14] demonstrated that the degradation rate of various devices prepared from the same PDLLA polymer depended on the size of the device with larger devices degrading at a faster rate than smaller ones. This is thought to be due to differences in length of the diffusion pathways within different sizes of polymer matrices which affects the diffusion rate of shorter chain

degradation products out of the polymer and autocatalysis rates within the polymer with the effect being more pronounced in larger devices [17, 20].

Other factors affecting degradation of PLGA are the nature of the release medium and the temperature of the conditions. PLGA degradation is affected by pH of the release medium. Hydrolysis of the ester linkages is catalyzed by protons and induced by bases [21] and both strongly acidic and strongly alkaline conditions accelerate degradation [2]. The rate of degradation and drug elution from PLGA microspheres also increases with increasing temperature [13, 21-22].

The temperature in which the device is degrading could be important in terms of the polymers glass transition temperatures (T_g) and hence, the drug delivery properties of the polymer, although given the intended application of many devices in the human body any temperature effects other than at temperatures of around 37°C are possibly unimportant. Park and Jonnalagadda [23] showed that T_g appears to decrease with increasing glycolic acid ratio and found T_g values of 55°C for PLGA 85:15 and 75:25 and 45-50°C for PLGA 65:35 and 50:50 which are greater than physiological temperatures of 37°C. T_g decreases as degradation proceeds. As average molecular weight decreases the degree of entanglement of the polymer chains is reduced and the mobility of the polymer chains increases leading to a reduction in T_g [20]. Blasi *et al* [24] showed that T_g decreased in hydrated PLGA devices to values below 37°C indicating that such devices may be in the rubbery state once they become hydrated when implanted in their target tissues or organs. The transition from the glassy state to the rubbery state should lead to increased rates of drug elution since the drug molecules can more easily diffuse through the polymer in the rubbery state

The release of drugs from such monolithic devices is via diffusion of the drug through the polymer coating, by its erosion, or by a combination of both [1]. Drug release profiles from PLGA devices is complex since the polymer phase properties change continuously during degradation resulting in changes in drug diffusivity and permeability [6] and polymer drug interactions can also be critical in controlling drug elution profile [1-2].

The nature of the drug will be important if it has an effect on polymeric properties such as degradation and hydration rate. Li *et al* [25] found that adding caffeine (a basic molecule) in low concentrations such that the molecule was dissolved and dispersed within the polymer matrix, accelerated PLLA degradation. Other basic molecules such as diazepam have been found to increase degradation rate of PLGA [2]. Lidobase was found to accelerate early degradation rate of PLGA as compared to lidosalt resulting in a bi-modal elution profile for the base as compared with a tri-modal release pattern for lidosalt [26]. Hydrophilic drugs such as aspirin may increase the hydration rate of PLGA devices but in a study by Siegel *et al* [27] this did not affect the degradation rate of the PLGA device. In that study the drugs Corticosterone, Haloperidol, Hydrochlorothiozide and Ibuprofen slowed PLGA degradation while Thiothixene accelerated degradation [27]. Haloperidol was found to change PLGA degradation from bulk eroding to surface eroding in this study. The chemical properties of the drug and their effect on the polymer degradation are therefore of critical importance in explaining drug-release mechanisms from PLGA polymers [2].

Drug release profile from PLGA devices is typically tri-modal consisting of an early burst release phase in which drug molecules deposited close to the surface of the polymer are rapidly released, followed by a slower release phase as drug molecules further within the matrix diffuse through the device. Finally there is a period of more rapid release as residual drug is eluted during the later stages of polymer degradation and erosion [6]. The rate of elution of a drug from any polymer may depend on the physicochemical properties of both the polymer and or the drug [1]. Release of Ganciclovir from a 75:25 PLGA polymer was tri-modal consisting of an initial burst as surface deposited drug was released followed by a slow release stage as deeper deposited drug diffused through the polymer matrix and a final rapid release during the later stages of degradation of the polymer [28]. Release of sirolimus from a PLGA multilayer system consisted of 2 stages – a slower initial diffusion controlled stage and then a faster stage associated with polymer mass loss. Elution of 5-Flouridine from simple monolithic PLGA film implants also showed a biphasic release profile but in this case it comprised an initial burst phase in which 24% of the drug was eluted followed by slower secondary release phase [1].

In this study we sought to examine the potential use of PLGA as a polymer coating for a paclitaxel DES by examining the degradation of the polymer and the release profile of the drug in an acellular *in-vivo* rat model as described in Chapter 2. We tested the hypothesis that increasing the glycolic acid component in PLGA would increase the hydrophilicity of the coating and introduce more sites susceptible to hydrolysis therefore increasing water ingress and hydrolysis resulting in different paclitaxel release profiles dependant on polymer degradation rate. Three blends of PLGA were chosen, 50:50, 75:25 and 85:15. Additionally the effect of coating thickness on degradation and elution properties was investigated using 50:50 PLGA. Effects of PLGA degradation on the pH of the surrounding media was determined using cellular exudate collected in the PMMA chambers.

3.2 Materials and methods

Chloroform: SpS Super Purity solvent, 99.9% stabilised (Romil Pure Chemistry).
Paclitaxel from *Taxus yannanensis* (Sigma-Aldrich)
50:50, 75:25, & 85:15 poly($_{D,L}$-lactide-*co*-glycolide) (Sigma-Aldrich)
PLGA coated Liberté™ WH 16mm stents obtained from Boston Scientific.
Implantation chambers: prepared from medical grade 20mm lengths of 10mm diameter medical grade polymethyl methacrylate (PMMA) (Goodfellows UK).
Chamber seals: 45μm cellulose nitrate millipore filters.

3.2.1 Preparation of stents and implant devices

For the comparison of polymer thickness and release of paclitaxel in the 50:50 blend 400μg of the PLGA with or without 5% paclitaxel (Sigma-Aldrich) and for one set 200μg 50:50 PLGA with 5% paclitaxel was coated onto a Liberté 16mmWH coronary stent. For the 75:25 and 85:15 blends 400μg of the PLGA with 5% paclitaxel was coated onto a Liberté™ 16mmWH coronary stent. The stents were placed into PMMA chambers which were sealed using a 0.45μm millipore filter as described in Chapter 2 page 41, Figure 2.2. Two test chambers each containing one stent were implanted subcutaneously into the backs of male Wistar rats, either side of the spine. The number of animals used for each time point is shown in the Appendix, Table A.1.

At the appropriate time points the implants were removed for analysis. The stents were washed by quickly dipping the stent twice into ELGA Purelab UHQ water. Excess moisture was quickly blotted off the stent and the sample was placed into a clean polystyrene vial, and dried under vacuum at room temperature. The samples were weighed every 24 hours until a stable weight was achieved and the samples were dry. The sample vials were then tightly capped and stored at 4°C in polythene sealed bags containing Silica desiccant.

3.2.2 Gel permeation chromatography:

For GPC analysis at early sampling times 2 stents were added to 1ml of chloroform in a capped glass vial and shaken gently for 3 hours to extract the polymer. For later time points more stents were combined and added to 1 ml of chloroform to ensure there was sufficient sample to get a detectable signal. GPC was performed using four 7.8x300mm, 5µm Waters Styragel columns (1. HR0.5 MWt range 0 to 1000, 2. HR2 MWt range 500 to 20,000, 3. HR3 MWt range 500 to 30,000 and 4. HR4 MWt range 5,000 to 500,000) with a flow rate of 1ml per minute with chloroform as a the mobile phase and using a Polymer Labs ELS 1000 evaporative light scatter detector (ELS) and Polymer Labs LC1200 UV/VIS ultra violet (UV) detector. Easi-Cal PS-2 (Polymer Labs) polystyrene standards, with a molecular weight range of 580 – 377,400 were dissolved in chloroform to give a 0.1% solution. Raw polymer was prepared by weighing an amount of polymer and adding chloroform to give a final concentration of 1mg/ml and gently mixing at 120rpm at room temperature for 3 hrs. The injection volume was 250µl with a polymer concentration of approximately 800µg/ml solvent for the stent samples and 100µl for the raw polymer and Easi-Cal standards) with three measurements per sample. Quantitative analysis and calculation of weight average molecular weight (M_w) was performed using Cirrius Software (Polymer Labs).

Paclitaxel concentration was measured using the area under the peak using data from the UV detector and comparing with standards prepared by dissolving paclitaxel in chloroform and diluted to give concentrations of 2, 5, 10, 20, & 50µg/ml. 250µl of each standard was injected and measured three times. Quality control (QC) checks

containing 0.8mg/ml 50:50 PLGA and either 10 or 20µg/ml paclitaxel were also prepared and analysed via GPC.

3.2.3 SEM and light microscopy

The explanted stents were examined by light microscopy by two methods; stereo dissection light microscope and upright reflected light microscopy using differential interference contrast microscopy and standard reflectance. SEM was performed using a Leo/Zeiss 1550 Field Emission Scanning Electron Microscope. One stent from each group was mounted onto an aluminium stub using double sided adhesive carbon tape and sputter coated with chromium using an EMITECH K575X coater.

3.2.4 pH measurements.

Cellular exudate recovered from the chambers was measured using a pH meter (Mettler Toledo) and the visual appearance of the exudate noted.

3.3 Results

3.3.1 GPC data

The chromatograms obtained from GPC revealed a monomodal plot for PLGA and a single sharp peak for the paclitaxel.

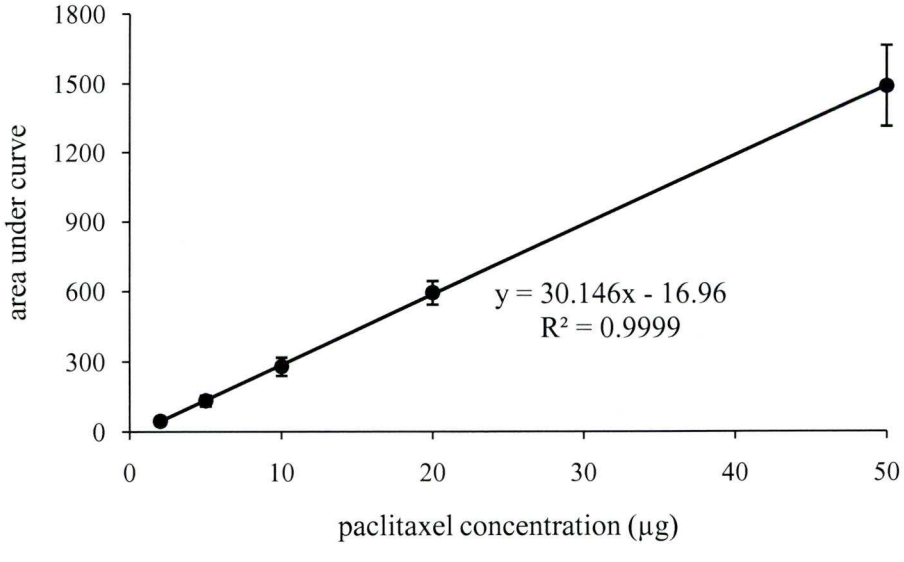

Figure 3.1 Paclitaxel Calibration (data from UV detector).
The graph shows the combined data from all of the GPC runs throughout the trial. Standard deviation is shown as error bars

Paclitaxel concentration on the 75:25 and 85:15 PLGA coated stents was measured using data from GPC from the UV detector. Combined calibration data from all the GPC runs performed during the trial is shown in Figure 3.1.

Control stents (not implanted) had paclitaxel concentrations of 19.85μg (stdev=1.61) and 20.97 (stdev=2.6) per stent respectively for the 75:25 and 85:15 polymer coatings (expected value was 20μg/stent). QC checks for 10μg/ml and 20μg/ml paclitaxel was 10.81μg/ml (stdev=0.89) and 20.71μg/ml (stdev=0.66) respectively.

Paclitaxel concentration on the 50:50 PLGA stents was derived using data from the ELS detector. A typical calibration curve is shown in Figure 3.2. Control (not implanted stents) had values of 17.68μg/stent (stdev=2.9) and 8.65μg/stent (stdev=1.11) respectively for the 400μg coating (expected value = 20μg) and 200μg coating (expected value = 10μg).

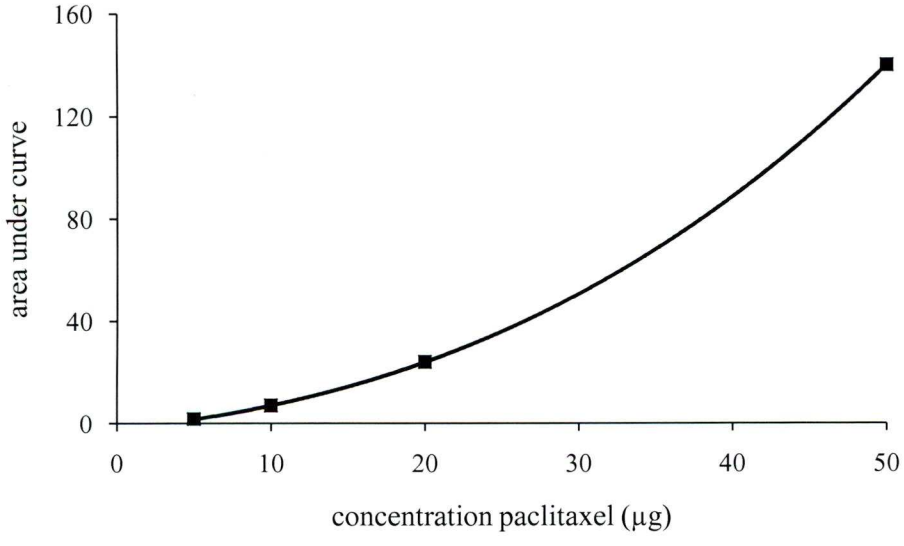

Figure 3.2 Paclitaxel Calibration Data.
Data from the ELS detector and used in paclitaxel determination for the 50:50 PLGA coated stents. Function: y= -205.9910+197.5421exp(0.0130*x)

3.3.1.1 Degradation and paclitaxel elution of 50:50 PLGA: Effect of paclitaxel and coating thickness.

The degradation of 50:50 PLGA and the elution profile of paclitaxel is shown in Figure 3.3. At the start of the trial the molecular weight of the polymer was 55,958, 56,098 and 58,236 for the stents with 400μg coating with and without paclitaxel and

the 200µg coating with paclitaxel respectively. During the trial period the degradation of the polymer in the 400µg coating per stent group was faster than in the 200µg coating per stent but with a slower elution rate of paclitaxel. Over the first 15 days the polymer degraded rapidly and the molecular weight of the polymer remaining on the stents had decreased to approximately 16,000, 18-19,000 and 23,000 in the 400µg coating with no drug and with paclitaxel and the 200µg coating with paclitaxel respectively.

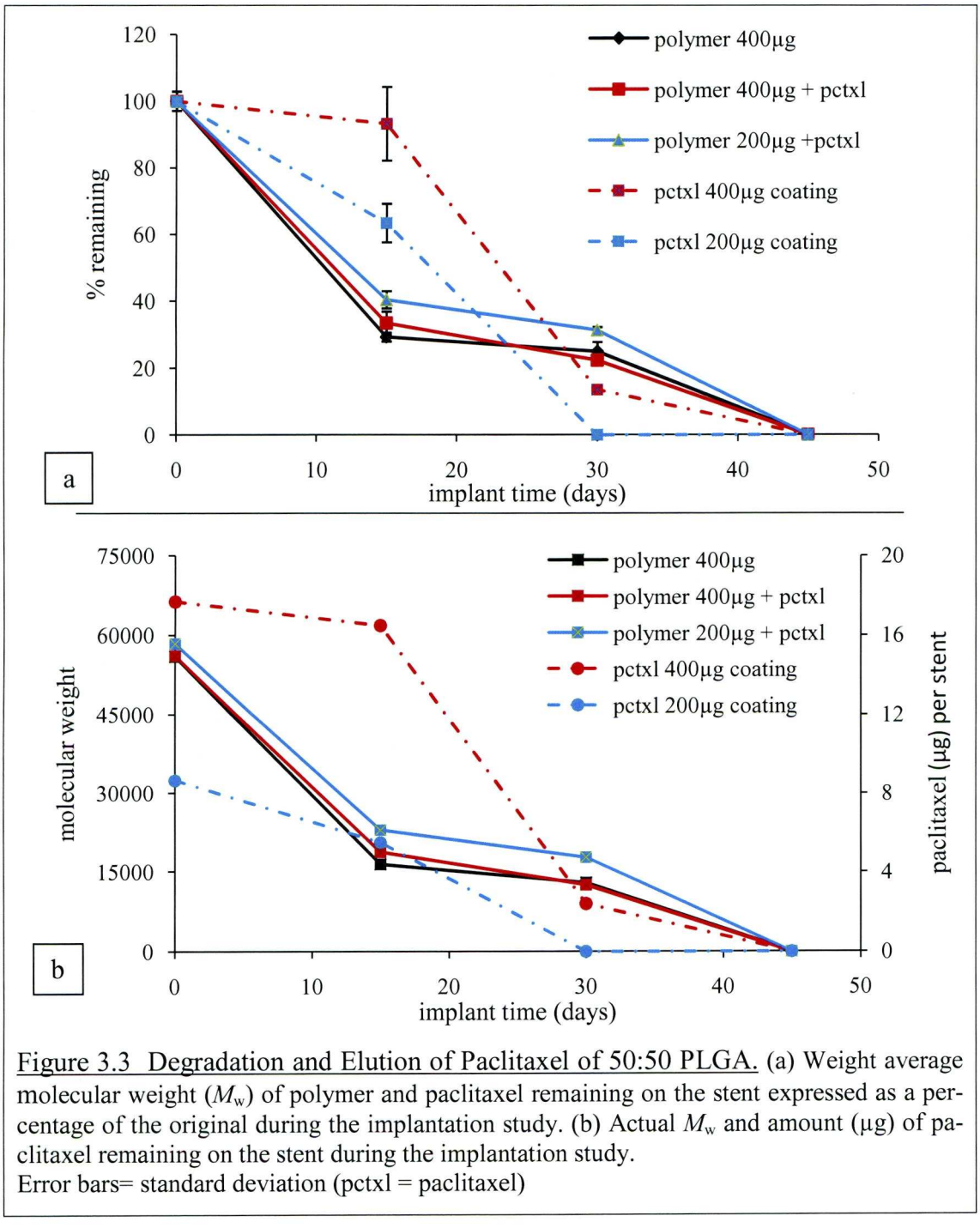

Figure 3.3 Degradation and Elution of Paclitaxel of 50:50 PLGA. (a) Weight average molecular weight (M_w) of polymer and paclitaxel remaining on the stent expressed as a percentage of the original during the implantation study. (b) Actual M_w and amount (µg) of paclitaxel remaining on the stent during the implantation study.
Error bars= standard deviation (pctxl = paclitaxel)

Paclitaxel was eluted faster from the 200μg coated stents with 64.4% of the paclitaxel remaining on the stent at 15 days while some 93.2% of the paclitaxel remained in the 400μg coated stents. After 15 days the degradation rate decreased and by 30 days the molecular weight was 23%, 22% and 31% of the original in the 400μg coated stents with and without paclitaxel and the 200μg coated stents with paclitaxel respectively. At 30 days no paclitaxel could be detected in the 200μg coating group but on some samples in the 400μg coating group 2.39μg of paclitaxel (13.5% of original) was still present on the stent. At 45 days implantation time neither polymer nor paclitaxel could be detected using GPC for any of the coating formulations. ANOVA on the molecular weight data for coating and implant time showed that there was no significant difference in degradation rate between the 400μg coating with and without paclitaxel, but over the time period from 15 to 30 days the molecular weight of the remaining polymer in the 200μg coating with paclitaxel was significantly higher than that in the 400μg coatings. This data suggests that the difference in degradation rate was not due to the presence of paclitaxel but is associated with initial coating volume.

3.3.1.2 Comparison of degradation and paclitaxel elution in 400μg stent coatings of 50:50, 75:25 & 85:15 PLGA.

At the start of the trial the M_w of the 50:50, 75:25 and 85:15 polymers was 56,098, 49,363 and 43,696 respectively (figure 3.4). The 50:50 polymer degraded fastest and by 30 days the molecular weight had reduced by 70% to around 12,000 to 13,000. The molecular weight of the 75:15 and 85:15 coatings had reduced by approximately 70% by 60 and 90 days respectively to a molecular weight of around 12-13,000. In both the 75:25 and 85:15 coatings the polymer molecular weight stabilized after falling to around 13,000. After 105 days implantation 75:25 polymer could not be detected by GPC while 85:15 polymer remained detectable with a molecular weight of approximately 13000 on the stent until the end of the study period at 120 days.

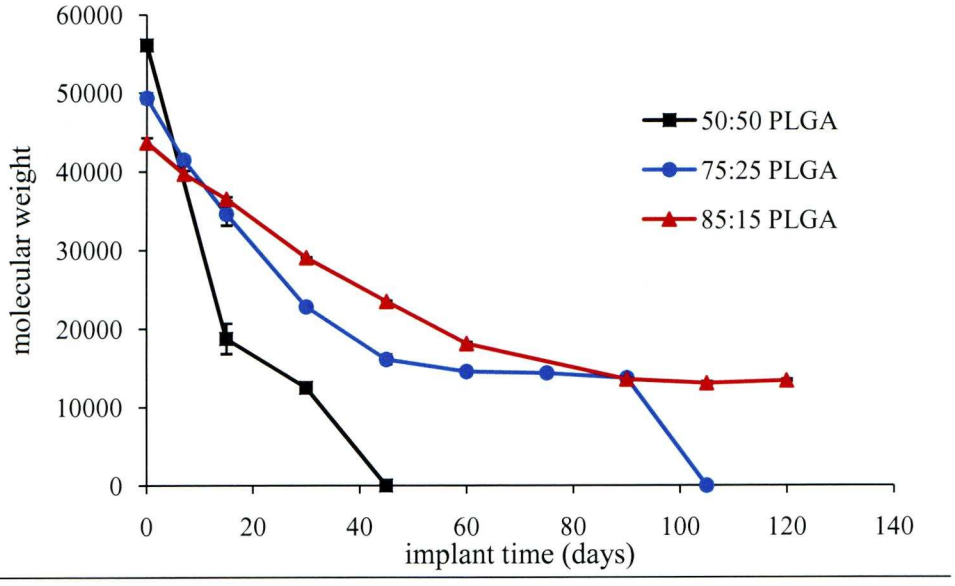

Figure 3.4 Degradation of 50:50, 75:25 & 85:15 PLGA
The graph shows the molecular weight (M_w) of the polymer remaining on the stent as implantation time increases. Error bars= standard deviation.

Elution of paclitaxel was fastest in the 50:50 PLGA and slowest in the 85:15 PLGA (Figure 3.5). Paclitaxel was released from the 50:50 and 75:15 PLGA stent coatings in a two phase manner: an initial period of slow release followed by a rapid loss of paclitaxel. At 15 days 93% of the paclitaxel remained on the 50:50 PLGA coated stent despite the degradation of the polymer to a molecular weight of 18,751. But by 30 days only 22% of the paclitaxel remained and the molecular weight of the polymer had further degraded to 12,483. At 30 days the 75:15 PLGA molecular weight of the polymer had degraded to approximately 22,000 and 84% of the paclitaxel remained on the stent.

More rapid release of paclitaxel occurred from the 75:15 polymer after 45 days as the molecular weight degraded to below 16,000 and paclitaxel could not be detected using GPC at 75 days despite polymer with a molecular weight of around 13,000 remaining on the stent. At 60 days the M_w of the 85:15 polymer was approximately 18,000 but there was very little measurable elution of paclitaxel during this period. At 90 days the polymer M_w had degraded to approximately 13,000 and around 50% (10µg) and 75% of the paclitaxel had been eluted by 90 and 105 days respectively. At 120 days 95% of the paclitaxel had been eluted with no further significant degradation of the polymer M_w. At 120 days data for paclitaxel was measurable in only 2

of the 3 samples (the paclitaxel in the third sample could not be read due to peaks from the cellular exudate or other sources overlapping the paclitaxel peak).

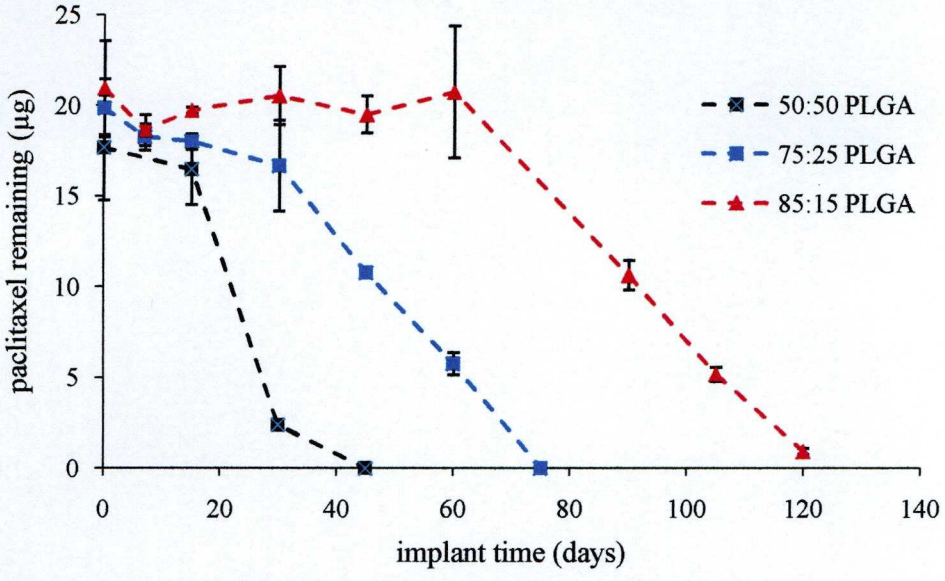

Figure 3.5 Elution of paclitaxel from 50:50, 75:25 & 85:15 PLGA stent coatings
The graph shows the amount of paclitaxel (in µg) remaining on the stent as implantation time increases. Error bars= standard deviation

3.3.2 SEM and light microscopy.

SEM and LM analysis are demonstrated in figures 3.6 to 3.12.

In the early stages of degradation the polymer was an intact coating (figure 3.6.a, 3.7.a & 3.7.e). With time polymer degradation and erosion occurred and the coating became compromised eventually revealing gaps in the coating, exposing the bare metal of the stent (Figure 3.6.b-d) & 3.7.b,d,f). The changes observed in the stent coating as degradation proceeded were similar in all three PLGA polymers but the changes occurred over different time scales (Figure 3.7).

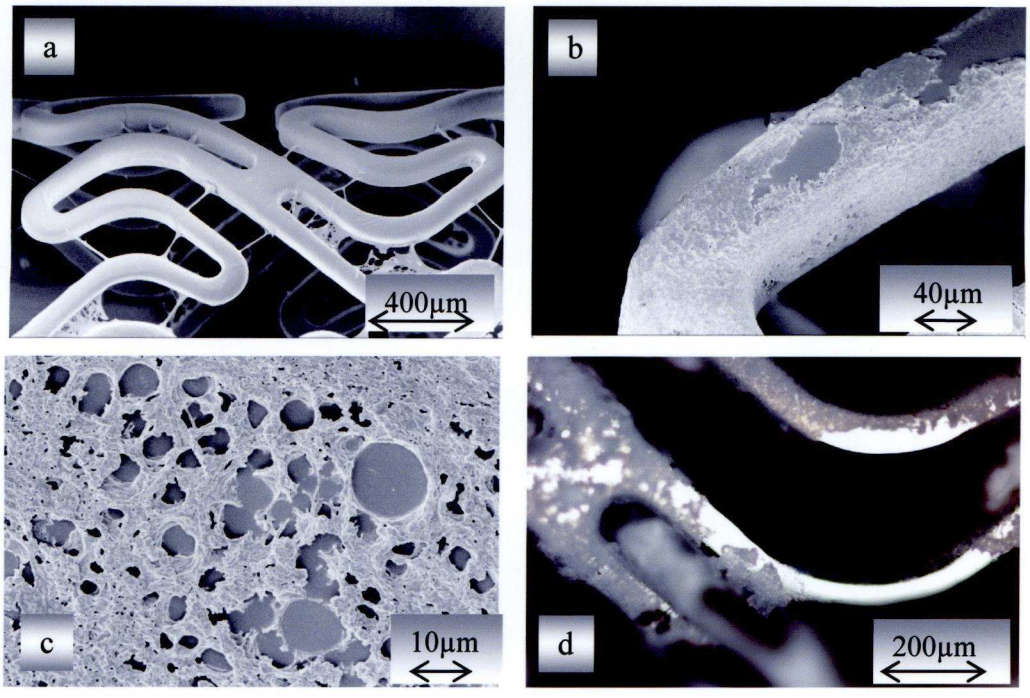

Figure 3.6 SEM and LM images showing deterioration of the polymer stent coating.
a) SEM micrograph 15 day implant time 50:50 PLGA ,b) SEM micrograph 30 day implant 50:50 PLGA, c) SEM micrograph 85:15 PLGA 120 day implant, d) LM image 85:15 PLGA 120 day implant

In the early stages of degradation the polymer was an intact coating (figure 3.6.a, 3.7.a & 3.7.e). With time polymer degradation and erosion occurred and the coating became compromised eventually revealing gaps in the coating, exposing the bare metal of the stent (Figure 3.6.b-d) & 3.7.b,d,f). The changes observed in the stent coating as degradation proceeded were similar in all three PLGA polymers but the changes occurred over different time scales (Figure 3.7).

As with the molecular weight data the visual appearance of the stents indicated a trend of a faster rate of degradation and mass loss from the 50:50 PLGA coating as compared with 75:25 PLGA while 85:15 PLGA coating degraded the slowest of the polymers on trial. (Figure 3.7 & 3.8).

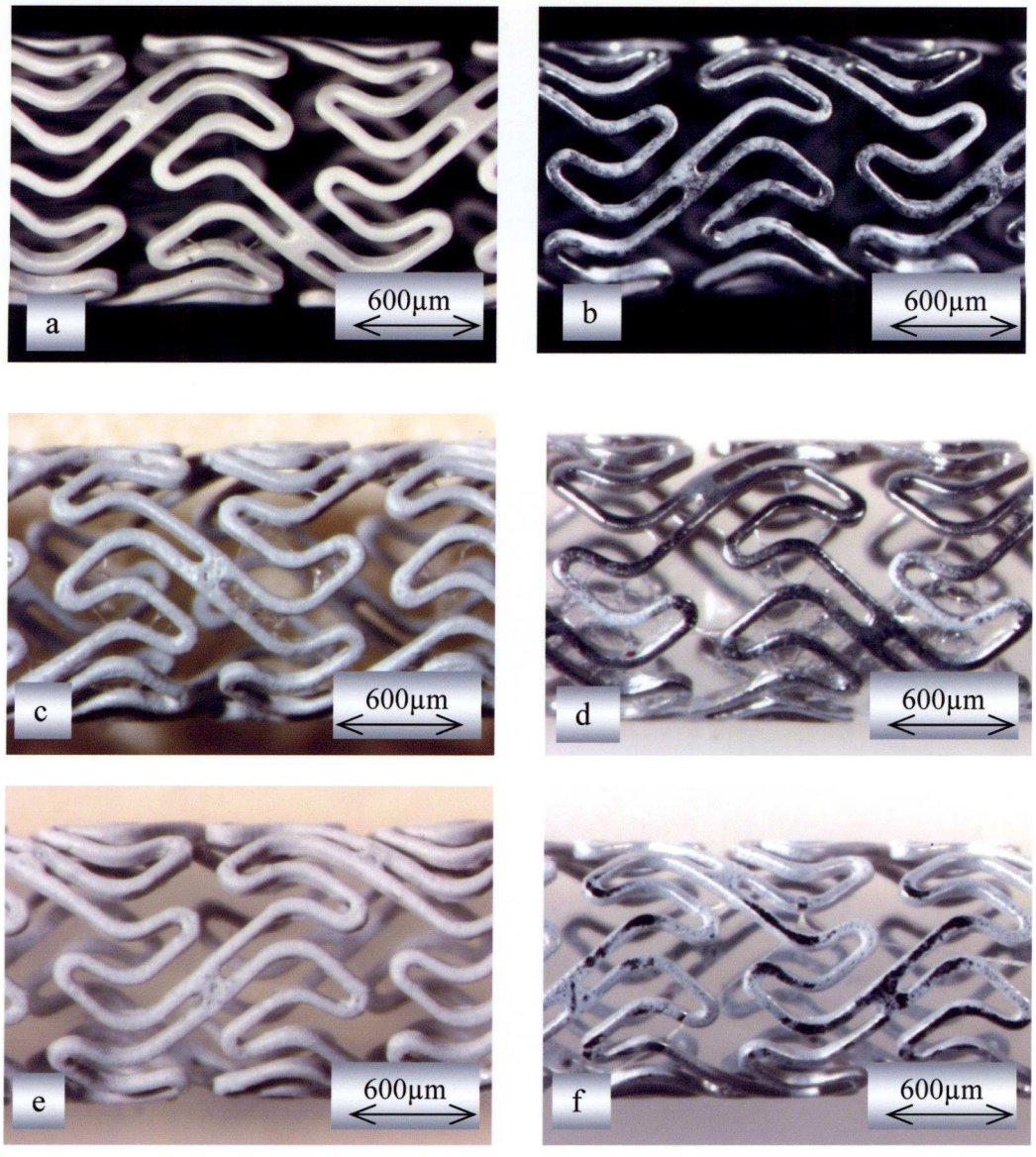

Figure 3.7 LM images showing changes in PLGA polymer coating integrity at different implantation times.
50:50 PLGA coated stent a) 15 day, b) 30 day
75:25 PLGA coated stent c) 45 day, d) 90 day
85:15 PLGA coated stent e) 90 day, f) 120 day

At 15 days implantation the polymer coating appeared to be intact on the 50:50 PLGA (Figure 3.7.a) despite the degradation of the polymer chains but surface pitting was evident at 15 days and at 30 days extensive loss of polymer bulk had occurred creating cavities and vacuoles in the polymer bulk (Figure 3.8.a&b) and gaps became evident in the coating exposing the metal struts of the stent (Figure 3.7.b).

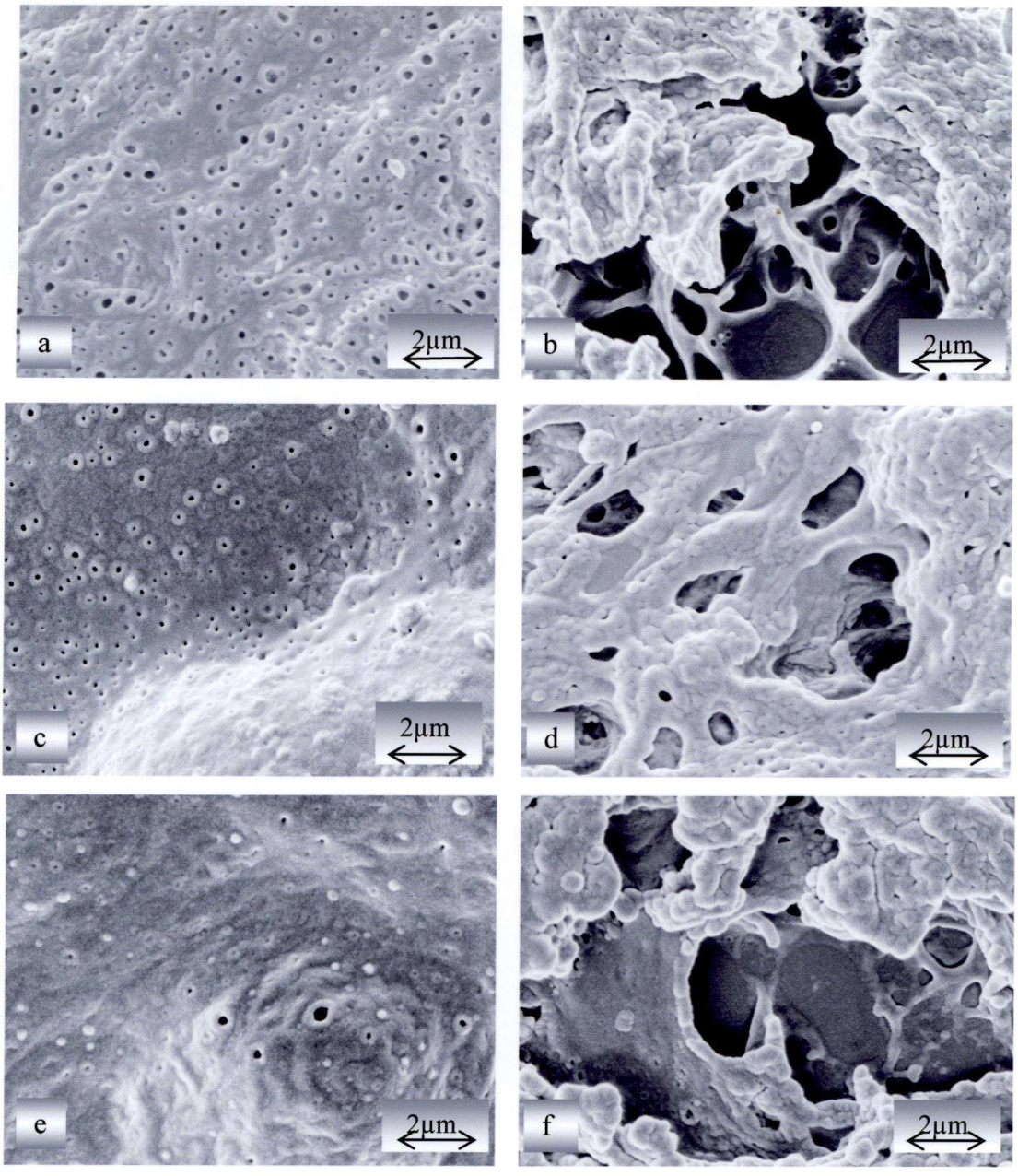

Figure 3.8 SEM images of the surface of different PLGA polymers showing different time scales and degradation. Magnification= 20k. a) 15 day 50:50 PLGA, b)30 day 50:50 PLGA, c) 75:25 PLGA 30 day, d) 60 day 75:25 PLGA, e) 60 day 85:15 PLGA, f) 105 day 85:15 PLGA.

At 45 days the bulk of the polymer coating is still intact in the 75:25 coated stents but by 90 days extensive areas of the bare metal of the stent were visible (Figure 3.7.c&d). Deterioration of the 85:15 coating was over a longer time period and at 90 days the polymer coating was still intact but by 120 days polymer erosion was advanced and large areas of bare metal of the stent were exposed (Figure 3.7.e&f). As compared with 50:50PLGA, surface pitting appeared later for the 75:25 and 85:15

PLGA at 30 and 60 days respectively while extensive loss of polymer bulk with the appearance of larger cavities and vacuoles in the polymer bulk was seen at 60 days and 105 days in the 75:25 and 85:15 PLGA respectively (Figure 3.8.c-f).

PLGA is a bulk eroding polymer and as such degradation proceeds faster within the body of the polymer than at the surface. Bulk degradation at 15 days was evident in the 50:50 PLGA stent coating where a fissure in the apparently intact polymer coat revealed a highly vacuolated area where polymer degradation and mass loss had occurred beneath the surface of the polymer (Figure 3.9).

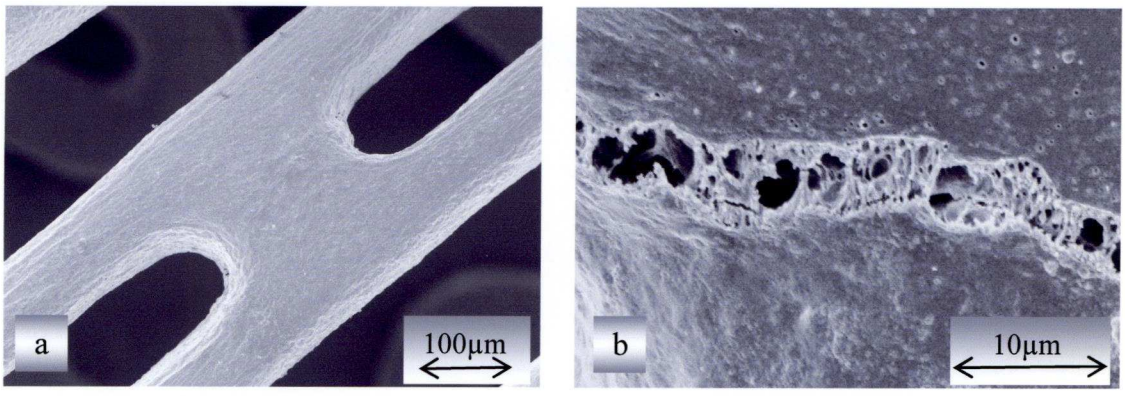

Figure 3.9. SEM images of 50:50PLGA after 15 days implantation. a) low magnification image of a stent showing intact coating. b) high magnification image of an area of (a) where the surface was scratched, showing bulk degradation within the bulk of the polymer.

Evidence of 'blistering' was seen on part of the surface of the 75:25 and 85:15 PLGA coating after 30 days (Figure 3.10.a). Rupture of the blisters at 30 days and 60 days on the 75:25 and 85:15 coating respectively revealed cavities below the polymer surface indicating bulk erosion and polymer mass loss (Figure 3.10.a&b). Similarly, blistering of the stent coating was seen in the 85:15 PLGA polymer at 45 days and becoming more extensive at 60 and 90 days implantation time (Figure 3.10.c&d).

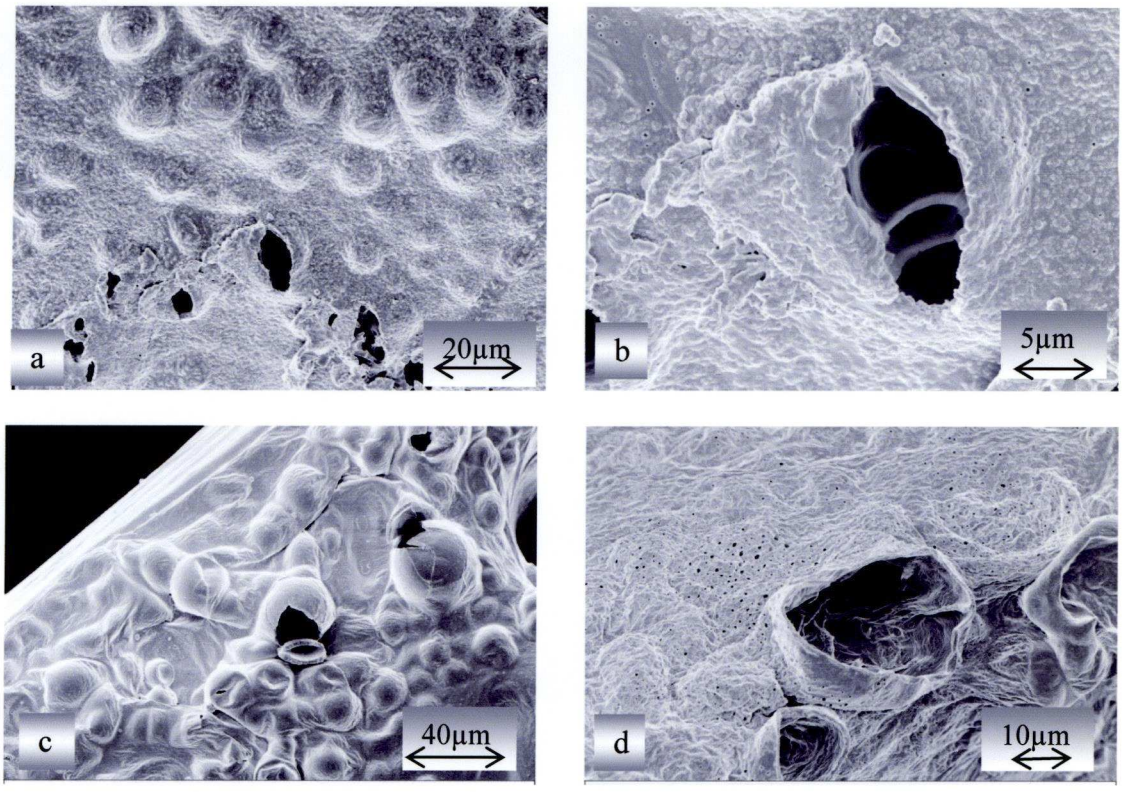

Figure 3.10. SEM images. Blistering on 75:25 and 85:15 PLGA stent coatings.
a&b 75:25 PLGA coating after 30 days implantation. c&d 85:15 PLGA coating after 60 and 90 day implantation respectively.

Progressive polymer degradation and erosion leading to mass loss in 85:15 PLGA coated stents can be seen in figure 3.11. At 45 days the polymer coating is intact but with areas of blistering and at 90 days the blistering is extensive but the coating remains intact (Figure 3.11.a&b). By 105 days polymer erosion had occurred and gaps in the polymer coat and rupture of the blisters was evident (Figure 3.11.c). By 120 days extensive polymer mass loss had occurred and the polymer coating appeared as a highly degraded covering with a mesh like appearance revealing extensive areas of the bare metal of the stent (Figure 3.11 c&d).

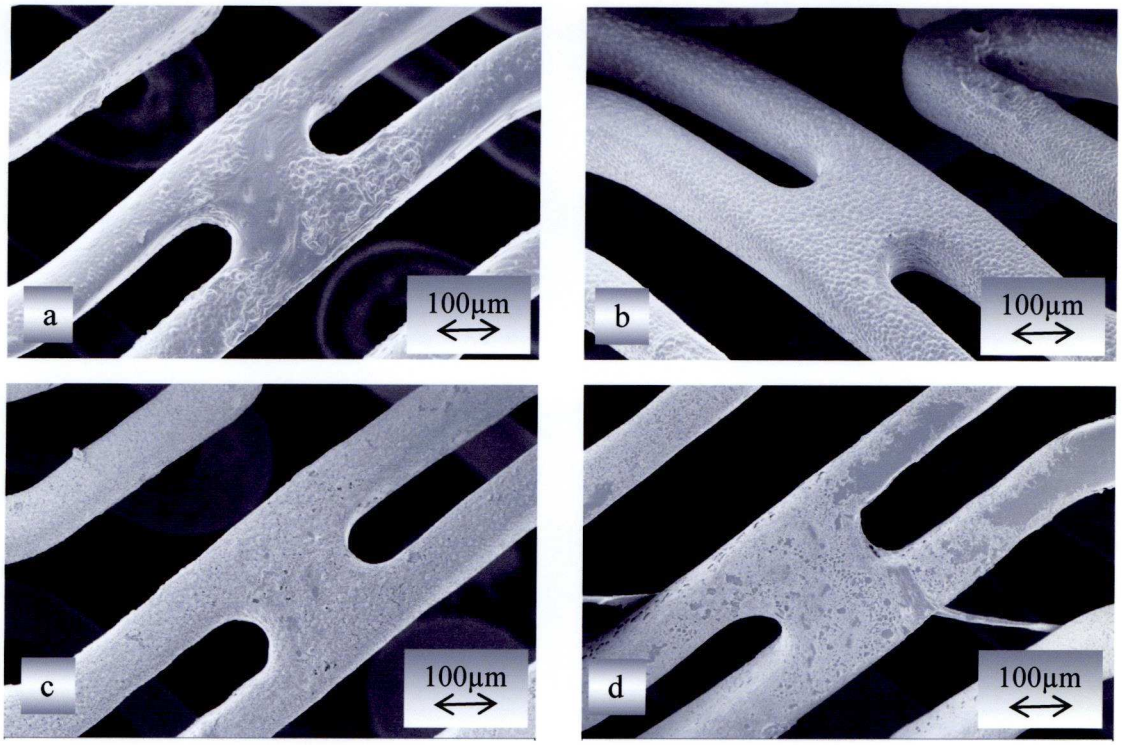

Figure 3.11. SEM Images of 85:15 PLGA coated stents
a) after 60 days implantation, b) after 90 days implantation, c) after 105 days implantation and d) after 120 days implantation.

After 45 days and 105 days for the 50:50 and 75:25 respectively, polymer could not be detected using GPC. Light microscopy gave the impression of a metallic stent with no polymer attached but at high magnification SEM showed residue of polymer still adhering to the stent (Figure 3.12.a-d). At 120 days 85:15 polymer could still be detected using GPC. But SEM and LM indicated that the polymer was extensively degraded, with evidence of advanced polymer erosion and. mass loss (Figure 3.12.c&d).

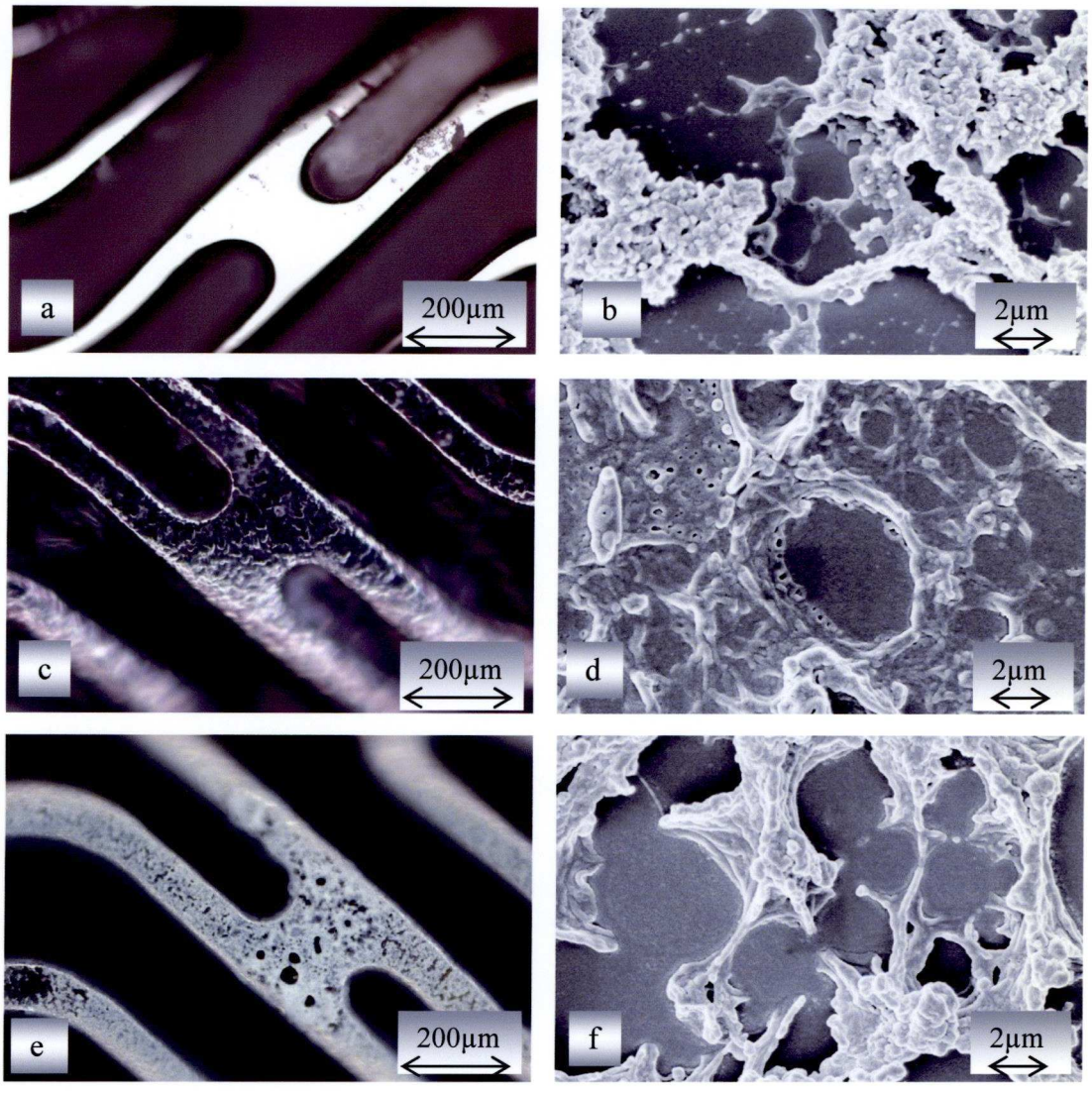

Figure 3.12 SEM and LM images: Later stages of degradation of the polymer coating. a) LM, b)SEM, 50:50 PLGA coating after 45 day implantation, c) LM, d) SEM, 75:25 PLGA coating after 105 days implantation, d) LM e) SEM, 85:15 PLGA coating after 120 days implantation.

3.3.3 Chamber exudate pH.

3.3.3.1 50:50 PLGA 200µg and 400µg coatings.

Figure 3.13 shows small changes in pH between the groups and over implantation time. Statistical analysis by ANOVA showed that at 15 and 30 days there was no

significant difference in pH between the groups, but the pH of the exudates was significantly higher in all groups at 15 days than at 30 days ($p<0.001$ for the 400µg coating with and without paclitaxel, $p=0.002$ for the 200µg + paclitaxel coating). ANOVA demonstrated that implant time significantly affected pH ($p<0.001$).

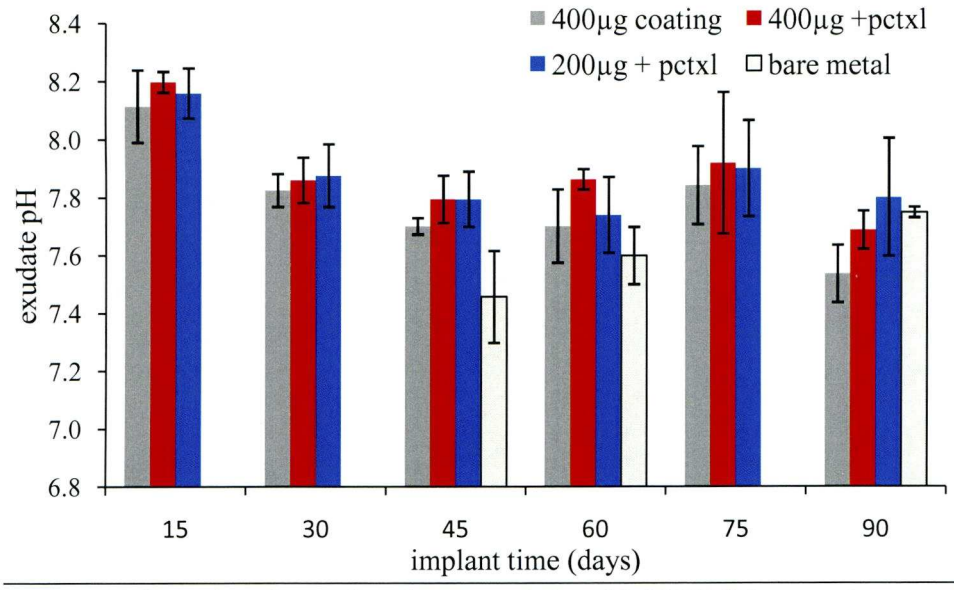

Figure 3.13 pH of exudate from chamber containing 50:50 PLGA coated stents.
A= 400µg coating, B= 400µg coating + 5%w/w paclitaxel, C= 200µg coating + 5% paclitaxel. nb After 45 days there was no polymer detected on the stents

At 15 days the pH across all groups was 8.16, but over the trial the pH gradually decreased to 7.69 by 90 days, although the data from the 75 day time points did not fit in with this trend. There was a slight decrease in the pH of the chamber exudates after 45 days but given that there was no detectable polymer on the stents after this time point the pH change is unlikely to be associated with polymer degradation products. Whether the change is associated with the presence of the exposed metal surface of the stent is unclear since there was a slight, but significant ($p=0.018$) increase in the pH of exudates from chambers containing BMS from 7.46 at 45 days to 7.75 at 90 days.

3.3.3.2 400µg stent coating with 5% paclitaxel.

An increase in pH over time was observed in exudates from chamber containing BMS (Figure 3.14) and analysis by ANOVA showed that at the pH at 45 days was significantly lower than at 90 and 120 days ($p<0.05$).

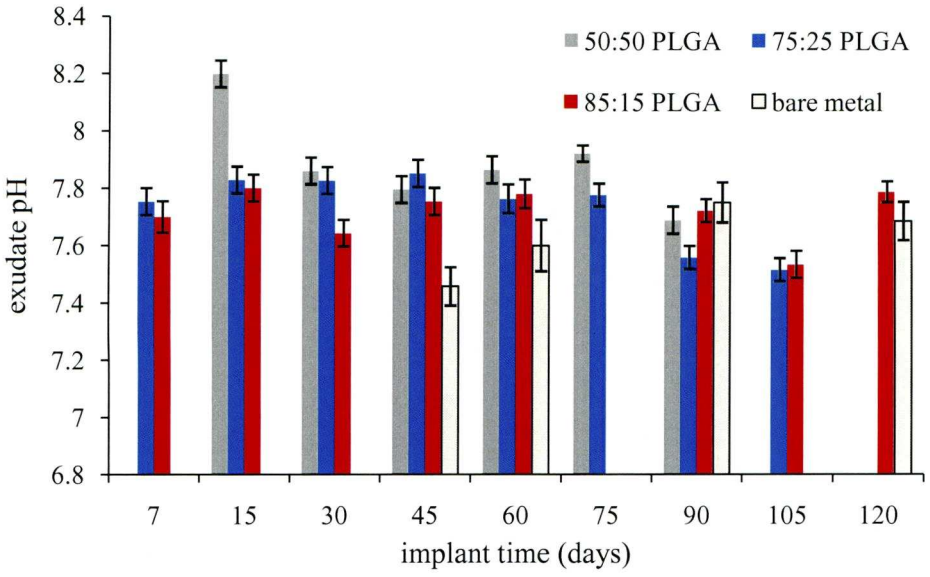

Figure 3.14 pH of chamber exudate from stents coated with 400µg polymer with 5% paclitaxel.
Dataset for 50:50 PLGA is taken from 400µg coating with 5% Paclitaxel.

The pH of the chamber exudates from 50:50 PLGA coated stents at 15 days was significantly higher than all the other sampling points and groups. There was no obvious trend in change of exudate pH from chambers containing 75:25 PLGA coated stents but the pH was significantly lower at 90 days ($p<0.001$) than the earlier time points. Likewise there was no obvious trend in exudate pH from chambers containing 85:15 PLGA coated stents but analysis by ANOVA showed that at 7, 30, 90 and 105 days the exudate pH was significantly lower than the other time points. Overall there was no difference in exudate pH and implant time between the 75:25 and 85:15 coated stents.

3.3.4 Mass loss data

Mass loss was to be determined by comparing the weight of polymer on the stent after implantation with the original weight of polymer on the stent. Following recovery of the stents from explanted chambers cellular exudate was found to adhere to the stent. This exudate was not completely removed by the washing protocol therefore making the weight measurement of the explanted stent unreliable. Data shown in

Figure 3.15 is from another trial using the same protocol for 400µg polymer coating using an *in vitro* model.

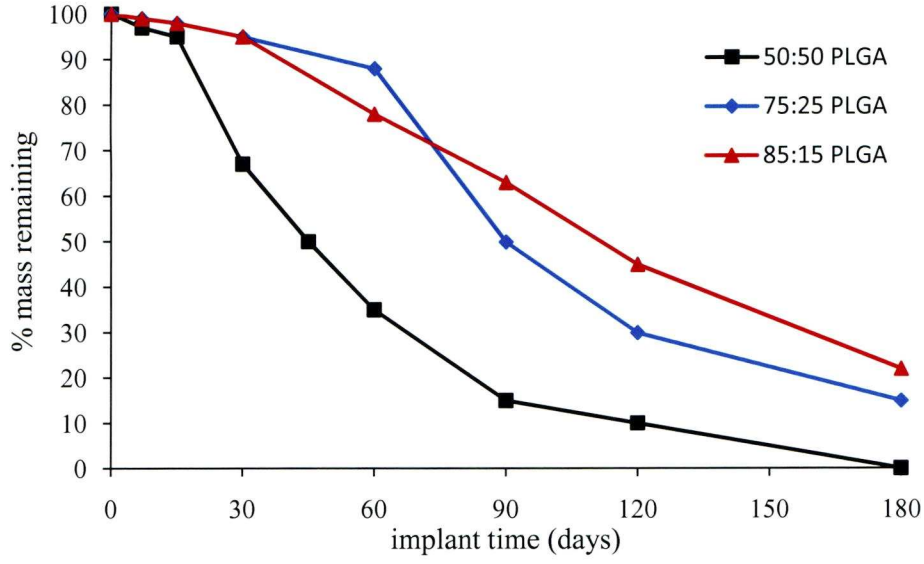

Figure 3.15 *In vitro* polymer mass loss from PLGA coated stents.
Graph shows the mass of polymer remaining on the stent after implantation.

Figure 3.15 shows that mass loss was fastest in the 50:50 PLGA and overall, was slowest in the 85:15 PLGA. In all three polymers mass loss was initially slow with a lag phase lasting approximately 15 days in the 50:50 PLGA and 30-60 days in the 75:25 and 85:15 PLGA. After 15 days implantation mass loss was more rapid in the 50:50 PLGA declining to around 30% of the original mass remaining by 60 days. In this model polymer could still be measured at 90 and 120 days by which time approximately 10% of the mass remained. In the 75:25 PLGA coated stent group the lag phase lasted 60 days by which time 85-90% of the mass remained after which the rate of mass loss increased and at 120 days approximately 30% of the polymer mass remained. 75:25 PLGA was still detectable on the stent at 180 days. Mass loss from 85:15 PLGA coated stents mirrored that from the 75:25 coated stents to 60 days. After 60 days mass loss declines steadily and faster in the 75:25 PLGA coatings as compared with 85:15 PLGA coating. As such the 60 days time point for 75:25 PLGA days showing slower mass loss than the 85:15 PLGA coatings is counterintuitive and may be erroneous. After 90 days mass loss from the 85:15 PLGA coated stents was

slower than the 75:25 PLGA coatings and approximately 45 and 25% of the mass remained at 120 and 180 days respectively.

3.4 Discussion.

The effect of glycolic acid content of PLGA and the thickness of coating applied to a coronary stent on the degradation of the polymer and the release of paclitaxel has been demonstrated. The suitability of any of the PLGA polymers tested as coronary stent coatings will depend on the degradation profile and residency time of the polymer, the drug delivery properties and that the erosion of the polymer coating is via steady removal of solubilised oligomers with the less degraded polymer remaining adhered to the stent with no peeling or loss of larger fragments of the coatings.

Using a 400μg coating of 50:50 PLGA there was no difference in the degradation of the polymer with or without 5% paclitaxel. Since the degradation of PLGA is via hydrolysis, degradation rate is governed both by the ingress of water into the device and the rate of hydrolysis. The presence of a drug within a PLGA matrix has been demonstrated to affect the degradation rate possibly by altering the rate of water diffusion into the polymer bulk. Paclitaxel is a hydrophobic drug but inclusion of 5% w/w of the drug into 50:50 PLGA had no effect on degradation of a 400μg stent coating. Hydrophilic drugs such as aspirin have been found to increase the ingress of water into a device and thus increase the polymer degradation rate whereas more hydrophobic drugs decreased water uptake and consequently reduced the degradation rate [27].

The degradation rate in a 200μg 50:50 PLGA + 5% paclitaxel coated stent was slower than in the 400μg 50:50 PLGA + 5% paclitaxel stent coating while the elution of the paclitaxel was faster. This effect has been observed in other studies on PLGA showing that increasing the size of a device increases the degradation rate of the polymer [2, 13-14, 20].

Differences in the degradation rate may be attributable to a couple of factors. The release rates of the short chain degradation products is slower in larger devices and their contribution to the average molecular weight of the polymer will be greater for

larger devices as compared with smaller ones. Consequently the average molecular weight of the thicker (400μg) coating will decrease faster than in the thinner (200μg) coating from which short chain degradation products will be more rapidly released [20]. Secondly rates of autocatalysis within the PLGA coating could be greater in the 400μg coating as compared to the 200μg coating. Short chain alcohols and acids produced from ester bond hydrolysis within the polymer can diffuse out of the matrix of the polymer into the release medium where they are neutralized. Additionally bases, such as hydroxide ions, from the fluids of the release medium can diffuse into the device neutralizing the generated acids. The rate of diffusion of these two processes will be slower in the thicker (400μg) coating as compared with the 200μg coating. Ester bond hydrolysis is catalyzed by protons and if the diffusion of bases into the device is not sufficient to neutralise the acids formed during PLGA degradation there will be a decrease in the micro pH within the polymer leading to increased rates of autocatalysis, the effect being more pronounced in larger devices [17, 20]. In the thicker coating there would be a larger diffusion pathway for soluble oligomers and an increased tendency for the oligomers to be trapped in the polymer matrix, thereby increasing the number of carboxylic acid groups in the coating giving an increased rate of autocatalysis as compared to the thinner coating. Additionally the shorter diffusion pathway of the thinner 200μg coating will enable faster diffusion of hydroxide ions and other bases from the external medium as compared to the 400μg coating. The neutralising effect would be expected to be greater in the 200μg PLGA coating contributing further to lower rates of autocatalysis as compared with the thicker coating [17, 20]. The elution of a drug is affected by a number of factors including polymer chain length with smaller chains offering less restriction for drug diffusion and the length of the diffusion pathway, with smaller diffusion pathways expected to enable faster drug diffusion out of the device [17, 20]. In our study faster rates of polymer degradation was seen in the 400μg coating as compared with the 200μg coating but paclitaxel was released faster from the thinner (200μg) coating. Faster elution of paclitaxel from the 200μg coating as compared to the 400μg coating possibly indicates the relative importance of diffusion distance and polymer chain length in the elution of paclitaxel from PLGA polymer matrices.

Comparison of the 50:50, 75:25 and 85:15 PLGA 400μg coatings revealed that morphological changes and degradation of the polymer were similar but occurred in a

time dependant manner related to the glycolic acid content of the copolymers and in this study 50:50 PLGA stent coating degraded the fastest and had the fastest mass loss and 85:15 PLGA coating the slowest with the slowest mass loss. Glycolic acid units constitute vulnerable points in the polymer chains and degradation occurs preferentially on the glycolic acid bonds [9] and increasing glycolic acid content of the polymer increases the degradation rate. Additionally increasing the glycolic acid content of the polymer increases hydrophilicity of the polymer coating and the consequent higher water uptake increases the rate of hydrolysis [29-30].

The relationship between molecular weight loss, paclitaxel elution and polymer mass loss are shown in Figure 3.16. Initially, degradation of the stent coating was via hydrolysis resulting in a reduction in the M_w of the PLGA to around 12,000 to 13,000 after approximately 30, 60 and 90 days for the 50:50, 75:25 and 85:15 PLGA respectively. Whereas the M_w of the coatings decreased steadily after implantation of the device there was an early phase in which the release of paclitaxel and mass loss was minimal. This was followed by a phase in which M_w reduction was slower but polymer erosion and paclitaxel elution was faster.

After 15 days implantation 95% of the polymer mass remained on the stent and only approximately 7% of the paclitaxel had been eluted in the 50:50 PLGA coated stents. After 30 days implantation the polymer had degraded to a molecular weight of around 12-13000 and some 30% of the polymer mass had been eroded in the 50:50 PLGA coated stents and SEM revealed a highly porous structure of the polymer coating (Figure 3.8). This period of rapid mass loss was associated with rapid elution of paclitaxel and after 30 days 85% of the paclitaxel had been eluted.

A similar pattern was seen for the 75:25 and 85:15 PLGA coated stents but over longer time periods. After 30 days implantation only 5% of the polymer mass had been eroded in the 75:25 PLGA coated stents despite the reduction in polymer M_w to around 22,000 and surface pitting was observed using SEM (Figure 3.8). At this point about 15% of the paclitaxel had been eluted. After 30 days a period of more rapid mass loss was associated with a faster rate of paclitaxel elution, and by 60 days average M_w was approximately 14,000, approximately 70% of the polymer mass remained on the stent and some 71% of the paclitaxel had been eluted in the 75:25

PLGA coated stents. At 60 days SEM revealed a surface with large pores and a highly vacuolated structure within the body of the coating (Figure 3.8).

Rate of mass loss increased in the 85:15 PLGA coatings after 30 days at which point the M_w of the polymer was approximately 30,000 and paclitaxel elution was negligible. At 60 and 90 days SEM revealed a polymer surface that was largely intact with only minimum pitting (Figure 3.8 and 3.11). After 60 days implantation mass loss and paclitaxel elution was rapid by which point the M_w had degraded to 14,500. After 105 days 80% of the paclitaxel had been eluted and only 50% of the polymer mass remained on the stent and SEM revealed a highly vacuolated and porous structure of the stent coating (Figure 3.8). The Average M_w of the 85:15 PLGA polymer remained at about 13,000 after 60 days.

At 45 days and 105 days polymer could not be detected on the 50:50 and 75:25 PLGA coated stents respectively using GPC. Polymer from the 85:15 PLGA coating was still detectable up to 120 days using GPC and was visible as a thin covering over the stent with areas of the bare metal visible.

Other studies [28, 31] have demonstrated a similar 2 phase pattern of degradation of PLGA matrices consisting of an initial phase of reduction in molecular weight followed by an erosion phase resulting in mass loss. Mass loss of PLGA is believed to occur when the molecular weight of the polymer fragments have decreased to a critical value such that they can dissolve in aqueous media and that the polymer bulk is sufficiently porous to allow the fragments to escape to the surrounding media [18, 29]. A 'critical molecular weight' of 10,000 was seen during the degradation of 50:50 and 75:25 PLGA microspheres after which mass loss of the PLGA occurred [29].

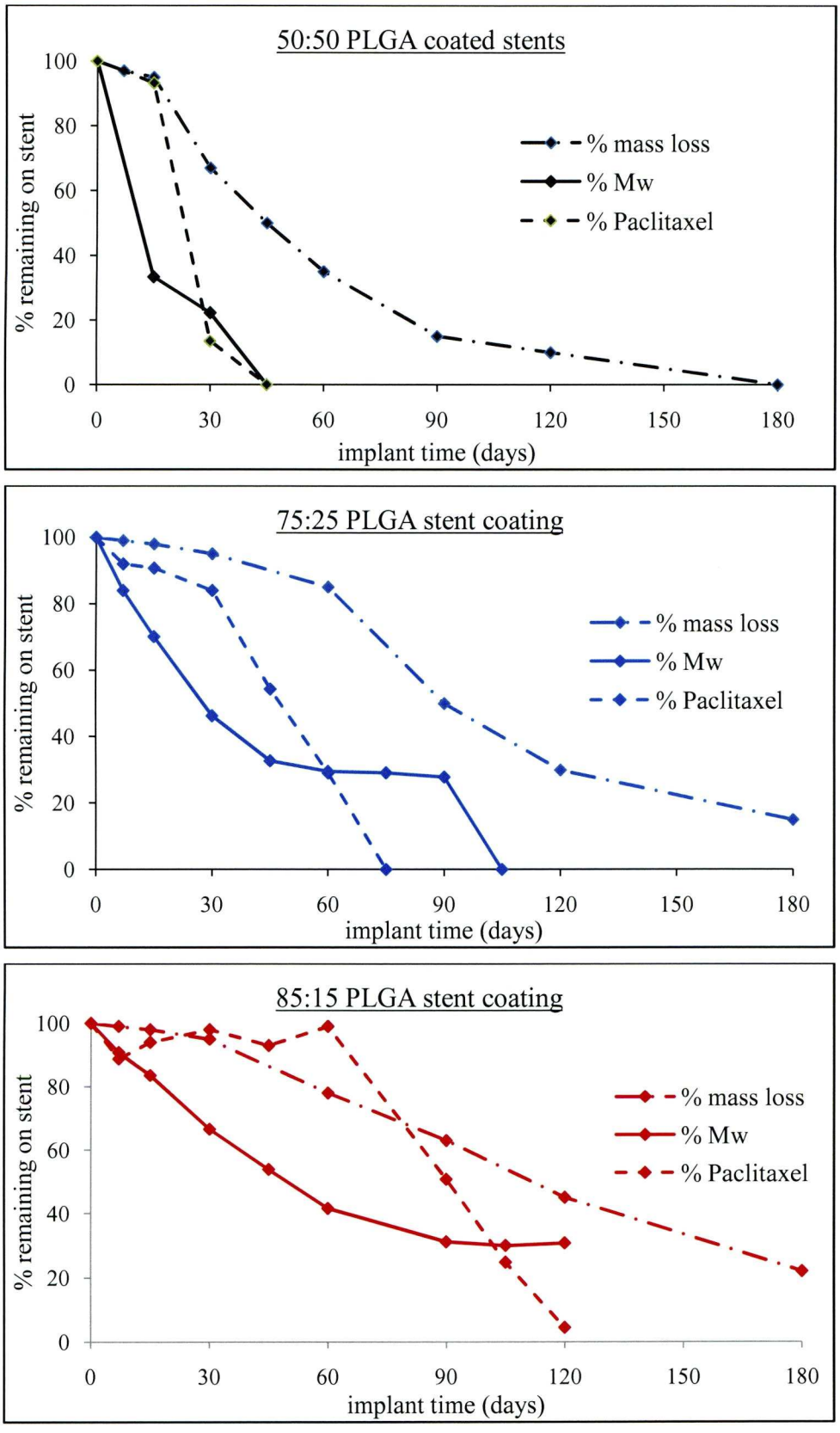

Figure 3.16. Relationship between weight average molecular weight (M_w), mass loss and paclitaxel elution in degrading PLGA stent coatings. The graphs show the molecular weight, mass and paclitaxel remaining on the stent expressed as a percentage of the original amount. Data for molecular weight and paclitaxel is taken from the *in-vivo* study. Mass loss data is from an *in vitro* study (unpublished data provided by Boston Scientific)

Data from this study and others (e.g. [8, 13, 18, 32-33]) indicate a mechanism whereby products from PLGA degradation are unable to diffuse out of the polymer matrix and that paclitaxel only initially diffuses out of the matrix very slowly. During the second phase of degradation, the M_w change is less rapid and becomes stable, but the structure of the coating becomes highly vacuolated and surface pitting appears on the stent coating. SEM micrographs reveal a highly vacuolated structure within the body of the coating and the existence of surface pitting at implantation periods associated with polymer erosion and paclitaxel elution. It is during this phase that paclitaxel elution is most rapid.

A possible explanation for this is given by Göpferich [33] and Park [18]. When the polymer chains degrade within the bulk of the coating they cannot erode if they have no connection to the external medium via a system of pores. Since PLGA is a bulk eroding polymer, degradation is occurs at a faster rate within the coating than at its surface. The surface layer acts as a semi-permeable diffusion barrier for the entrapped oligomers but allows diffusion of low molecular weight molecules such as water. A system of cavities within the polymer bulk is created as the polymer degrades and osmotic pressure builds up within the polymer bulk. As the surface layer gradually degrades and becomes thinner it will break at some point when a critical osmotic pressure builds up within the device due to the accumulation of degradation products [32]. Only after a critical degree of degradation is reached and a network of pores and cavities are created within the device can the release of degraded polymer (resulting in mass loss) and the erosion phase of drug release commence [32].

Elution rate of paclitaxel was dependant on the degradation and mass loss of the PLGA stent coating. Release of the paclitaxel consisted of an initial lag phase in which only small amounts of the drug was released followed by a more rapid phase during which the remainder of the drug was released. The elution of the drug was similar in all three PLGA polymers but occurred over different time scales with the shortest lag phase occurring in the 50:50 PLGA coating and the longest in the 85:15 PLGA coating. The length of the lag phase was related to the initial phase of polymer degradation via hydrolysis and during this period elution of paclitaxel would be via diffusion through the polymer matrix. As the PLGA entered the second phase of degradation in which mass loss was occurring, the formation of cavities and larger air

spaces created a more porous structure facilitating a more rapid release of paclitaxel. Wang *et al* [31] observed a similar pattern for release of sirolimus from PLGA films. Interestingly in our study and that of Wang *et al* [31] there was no initial burst of drug release as has been seen in other studies (e.g. [1, 28, 34]) but the reason for this is unclear.

The effect of polymer degradation and erosion on the integrity of the stent coating could be seen using SEM and Light microscopy images. Good adherence of the stent coating was observed throughout the study in all PLGA blends used. The polymer coating initially appeared intact but as time progressed areas of degradation could be seen and eventually areas of bare metal could be observed resulting in an appearance of a light meshwork of polymer coating with extensive patches of bare metal visible. The rate of deterioration was related to the rate of polymer degradation. During the study period the coatings remained attached to the stents, with no obvious areas of peeling or flaking occurring in any of the PLGA blends but at later sampling points the coating often appeared to have areas where the coat was only loosely attached to the metal stent.

Degradation of PLGA can increase the acidity of the release medium. Wu and Wang [12] found that when PLGA was incubated in distilled water, the pH of the distilled water decreased to a pH2, while Li *et al* [8] found that the pH of an saline incubating medium dropped after 5 weeks eventually falling to pH2.6 at 10 weeks. Li *et al* [9] found that the pH of an incubating medium of PBS (pH7.4) remained stable for 7 weeks after which a slight drop in pH to 6.4 was observed. Although there were small differences in pH in the cellular exudate in the PMMA chambers between groups (some of which were statistically significant) it was difficult to discern any real pattern to the changes or to demonstrate any significance to the observations. There was a small but significant change in pH from 8.16 to 7.69 by 90 days in the exudate from 50:50 PLGA chambers and the pH of exudate from 75:25 PLGA chambers was significantly lower at 90 days than at earlier time points whereas there was no significant change in pH exudate from 85:15 PLGA chambers over time. To further complicate matters, the exudate from the chambers containing BMS was more acidic than those containing PLGA coated stents. It may be expected that the production of lactic and glycolic acid during degradation of PLGA would affect the

exudate pH if the production of acidic groups exceeded the buffering capacity of the cellular exudate and perhaps the changes seen reflect differing degradation rates. This was not the case in this study and the pH remained within the normal physiological range for interstitial fluid.

Delivery of the paclitaxel from the coating must be at a sufficient concentration and duration to be effective in preventing restenosis but preferably not cause toxic effects or delay arterial healing. A minimum period of 30 days elution of paclitaxel from the implanted stent has been suggested and during that period between 8- 8.5μg (7.5% of total drug loading) and 23.6μg (11.9% total drug loading) respectively of paclitaxel would be delivered from the currently used SR and MR Taxus™ coronary stents [34-35]. Of the PLGA polymers studied, the 400μg 50:50PLGA and 75:25 PLGA coating delivered comparable amounts of paclitaxel over the first 30 days but drug elution did not follow zero order kinetics and only small amounts of paclitaxel were delivered up to 15 days: the consequences of this on restenosis is unknown but data from Kammath [34] indicate that only small amounts of the SR formulation were released from the Taxus™ coronary stent during the first 10 days and yet this formulation is effective in reducing ISR. But all of the paclitaxel (20μg) is eluted within 45 and 75 days from the 50:50PLGA and 75:25 PLGA coatings respectively. 85:15 PLGA coatings delivered its paclitaxel loading over a period of 120 days, but the effectiveness of this formulation in preventing ISR may be impaired due to the extended lag phase of about 60 days. Further studies would be required to determine the required duration of drug elution from these PLGA polymer coatings.

3.5 Conclusions

The effect of varying glycolic acid content on degradation rate and drug eluting profile in PLGA stent coatings was determined. Additionally the effect of adding 5% paclitaxel to a 400μg coating of 50:50 PLGA on the degradation rate and the differences in size of coating of 50:50 PLGA was determined. The addition of 5% paclitaxel to a coating of 50:50 PLGA had no effect on rate of degradation. But a 200μg coating of 50:50 PLGA degraded more slowly than a 400μg coating, but elution of paclitaxel was faster from the thinner coating.

Increasing the glycolic acid content of the PLGA coating increased the degradation rate of the polymer and the elution of paclitaxel by increasing the hydrophilicity of the polymer and the number of sites with increased susceptibility to hydrolytic cleavage. Reduction in polymer molecular weight, mass loss and visual signs of morphological deterioration occurred in all PLGA polymers investigated but occurred over differing time scales. Degradation of polymer was fastest in the 50:50 PLGA coating and slowest in the 85:15 PLGA coating. Using GPC, no detectable polymer remained on the stents coated with 50:50 PLGA and 75:25 PLGA after 45 and 105 days respectively but small quantities of 85:15 PLGA coating were detected after 120 days.

The elution rate of paclitaxel was dependant on the degradation state of the PLGA. A two phase profile was observed with an initial diffusion controlled slow release phase as the molecular weight of the PLGA decreased by hydrolysis and a second faster phase associated with mass loss of the polymer and morphological changes in the stent coating with the appearance of cavities and air spaces creating a more porous structure. Paclitaxel was eluted fastest from the 50:50 PLGA and slowest in the 85:15 PLGA coating. Complete elution of the drug had occurred by 45 and 75 days from the 50:50 and 75:25 PLGA coatings respectively. An extended lag phase of 60 days was observed for the 85:15 PLGA coating but by 120 days 95% of the paclitaxel had been eluted.

Small changes in pH in the chamber exudate were observed during the study and may have been associated with polymer degradation but no clear patterns were observed and the pH of exudate from chambers containing BMS was more acidic than those containing PLGA coated stents.

3.6 References

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The Effect of Blending Different PLGA Polymers and Addition of Pluronics on Polymer Degradation and Paclitaxel Elution.

4.1 Introduction

The goal for successful drug delivery from polymer coated devices and microspheres is a constant and controlled delivery of the contained drug at the required concentration at the appropriate time and for a sufficient duration (the therapeutic window). Release of drugs from PLGA is closely associated with the degradation of the polymer and typically shows a tri-phasic release profile consisting of an initial rapid release phase followed by a second phase of slower release and a final rapid release phase, or a biphasic release profile consisting of either an initial burst phase followed by a phase of sustained release [1] cited by [2], [3] or a initial lag phase followed by a period of faster drug elution [4]. The initial rapid release or burst phase is a frequent feature of drug release from monolithic polymer controlled release systems and is considered to be due to the dissolution or diffusion of drug particles located on or near to the surface of the polymer device [2]. The second phase (initial lag phase in some bi-phasic release profiles) is generally attributed to diffusion controlled release of the drug from within the polymer matrix and the final faster release phase is associated with polymer erosion and breakdown of the polymer matrix [4-5]. Kunou *et al* [6] found that release of ganciclovir from PLGA microspheres was tri-phasic consisting of an initial fast release phase (burst phase), a second slower diffusion release controlled phase followed by final rapid release phase. Release of sirolimus from a PLGA device consisted of 2 stages – but in this case a slower, initial diffusion controlled stage was by followed a second phase of faster drug release [4]. Data from chapter 3 of this thesis demonstrated a biphasic release profile of paclitaxel from PLGA comprising of an initial lag phase in which very little paclitaxel was eluted followed by a second phase of more rapid drug release.

For coronary stents sufficient anti-restenoic drug needs to be delivered for the first few days following stent deployment and for a period of at least 3 weeks to prevent restenosis [7], but the lag phase observed in PLGA coatings may result in insufficient drug being delivered to the vascular wall during this critical period. In chapter 3 of this thesis, shorter lag phases were seen using 50:50 PLGA coatings but the duration

of paclitaxel delivery was probably insufficient to prevent restenosis over the longer term. Paclitaxel delivery from 85:15 PLGA coatings was of a suitable duration but insufficient drug may have been released in the first few days following implantation due to the extended lag phase that was observed.

A number of approaches have been used to modify the degradation rate and drug elution properties of PLGA to eliminate the phases seen in the drug elution profile and to fine tune release profiles to suit the clinical requirements of the device. Methods include co-polymerizing PLGA with polyethylene glycol (PEG), blending different molecular weights of the same polymer and blending with polymers of different hydrophilicity /hydrophobicity [8]. A summary of some of the trials and approaches used to alter the drug elution profile from PLGA polymers is shown in Table 4.1.

The rate of drug release is predominantly determined by the rate of hydrolysis of the polymer matrix and the ability of the drug to diffuse through the pores and spaces as the polymer degrades [5]. Degradation of PLGA polymers is via hydrolytic breakage of the ester bonds in the polymer chains and the accessibility of water to these bonds will determine the rate of degradation [9]. The drug eluting properties of PLGA are also affected by the glass transition temperature (T_g) of the polymer and faster drug elution occurs when the polymer is in the rubbery state due to the high mobility of the polymer chains in this state. The T_g of a glassy polymer can be decreased by blending low molecular weight substances (plasticizers) with the PLGA. If the T_g of the plasticized polymer is lower than the environment the polymer will be in the rubbery state [10].

Addition of hydrophilic copolymers such as polyethylene glycol (PEG) to the PLGA matrix can have a two fold effect on drug release profile: increased water uptake and therefore potentially increasing hydrolytic degradation of the polymer and secondly decreasing the T_g of the polymer which may result in transition of the polymer from a glassy to a rubbery state [4]. Water itself has been shown to have a plasticizing effect on 50:50 PLGA [10]. Attempts to alter drug release profiles using copolymers as plasticisers has had mixed results (see Table 4.1). Wang *et al* [4] found that sirolimus elution from 53:47 PLGA occurred over two-phases with an initial slow release

phase followed by a faster second phase. Addition of 5% or 10% polyethylene glycol (PEG) increased the water uptake of the polymer while decreasing the glass transition temperature (T_g). This increased the initial rate of polymer degradation and consequently increased the initial rate of sirolimus release making the release profile linear [4]. But addition of 5%w/w PEG to 80:20 PLGA films did not alter the drug release profile of heparin [11].

Paclitaxel release from PLGA films containing up to 20%w/w plasticisers such as methoxypolyethylene (MePEG) or a diblock copolymer composed of PLLA-MePEG was very slow with less than 5% of the paclitaxel being released over 2 weeks, although addition of 30% w/w PLLA-MePEG to the films substantially increased paclitaxel release [12]. Addition of a more hydrophobic diblock copolymer poly (ϵ-caprolactone) (PCL)-MePEG increased paclitaxel elution as compared to PLGA alone or PLGA blended with MePEG-PLLA [13].

Blending of PLGA polymers with differing lactide/glycolide (LA:GA) ratios and/or differing molecular weights is another strategy used to modify drug release profiles and some of the studies utilising blends of PLGA polymers is shown in Table 4.1. The biodegradation rate of PLGA is affected by the composition of the PLGA. Increasing the GA content of a polymer has a twofold effect on degradation: GA is more hydrophilic than LA and hydration increases as the GA moiety content increases resulting in faster degradation [14] and since GA units are more vulnerable than the LA units to hydrolytic degradation, increasing GA content increases the polymers degradation rate.

Blending polymers of different LA:GA ratio or molecular weight may be expected to produce a polymer with a degradation rate and drug release profile that is a compromise between that of the PLGA polymers alone. Ganciclovir was released rapidly from low molecular weight (8 kDa Daltons) 50:50 PLGA microspheres over approximately 10 days but by blending low molecular weight 50:50 PLGA with 65:35 PLGA an initial fast release occurred over 5 days followed by a slower phase over approximately 15 days with a final rapid release phase [15]. Blending low molecular

Table 4.1 Plasticisation and blending of PLGA polymers: Summary of trials

Drug	device	PLGA copolymer ratio	Drug elution profile	Additive or blend	Effect on drug elution profile	reference
Sirolimus	films	53:47	bi-phasic 2 : lag phase, then faster release phase	PEG (5 or 10% w:w)	bi-phasic to linear	[4]
ganciclovir	microspheres	PLLA	tri-phasic	PLLA, 130kDa blended with PLLA 5kDa	At ratio 80:20 (130k:5kDa) profile became bi-phasic 1	[16]
ganciclovir	microspheres	75:25	tri-phasic with long lag phase	75:25 PLGA with 50:50 (MWt 5kDa) PLGA	Increasing 5kDa 50:50 PLGA faction reduces lag phase and overall duration of release of drug	[5]
paclitaxel	films	50:50	bi-phasic 1: initial burst phase & 2nd slower release phase	MePEG (10%w:w)	no significant effect	[12]
paclitaxel	films	50:50	bi-phasic 1: initial burst phase & 2nd slower release phase	PDLLA-MePEG (10-30%w:w)	20% -small effect 30%- increased initial the burst phase	[12]
paclitaxel	microspheres	50:50	bi-phasic 1: initial burst phase & 2nd slower release phase	PCL-MePEG (10-30%w:w)	20& 32% w:w increased the initial burst phase	[13]
heparin		80:20	bi-phasic 1: initial burst phase & 2nd slower release phase	PEG (5%w:w)	no significant effect	[11]
leuprolide	microspheres	50:50	28.6kDa – triphasic 8.6kDa-biphasic2	28.6kDa & 8.6kDa	A 3:1 blend ratio resulted in profile intermediate between the initial profile.	[17]

Tri-phasic: burst, 2nd slow release phase, 3rd fast release or second burst
Bi-phasic 1: burst phase, 2nd linear release phase
Bi-phasic 2: initial lag phase, 2nd faster release phase

weight 50:50 PLGA (8 kDa) with 75:25 PLGA increased the release of ganciclovir as compared with 75:25 PLGA alone: blending at a ratio of 1:1 reduced the lag phase from approx 50 days to approx 8 days and all the drug was released by 20 days as opposed to 100 days for 75:25 PLGA alone [5].

Within a homogenous blend of two PLGA polymers gelling and solidification of the constituent polymers occurs separately within the polymer matrix [18-19] cited by [17]. Phase separated polymers can be obtained by blending polymers creating morphologies, matrix characteristics and drug release profiles that are different to the constituent polymers alone [9]. In a degrading polymer device comprising a blend of polymers with different degradation profiles, hydrolysis of the domains of the faster degrading polymer may lead to the creation of cavities and spaces within the polymer matrix through which contained drugs may diffuse out into the external medium [20].

Pluronics are surfactants made up of copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) [9]. Hydration of PEO/PPO/PEO triblock copolymers is dependant on the PEO:PPO ratio and increasing PEO content increases the hydrophilicity of the copolymer [8]. When blended with PLGA they can form phase separated morphologies with an intact surface morphology and different levels of hydration depending on the hydrophilicity of the pluronic and its concentration in the blend [9]. Pluronic copolymers have been used in blends with PLGA to produce films for controlled delivery of proteins [21] and have minimal toxicity and some are presently in clinical use [9].

F-127 is a hydrophilic triblock pluronic [9] and has a molecular weight of approximately 12,300 daltons of which about 70% is PEO [8]. Yeh *at al* [21] found that blending F-127 into PLGA microparticles resulted in a burst phase of protein release followed by a short lag phase of 5 days and then a period of sustained protein delivery. Overall there was little difference in percentage cumulative release of protein between 50:50 PLGA and 50:50 PLGA/F-127 (blend ratio3:1) but percentage cumulative release was faster at blend ratios of 1:2 PLGA:PLGA/F-127. Raiche and Puleo [8] found that blending 8% w/w F-127 was found to be optimum for hydration of

50:50 PLGA microspheres and increased the *in vitro* degradation rate and protein release as compared with 50:50 PLGA microspheres.

The prolonged lag phase seen in the release of paclitaxel from 85:15 PLGA limits its use as a potential coating for coronary stents. In this chapter the hypothesis that faster degradation and early creation of a porous structure within the matrix of 85:15 PLGA devices should increase the initial release rate of paclitaxel thereby shortening the lag phase resulting in a more linear release profile of the drug. Addition of soluble hydrophilic copolymers such as pluronic F-127 to PLGA polymers should increase the hydration of the polymer matrix resulting in faster degradation and fast degradation and dissolution of the copolymer within the device should lead to creation of pores and cavities through which the drug molecules can more easily diffuse. Blending of low molecular weight PLGA and PLGA polymers with a higher GA content than 85:15 PLGA should also introduce faster degrading domains resulting in cavities and pores within the polymer matrix through which paclitaxel molecules could be released more quickly. The above strategies should increase the early release of paclitaxel after the device is implanted or immersed in the release medium while paclitaxel molecules entangled in the slower degrading 85:15 PLGA domains will be released more slowly thereby maintaining a steady elution of paclitaxel over the study period.

The study comprised two trials. In Teflon Disc Trial 1(TD1) the effect of adding 8% w/w Pluronic F-127 to 85:15 and 50:50 PLGA, and blending 85:15 PLGA with 50:50 PLGA (1:1) on the elution of paclitaxel was studied. The second trial (TD2) tested the effect of adding low molecular weight (LMWt) 50:50 PLGA to 85:15 PLGA compared with a blend of 50:50/85:15 PLGA and 65:35 PLGA.

4.2 Materials and methods

4.2.1 Materials.

Poly($_{D,L}$-Lactide-*co*-Glycolide) (PLGA); 50:50 MWt 40-75kDa, 65:35 MWt 40-75kDa, 85:15 MWt 90-126kDa, 50:50MWt 5-15kDa: from Sigma-Aldrich. Pluronic F-127, sodium azide (99.5%) and phosphate buffered saline (pH 7.4) from Sigma-

Aldrich. Chloroform (SpS 99.9% Super Purity solvent, stabilised) from Romil Pure Chemistry, UK. The paclitaxel was kindly donated by Boston Scientific Corporation, USA.

4.2.2 Methods.

4.2.2.1 Preparation of polymer films

13mm diameter discs were cut from Teflon sheeting (Goodfellow 1.0mm thick polytetraflouroethylene sheets). The discs were cleaned in 50% v:v ethanol water mixture and allowed to dry. The dry discs were weighed using a Sartorius CP2 fine balance and the weight was recorded.

Polymer blends containing 5% (w:v) Paclitaxel were prepared as follows PLGA, F-127 and paclitaxel were dissolved in chloroform at a concentration of 100mg/ml. PLGA, paclitaxel and F-127 were dissolved separately in chloroform by mixing on a stirrer for 1 hour. The samples were then mixed in the proportions indicated in Table 4.2 and mixed for a further hour.

Table 4.2. Preparation of polymer films: the volume (mls) of each ingredient used in each blend.

	Polymer/ingredient (100mg/ml)				
Blend	85:15 PLGA	50:50 PLGA	65:35 PLGA	F-127	Paclitaxel
85:15 PLGA	9.5				0.5
50:50 PLGA		9.5			0.5
65:35 PLGA			9.5		0.5
85:15 + 50:50 PLGA	4.75	4.75			0.5
85:15 + F-127	8.7			0.8	0.5
85:15 + LMWt 50:50 PLGA	4.75	4.75			0.5
50:50 + F-127	8.7			0.8	0.5

The mixture was then sonicated using a VWR ultrasonic cleaner water bath for 30 minutes to aid mixing and to prevent formation of air bubbles within the polymer solution. 100µl of polymer was added to a 13mm dia Teflon disc and coated using a WS-400B-6NP Lite spin coater (Laurell Technologies Corporation) for 15 seconds each at 1,000, 2,000, 3,000 and 4,000 rpm followed by 15 seconds each at 3,000,

2,000, and 1,000 rpm. The samples were then left in the open overnight and then dried under vacuum at room temperature until a constant weight had been achieved. The dry weight of the polymer coated disc was recorded and the mass of polymer on each disc determined. Samples were placed in 12 well polystyrene plates and 4 mls of PBS containing 0.1% (w:v) sodium azide was added and placed on a shaker at 120 rpm at 37°C. The PBS release media was changed weekly. Samples were collected at 3, 7, 14, 21, 30, 45 and 60 days in TD1 and 14, 21, 30, 45, 60 and 75 days in TD2.

4.2.2.2 Water content.

At the appropriate time points samples were recovered from the release media. Samples were carefully blotted dry using Kimwipes tissue (Kimtech Science) and the wet weight of the sample taken. Samples were then dried under vacuum at room temperature until a constant weight had been reached. The dry weight of the polymer was recorded and used to calculate polymer mass loss and water content of the film using the formulae:

Percentage polymer mass remaining =100*(mass of polymer at recovery/original polymer mass)
Percentage water content = 100*(mass of water in the film/wt of polymer remaining).

4.2.2.3 Gel permeation chromatography (GPC):

Equipment, columns and polymer standards used were as used in chapter 3. The polymer remaining on each disc was recovered by adding 1ml of chloroform to the disc(s) in a capped glass vial and shaking gently for 3 hours. The number of discs used for each GPC sample is shown in Table 4.3. In samples recovered at 3 and 7 days 1 disc was used for each GPC sample. At later times points and in TD2, two discs were added to 1ml of chloroform for each GPC sample to ensure that sufficient polymer and paclitaxel would be present for quantification.

Table 4.3 Number of discs used per sample for GPC

	sampling time (days)							
Trial N°	3	7	14	22	30	45	60	75
TD1	*1*	*1*	*2*	*2*	*2*	*2*	*2*	
TD2			*2*	*2*	*2*	*2*	*2*	*2*

GPC was performed using four 7.8x300mm, 5μm Waters Styragel columns (1. HR0.5 MWt range 0 to 1000, 2. HR2 MWt range 500 to 20,000, 3. HR3 MWt range 500 to 30,000 and 4. HR4 MWt range 5,000 to 500,000) with a flow rate of 1ml per minute with chloroform as a the mobile phase and using a Polymer Labs ELS 1000 evaporative light scatter detector (ELS) and Polymer Labs LC1200 UV/VIS ultra violet (UV) detector. Easi-Cal PS-2 (Polymer Labs) polystyrene standards, with a molecular weight range of 580 to 377,400 were dissolved in chloroform to give a 0.1% solution.

Raw polymer was prepared by weighing an amount of polymer and adding chloroform to give a final concentration of 1mg/ml and gently mixing at 120rpm at room temperature for 3 hrs. The injection volume was 250μl with a polymer concentration of approximately 800μg/ml solvent for the stent samples and 100μl for the raw polymer and Easi-Cal standards) with three measurements per sample. Quantitative analysis was performed using Cirrius Software (Polymer Labs) and molecular weight data is expressed as weight average molecular weight (M_w).

Paclitaxel concentration was measured using the area under the peak using data from the UV detector as in Chapter 3 using a calibration curve of 10, 20, 50, 100 and 200 μg/ml paclitaxel. Quality control (QC) checks containing 0.8mg/ml 85:15 PLGA and 20 or 50μg/ml paclitaxel in chloroform were also prepared and analysed via GPC.

4.2.2.4 SEM

Two discs from each sample point were mounted onto an aluminium stub using double sided adhesive carbon tape and sputter coated with chromium using an EMITECH K575X coater. SEM was performed using a Leo/Zeiss 1550 Field Emission Scanning Electron Microscope.

4.2.2.5 pH measurements.

The pH of the release medium from film samples and blank teflon disc samples (controls) at each of the sampling time points was measured using a Mettler Toledo pH meter.

4.3 Results.

4.3.1 Coating of PTFE discs: amount of PLGA and concentration of paclitaxel.

The amount of polymer and paclitaxel initially added to each disc by group is shown in Table 4.4. Overall the mean weight of polymer applied to each disc was 794μg ±139μg but there was variability within groups and between groups in the amount of polymer on each disc with relative standard deviations of between 14-18%. The initial amount of 85:15/LMWt 50:50 PLGA at 680μg (stdev=114) was significantly lower than that found on the other groups ($p<0.001$). But once the discs were randomized with the respect to the sampling date it was found that there was no significant difference in the weight of polymer between or within the groups.

Table 4.4 Initial Coating of Teflon discs. The table shows the mean and standard deviation (stdev) of the amount of polymer and paclitaxel coated onto the Teflon discs in each group.

		polymer (μg)		Paclitaxel (μg)	
group	label	mean	stdev	Mean	Stdev
85:15 PLGA	A	831	151	49.6	8.8
50:50 PLGA	B	766	106	49.5	4.4
85:15/50:50 PLGA	C	841	118	50.2	4.2
85:15 PLGA/F-127	D	892	132	54.4	6.1
50:50 PLGA/F-127	F	783	120	55.0	4.4
85:15/LMWt 50:50 PLGA	G	680	115	45.8	9.8
65:35 PLGA	H	820	154	57.0	13.0

Data for the original amount of paclitaxel was derived from GPC studies and is shown in Figure 3.1. The mean amount of paclitaxel was 52.6μg (stdev=10) per Teflon disc and there was no significant difference ($p>0.05$) between the groups in the initial concentration of paclitaxel on each Teflon disc.

4.3.2 pH data.

Figure 4.1 shows the pH change in the release media in TD1.

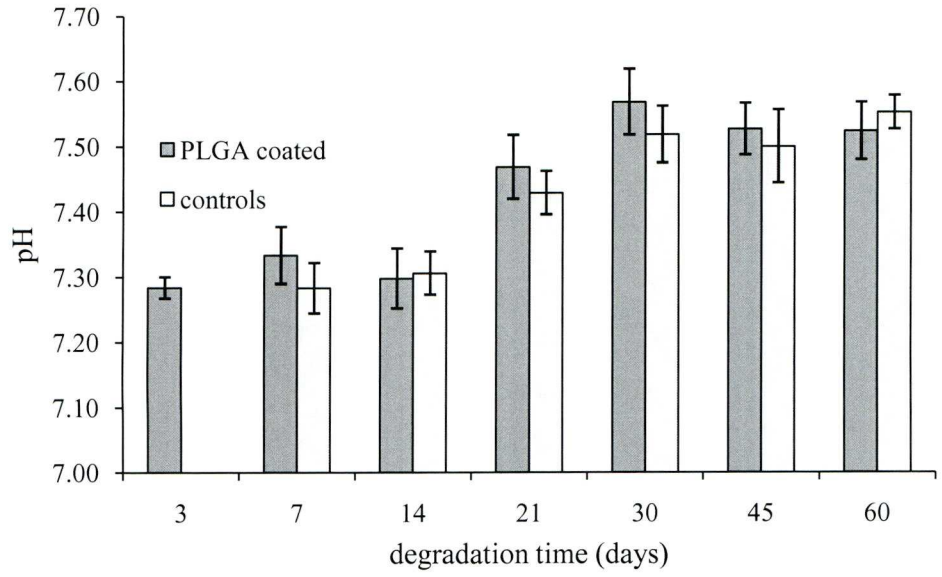

Figure 4.1. pH change in the incubation media from polymer coated discs and controls in trial TD1.

The pH change is similar in all groups and steadily rises from approx 7.27 at the start of the trial to approx 7.58 at the end of the study period. Analysis by ANOVA showed that there was no significant difference between the groups at the various time points ($p>0.05$) and that there was no significant difference between the control media and that of the samples at any given time point ($p>0.05$).

The pH increased over time in all groups and controls. In the trial samples in all the groups pH had increased significantly at 21 days ($p<0.001$). This increase was also seen in the control media. Stock control media had a mean pH of 7.3. By 21 days the pH in the control wells had risen significantly ($p<0.001$) to 7.43. This would indicate that the change in pH observed in the media from the PLGA coated samples may not be due to release of degradation products, which tend to result in a slight decrease in pH, but is due to other factors.

The pH changes seen in the release media from trial TD2 are shown in Figure 4.2. There was no significant difference in the pH of the release media between the groups of the polymer coated discs or between the controls and the polymer coated discs in trial TD2. The pH of the release media in trial TD2 in both polymer coated discs and controls was 7.36 and 7.37 respectively at 7 days. But at 22 days the pH had risen significantly ($p<0.001$) as compared with 7 days to 7.73 and 7.71 respectively in the controls and the polymer coated groups. There was no further significant

changes in pH in either the controls or the polymer coated groups and pH was >7.7 for the remainder of the study.

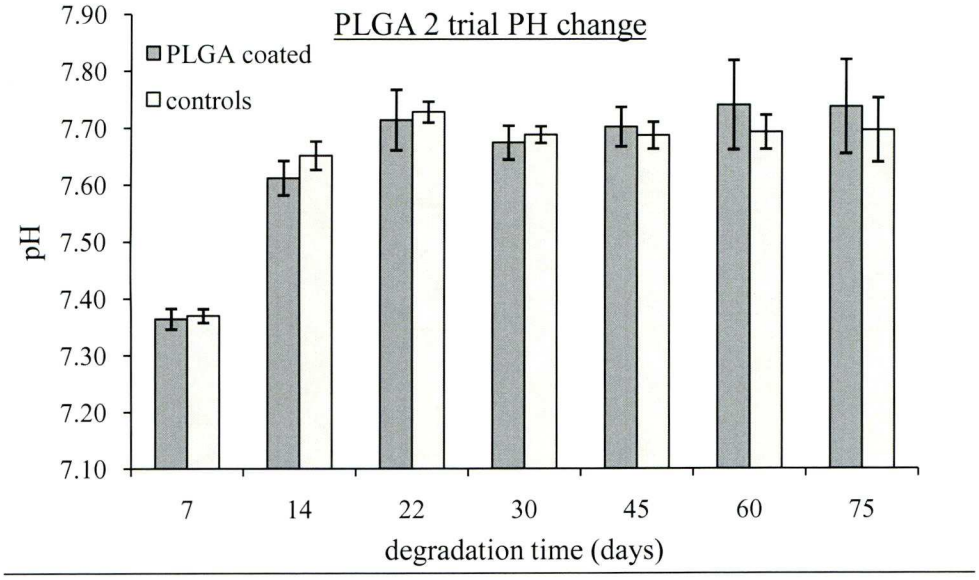

Figure 4.2 Change in incubation media pH during polymer degradation in trial TD2. data uses combined figures from the all of the groups

4.3.3 Paclitaxel assay

The combined calibration data for the paclitaxel assay is shown in Figure 4.3. The calibration was reproducible across all runs and the mean values (from all GPC runs) for the QC checks (see below) indicated that the assay gave acceptable sensitivity.

- 20µg= 18.24µg (stdev =3.28), recovery = 91.22%
- 50µg= 49.93µg (stdev = 2.48), recovery = 99.86%

Given the variation in original polymer mass on each disc, the following method was used to calculate paclitaxel per disc for comparison between groups etc: the mean concentration of paclitaxel (µg paclitaxel per mg polymer) was determined using the paclitaxel data derived from control discs for each group. This figure was then used to determine the original amount of paclitaxel per disc on the incubated discs using the initial polymer weight. The actual amount of paclitaxel remaining on each disc after incubation was then calculated as a percentage of its original concentration.

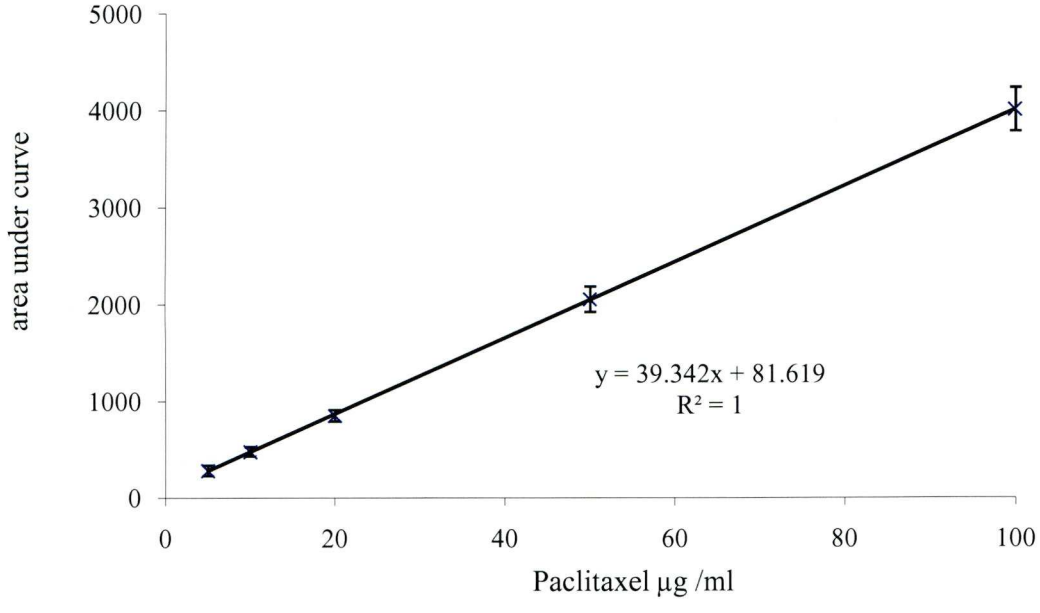

Figure 4.3 Paclitaxel Calibration Curve. Data is derived from GPC chromatograms of varying concentrations of paclitaxel. The figure represents the combined data taken from all the GPC runs in this trial.

4.3.4 Effect of pluronics on water uptake, polymer erosion, degradation and paclitaxel elution

4.3.4.1 Water content

The water content of 85:15 PLGA and 50:50 PLGA with and without F-127 is shown in Figure 4.4. In most cases the data has a large standard deviation but trends in water uptake can still be discerned. Initially the water content of the PLGA films was lower than in films containing F-127; 50:50 and 85:15 PLGA films had a water content of 2.5% and 3.3% water content whereas the 50:50 and 85:15 films with F-127 had a significantly higher ($p=0.040$ and $p=0.013$) water content of 8.5% and 9.5% respectively. At 14 days the water content of the films containing F-127 decreased to approximately 5% and remained at between 4.5-6% water content to the end of the study period. After 14 days there was no significant change in the hydration of the films containing F-127. The water content of the 85:15 PLGA films increased steadily to 45 days to 14% after which it fell to just under 6%. Water content in the 50:50

PLGA films increased to 8.6% after 30 days incubation and was 7.6% at the end of the study period (45 days).

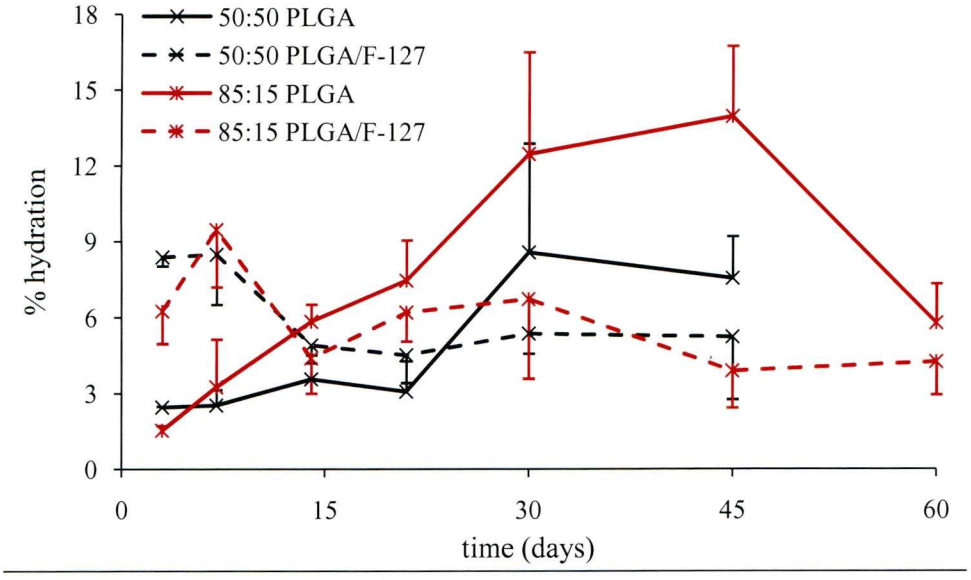

Figure 4.4 Effect of degradation time on water content of PLGA films, with and without pluronic F-127. Error bars= standard deviation

There was no significant difference (p=.996) between hydration in 85:15 and 50:50 PLGA films to 7 days, but from 14 days until the end of the study period the hydration of 85:15 PLGA was significantly higher ($p<0.001$) than the 5050 PLGA (except at 30 days where p=0.208). At 30 and 45 days water content of the 50:50 PLGA films was higher than with the addition of F-127 but the effect was not significant (p =0.324 and p=0.193 at 30 and 45 days respectively). The water content of 85:15 PLGA films was significantly higher ($p<0.05$) at 30 and 45 days as compared with films containing F-127.

4.3.4.2 Polymer erosion (mass loss).

The effect of adding of 8% w/w F-127 on the mass loss of 85:15 and 50:50 PLGA is shown in Figure 4.5. After 3 days 7-8% of the polymer mass had been eroded in the 85:15 PLGA films with and without F-127. Mass loss was slow in these two groups and at the end of the study period (60 days) 90% of the polymer remained on the PTFE disc. Addition of F-127 to 85:15 PLGA films had no significant effect on polymer mass loss over the study period.

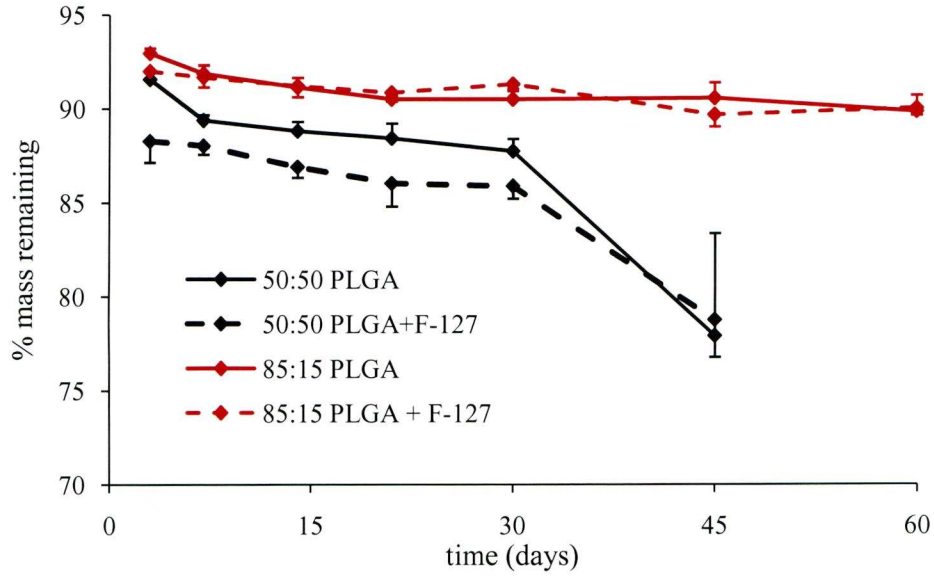

Figure 4.5 Effect of addition of pluronic F-127 to PLGA films on mass loss. Mass remaining on the film (as a percentage of the original amount of polymer) during polymer degradation. Error bars show the standard deviation.

Mass loss was faster in the 50:50 PLGA films with and without F-127 as compared with 85:15 PLGA films (with and without F-127). The mass loss profile for films of 50:50 PLGA was similar to that 50:50 PLGA/F-127 films. At 3 days 91% and 88% of the polymer remained on the PTFE disc in the 50:50 PLGA and 50:50 PLGA/F-127 films respectively. Polymer erosion then proceeded slowly in these groups until 30 days after which mass loss was more rapid and at 45 days (the end of the study period) the amount of polymer remaining on the PTFE films had fallen to 78% in both the 50:50 PLGA films and the 50:50PLGA/F-127 films. Mass loss occurred significantly ($p<0.001$) faster in 50:50 PLGA films containing F-127 to 30 days but at 45 days there was no significant difference ($p=0.976$) in the mass remaining.

4.3.4.3 GPC data: effect of pluronics on polymer degradation and paclitaxel elution.

The change in molecular weight of films of PLGA with and without F-127 are shown in Figure 4.6. 50:50 PLGA films degraded faster than the 85:15 PLGA films and addition of F-127 to either 50:50 PLGA or 85:15 PLGA slowed the rate of degradation. The initial molecular weight of the 50:50PLGA and 50:50PLGA/F-127 films were 44,000 and 40,254 respectively. As degradation proceeded the molecular weight of

the 50:50 PLGA films decreased to 80% to 8,700 and by 70% to 12,100 in the 50:50 PLGA/F-127 films respectively at 45 days. Addition of F-127 to 50:50 PLGA significantly slowed the rate of polymer degradation as measured by reduction in percentage molecular weight remaining on the disc from 14 days incubation ($p=0.022$) and the effect remained significant at 45 days ($p=0.011$).

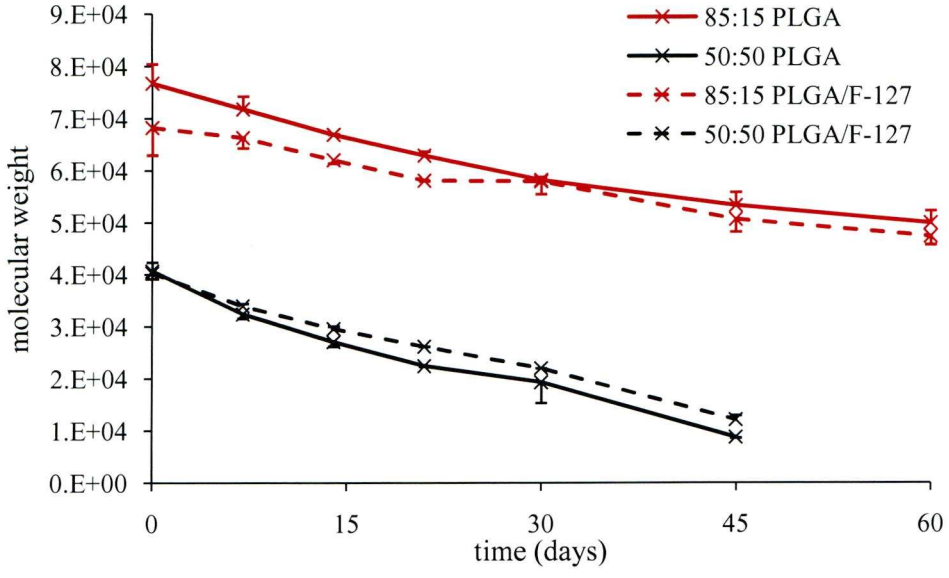

Figure 4.6 Molecular weight change: Degradation of 50:50 and 85:15 PLGA– effect of adding pluronic F-127.Degradation is shown as the change in molecular weight of the polymer remaining in the film. Error bars=standard deviation

The initial molecular weight of the 85:15 PLGA and 85:15/F-127 films was 76,734 and 68,222 respectively. Degradation of these films was slow in comparison with the 50:50 PLGA films and at the end of the study period (60 days) the 85:15 PLGA films had degraded by 35% to 49,900 and the 85:15 PLGA/F-127 films had degraded by 31% to 47,400. Addition of F-127 to the 85:15 PLGA films decreased the degradation rate but the difference was not significant at any of the time points ($p>0.05$).

Overall, paclitaxel was released more rapidly from films containing 50:50 PLGA (with and without F-127) as compared 85:15 PLGA and 85:15 PLGA/F-127 films but the difference was only significant at 45 days ($p<0.001$).

An initial rapid release of paclitaxel occurred and after 7 days incubation approximately 12% of the paclitaxel had been released from the 50:50 PLGA films. Paclitaxel elution was then very much slower and at 30 days 16% of the paclitaxel had

been eluted from the films but the difference was not significant (p=0.989) as compared with 7 days incubation. After 45 days degradation approximately 43% of the paclitaxel had been released from the 50:50 PLGA films and the difference as compared with 30 days was significant (p<0.05, p=0.002). F-127 had no effect on paclitaxel release to 30 days. But at 45 days 29% of the paclitaxel had been eluted from the 50:50 PLGA/F-127 which was significantly less than that eluted from the 50:50 PLGA films (p<0.05, p=0.015).

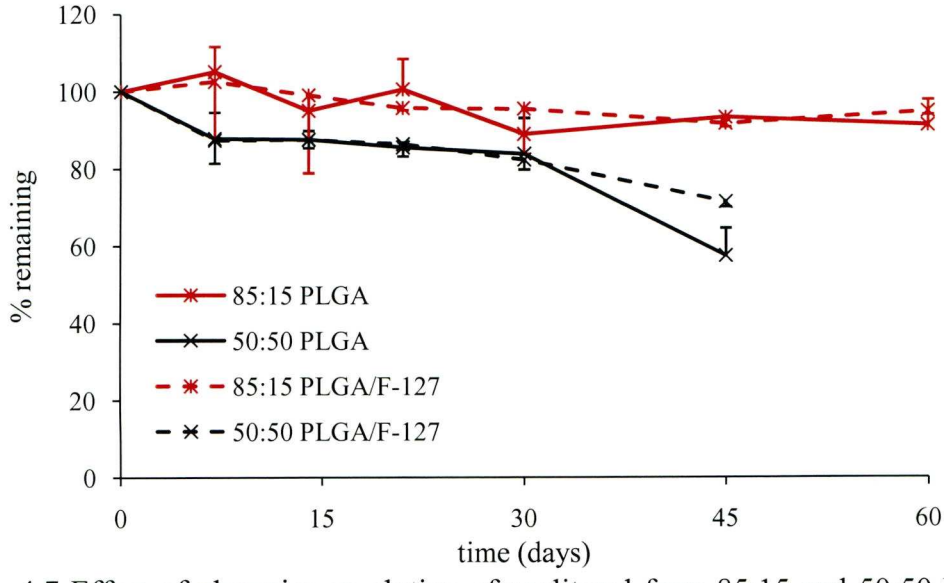

Figure 4.7 Effect of pluronics on elution of paclitaxel from 85:15 and 50:50 PLGA films. The paclitaxel concentration is expressed as the amount of paclitaxel remaining in the film as a percentage of the paclitaxel concentration at the start of the trial. Error bars = standard deviation.

There was very little elution of paclitaxel from the 85:15 PLGA and 85:15/F-127 films. There was no noticeable burst release over the first 7 days of degradation of paclitaxel as seen in the 50:50 PLGA films and over the whole of the study period of 60 days only 9% and 5% of the paclitaxel was released from the 85:15 PLGA and 85:15PLGA/F-127 films respectively. Addition of F-127 to 85:15 PLGA films had no significant effect on paclitaxel release at any time over the study period (p>0.05).

4.3.4.5 Effect of pluronics on polydispersity.

Changes in the polydispersity (PDI) of the polymer films as the polymers degrade is shown in Figure 4.8. At the start of the trial the PDI of the 50:50 and 85:15 PLGA films was 1.598 and 1.610. Addition of F-127 increased the PDI of the 50:50 PLGA

and 85:15 PLGA films to 1.634 and 1.598 respectively but there was no significant difference in PDI between any of polymer films prior to the start of degradation.

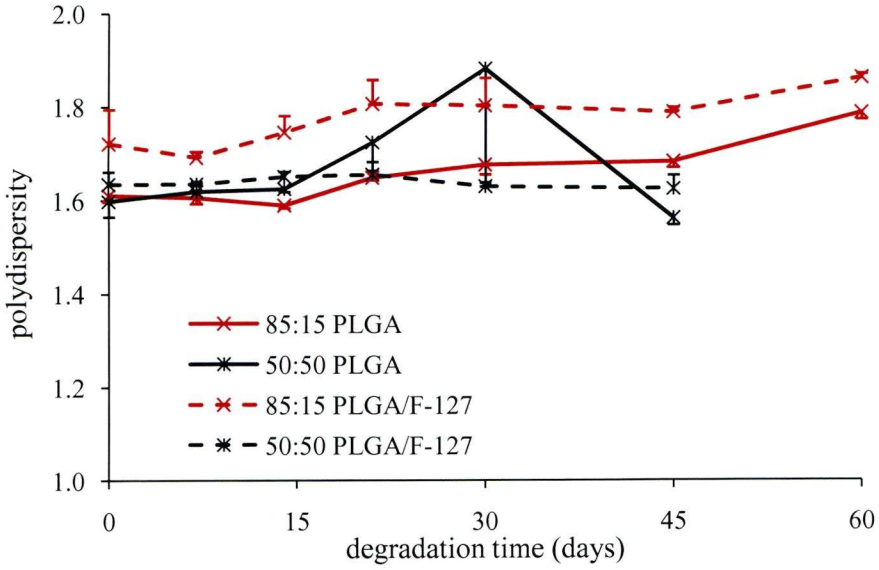

Figure 4.8 Effect of pluronic F-127on polydispersity (PDI) of 50:50 PLGA and 85:15 PLGA films. The graph shows the changes in polydispersity during polymer degradation. Error bars show the standard deviation.

There was very little change in the PDI of 50:50 PLGA and 50:50 PLGAF-127 films over the study period and at 45 days the PDI was 1.563 and 1.625 which was not significantly different to the controls (p=1.563 and p=1.625 respectively). The PDI of films containing 85:15 PLGA and 85:15 PLGA/F-127 increased over the study period (60days) to 1.788 and 1.863 respectively which was significant as compared with the controls (p=0.024 and p=0.025 respectively).

4.3.5 Effect of polymer blending on water uptake, polymer erosion, degradation and paclitaxel elution

The effect of blending 85:15 PLGA with 50:50 PLGA was tested in trial TD1. In trial TD2 the effect of blending a low molecular weight 50:50 PLGA with 85:15 PLGA on polymer degradation and drug elution was compared with 65:35 PLGA and 85:15/50:50 PLGA films.

4.3.5.1 Water content

The percentage hydration of polymer films of 50:50/85:15 PLGA and 65:35 PLGA is shown in Figure 4.9. The hydration of 65:35 films was very similar to that of 85:15 PLGA films and water content increased from 6.7% at 14 days to 12.6% at 45 days after which water content decreased to 7.6% at 60days. Despite the changes in hydration of 65:35 PLGA films from 14 to 45 days the differences were not significant ($p>0.05$). The hydration of a 1:1 blend of 85:15/50:50 PLGA films was similar to that of 50:50 PLGA films, with water content increasing significantly ($p<0.001$) to 9.8% at 30 days after which the water content decreased to 2.9% and 1.6% at 45 and 60 days respectively.

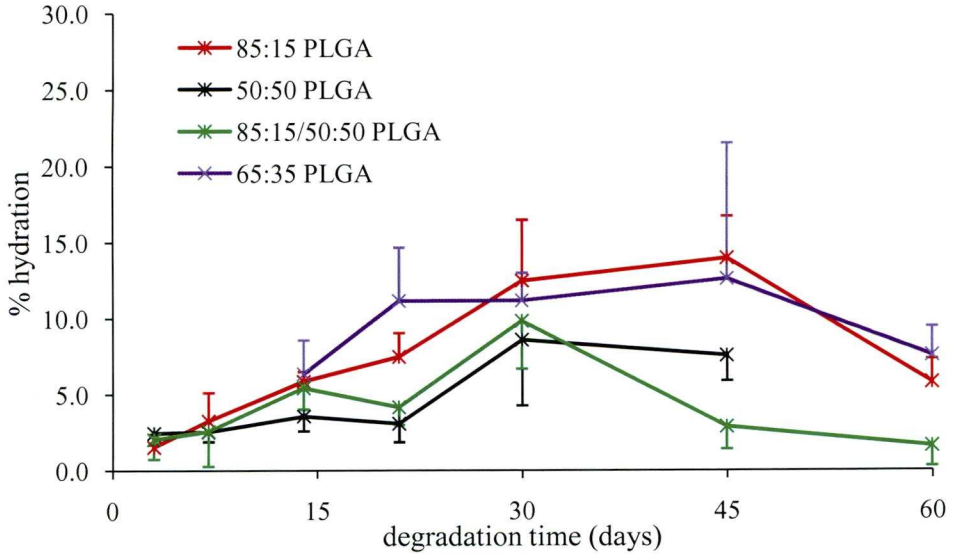

Figure 4.9 Change in percentage water content of 85:15, 65:35, 50:50 and 85:15/50:50 PLGA films during degradation. Standard deviation is represented by the error bars

At 30 days the percentage hydration of the 65:35 PLGA films and 85:15/50:50 PLGA films was 11.2% and 9.9% but the difference was not significant ($p=0.988$), but at 45 days the difference was significant ($p=0.004$). There was no significant difference in the hydration of the 65:35 PLGA and 85:15/50:50 blend films to 30 days ($p>0.05$) but the difference was significant at 45 and 60 days ($p<0.001$).The percentage water content in the 85:15/LMWt 50:50 PLGA films (data not shown) increased with degradation time to 38.7% ±12.5.

4.3.5.2 Polymer erosion (mass loss)

The mass loss during degradation on 85:15, 50:50, 65:35 PLGA, and blends of 85:15/50:50 PLGA and 85:15/LMWt 50:50 PLGA is shown in Figures 4.10 and 4.11.

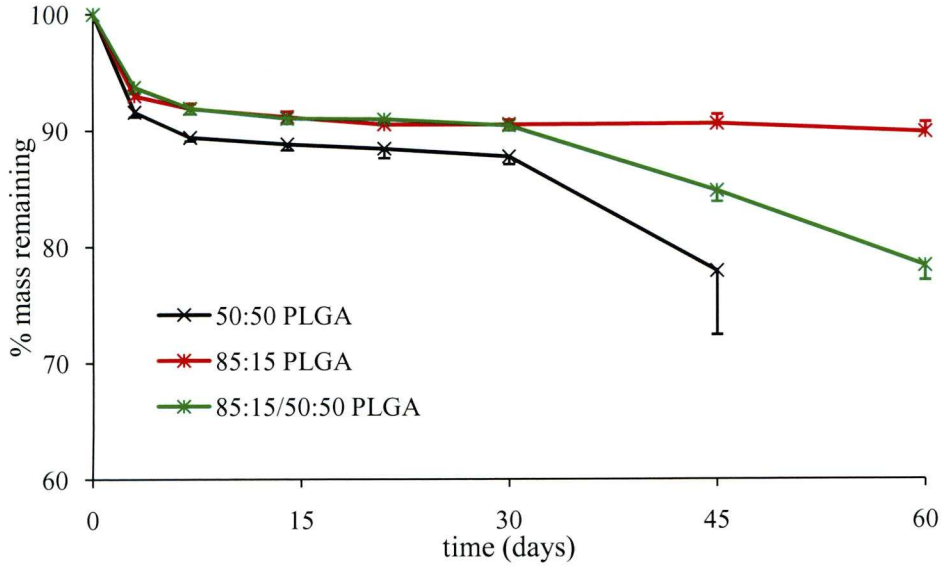

Figure 4.10. Percentage Mass Loss in 50:50, 85:15 and 50:50/85:15 blend PLGA polymer films during degradation. Data taken from trial TD1. Mass remaining on the film (expressed as a percentage of the original polymer weight) during polymer degradation. Error bars = standard deviation.

Initially there was a loss of between 7-8% of the polymer mass in 50:50, 85:15 and 50:50/85:15 blend PLGA polymer films during the first three days of incubation. This was followed by a period of much slower mass loss. At 30 days 87.8% of the original polymer films mass remained on the discs in the 50:50 PLGA group and 90.5% remained in the 85:15 PLGA and 50:50/85:15 PLGA blend. At 30 days there was no significant difference in mass loss between the 85:15 PLGA and the 85:15/50:50 PLGA blend ($p=0.999$) but the mass loss from the 50:50 PLGA films was significantly higher ($p<0.001$) than the other two groups at 30 days. After 45 days degradation rate of mass loss was accelerated in the 50:50 PLGA and 50:50/85:15 PLGA films. Blending 50:50PLGA into 85:15 PLGA increased the rate of mass loss after 30 days and at 45 days 85% of the mass of the 85:15/50:50 PLGA blend remained as opposed to 90% in the 85:15 PLGA films and the difference was

significant (p=0.015). Further mass loss in 85:15/50:50 PLGA blend occurred and at 60 days 22% of the polymer mass had been eroded.

Mass loss data from trial TD2, comparing 65:35 PLGA, 50:50/85:15 PLGA blend and 85:15/50:50 PLGA blend is shown in Figure 4.11. There was an initial rapid loss of polymer bulk from all three types of films and after 14 days degradation, 93%, 91.3% and 92.4% of the polymer remained on the 85:15/50:50 PLGA, 85:15/LMWt 50:50 PLGA and 65:35 PLGA respectively.

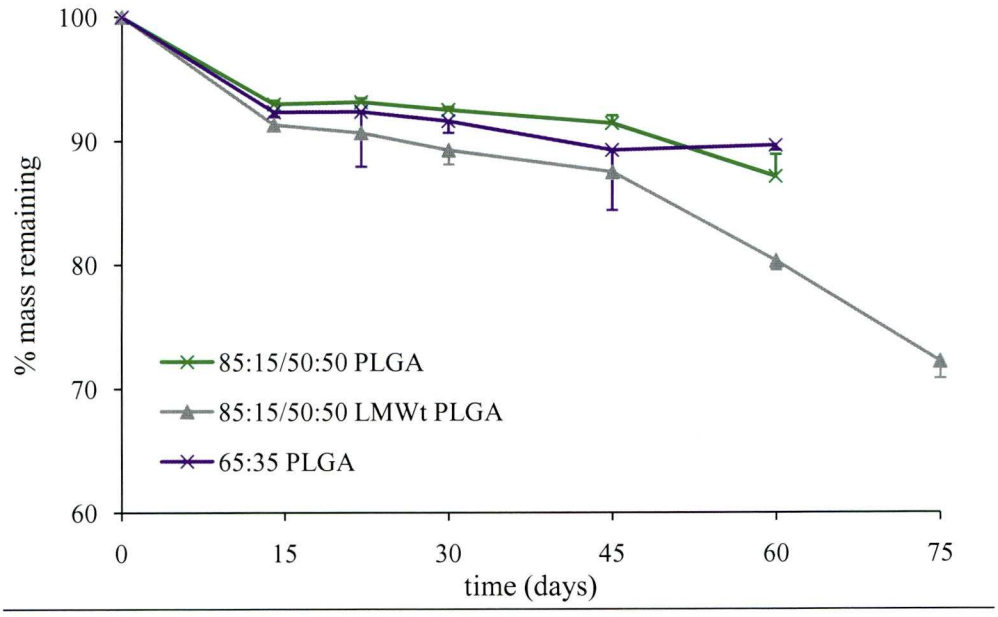

Figure 4.11 Polymer Mass Loss in 65:35, 85:15/50:50 blend and 85:15/LMWt 50:50 PLGA during degradation. Data fromTD2. Mass loss is shown as weight of polymer remaining on the film as a percentage of the original polymer weight. Error bars = standard deviation.

After the initial phase of rapid mass loss, polymer erosion was much slower from the 85:15/50:50 PLGA and 65:35 PLGA films and at 60 days 87.1% and 89.7% respectively of the polymer remained on the films. The change in percentage mass remaining from 14 to 60 days was not significant in the 85:15/50:50 PLGA and 65:35 PLGA films (p=0.254 and p=0.213 respectively) but at 60 days significantly more mass loss had occurred from the 85:15/50:50 PLGA films as compared with the 65:35 PLGA films (p=0.015).

Polymer erosion was more rapid from the 85:15/LMWt 50:50 PLGA films as compared with the other polymer films in TD2 and blending LMWt 50:50 PLGA with 85:15 PLGA significantly increased polymer erosion from 22 days onwards as compared with the 85:15/50:50 PLGA films. From 14 days to 45 days degradation time the percentage polymer mass remaining on the 85:15/LMWt 50:50 PLGA films decreased from 91.3% to 87.5% after which the rate of mass loss increased and at 60 and 75 days the mass remaining decreased to 80.3 and 72.2% respectively. The change in percentage mass remaining on the 85:15/LMWt 50:50 PLGA films was significant ($p<0.001$) between 45 to 60 days and 60 to 75 days degradation time.

4.3.5.3 GPC data: effect of polymer blending on degradation and paclitaxel elution.

Polymer degradation, as expressed as percentage change in molecular weight in the polymer remaining on the film is shown in Figures 4.12 &13. The degradation of 85:15 PLGA and 50:50 PLGA films is discussed in section 4.3.4.3

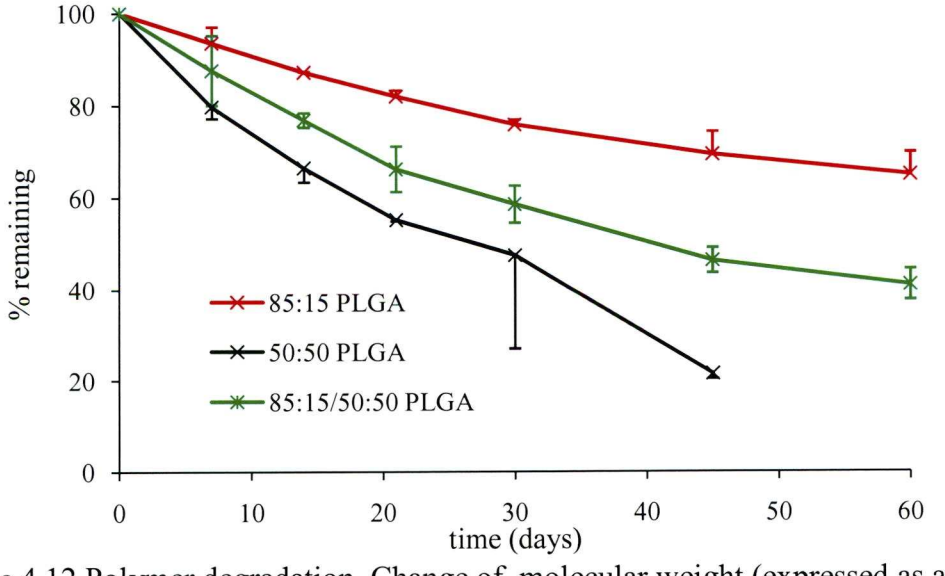

Figure 4.12 Polymer degradation. Change of molecular weight (expressed as a percentage of the original M_w, remaining on the film) in 85:15, 50:50 and 85:15/50:50 blend PLGA polymer films. Data from trial TD1 trial error bars = standard deviation

Blending 85:15 and 50:50 PLGA resulted in an original molecular weight of 56,372 but by 7 days the polymer had degraded significantly ($p=0.007$) to 49,399 and by 60 days the molecular weight was approximately 23,000 (Figure 4.12). The degradation rate of 85:15/50:50 PLGA blend films was intermediate as compared to 50:50 PLGA

and 85:15 PLGA films. After 7 days degradation time the M_w of the 85:15/50:50 PLGA blend films were 87% of the original as compared with 80% on the 50:50 PLGA films and 93% on the 85:15 PLGA films. At this sampling point there was no significant difference in the rate of degradation of the 85:15/50:50 PLGA blend as compared with the other two groups (p=0.119 and p=0.352 respectively). At 45 days the percentage M_w remaining on the 85:15/50:50 PLGA blends (46.5%) was significantly different to the other films (p<0.001 in both cases): the 85:15/50:50 PLGA blend was more degraded than the 85:15 PLGA films where 69.5% of the original molecular weight remained but less degraded than the 50:50 PLGA films which had degraded to 21.5% of the original M_w. At 60 days the 85:15/50:50 PLGA blends had degraded further to 41.2% of the original M_w as compared with 65% remaining on the 85:15 PLGA films (p<0.001).

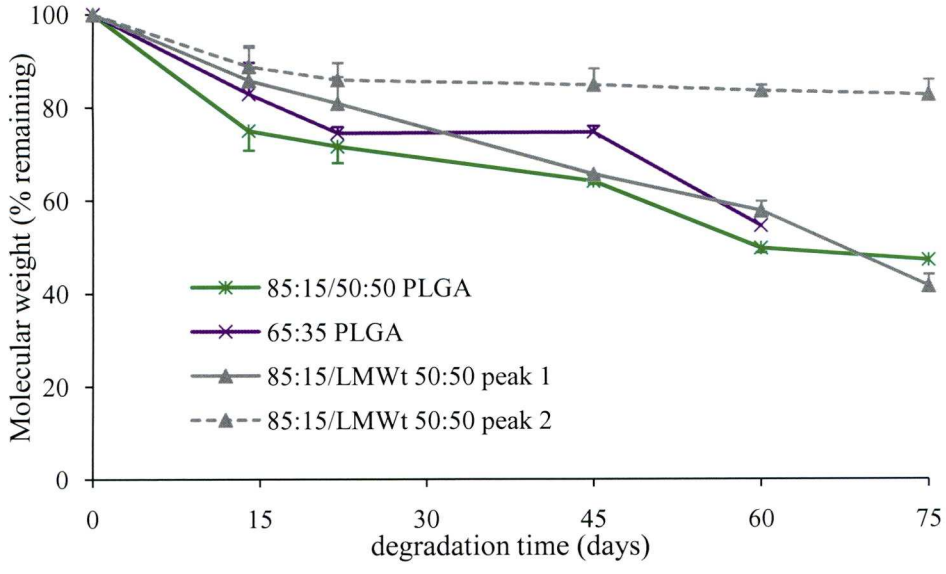

Figure 4.13 Polymer degradation. Change of molecular weight (expressed as a percentage of the original M_w, remaining on the film) in 65:35, 85:15/50:50 blend and 85:15/LMWt 50:50 blend PLGA polymer films. Data from trial TD2. error bars = standard deviation. The blend of 85:15/LMWt 50:50 PLGA produced two peaks on the GPC chromatograms.

The data comparing molecular weight change in films comprising 85:15/50:50 PLGA, 65:35 PLGA and 85:15/LMWt 50:50 PLGA is shown in Figure 4.13.

At the start of the trial the M_w of the 85:15/50:50 PLGA blend and 65:35 PLGA films was 60,360 and 50,262 respectively. After 14 days degradation the 85:15/50:50

PLGA films the M_w of the films had degraded by 25%. After 14 days the films degraded significantly to 75 days ($p<0.001$) to a M_w of 28,501 (47% of the original). At 14 days the M_w of the 65:35 PLGA films had decreased by 17% to 41,685. After 60 days degradation the M_w of the 65:35 PLGA films was 27,432 (54.5% of the

original) which was significantly lower than at 14 days ($p<0.001$). The rate of degradation of the 85:15/50:50 PLGA films was faster than the 65:35 films over the trial with significant differences in percentage M_w change at 45 and 60 days ($p<0.001$).

The chromatograms from 85:15/ LMWt 50:50 PLGA films were bi-modal consisting of two polymer peaks which were measured as distinct distributions (Appendix, Figure A.5). But the degradation of the peaks was very different. Peak 1 had an initial M_w of 86,669 which degraded by 14% after 14 days to 72,800 and by approximately 40% over the 75 days of the trial to 35,293. The percentage molecular weight change was significant from 14 days to 45 days and onwards ($p<0.001$). Degradation was slower in peak 2. The M_w of peak 2 was 6,605 and the start of the trial and at 14 days the M_w was 89% of the original. After 75 days degradation the M_w of peak 2 had reduced by 17% to 5,523. The percentage M_w change in peak 2 was significant at 75 days ($p=0.025$) as compared with 14 days but not at earlier time points ($p>0.05$).

Release of paclitaxel from 85:15/50:50 PLGA films was slow over the first 45 days of the trial followed by a phase of faster release by 60 days (Figure 4.14). After 7 days degradation there was no measurable release of paclitaxel but at 45 days approximately 7.5% of the paclitaxel had been released from 85:15/50:50 PLGA films but there was no significant difference in the amount of paclitaxel released from 7 to 45 days degradation time ($p>0.05$). After 60 days 20% of the paclitaxel had been released from the 85:15/50:50 PLGA films and the difference was significant as compared to 7 and 45 days ($p<0.001$ and $p=0.043$ respectively).

Elution of paclitaxel to 45 days from 85:15/50:50 PLGA films was similar to that of 85:15 PLGA films with 92.4% and 93.4% respectively of the paclitaxel remaining on the film at 45 days and the difference was not significant ($p=0.999$). At 60 days more paclitaxel had been released from the 85:15/50:50 blend films compared to the 85:15

films (20% and 8.5% respectively) but surprisingly ANOVA showed that the difference was not significant (p=0.054).

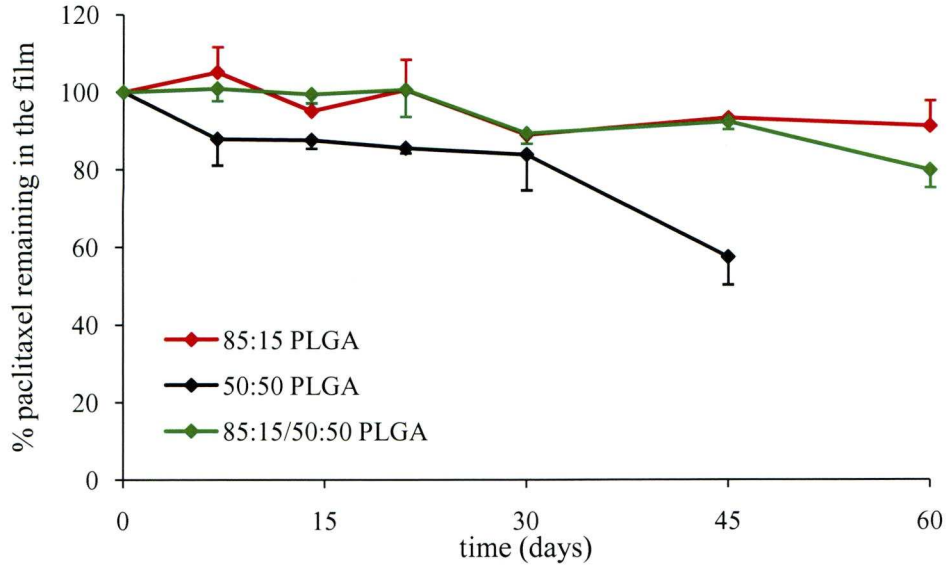

Figure 4.14 Release of Paclitaxel from 85:15, 50:50 and 85:15/50:50 PLGA films. Data taken from trial TD1. Error bars = standard deviation. Graph shows the concentration of paclitaxel remaining on film expressed as a percentage of original (control) concentration.

Elution of paclitaxel was slower from the 85:15/50:50 PLGA films as compared to the 50:50 PLGA films but the difference was only significant ($p<0.001$) at 45 days when 7.5% and 42.5% of the paclitaxel had been eluted from the 85:15/50:50 PLGA films and 50:50 PLGA films respectively.

Figure 4.15 shows the elution of paclitaxel from 85:15/50:50 PLGA, 65:35 PLGA and 85:15/LMWt 50:50 PLGA films from study TD2. The data for paclitaxel release in TD2 was subject to large standard deviations at some sampling points but trends could still be discerned in the data. In TD2 there was no measurable release of paclitaxel during the study period (75 days) from 85:15/50:50 PLGA films. There was no measurable release of paclitaxel from 65:35 PLGA films up to 30 days but at 60-days 10.3% of the paclitaxel had been released from the 63:35 PLGA films but this was not significant ($p>0.05$) as compared with earlier sampling points. There was no significant difference in release of paclitaxel from 85:15/50:50 PLGA films and 65:35 films up to 45 days degradation time but at 60 days significantly ($p=0.033$) more paclitaxel had been released from the 65:35 PLGA films.

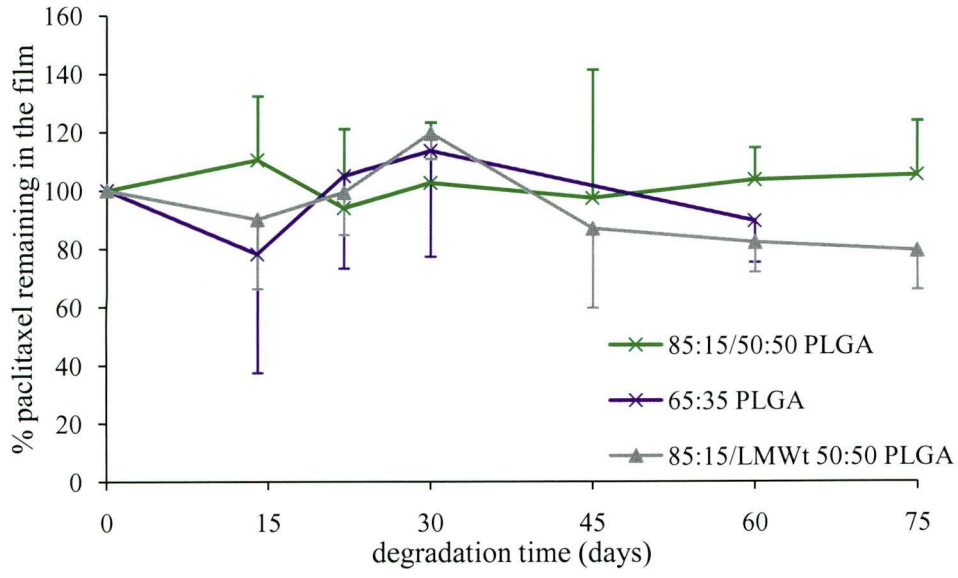

Figure 4.15. Release of Paclitaxel from 85:15/50:50 blend, 65:35 and 85:15/LMWt 50:50 blend PLGA films. Data taken from trial TD2. Error bars=standard deviation. Graph shows the concentration of paclitaxel remaining on film as percentage of the control concentration.

There was no significant release of paclitaxel from the 85:15/LMWt 50:50 PLGA films up to 45 days. But after 60 and 75 days degradation time the paclitaxel remaining on the films decreased to 82.3% and 79.5% of the original amount respectively but despite this decline there was no significant difference in the amount of paclitaxel remaining on the films as compared to earlier sampling time points. At 60 and 75 days release of paclitaxel was significantly higher from the 85:15/LMWt 50:50 PLGA films ($p=0.021$ and $p= 0.19$ respectively) as compared with 85:15/50:50 blend films but was not significantly different to the paclitaxel release from 65:35 PLGA films at 60 days ($p=0.906$).

4.3.5.4 GPC data: polydispersity index (PDI)

Figure 4.16 shows the effect of degradation of the PLGA films on the polydispersity index (PDI) of the polymer films. The PDI of the 85:15 PLGA and 65:35 PLGA films at the start of the trial was 1.651 and 1.552 respectively. The PDI increased as the polymer films degraded and at 60 days the PDI was 1.788 and 1.761 for the 85:15 PLGA and 65:35 PLGA films respectively: a significant increase as compared to the controls (85:15 PLGA, $p=0.024$ and 65:35 PLGA $p<0.001$). There was no sig-

nificant difference between the PDI of the 85:15 PLGA films and the 65:35 PLGA films as the polymers degraded.

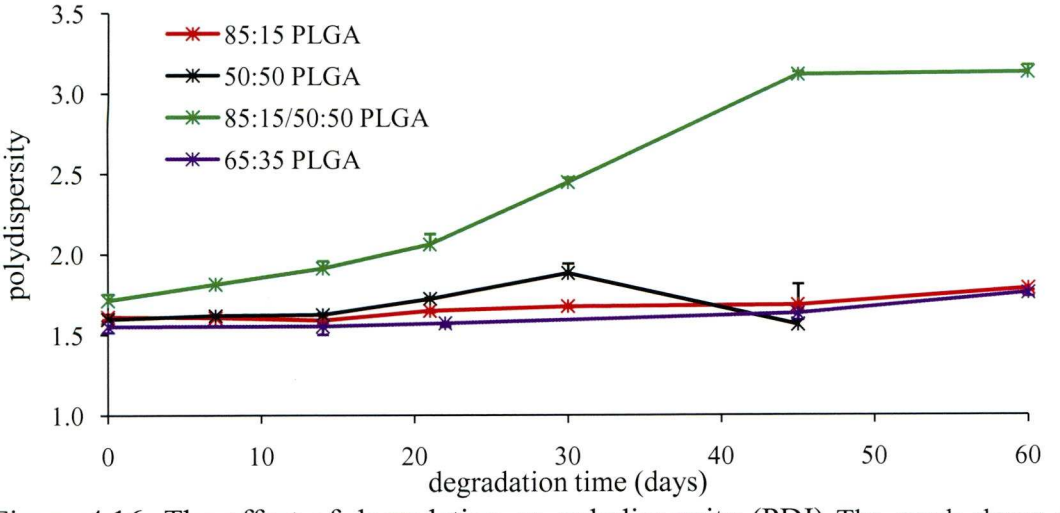

Figure 4.16. The effect of degradation on polydispersity (PDI) The graph shows the changes in polydispersity during polymer degradation. Error bars show the standard deviation.

The PDI of the 50:50 PLGA films at the start of the trial was 1.639 but as the films degraded the PDI increased significantly (p=0.038) at 30 days to 1.882. But after 45 days degradation time the PDI was 1.562 which was not significantly different (p=0.924) to the controls and was not significantly different to the 85:15 PLGA or 65:35 PLGA films at 45 days.

Degradation of the 85:15/50:50 PLGA films was associated with increased PDI. PDI increased significantly (p=0.002) from 1.768 at the start of the study to 2.063 at 21 days. Rate of change of PDI increased after 21 days and at 45 days the PDI was 3.119 at 45 days. From 45 to 60 days degradation time there was a slight increase in PDI to 3.133. There was little change in the PDI of peak 1 and 2 from the 85:15/LMWt 50:50 PLGA films over the study period. At the start of the trial the PDI of peak 1 was 1.378 and after 75 days degradation the PDI was 1.380, while the PDI of peak 2 was 1.311 and 1.318 in the controls and after 75 days respectively (data not shown).

4.3.5 SEM analysis

The SEM analysis was carried out using the In-Lens at EHV=5kv. The most revealing images are those taken at X20,000 and the results are displayed below.

Figure 4.17 shows a low magnification (X500) image of an 85:15 PLGA film. The surface of the films prior to start of degradation appears as a smooth surface. But at higher magnification the surface is revealed to have a rippled texture (Figures 4.18 - 4.22). The appearance of the 50:50 PLGA control films is of a rippled surface comparable with those seen on the 85:15 PLGA films at 20K magnification (Figures 4.18a and 4.19a).

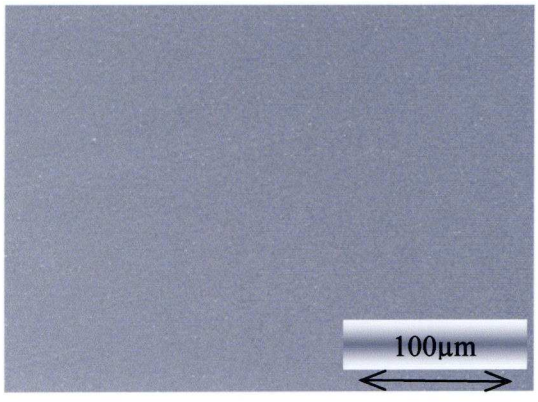

Figure 4.17 Low magnification SEM image: Control 85:15 PLGA film. The film appears as a smooth flat surface at low magnification

The effect of polymer degradation on the appearance of 50:50 PLGA films with and without F-127 is shown in Figure 4.18. The control films (4.18 a and b) have a textured, rippled surface and the ripples appear more pronounced in the 50:50 films. At 22 days (Figure 4.18 c and d) the rippled texture of the surface is evident but areas of the 50:50/F-127 PLGA films have rippled areas and areas where the ripples are less prominent. At 45 days (Figure 4.18 e and f) the surface of the films show extensive pitting, with a highly porous structure within the bulk of the films. Small surface pits are evident in the control and 22 day 50:50/F-127 films PLGA but there was no visual evidence that they were part of a network of pores connected to the inner regions of the film at those stages of the trial.

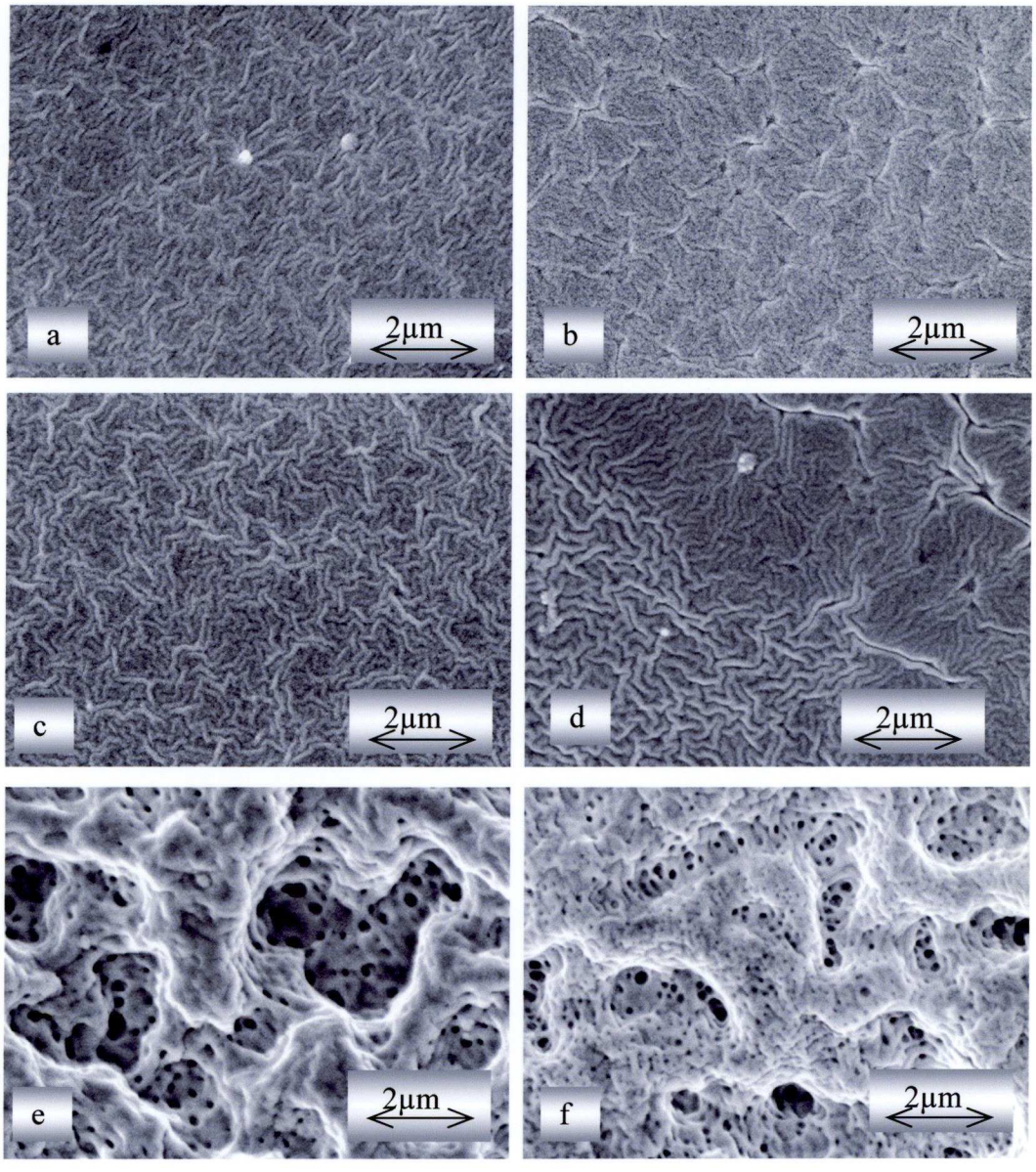

Figure 4.18 SEM Images. 50:50 PLGA with and without F-127showing the effect of degradation as incubation time increases. a,c,e 50:50 PLGA films b,d,f 50:50/F-127 PLGA films. Controls (a and b), 22day (c and d), 45 day (e and f). The films have a rippled appearance up to 45 days and after 60 days the surface of the films becomes more pitted and degraded.

The effect of polymer degradation on the appearance of 85:15 PLGA with and without F-127 is shown in Figure 4.19. There is little obvious change in the appearance of the 85:15 PLGA films over the trial, with the rippled surface seen in the controls still apparent at 60 days.

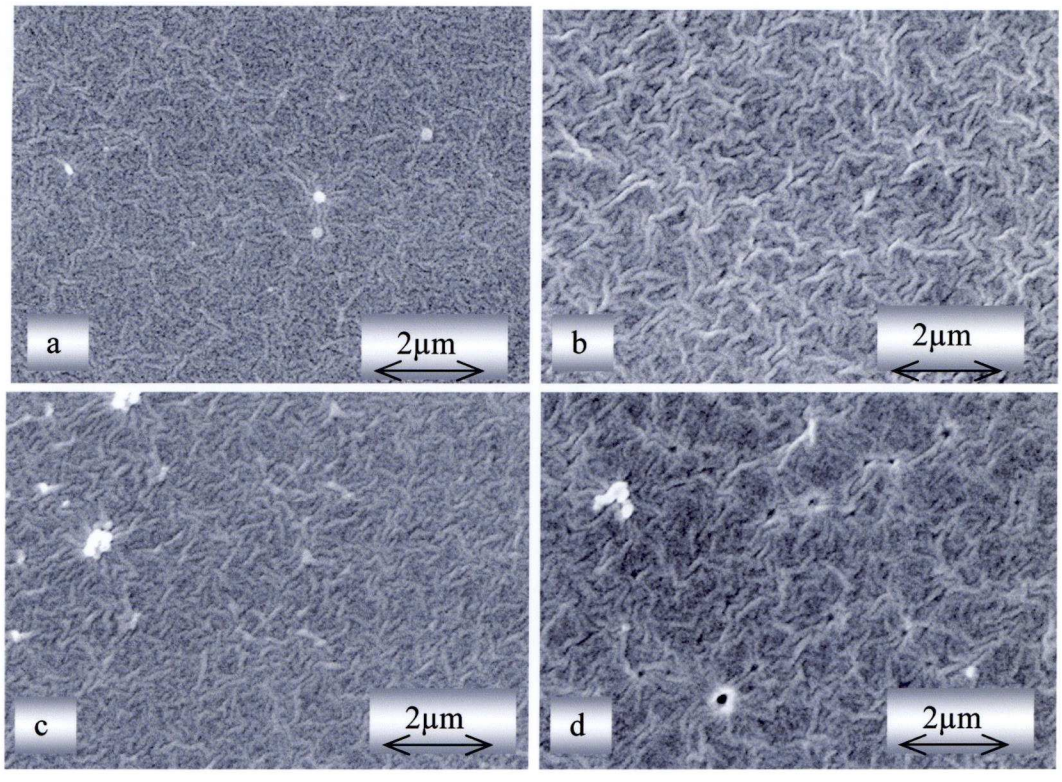

Figure 4.19 SEM Images. 85:15 PLGA with and without F-127 showing the effect of degradation time on the surface of the polymer films. a,c, 85:15 PLGA films, b,d, 85:15/F-127 PLGA films. Controls (a and b), 60day (c and d).The control films have a rippled appearance and there is little change in the appearance of the films the degradation time increases.

Control 85:15/F-127 films (Figure 4.19b) have a similar appearance to those of the 85:15 PLGA films, but after 60 days degradation the ripples are not as pronounced surface indentations are apparent and possible evidence of pore formation at the surface of the film (Figure 4.19d).

Changes in the surface morphology were seen in films composed of a blend of 85:15/50:50 PLGA are shown in Figure 4.20. The control films had a rippled surface similar to that seen in 50:50 and 85:15 PLGA control films. At 30 days the surface appeared to be covered with small pits but at this stage they did not appear to form a network of spaces with the inside of the film.

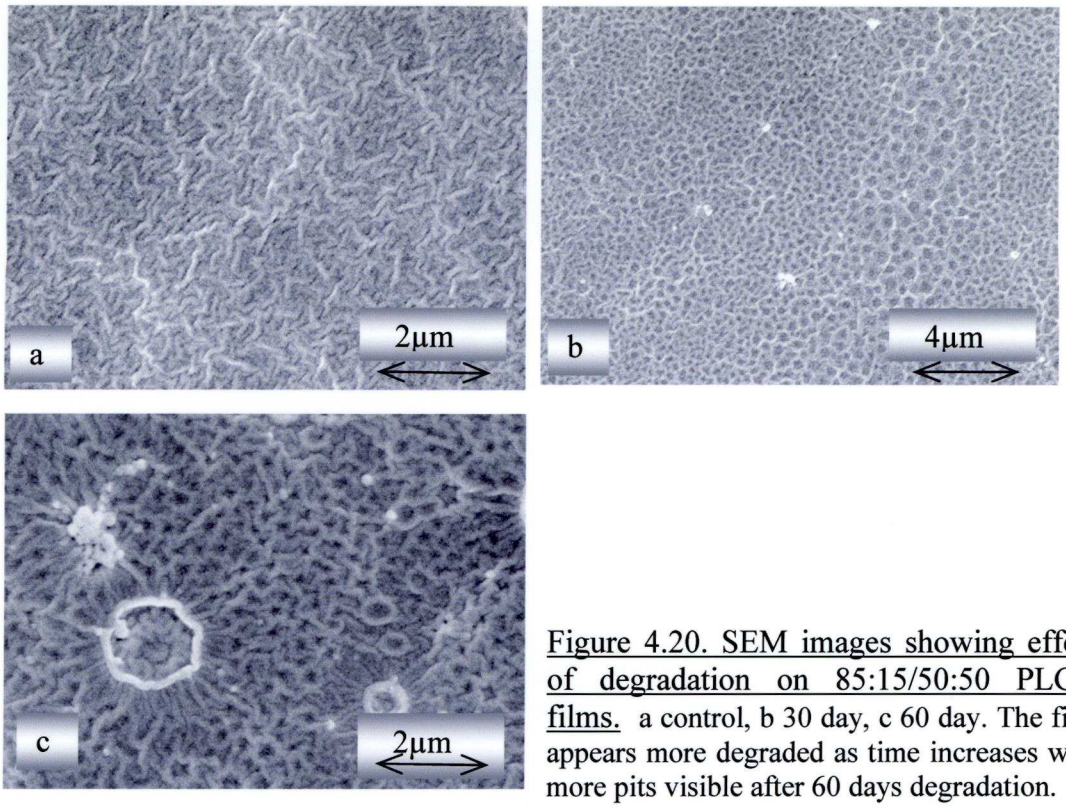

Figure 4.20. SEM images showing effect of degradation on 85:15/50:50 PLGA films. a control, b 30 day, c 60 day. The film appears more degraded as time increases with more pits visible after 60 days degradation.

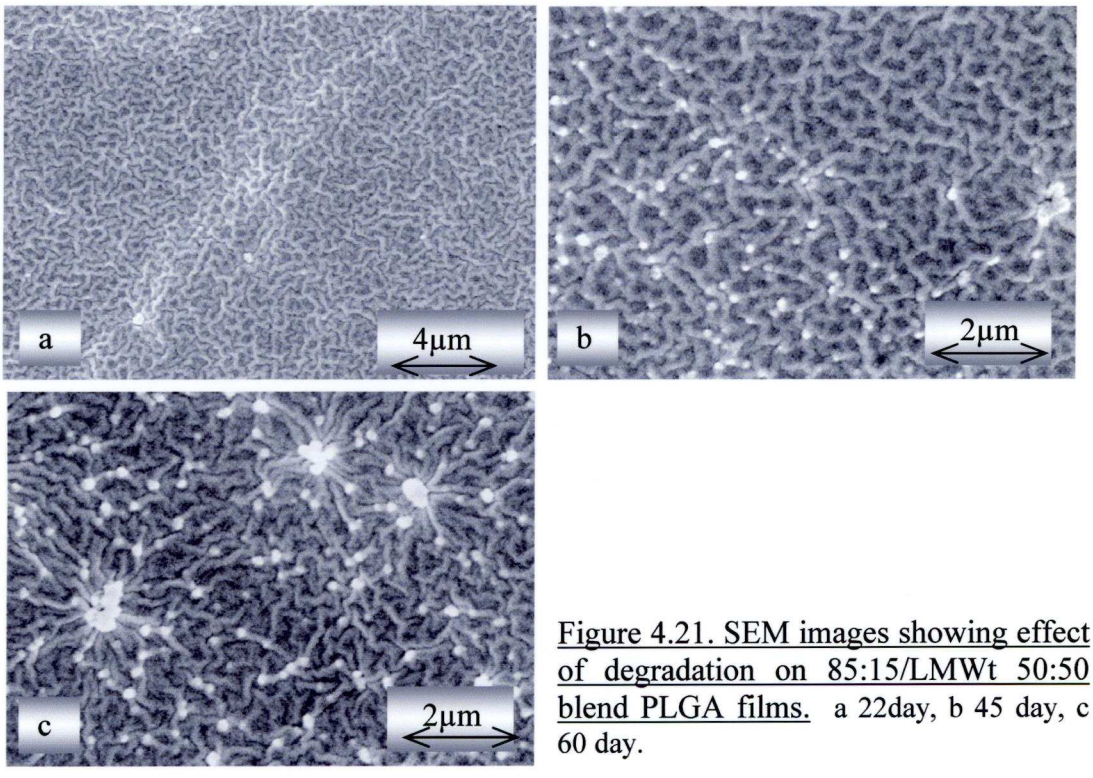

Figure 4.21. SEM images showing effect of degradation on 85:15/LMWt 50:50 blend PLGA films. a 22day, b 45 day, c 60 day.

By 60 days evidence of pitting was present revealing the inner portions of the polymer film (Figure 4.20c).

At 22 days the surface of the 85:15/LMWt 50:50 films were similar to those in the 85:15/50:50 blend at 45 days. The surface was rippled but the ripples formed shallow pits which did not appear to connect to the inner regions of the film. Pits and pores were not seen in the 85:15/LMWt 50:50 films during the study period but at 45 and 60 days increasing amount of very small white deposits were visible on the surface of the films. Similar deposits were seen in the other types of films but were less numerous and their appearance was not associated with a particular stage of degradation.

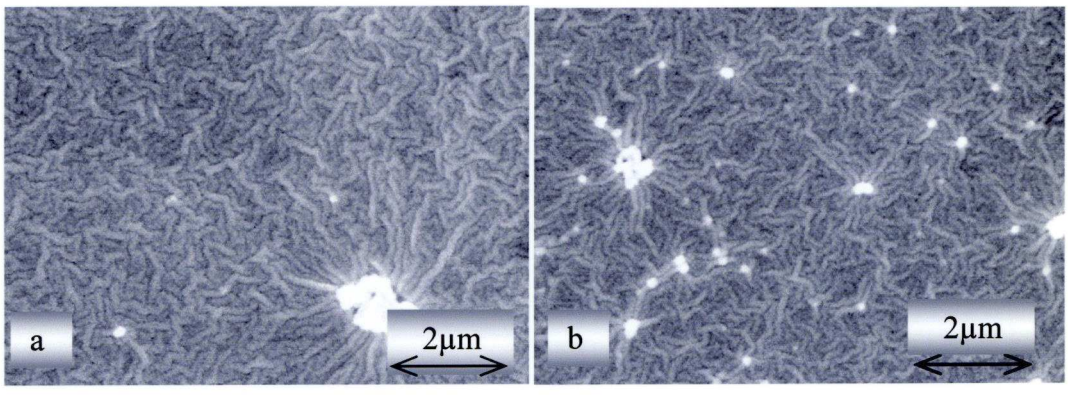

Figure 4.22 SEM images showing effect of degradation on 6535 PLGA films.
After a, 30 days & b, 60 days. There is little change in the surface of the films from 30 days to 60 days degradation time.

The surface morphology of the 65:35 PLGA films did not change appreciably during the study period. At 22 days the surface was rippled and was similar to control films and at 60 days there was little change, although there was some evidence of the white deposits seen in the 85:15/LMWt 50:50 blend PLGA films.

4.4 Discussion

The release rate of drugs such as paclitaxel from bioerodible devices is both diffusion-controlled and erosion-controlled [3, 22]. Chapter 3 of this thesis and other studies e.g. [4, 23-24] have shown that the diffusion controlled phase is associated with a

decrease in M_w of the polymer but with no corresponding mass loss and slow release of the contained drug. As degradation of the polymer continues the M_w decreases to 12-13,000 and soluble monomers are formed, and faster mass loss and polymer erosion occurs increasing the porosity of the device resulting in a phase of faster drug release.

The aim of the studies in this chapter was to alter the degradation profile of 85:15 PLGA to reduce the lag phase in paclitaxel release seen in the diffusion controlled release phase and to achieve a linear release profile of the drug.

The methods used aimed to

I. Increase the hydration of the polymer by addition of more hydrophilic co-polymers and thereby increase hydrolytic degradation rates of the polymer.
II. Increase the degradation by blending 85:15 with more hydrophilic, higher GA content, faster degrading PLGA polymers.

In this study degradation and release of paclitaxel from 50:50 PLGA films was faster than that seen in 85:15 PLGA films the reasons for which are described in Chapter 3 pp 86-91. Degradation of the 50:50 PLGA was slow over 30 days but at 45 days the molecular weight of the polymer had reduced to approximately 8,000 at which point the monomers can solubilise, leading to a period of faster mass loss and drug elution. At the end of the study period (60 days) the M_w of the 85:15 PLGA films was 49,900 and degradation of the polymer chains was not sufficient to produce soluble monomers. Mass loss was therefore minimal and release of paclitaxel was diffusion-controlled and occurring at a very slow rate.

4.4.1 Effect of pluronics

F-127 is a hydrophilic copolymer and addition of up to 8% w/w to PLGA microspheres has been shown to increase polymer hydration [8]. Degradation of PLGA polymers is via hydrolytic breakage of the ester backbone bond. Increasing the hydration of the matrix in the vicinity of the hydrolytically labile ester bond should increase polymer degradation [9]. F-127 was added to films of 85:15 and 50:50 PLGA films with the intention to increase polymer hydration and consequently the polymer

degradation. The addition of F-127 to 50:50 and 85:15 PLGA increased the hydration of the polymer films for the first week following immersion of the films in PBS. But after 14 days the water content PLGA/F-127 films decreased to 5-6% while the water content of the 85:15 and 50:50 PLGA films continued to increase reaching a maximum of 14% and 8.6% respectively. The increased amounts of hydration in the first week of the trial did not affect the early degradation rate of the films and the films containing F-127 degraded more slowly than films containing 85:15 or 50:50 PLGA alone.

Addition of F-127 decreased the rate of polymer degradation of 85:15 PLGA (the effect was not significant) and had no effect on mass loss and paclitaxel elution of 85:15 PLGA films. Little change was seen in the surface morphology of the films using SEM. Mass loss was faster over the first 30 days of the trial in 50:50 PLGA films containing F-127 films but this was not associated with an increase in degradation rate of the polymer or faster paclitaxel elution. At 45 days similar mass loss and extensive erosion of the 50:50 PLGA films with and without F-127 had occurred (Figures 4.5 and 4.18) but more paclitaxel had been released from the 50:50 PLGA films without F-127.

Wang *et al* [4] found that the addition of PEG (5 & 10%) increased the hydration and decreased the T_g of 53:47 PLGA resulting in a faster initial degradation rate of the polymer and eliminated the early lag phase associated with sirolimus release from PLGA polymers. The lowering of the T_g increased the free volume within the polymer matrix which is favourable for water diffusion into the polymer and the increase in hydration increased the degradation of the ester bonds resulting in a faster mass loss and sirolimus elution.

Raiche and Puleo [8] found that adding 8% w/w F-127 increased the hydration of 50:50 PLGA microspheres. Release of lysozyme was triphasic but the lag phase during diffusion controlled release was shorter for the microspheres containing F-127.
In that study hydration of >20% was found in microspheres containing 6% F-127 but it is unclear how hydration varied (if at all) as the polymer degraded. Yeh *at al* [21]

used a ratio of 1:2 PLGA:F-127 to substantially increase protein release from microspheres.

In this study F-127 did not affect the degradation rate of the polymer despite increasing the hydration of the polymer matrix in the early stages of the trial. Park *et al* [9] found that the degradation rate of PLLA films was not affected by the addition of a pluronic despite increasing the hydration of the polymer devices. This may be explained by two opposing effects occurring within the PLGA/pluronic blend. Addition of the pluronic increases the hydration of the film which should increase the degradation rate of the polymer. But hydrogen bond formation between the pluronic and the terminal carboxylic groups of PLLA/PLGA polymers reduces the contribution of autocatalysis from free carboxylic acid resulting in a reduced rate of degradation. Despite increased hydration resulting in hydrolytic cleavage of the ester linkage in the PLGA, the terminal carboxylic groups generated by hydrolysis are not available for further catalytic degradation because of immediate hydrogen bonding with the ether bonds of the pluronic [9].

Hydrophilic pluronics such as F-127 can quickly leach out of PLLA films [9] and this may explain the increased mass loss from 50:50 PLGA films containing F-127. But there was no difference in mass loss seen between 85:15 PLGA films with and without F-127. A possible explanation for this observation is that pluronics can accumulate towards the surface of PLGA films and the extent to which this occurs is dependant on the type of PLGA and the hydrophobicity of the pluronic [25]. Surface enrichment of pluronics in PLGA films decreases with increasing GA content [25] and so there may have been less accumulation at or towards the surface of the 85:15 PLGA films as compared with the 50:50 PLGA films. If molecules of F-127 were distributed more towards the surface of the 50:50 PLGA films it is possible that these molecules could have been leached at a faster rate than F-127 molecules from within 85:15 PLGA films where there would be less surface enrichment of pluronic. The molecules of F-127 may have been entangled with the 85:15 PLGA molecules within the film and been unable to diffuse out into the release medium. Addition of F-127 to 85:15 PLGA films resulted in higher PDI and lower molecular weights (Figure 4.6 & 4.8) during the study period indicating a wider distribution of molecular weights of

polymer chains in the film which may be due to the retention of F-127 molecules. Rapid leaching of F-127 at or near to the surface of 50:50 PLGA films may explain the initial high loss of polymer mass over the first 3 days which was higher than in the other films (Figure 4.5). But, interestingly this early mass loss was not associated with an increased early burst release of paclitaxel (Figure 4.7). Overall there was no difference in the PDI of 50:50 PLGA films with and without F-127, perhaps due to early loss of the pluronic from the film.

Formation of liquid-crystalline regions within the polymer matrix of PLGA/F-127 blends occurs as the polymer becomes hydrated affecting the diffusivity of a drug through the pores and channels of the polymer [9]. The PPO block of F-127 has been found to form a strong hydrophobic interaction with PLGA while the PEO segments orient way from the surface into the aqueous areas of the matrix [9, 26]. Hydrophobic drugs such as doxorubicin can be localised into pluronic micelles by hydrophobic interaction between the drug and the PPO blocks. Paclitaxel, being a hydrophobic drug could similarly become associated with the PPO segment of a F-127/PLGA blend affecting the ability of the drug to diffuse through the film into the release medium [26]. In this present study 45% of paclitaxel was released from the 50:50 PLGA films at 45 days. Addition of F-127 significantly reduced the release of paclitaxel in 50:50 PLGA films with only 29% being released at 45 days despite similar polymer erosion rates to that of the 50:50 PLGA films. While it is unclear what the nature of liquid-crystalline phases formed in the pluronic blend films in this study, if such interactions between paclitaxel and the PPO blocks was present then this may explain the difference between paclitaxel elution in 50:50 PLGA and 50:50 PLGA/F-127 blend films.

Addition of PEG had no affect heparin release from 80:20 PLGA devices [11]. In this case plasticisation occurred in the polymer reducing the T_g in the plasticized polymer but this did not result in faster drug elution despite a significant reduction of M_w of the polymer. The authors attribute this to an increase in the melting enthalpy of the polymer which in turn caused re-crystallization of residual polymer units that were richer in lactide units which was not favourable to drug release [11]. Addition of 10% w/w MePEG to 85:15 and 50:50 PLGA resulted in reduction of the T_g of the

polymers to around room temperature but the effect on paclitaxel elution was minimal. 30% w/w MePEG was required to significantly increase the release of paclitaxel from 50:50 PLGA when the release profile became biphasic with a fast release phase of around 7 days followed by a slower phase of diffusion controlled release. The addition of hydrophilic copolymers such as pluronics can hydrate and cause plasticization of a polymer. But the effect on drug release behaviour is often a compromise of a number of processes within the matrix some of which enhance release while others impede drug elution [9].

4.4.2 Effect of PLGA blending

The degradation rate and mass loss profile of the 85:15/50:50 PLGA films was almost an average of that seen in the 85:15 and 50:50 PLGA films (Figures 4.10 & 4.12). The 85:15/50:50 PLGA films degraded at a faster rate than the 85:15 PLGA films and onset of polymer mass loss occurred after 30 days whereas there was little change in polymer mass of the 85:15 PLGA films. The delay in the onset of polymer mass loss (30 days) was similar in the 50:50 PLGA films to that seen in the 85:15/50:50 blend but the rate of mass loss was then faster in the 50:50 PLGA films: approximately 22% of the mass had been lost in the 50:50 PLGA films at 45 days whereas comparable amounts of mass loss was only observed in the 85:15/50:50 PLGA blend films at 60 days.

Elution of paclitaxel from 85:15/50:50 PLGA films commenced after the onset of polymer erosion and at 60 days 22% of the paclitaxel had been released as compared with 8.5% from the 85:15 PLGA films. But paclitaxel release was delayed as compared with the 50:50 PLGA films where approximately 43% of the paclitaxel had been released by 45 days.

Increasing the GA content of a polymer increases the hydrophilicity of a polymer and the hydration of 85:15 PLGA films should be increased by blending 50:50 PLGA into the film. But the water content of the 85:15 PLGA films was higher than that seen in the 50:50 PLGA and the 85:15/50:50 blend PLGA films in this trial (Figure 4.9). Since the rate of water diffusion into a PLGA film is generally greater than the

rate of hydrolysis, the polymer degradation may not be affected by the hydration rate [8]. The GA units are more hydrophilic than the LA units and hydrolysis occurs preferentially on the GA linkages because the methyl pendant group on the LA unit sterically hinders hydrolysis [14, 24, 27]. Hence faster degradation is associated with higher GA content. In this study overall matrix hydration appears to be less important to PLGA degradation than the GA content of the polymer film and the ability of the water molecules within the hydrated device to attack the ester linkages.

The rationale behind blending 50:50 PLGA into 85:15 PLGA films was to create a matrix with areas of higher GA content to create faster degrading regions within the polymer matrix. Faster degradation of these regions would lead to spaces and cavities through which the paclitaxel could diffuse at a faster rate relative to 85:15 PLGA films. The overall GA:LA ratio in a 1:1 blend of 85:15/50:50 PLGA is 32.5:67.5, comparable to 65:35 PLGA. But there was no obvious difference in polymer mass loss and paclitaxel elution between the 85:35/50:50 blend PLGA films and 65:35 PLGA films during the course of this study. However the PDI change and surface morphology of the 85:15/50:50 blend is different to that of the other films.

Figure 4.16 shows the change in PDI during the degradation study. At the start of the trial the 85:15/50:50 blend PLGA, 85:15, 65:35 and 50:50 PLGA films had a similar PDI. As degradation proceeded the PDI of the 85:15/50:50 blend PLGA films increased significantly to 3.133 at 60 days. The PDI of the other films increased slightly during degradation with a PDI of 1.788 and 1.761at 60 days for the 85:15 and 65:35 PLGA films respectively. This indicates a wider distribution of MWt's of the polymer chains within the 85:15/50:50 blends and the distribution increases with degradation time. It has been shown that within a degrading polymer the slow degrading surface of the polymer matrix can act as a semi-permeable diffusion barrier leading to entrapment of degraded oligomers within the device [28]. It can be envisaged that in a PLGA blend, lower molecular weight fragments created by hydrolysis of the 50:50 PLGA chains may become entrapped within the polymer bulk by a surface of less degraded 85:15 polymer chains resulting in a wider distribution of MWt's of the polymer chains in the film and hence an increased PDI.

The surface morphology as revealed by SEM showed differences between the 85:15/50:50 blend PLGA films as compared with the other films. At the start of the trial the films had a textured and rippled appearance. There was little change in the surface of the 85:15 and 65:35 PLGA as the films degraded in this study (Figure 4.19 and 4.22). But as degradation proceeded the surface of the 85:15/50:50 blend films changed and the textured appearance of interlocking ripples was replaced by a more open, cratered appearance at 30 days (Figure 4.20) and by 60 days larger pores could be seen connecting to the inner regions of the film. The reason for the appearance of the 'craters' is unclear, but it may represent faster solubilisation of polymer from 50:50 PLGA that was located at the surface of the film while the slower degrading 85:15 polymer retains its structure. There were no pits or pores seen on the 65:35 or 85:15 PLGA films and it is conceivable that had the degradation time been extended faster mass loss and paclitaxel elution may have become apparent in the 85:15/50:50 blend films.

Blending of polymers of differing composition or molecular weight may alter drug diffusion properties of the device by creation of water channels in the device created by faster degrading regions of the device where faster degrading polymers are present. Kunou *et al* [16] found that in a PLLA blend of 5kDA and 70kDa PLLA, in the faster degrading 5kDa PLLA regions water channels were formed connecting the surface to the inner regions of the implant device resulting in faster ganciclovir elution as compared with PLLA (70kDa). Faster degradation of LMWt 50:50 PLGA regions in a blend with 65:35 PLGA resulted in the creation of cavities and spaces within the polymer matrix through which the ganciclovir molecules could diffuse out into the external medium [20]. Duvvuri *et al* [5] reduced the diffusion controlled release phase of ganciclovir from 79 days to just under 10 days by blending a 1:1 ratio of 8 kDa 50:50 PLGA with 75:25 PLGA. Addition of 8 kDa 50:50 PLGA had a plasticizing effect on 75:25 PLGA and the biggest reductions in T_g occurred with higher content of 8 kDa 50:50 PLGA in the blend.

GPC data from degrading 85:15/LMWt 50:50 PLGA was bi-modal with 2 distinct peaks (Appendix, Figure A.5) and molecular weight change in both peaks was determined. The faster eluting peak was from the 85:15 PLGA component and this de-

graded from 86kDa to 35kDa over the 75 day study period. The slower eluting peak was from the 50:50 PLGA component which degraded slowly from a M_w of 6.6kDa at the start of the study to 5.5kDa after 75 days degradation. Mass loss only takes place when the MWt of the PLGA has degraded to a critical value, usually around 10kDa [28-29]. The polymer chains from the 50:50 PLGA component in the blend films would be below this critical value and would therefore be expected solubilise readily and create spaces and cavities within the film to enable release of paclitaxel.

Addition of low molecular weight (LMWt) 50:50 PLGA to 85:15 PLGA resulted in faster mass loss and paclitaxel elution as compared with 65:35 PLGA and the 85:15/50:50 blend PLGA films. The surface morphology after 22 days incubation was comparable with that of the 85:15/50:50 blend at 30 days with a cratered appearance on the surface of the films. There was no evidence of pores or pitting on the films, as degradation proceeded but increasing mounts of a white deposit was visible at 60 and 75 days. It was not possible in this study to determine the nature of the white deposits.

Acid degradation products have been shown to lower the pH of release media containing degrading PLGA devices [27]. Although the PBS release media was changed weekly, the pH of the release medium in trials TD1 & 2 was found to increase slightly as degradation time increased (Figures 4.1 and 4.2). The pH change was the same in all groups and was also seen in the controls (where a blank Teflon disc had been incubated in PBS) and so is unlikely to be an effect of PLGA degradation. The pH of release media can have an effect on PLGA degradation, but such effects are only seen with release media with very acid or very basic pH [27] and the changes seen in TD1 and TD2 are unlikely to have had an effect on polymer degradation or drug elution.

The degradation rate of 85:15/50:50 blend was measured in both TD1 and TD2 and the data shows that in TD2 degradation rate was slower with equivalent stages in degradation, mass loss and paclitaxel elution being delayed by approximately 15 days. The reason for this difference is possibly due to problems encountered in the

warm room and for a large portion of TD2 the incubation temperature was a few degrees below 37°C.

Despite this standard procedure of film production there was some difference both between the groups and within groups in terms of original mass of polymer. However once the samples had been randomised into the various time points there was no difference in the original amount of polymer. Thus it is safe to assume that differences in the degradation rate and drug release were due to the composition of the polymer and not due to differences in thickness of the film or drug loading.

4.5 Conclusions.

The addition of F-127 had no effect on PLGA degradation and may have reduced paclitaxel elution in this study. Addition of 8% w/w Pluronic F-127 to 85:15 or 50:50 PLGA can increase the hydration of polymer films. The increased hydration may increase the rate of hydrolysis of the polymer film but rates of autocatalysis within the matrix of the polymer are reduced due to hydrogen bonding between the carboxylic end groups of PLGA and the PPO segment of F-127 resulting in no overall effect on the degradation rate. Release of paclitaxel from PLGA films containing F-127 may be reduced due to a hydrophobic interaction between the drug and micelles of PPO and the polymer surface. Inclusion of F-127 in PLGA polymers does not appear to be an effective method to reduce the diffusion controlled release phase of paclitaxel.

Blending a 1:1 ratio of 50:50 PLGA into 85:15 PLGA advanced the onset of polymer erosion and reduced the length of diffusion controlled release (lag phase) of paclitaxel as compared with 85:15 PLGA. The onset of polymer erosion was further advanced and the diffusion controlled release phase of paclitaxel was shortened by using a low molecular weight 50:50 PLGA blended with 85:15 PLGA. This demonstrates that polymer blending can be used to effectively increase the release of paclitaxel from PLGA devices. Further work could be done to fine tune the ratio of polymer blends utilized in order to optimise release profile for coronary stent applications.

4.6 References.

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Discussion

Despite the successful use of DES since their introduction in 2002 in reducing rates of in-stent restenosis (ISR) and the need for subsequent revascularisation (TVR) procedures as compared with BMS and balloon angioplasty [1-2], concerns have arisen regarding late stent thrombosis (LST) which may be due to delayed arterial healing and re-endothelialisation of the artery wall [1, 3-5] which may be an unintended result of the action of the drugs currently used in DES. Additionally in a small number of cases hypersensitivity reactions to the polymer coatings used on DES have been reported [6-9].

For paclitaxel, differing release profiles can have a profound impact on efficacy [10] and a principal target of current research is on the development of biocompatible, biodegradable polymers that would permit controlled drug release whilst minimizing effects such as delayed arterial healing [1]. Complete bioresorption of the stent coating would reduce the tissue exposure to the polymer and reduce any short and long term inflammatory and thrombotic events associated with exposure to the currently used durable polymers [11]. Additionally complete elution of the contained drug would ensure that there are no long term consequences with the large amount of drug that remains in the polymer coating of the currently used Taxus DES [10].

In this study the degradation and paclitaxel elution profile of two biocompatible, biodegradable polymers, TyRx, a tyrosine based polyarylate and Poly($_{D,L}$-lactide-co-glycolide) was assessed for their potential as coatings for DES. The degradation of the polymers was determined using GPC and related to polymer erosion and paclitaxel elution and to the physical appearance of the stent coating.

5.1 *In vivo* tissue chamber models and *in vitro* testing of DES.

The rat subcutaneous tissue chamber model facilitates the study of degradation and drug elution of DES coatings in complete interstitial exudates fluid environment. In this model polymer degradation and drug release is in *in vivo* physiological medium at body temperature and pH. However this model, in common with *in vitro* models, does not take into account all of the conditions experienced by a stent *in situ* and

does not replicate the effect of factors such as flow dynamics, shear stress, continuous pulsatile effects and strut encapsulation by SMC.

The polymer under test in this study, namely TyRx and PLGA, are degraded via hydrolysis. Some polymers are also susceptible to degradation *via* enzymatic cleavage. There are no studies indicating the susceptibility of TyRx to enzymatic degradation. There are conflicting reports on the role of enzymes in degradation of PLGA with some suggesting enhanced degradation rates in the presence of enzymes while other have demonstrated no effect (for review see [12-13]). However there was no difference in the degradation and paclitaxel elution profile of PLGA or TyRx coated stents from the rat tissue chamber model as compared with an *in vitro* study using a release medium comprising PBS and Tween, pH 7.4 at 37°C (unpublished data). This suggests that in this study degradation was predominantly by hydrolysis with negligible effects due to enzymatic cleavage.

The effects of flow dynamics are not assessed in the tissue chamber model (or in most *in vitro* models). Models incorporating flow have shown that degradation of PLGA films and scaffolds is faster under static conditions than in flow conditions [14-15]. Additionally the tissue chamber model is unable to replicate the effect of placement of the stent adjacent to the artery wall on polymer degradation and drug delivery. Models have been developed to replicate the effects of arterial contact by placing the stent in flow chambers and using hydrogels as a simulated artery wall [16]. Lower release rates of doxorubicin were observed when the stent was placed adjacent to the hydrogels as compared with the stent having no adjacent compartment in the flow chamber [16].

However despite these limitations with the *in vivo* tissue chamber model and standard *in vitro* models these tests have a high value for anticipating in vivo drug release in a clinical setting [16]. Kammath *et al* [17] found that the release rates of paclitaxel from the Taxus coronary stent in *in vitro* studies using PBS/Tween 20 medium was comparable to that observed in stents implanted into rabbit iliac arteries. DES which posses favourable properties in terms of drug release, polymer coating integrity and dissolution in the rat tissue chamber model are promising candidates for further *in vivo* testing in animal artery models. The tissue chamber model is an equivalent pre-

dictive model to the *in vitro* models using physiological conditions in predicting the degradation and drug elution of PLGA and polyarylate stent coatings. The model is likely to be a better predictor of *in vivo* pharmacokinetics when the test polymer is degraded both hydrolytically and enzymatically.

5.2 Degradation and paclitaxel elution of TyRx stent coatings.

Tyrosine derived polyarylates have been used for the release of water soluble peptides [18], anticoagulants [19] and show a diffusion controlled release mechanism of low molecular weight substances [20]. Tyrosine derived polymers tend to have a low uptake of water and since the monomers created during degradation are not readily water soluble polymer erosion is very slow and occurs only at the end of the degradation process [19].

In chapter 2 the degradation and drug elution profile of a 400μg coating of a tyrosine based polyarylate (TyRx) containing 2.5%w/w (10μg) paclitaxel on a16mm Liberté stent was determined using an acellular *in-vivo* model. As the polymer degraded the molecular weight (Mw) of the polymer decreased from around 36,000 to 12-13,000 after 60 days implantation (Figure 2.4). After 100 days the degradation rate decreased and the Mw remained at around 4,000 from 150 days onwards. Addition of paclitaxel had no effect on the degradation rate of the polymer (Figure 2.4), but paclitaxel elution was very slow due to the slow erosion of polymer from the stent (Figure 2.9). There was no measurable release of paclitaxel after 15 days degradation and after 250 days 6.87 ±2.6 μg of paclitaxel remained on the stent. Release was faster after 250 days as polymer erosion occurred and at 280 days approximately 7 μg of the paclitaxel had been released.

5.3 Degradation and paclitaxel elution from PLGA stent coatings.

PLLA and PLGA are among the most commonly used biodegradable polymers for sustained drug release. They are biocompatible and degrade by simple hydrolysis of the ester bonds into natural metabolites, lactic and glycolic acid, which are easily removed from the body by normal metabolic pathways [21].

The elution of hydrophobic drugs such as paclitaxel is diffusion controlled and erosion controlled from PLGA devices and the duration of each phase is dictated by the degradation rate of the polymer matrix. Increasing the GA content of PLGA increases the degradation rate and the drug elution properties of the device [12]. Increasing the thickness of the stent coating increases the polymer degradation rate but reduces the elution rate of paclitaxel (for the same drug: polymer ratio). The drug itself can affect the degradation of the polymer and thus its elution [22].

In chapter 3 the effect of adding 5%w/w (20μg) paclitaxel on the degradation of a 400μg coating of 50:50 PLGA on a 16mm Liberté stent was examined and addition of the drug was found to have no effect on the degradation rate of the polymer (Figure 3.3) or cause cracking and delamination of the coating as the polymer degraded. Secondly it was shown that elution of paclitaxel was bi-phasic with an initial lag phase in which elution was diffusion controlled followed by a faster release phase (erosion controlled) associated with polymer mass loss. The lag phase lasted about 15 days, and only about 1μg of paclitaxel was released during that period. After 30 days implantation approximately 15.5μg of paclitaxel had been released and at 45 days all the polymer had been solubilised from the stent along with the remaining paclitaxel.

A lag phase was not detected using a 200μg coating of 50:50 PLGA with 5%w/w paclitaxel possibly because at the first sampling time erosion controlled release had already commenced. Elution of paclitaxel was faster in the thinner coating and at 15 days approximately 4.8μg of paclitaxel had been eluted. All of the paclitaxel (8.5μg) had been eluted after 30 days and the polymer had been completely solubilised at 45 days.

Increasing the GA content of the polymer increased the duration of the lag phase and the residency time of the polymer (Figure 3.4 & 3.5). A lag phase of 30 and 60 days was seen for the 75:25 and 85:15 PLGA coated stents respectively. During this phase there was no measurable release of paclitaxel from the 85:15 coatings but around 3μg of paclitaxel was released from the 75:25 coatings. The remainder of the paclitaxel (approx 17μg) was eluted over the following 45 days and after 75 days paclitaxel could not be detected on the 75:25 PLGA coated stents. The paclitaxel was re-

leased over 60 days from the 85:15 PLGA coatings in a linear release profile following the lag phase. Complete solubilisation of the 75:25 PLGA coatings had occurred by 105 days but 85:15 PLGA was still detectable on the stent at 120 days which was the end of the study period.

The elution of paclitaxel was dependent on the degradation of the PLGA coating. During the lag phase the Mw of the polymer was decreasing rapidly but there was little loss of polymer mass during this period (Figure 3.16) and the polymer coating remained intact (Figure 3.7 and 3.8) and the release of paclitaxel was slow. Once the polymer Mw had decreased to around 12,000-13,000 the polymer fragments become soluble in the aqueous media and the rate of polymer erosion increased creating a porous structure within the stent coating which facilitated faster diffusion of paclitaxel. The dependence of polymer mass loss and drug elution on the degradation state of polymer has been demonstrated in other studies [23-26].

5.4 Paclitaxel elution profiles of DES compared with PLGA and TyRx coated stents.

Inhibition of ISR using stents with high doses of paclitaxel is associated with incomplete arterial healing and cytotoxic effects of paclitaxel [27]. The rapid elution (63% within 14 days) of paclitaxel from stents coated with poly(lactide-co-Σ-caprolactone) with an initial drug content of 207μg of paclitaxel resulted in reduced rates of ISR but was associated incomplete healing and low rates of re-endothelialization [28]. A 10μg dose of paclitaxel released over 10 days was found to have higher indexes of injury (such as fibrin deposition and eosinophilic deposits) as compared with the same dose released over 30 days [10].

Lower doses of paclitaxel delivered over a minimum period of time may provide similar efficacy as the existing paclitaxel DES in preventing ISR but with reduced effects such as delayed endothelial healing, inflammation, and LST, while complete bioresorption of the polymer coating may reduce short and long term inflammatory and thrombotic events caused by hypersensitivity reactions to the durable polymer coatings currently used [11].

The amount and duration of paclitaxel released from the DES has an effect on both the efficacy of the stent in reducing ISR and on delayed arterial healing and vascular re-endothelialization. Rapid release of a 8.6μg dose of paclitaxel over about 1 week from a chondroitin coated stent did not reduce neointimal thickening in New Zealand White rabbits but a higher dose (20μg) of paclitaxel released within 1 week was found to reduce ISR for up to 90 days but was associated with incomplete healing, local artery inflammation and fibrin deposition [29]. Jabara *et al* [30] found that release of approximately 10μg of paclitaxel from a PLGA coated stent over 80-100 days was effective at reducing ISR in a porcine model at one month, but was not better than BMS at 3 months. Release of the drug was via a rapid release phase over 20 days in which approximately 3.5μg of paclitaxel was released, followed by a period of slower release to 60 days after which the remainder of the paclitaxel was released. Slowing the rate of release was found to mitigate the toxic effects of paclitaxel seen in higher drug loadings [30]. Studies from animal trials do not translate directly to humans and there is a 1:6 ratio for time effects in pigs to humans (i.e. an effect of 90 days in pigs is equivalent to 18 months in humans) [31-32].

The PICES trial explored the effect of variable dose and release rates of paclitaxel on humans using the Conor strut-filled paclitaxel eluting stent. The results showed that shorter delivery of a 10μg dose over a week resulted in no improvement over BMS but the same dosage released over 30 days resulted in inhibition of ISR. The results were confirmed in the EUROSTAR and COSTAR I trials but the formulation was inferior to the Taxus SR stent after 30-90 days in reducing in-stent neointimal hyperplasia in the COSTAR II trials [10-11, 32]. The slow release (SR) Taxus stent contains a total of 108μg of paclitaxel and most of the drug that is released is in the form of an initial burst release over the first few days after implantation and after 14 days approximately 1-2μg of paclitaxel are released from the SR formulation [33]. By 30 days approximately 7.5% (8μg) of the paclitaxel is released [10, 32] but approximately 90% of the drug remains sequestered within the polymer coating without further measurable loss at 6 months after implantation [32, 34]. There appears to be little difference in paclitaxel dose and release rate between the Conor stent and the Taxus SR stent in COSTAR II, and it is unclear if the difference in efficacy is due to differences in stent designs or due to any further anti-restenoic effect of the paclitaxel remaining on the Taxus SR stent after 6 months.

The ideal elution rate for paclitaxel has yet to be elucidated [32], but it would appear from the studies outlined above that between 10-20μg of paclitaxel needs to be eluted in a steady manner during the first 30 days following implantation and perhaps further release is required over 90 days to maintain performance.

The paclitaxel elution data from the TyRx and PLGA studies is summarised in Table 5.1. In the formulation used in chapter 2, TyRx would not appear to be a good candidate as a coronary stent coating. Although tyrosine based polyarylates have demonstrated good biocompatibility and are not cytotoxic [19] complete bioresorption of the polymer is very slow and polymer was still evident on the stent at 280 days. This leaves the possibility of polymer fragmentation causing blockage of small capillaries and may lead to long term inflammatory and thrombogenic events [35].

Table 5.1 Degradation of polymer stent coatings. Summary of effect of polymer on elution of paclitaxel.

polymer	**μg Paclitaxel /stent**	**Duration of lag phase (days)**	**Paclitaxel (μg) eluted in lag phase**	**Duration of erosion controlled phase(days)**	**Complete Elution of paclitaxel (days)**	**Complete polymer solubilisation (days)**
TyRx 400μg coating	10	240	3.1	>50	>290	>290
50:50 PLGA 200μg coating	10	none detected	not applicable	30	30	45
50:50 PLGA 400μg coating	20	15	1.4	15	45	45
75:25 PLGA 400μg coating	20	30	3.2	45	75	105
85:15 PLGA 400μg coating.	20	60	Non detected	60	120	>120

Paclitaxel per stent refers to the initial amount of paclitaxel on the stent. The amount of paclitaxel eluted in the lag phase is shown in μg. The duration of the erosion controlled phase is the time from the end of the lag phase to the complete elution of paclitaxel. Complete solubilisation of the polymer refers to the time at which no polymer could be detected on the stent using GPC.

No burst release of paclitaxel was observed but, significantly there was no measurable release of paclitaxel in the first 15 days of the trial and at 90 days only about 2.25 μg had been released. Based on previous studies, this would suggest that insufficient amounts of paclitaxel are released over the desired timescale to be effective in reducing ISR.

Elution of paclitaxel from PLGA was much faster than from TyRx. The higher drug loading in PLGA as compared to TyRx (5% and 2.5% respectively) may be partially responsible but it is more likely to be due to the faster degradation with more rapid mass loss due to the higher water solubility of the degraded monomers of the PLGA polymer coatings as compared with TyRx [19].

A 5%w/w dose of paclitaxel in a 200μg coating of 50:50 PLGA had been eluted by 30 days with complete polymer dissolution by 45 days which was similar to that seen in COSTAR I, EUROSTAR and PISCES trials which were efficacious in reducing ISR [10-11]. But based on the observations of the COSTAR II trial [11] and the work of Jabara *et al* [30] this may be insufficient to maintain prevention of intimal hyperplasia for longer periods up to 90 days and beyond. Additionally, approximately 4.8μg of paclitaxel was eluted over the first 15 days of implantation which is higher than that from a SR Taxus DES and as such may cause problems associated with delayed arterial healing and slow re-endothelialization although release rates of a similar magnitude from PLGA coated stents did not have a detrimental effect on healing in porcine arteries [30]. Additionally, assuming a steady release of drug from a 10μg/30 day DES formulation, 5μg of paclitaxel would be expected to be released by 15 days and no adverse effects were reported from such a release profile from the PISCES trial [10].

Release of paclitaxel (approximately 15μg) from the 400μg coating of 50:50 PLGA with 5%w/w paclitaxel over 30 days, is greater than the 10μg/30 day duration dose released from Conor stents [10-11, 36] and from PLGA coated stents in a porcine model [30] and so would be expected to significantly reduce ISR at least to 3 months. But paclitaxel release was slow to 15 days when only 1μg of paclitaxel was released which may reduce the formulations effectiveness in reducing ISR while the rapid release phase of approximately 15μg of the paclitaxel over the following 15

may have adverse effects such as delayed arterial healing and re-endothelialization. The effectiveness of the formulation may also be limited due to the duration of paclitaxel release since all the paclitaxel had been released from the stent by 45 days.

A longer duration of paclitaxel release of 75 and 120 days respectively for 75:25 and 85:15 PLGA coatings was observed using 400μg polymer coating with 5%w/w paclitaxel. But there was little or no release of paclitaxel from these two formulations during the first 30 days following implantation and it is during this period that the drug needs to be released to be effective in preventing proliferation of the smooth muscle cells [10].

5.5 Modification of paclitaxel elution from PLGA devices.

The release profile of paclitaxel from PLGA in the study by Jabara *et al* [30] consisted of a rapid release phase over the first 20 days of implantation during which approximately 30% of the drug was released This was followed by a slower phase of release lasting for about 25 days, a second rapid release phase of approximately 10 days in which some 40% of the drug was released and a final period of slow release in which the remainder of the paclitaxel was released. This is at odds with the biphasic release profile from PLGA coatings seen in chapter 3 of this study and from other drug release studies e.g. [26]. The GA:LA ratio is not given in the study by Jabara *et al* [30] and so direct comparison with the paclitaxel release studies from PLGA in chapter 3 is difficult. However the release profile of PLGA can be modified to reduce or eliminate the lag phase and produce a more even elution of the drug over the desired release period [26, 37-39]. A reduction in the duration of the lag phase while maintaining the overall duration of drug release would improve the usefulness of both the 85:15 and 75:25 PLGA coatings. 85:15 PLGA was chosen for further study due to its longer duration of drug release of over 90 days.

Blends of polymers form phase separated regions within the polymer matrix and can exhibit advantageous properties for the desired drug release profile that the individual polymers do not posses [40]. In chapter 4 of this study two approaches were used to modify the paclitaxel elution profile from 85:15 PLGA films by (i) blending low molecular weight hydrophilic copolymers (ii) blending PLGA polymers with higher

GA content with different molecular weights, into 85:15 PLGA films. The study was carried out *in vitro* using polymer films on coated onto PTFE discs incubated at 37°C in PBS at pH7.4.

The rationale behind polymer blending was that including more hydrophilic copolymers into the films would increase the hydration of the films and so increase the rate of ester bond hydrolysis within the PLGA polymer, leading to faster early degradation rates and consequently faster drug release. Blending 85:15 with PLGA polymers of higher GA content would increase degradation rate due to the increased hydrophilicity of the GA units as compared with the LA component, and since the GA component is more easily degraded than the LA units increasing GA content should lead to faster degrading areas within the polymer matrix. Additionally, the solubilisation of the faster degrading PLGA domains or leaching out of the matrix of the soluble F-127 chains would create a series of small pores and channels through which paclitaxel would more quickly and easily diffuse out of the film and into the incubating medium [41].

The pluronic F-127 is a hydrophilic triblock copolymer of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) with a MWt of 12,300, 70% of which is made up from PEO, and hydrophilic and hydrophobic pluronics been used to modify release of proteins from PLGA matrices [40, 42-43].

Raiche and Puleo [42] found that blending 8%w/w F-127 was optimum for improving water uptake into PLGA devices and so a ratio of 92% PLGA:8% F-127 was chosen for this study. In chapter 4 of this thesis it was found that the addition of F-127 increased the hydration rate of the films but after 14 days incubation the blended films had lower water content than the 85:15 or 50:50 PLGA films. But despite the increase in hydration over the first 14 days of the trial, addition of F-127 had no significant effect on the degradation rate, polymer erosion or paclitaxel elution of 85:15 PLGA films. Increased mass loss occurred in 50:50 PLGA films blended with F-127 but this was not associated with an increase in polymer degradation or paclitaxel elution. Higher concentrations of pluronics of up to 75% w/w have been used in PLGA matrices to alter degradation and drug release profiles resulting in more release of the contained drug during the burst period followed by a period of slow drug release, the

extent of the burst being dependant on the content of pluronic in the PLGA device [40, 43]. Such an elution profile would not be suitable for paclitaxel release from coronary stents especially if toxic amounts of drug were released during the burst period. There is also concern about the creation of domains of pluronics due to loading high fractions of pluronic into PLGA devices. F-127 is highly water soluble and will easily dissolve creating a highly porous structure [42]. While such a structure may enhance paclitaxel elution there is a risk that this would weaken the remaining polymer coating making it more prone to delamination.

The effect of blending polymers with differing GA content and MWt on the degradation and paclitaxel elution of 85:15 and 50:50 PLGA was studied in trial TD1. The length of the lag phase in paclitaxel release and the time for the onset of polymer erosion was slower by about 15 days from 50:50 PLGA films in the PTFE *in vitro* trial as compared to that seen in 50:50 PLGA coated stents in the *in vivo* trial in chapter 3 (Figure 3.4, 3.5, 4.12 and 4.14). The reasons for the slower degradation rate seen in TD1 are unclear. The *in vivo* degradation rate and drug release profile of PLGA has been shown to be faster than in *in vitro* models due to the presence of lipids and enzymes in the former system [30] but this is contentious [12]. The drug:PLGA ratio was the same for both trials but there was about 3.7μg/mm^2 of polymer on the stents as compared with approximately 6μg/mm^2 of polymer on the PTFE discs and as shown in chapter3, polymer erosion and drug release is faster from thinner coatings. Additionally due to the geometry of the stent there may be a higher surface area of the polymer exposed to the aqueous media on the stent as compared with the PTFE films which may facilitate faster diffusion of degraded soluble oligomers into the release medium and hence increase paclitaxel elution.

A 1:1 ratio blend of 50:50PLGA and 85:15 PLGA increased the degradation rate, rate of mass loss and reduced the length of the lag phase of paclitaxel elution of the films as compared with 85:15 PLGA (Figures 4.10, 4.12, 4.14). The lag phase before onset of paclitaxel elution was 45 days, some 15 days slower than for the 50:50 PLGA films. There was a lag phase in paclitaxel release from the 50:50 PLGA and 85:15/50:50 blend PLGA films of 30 and 45 days respectively. Onset of mass loss was after 30 days degradation and was slower in the blended films than in 50:50 PLGA films. Taking into account the delay in the onset of drug release and polymer

erosion of about 15 days in the PLGA films as compared with PLGA coated stents, a lag phase in paclitaxel release of a 1:1 blend of 85:15/50:50PLGA on a stent would be about 30 days in a comparable *in vivo* model as used in chapter 3. While this would represent a large reduction in the length of lag phase in paclitaxel release as compared to 85:15 PLGA it may still be too long for an effective formulation for a coronary stent application.

The overall ratio of GA:LA of 65:35 PLGA is similar to that in a 1:1 blend of 85:15/50:50 PLGA but it may be expected that in the blend polymer there may be areas of higher GA:LA ratio within the polymer matrix where 50:50 polymer chains are located resulting in faster degrading areas within the film. But there was no difference in the duration of the lag phase in paclitaxel elution and onset of polymer erosion in 85:15/50:50 PLGA films as compared with 65:35 PLGA films, possibly due to the miscibility of the two polymers resulting in good dispersion of the polymer chains within the film matrix with no areas with predominantly 50:50 PLGA chains.

Replacing the 50:50 PLGA (MWt=40,000) with a low molecular weight (LMWt) 50:50 PLGA (MWt=6,600) in the blend with 85:15 PLGA (85:15/LMWt 50:50 PLGA) resulted in faster onset of paclitaxel elution and polymer mass loss as compared with blended 85:15/50:50 films and 65:35 PLGA films (Figures 4.11 and 4.15). Onset of polymer mass loss and paclitaxel release was advanced by about 15 days in the 85:15/LMWt 50:50 PLGA blend films as compared with the 85:15/50:50 PLGA films. This would indicate that comparable early release rates of paclitaxel from a coronary stent coated with 50:50 PLGA could be achieved using a blend of 85:15/LMWt 50:50 PLGA. The formulation could be useful if a satisfactory duration of paclitaxel elution is also maintained.

Other studies have shown that drug release profiles from PLGA devices can be modified by blending LMWt PLGA polymers. The duration of the lag phase seen in the release of ganciclovir from 75:25 PLGA microspheres was reduced from 60 days to around 7 days by blending low molecular weight (8,000) 50:50 PLGA into the microspheres at a ratio of 1:1. But the duration of ganciclovir elution was reduced by a quarter to around 25 days. A blend of 3:1 75:25PLGA:LMWt 50:50 reduced duration

of the lag phase to approximately 15 days and maintained duration of release for around 65 days [39]. Addition of LMWt 50:50 PLGA to 75:25 PLGA microspheres increased the amount of peptide released in the initial burst phase in a dose dependant manner but had little effect on the duration of the subsequent lag phase [38].

Blending fast degrading LMWt PLGA into films of a tyrosine polyarylate (poly(DTH adipate) reduced the duration of the lag phase in the release of a water soluble peptide (integrilin) [18]. Further work would be required to determine if blending water soluble LMWt copolymers such as PEG or pluronics or LMWt 50:50 PLGA would alter the paclitaxel elution profile from TyRx to make it a suitable coating for coronary stent coatings. Thinner coatings and higher drug loadings would alter the drug release profile but further work would be required to determine if a satisfactory drug elution profile can be achieved by altering these parameters.

Blending LMWt 50:50 PLGA into 85:15 PLGA polymer coatings offers a promising method to fine tune paclitaxel elution from coronary stents. Further work would be required using polymer coated stents to determine the ratio of LMWt 50:50 PLGA required to achieve the desired elution profile. Alternatives to blending polymers would be to create a layer of fast eroding PLGA such as LMWt 50:50 PLGA to deliver a dose of paclitaxel in the early days following implantation, over a layer of slower degrading PLGA such as 85:15 PLGA containing paclitaxel to deliver the drug for the more extended periods that may be required. A similar mechanism is currently in use in the Cypher stent where an outer layer of polymer acts as a barrier to reduce the amount of sirolimus released in the early burst phase following implantation [32]. Kothwala *et al* [44] used layers of 50:50 PLGA and 75:25 PLGA and poly vinyl pyrrolidone to control release of paclitaxel from a coronary stent. In this system there was no lag phase in paclitaxel release but the initial amount of drug on the stent was high (200μg) which can effect release kinetics and further work would be needed to determine if the release profile is maintained at lower drug loadings. Further work would be required to determine the drug loadings and thickness of each layer for a stent comprising LMWt 50:50 PLGA and 85:15 PLGA required to obtain the desired release profile.

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Conclusions

PLGA and Tyrosine derived polyarylates, such as TyRx (P22-10) are biocompatible and biodegradable polymers that are suitable for use in biomedical applications and some drug delivery devices. Polymer coatings for a DES must provide good structural integrity throughout degradation, consistent and controlled drug delivery at the desired concentration, have good vascular compatibility, be non-thrombogenic and generate as benign a response from the host as possible or produce no more adverse effects as that from a BMS. Complete solubilisation of the polymer coating is also desirable to avoid any late thrombotic events that may be due in part to the presence of undissolved polymer on the stent. For DES a steady release of paclitaxel during the first 30 days following implantation and complete elution of the drug over a period of 30 to 90 days is probably required to prevent ISR over the long and short term.

The elution profile of paclitaxel from PLGA or TyRx stent coatings is dependant on the degradation of the polymer and comprises two phases: an initial diffusion controlled slow release phase (lag phase) as the molecular weight of the polymer coating is degraded by hydrolysis and a second faster phase associated with mass loss of the polymer and morphological changes in the stent coating with the appearance of cavities and air spaces creating a more porous structure within the stent coating.

Mass loss from tyrosine derived polyarylates occurs very slowly and only at the end of the degradation process due to low water solubility of the degraded monomers and the release of paclitaxel from such polymers will only occur when degradation is advanced and polymer erosion is occurring. The rates of degradation and mass loss for TyRx P22-10 were more rapid than that seen in other tyrosine containing polymers and after 30 days implantation the polymer had degraded by some 50% to approximately 25,000. But due to the low solubility of the degraded polymer chains mass loss was minimal until the polymer had degraded to a molecular weight of 4,000-5,000 after 150 days. Polymer dissolution was incomplete and the stent retained a coating of low molecular weight polymer but with areas of exposed bare

metal where the polymer had been eroded. As a result of the slow rate of polymer erosion, paclitaxel elution was slow with an extended lag phase of 240 days in which only approximately 3µg of the paclitaxel was released. Release of paclitaxel occurred over the 280 day study period but approximately 3µg of the drug was still retained in the polymer coating of the stent at the end of the study period.

There was no significant change in the pH of the interstitial fluid as a consequence of degradation and TyRx P22-10 is expected to have good tissue compatibility as has been seen in other tyrosine containing polyarylates. TyRx P22-10 exhibited good structural integrity but the presence of undissolved polymer on the stent over a time scale of 9 months reduced its suitability as a polymer coating for DES. Additionally since the release of paclitaxel was minimal over the first 30 days after implantation, the elution of paclitaxel may not be at a suitable dosage or duration to prevent ISR while the presence of paclitaxel in the stent coating in excess of 9 months may have unknown consequences. Further work on the polymer to enhance degradation, mass loss and drug elution would be required to address these issues.

Faster degradation rates and drug elution was seen using PLGA polymer coatings. The degradation of PLGA coated stents and drug elution profile of paclitaxel from PLGA coated stents can be controlled by varying the GA:LA ratio and by the coating thickness. Hydrolysis occurs more readily at the GA bonds and the increased hydrophilicity of PLGA polymers with higher GA moieties results in increased rates of hydrolysis and faster degradation of the polymer matrix. Release of paclitaxel is slow until the hydrolytic cleavage of the polymer chains has reduced the molecular weight of the monomers to a critical point and the onset of mass loss and polymer erosion occurs. Polymer erosion and mass loss occurs when the polymer chains have degraded to a molecular weight of around 12,000 resulting in the formation of pits and larger areas of erosion within and on the surface of the stent coating. As polymer erosion proceeds increasing amounts of pitting is evident with formation of larger cavities connecting the bulk of the polymer matrix to the surface of the coating resulting in faster diffusion of paclitaxel from the polymer matrix into the external medium.

Polymer degradation, erosion and visual signs of morphological deterioration occurred in all PLGA polymers investigated but occurred over differing time scales. Of the three PLGA polymers studied, 50:50 PLGA had the highest GA content and consequently degraded the fastest with the shortest time to onset of mass loss and lag phase in paclitaxel elution. 85:15 PLGA coatings degraded the slowest with the longest time to the onset of polymer mass loss resulting in the longest lag phase in paclitaxel elution. The integrity of the polymer during degradation was maintained in the PLGA coated stents with no obvious loss of larger polymer fragments or delamination and there were only minor, insignificant pH changes in the interstitial fluid. Complete solubilisation of 400μg coatings had occurred from the 50:50 and 75:25 PLGA coatings after 45 and 105 days of implantation respectively while only traces of polymer could be detected on the 85:15 PLGA coatings at 120 days which was the end of the study period.

None of the three PLGA blends studied with a coating of 400μg containing 20μg of paclitaxel produced a completely satisfactory release profile of the drug. 50:50 PLGA coatings have a shorter duration lag phase of around 15 days but complete elution of the drug had occurred by 30-45 days which may be an insufficient duration to effectively prevent ISR over longer time periods. No lag phase was observed for paclitaxel elution from the 200μg coating of 50:50 PLGA but complete elution of the drug had occurred by 30 days which may limit the ability of that formulation to prevent ISR over longer periods of time. Paclitaxel elution was over a longer period of time, 75 and 120 days respectively, from 75:25 and 85:15 PLGA coatings. However, the extended lag phase of paclitaxel release from the 75:25 and 85:15 coatings of 30 and 60 days respectively would be likely to severely limit their effectiveness since studies have indicated that a minimum amount of paclitaxel, possibly between10-20μg, needs to be released over the first 30 days of implantation to prevent ISR.

However the drug release properties of PLGA can be modified by blending PLGA with low molecular weight hydrophilic copolymers or with PLGA polymers with different GA:LA content or low molecular weight PLGA polymers. In this study the addition of a pluronic, F-127, had no effect on PLGA degradation and may have reduced paclitaxel elution. Addition of 8% w/w F-127 to 85:15 or 50:50 PLGA can

increase the hydration of polymer films. But any increase in hydrolysis as a result of faster hydration are nullified by reduced rates of autocatalysis within the matrix of the polymer due to hydrogen bonding between the carboxylic end groups of PLGA and the PPO segment of F-127 resulting in no overall effect on the degradation rate. Furthermore paclitaxel elution from PLGA films containing F-127 may be reduced due to a hydrophobic interaction between the drug, micelles of PPO and the polymer surface. Therefore inclusion of F-127 in PLGA polymers does not appear to be an effective method to reduce the lag or diffusion controlled release phase of paclitaxel.

Blending 50:50 PLGA into 85:15 PLGA matrices increases the overall GA content of the device thereby increasing the hydrophilicity of the device and introducing more sites in the polymer chain susceptible to hydrolytic cleavage. The onset of polymer erosion and the duration of the lag phase in release of paclitaxel from 85:15 PLGA matrices can be reduced by blending a 1:1 ratio of 50:50 PLGA with 85:15 PLGA. But the elution profile of the drug and rate of mass loss was no different to that for a 65:35 PLGA coating which has a similar ratio of GA:LA units. But the onset of polymer erosion was further advanced and the lag phase of paclitaxel release was shortened by using a low molecular weight 50:50 PLGA blended 1:1 with 85:15 PLGA. Blending low molecular weight 50:50 PLGA with 85:15 PLGA resulted in faster paclitaxel elution and polymer erosion than in 65:35 PLGA devices and produced a similar early release profile to that of 50:50 PLGA alone.

In conclusion, TyRx P22-10 and PLGA (50:50, 75:25 and 87:15) are polymers that have good biocompatibility and coating properties but their paclitaxel elution profile would limit their usefulness as coatings for DES. Blending low molecular weight 50:50 PLGA with 85:15 PLGA can be used to effectively modify the release of paclitaxel from PLGA devices but further work would be required to fine tune the ratio of polymer blends utilized in order to optimise the paclitaxel release profile for coronary stent applications.

GPC Chromatograms.

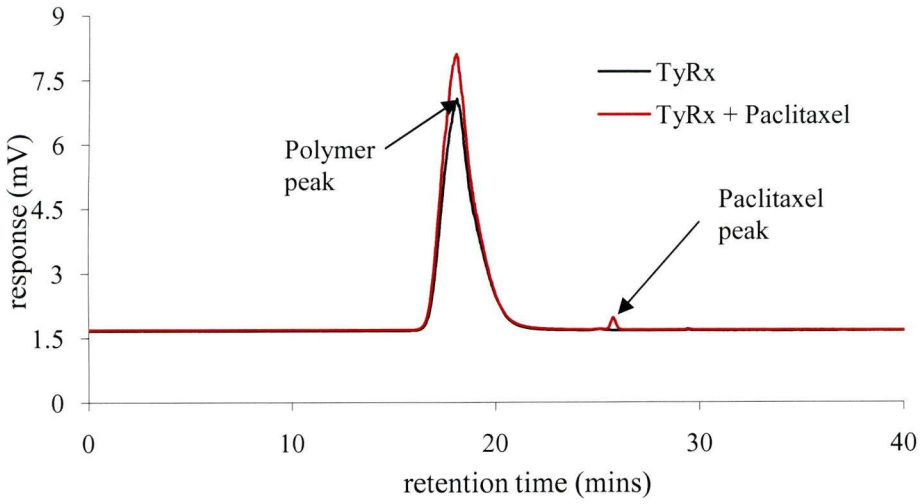

Figure A.1 GPC chromatograms for Control (not implanted) TyRx P22-10 coated stents with and without paclitaxel. Retention time is the time taken for the peak to be eluted from the columns. The response on the ELS detector is measured in millivolts. The TyRx + paclitaxel chromatogram shows the paclitaxel peak which is small relative to the polymer peak, but distinct from the baseline allowing measurement of the peak area and is distinct from the TyRx chromatogram

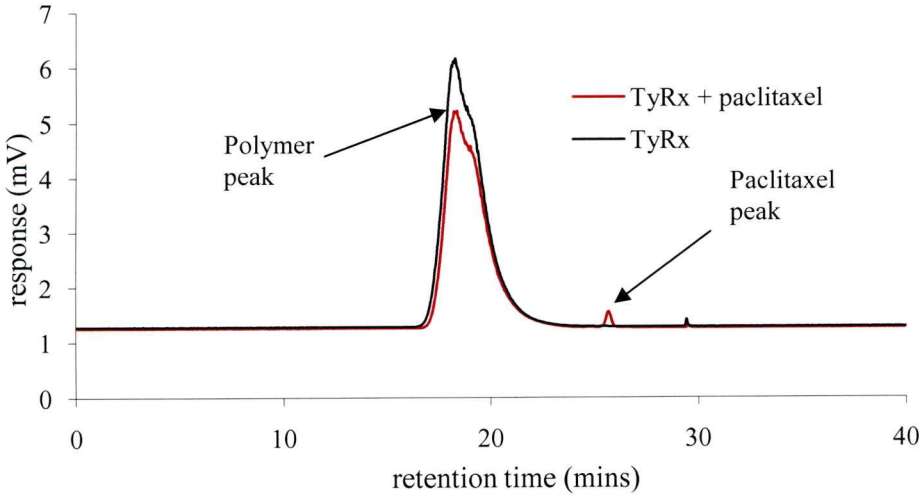

Figure A.2 GPC chromatograms from 14 day implanted TyRx P22-10 coated stents with and without paclitaxel. Axes as in Figure 18. The 'TyRx + paclitaxel' chromatogram shows that the paclitaxel peak is still visible at this time point and distinct to the TyRx alone chromatogram.

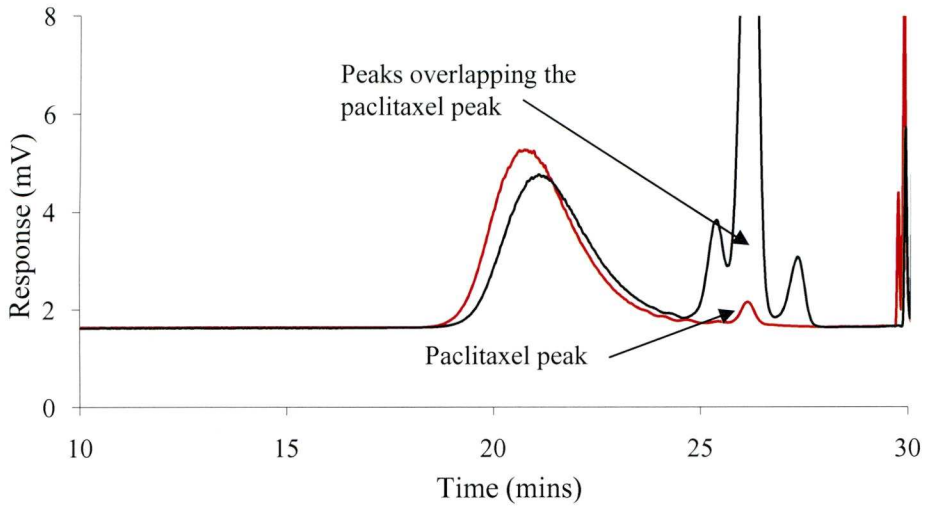

Figure A.3 GPC chromatograms from 60 day implanted TyRx P22-10 coated stents with paclitaxel. Axes as in Figure 18. The chamber seal from one stent remained intact (red line) gave a good plot for both the polymer and paclitaxel with no overlapping peaks and allowed for paclitaxel measurement. The black line shows the chromatogram from a stent where there was loss of chamber seal integrity allowing ingress of cellular elements and tissue onto the stents resulting in extra peaks on the chromatogram which overlapped and obscured the paclitaxel peak and paclitaxel could not be measured in this stent.

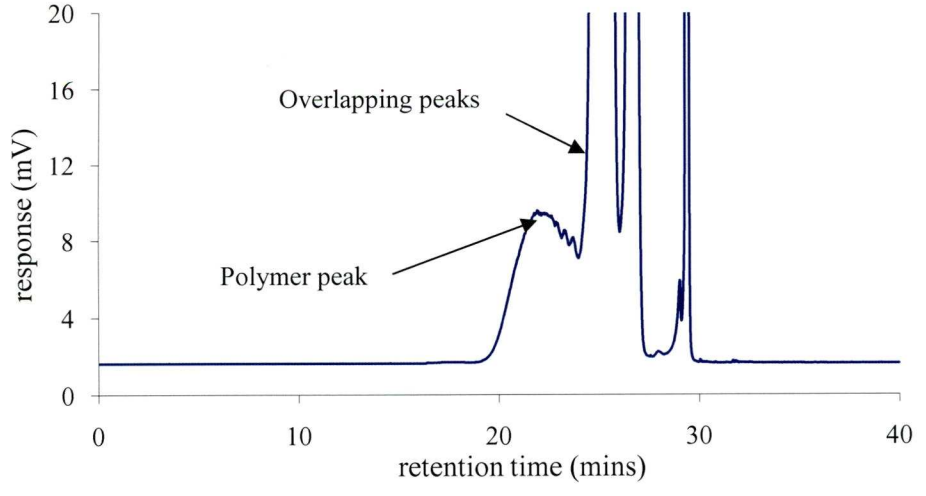

Figure A.4 GPC chromatograms from 90 day implanted TyRx P22-10 coated stents with paclitaxel. Axes as in Figure 18. Loss of integrity of the chamber seal allowed excessive ingress of cellular elements and tissue onto the stents resulting in extra peaks on the chromatogram which obscured the paclitaxel peak and overlapped with the tail of the polymer peak. In such a chromatogram neither paclitaxel nor polymer peak area could be measured.

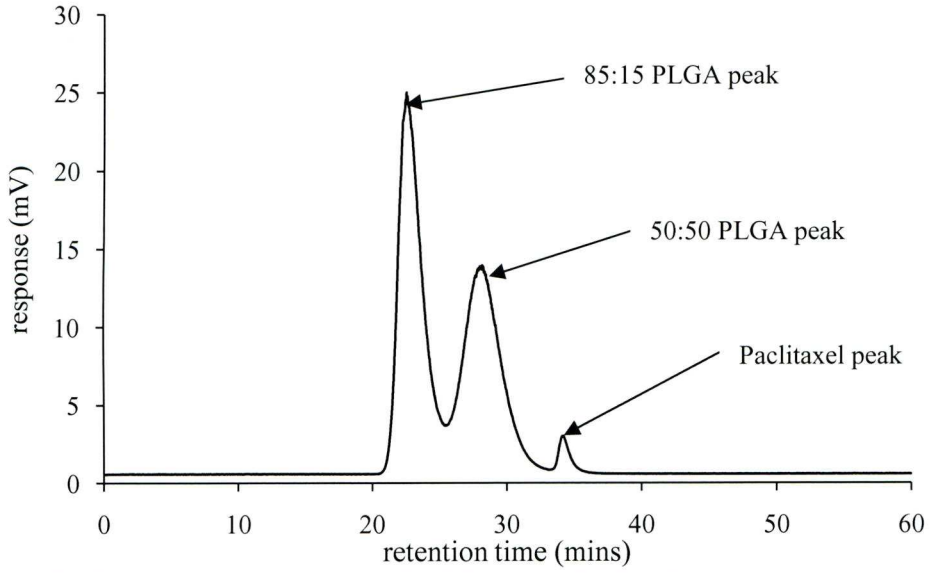

Figure A.5 Chromatogram from 85:15/LMWt 50:50 PLGA film after 14 days incubation in PBS. Data from the ELS detector. The distribution of molecular weights is Bi-modal with respect to the polymer and a smaller lower molecular weight peak for paclitaxel.

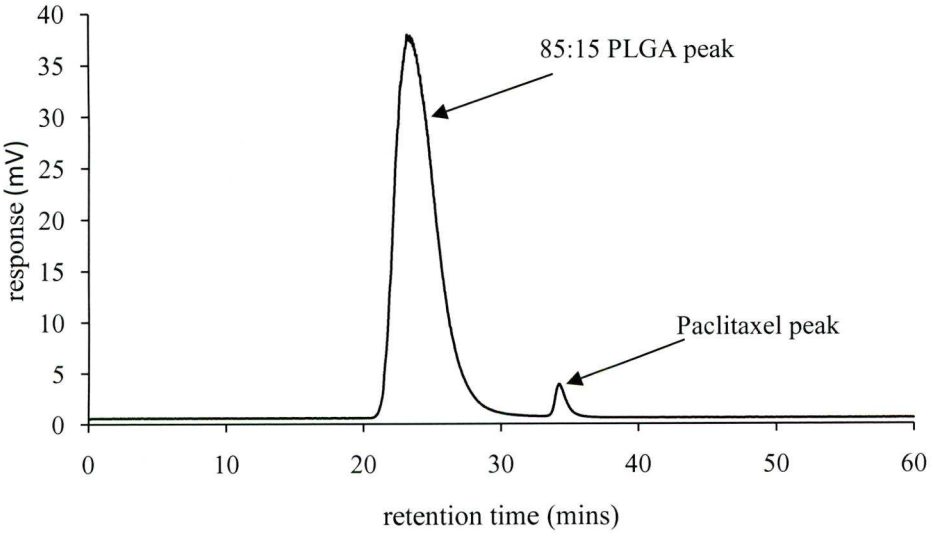

Figure A.6 Chromatogram from 85:15/ 50:50 PLGA film after 22 days incubation in PBS. Data from the ELS detector. The distribution of molecular weights is Uni-modal with respect to the polymer and a smaller lower molecular weight peak for paclitaxel.

	PLGA stent coating			
implant time (days)	**50:50 (400μg)**	**50:50 (200μg)**	**75:25 (400μg)**	**85:15 (400μg)**
7	0	0	3	3
15	3	4	3	3
30	3	4	3	3
45	4	5	4	4
60	4	5	4	4
75	4	5	6	0
90	4	5	6	6
105			6	6
120			6	6

Table A.1. The number of animals used for each time point in the degradation and drug elution study of PLGA coated coronary stents. Two chambers, each containing one stent were recovered from each animal at the designated time points.