"Developing Polypyrrole coated coronary stents for extended release of Sirolimus"

Niloofar Allahverdi (201256507)

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University of Strathclyde, Department of

Biomedical Engineering

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Declaration of Authenticity and Author's Rights

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ABSTRACT

Coronary heart disease remains the biggest killer worldwide. Amongst all the treatment options, drug-eluting stents (DES) are thought to be the most effective method of treatment for the majority of patients.

Despite the early success of DES in treating coronary artery disease, concerns and uncertainties were raised. The most critical limitations are poor drug release profiles, hypersenisvity reactions and delayed endothelium healing which can lead to fatal thrombotic events. Therefore there is a need to develop a more effective DES. The aim of this work was to develop a novel DES coating with an improved drug release profile.

This project investigated the potential use of conducting polymers for stent coatings in order to improve drug release profiles. Polypyrrole is a biocompatible conducting polymer with distinct chemical/physical properties. Sirolimus is a hydrophobic anti-proliferative drug which retains in the vessel wall to inhibit SMC proliferation. During this investigation polypyrole coated devices were created with the ability to release sirolimus over a therapeutically relevant period of time.

Electrochemical electropolymerisation technology was used to produce a series of poylyrrole coatings on stainless steel wires. Different voltages were used during the potentiostatic electropolymerisation process. Then the coated wires were immersed in different concentrations of sirolimus to determine the relationship between electropolymerisation voltage, drug concentrations and the drug release profiles generated. To achieve this, drug-polymer coated wires were immersed in a physiological release medium and samples were taken at various time points up to 28 days, and sirolimus release was analysed using UV spectroscopy.

It was found that there is a direct relationship between the concentration of the drug used in the coating solution and the drug release profile. Therefore by varying the concentration of sirolimus, an optimal drug release profile could be achieved. However

no direct relationship was found between the electropolymerisation voltage level and the sirolimus release profiles. This is a preliminary study and with further research it may be possible to modify drug release profiles by varying the voltage levels.

CONTENTS

“There are different wells within your heart.
Some fill with each good rain,
Others are far too deep for that.

In one well
You have just a few precious cups of water,
That "love" is literally something of yourself,
It can grow as slow as a diamond
If it is lost.”

Hafiz

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ABBERVIATIONS

ACE Angiotensin converting enzyme

CABG Coronary artery bypass graft

BMS Bare metal stents

CAD Coronary artery disease

DES Drug-eluting stent

DNA Deoxyribonucleic acid

FDA Food and drug administration

ISR In-stent restenosis

Ir Iridium

LST Late stent thrombosis

MHRA Medicine and healthcare product regulatory agency

MICAB Minimally invasive coronary artery bypass

NaSa Sodium Salicylate

P Phosphate

PEVA Polyethylene-co-vinyl acetate

PBMA Poly n-butyl methacrylate

Py pyrrole

Ppy Polypyrrole

PBS Phosphate buffered saline

PTCA Percutaneous transluminal coronary angioplasty

PCI Percutaneous coronary intervention

PDGF Platelet derived growth factor

PLLA Poly-l-lactic acid

PLGA Poly lactic-co-glycolic acid

Sr Strontium

SS Stainless steel

SEM Scanning electron microscope

SMC Smooth muscle cell

TXA_2 Thrombaxane A_2

VWF Von willebrand factor

W/V Weight by volume

CHAPTER ONE

LITERATURE REVIEW

1.1 Coronary Artery Disease

1.1.1 The heart and coronary arteries

The heart is one of the most important organs of the body along with the brain, lungs, liver and kidneys. They are given the term “vital organs”. The heart is a muscular organ responsible for pumping the blood throughout the body by repeated and rhythmic contraction.

Coronary arteries are hollow vessels distributed throughout the heart. Blood flows freely inside each vessel, thus providing the heart with a steady supply of oxygen and nutrients essential for normal function. The inner walls of the coronary arteries are lined with a single layer of endothelial cells, known as the endothelium (Fig 1.1). The middle layer of the artery, the media, is composed of smooth muscle cells and elastin. The adventitia is the outer most layer of the artery, consisting of fibroblasts and vaso vasora. Both the endothelium and adventitia play important roles in regulating the function of the arteries. They do this by the releases of a number of chemical signals in response to various stimuli (Click et al., 1989).

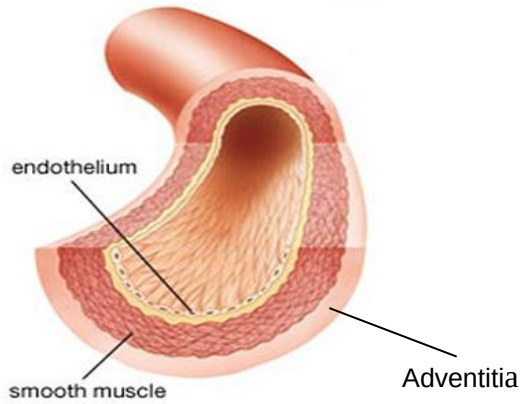

Figure 1.1 Illustration of a healthy coronary artery. The inner lining is made of endothelial cells forming the endothelium, the middle layer is made up of smooth muscle cells and the outer layer (adventitia) is composed of extracellular matrix, fibroblasts and vaso vasora (original picture available on (www.health.net).

1.1.2 Coronary Artery Disease

Coronary heart disease (CHD), also known as coronary artery disease (CAD) is the most common type of heart disease. CAD is the biggest killer in the United Kingdom causing around 82,000 deaths each year (NHS, 2012). In 2012, it was estimated that approximately 3 million people are living with the condition and it costs the UK economy around £9 billion per year in treatment costs and lost economic activity (British Heart Foundation, 2012).

CAD is the narrowing or blockage of the coronary arteries. This usually happens due to a build-up of fatty deposits, cholesterol, calcium and other substances. These materials, collectively referred to as plaques, build up on the wall of the coronary arteries which results in thickening of the artery wall, irritation of the endothelium and loss of elasticity of the vessel (MacMahon et al., 1990). The thickened wall of the artery restrict or blocks blood and oxygen flow to the heart muscles, main organs and other parts of the body (Figure 1.2).

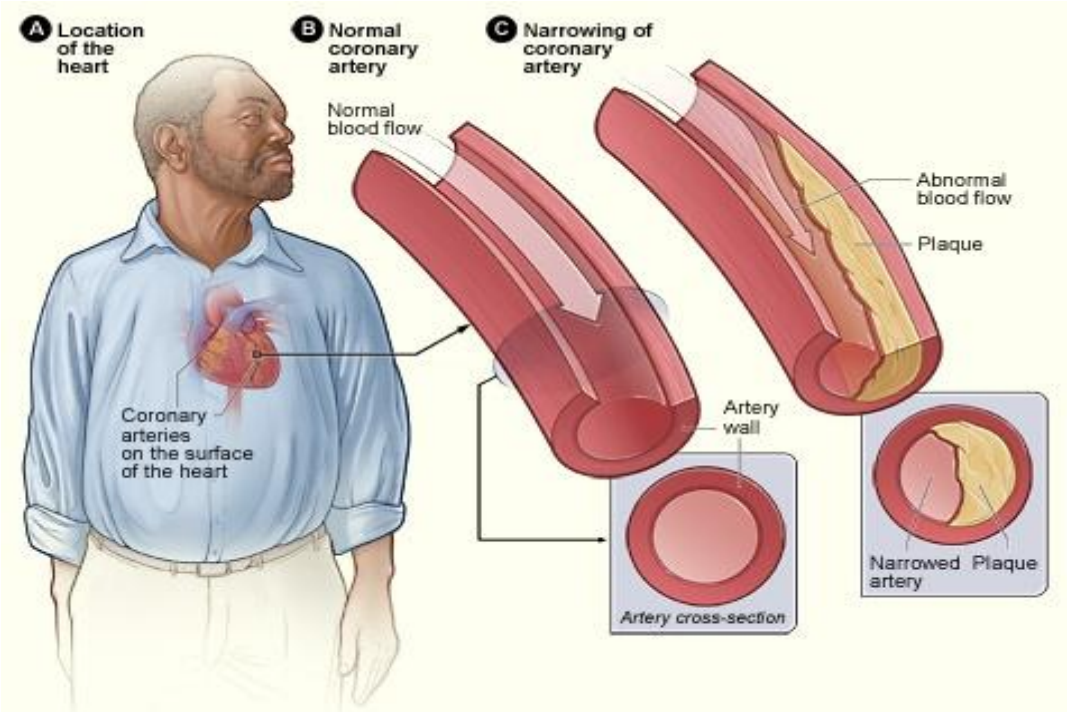

Figure 1.2 A- Shows the location of the heart in the body. B- Shows a normal coronary artery with normal blood flow and cross section of the normal artery. C- Shows a coronary artery narrowed by build-up of plaque with an abnormal blood flow. Cross section of the narrowed artery is also shown (Picture extracted, with permission from the National Heart, Lung and Blood institute website (as a part of the NIH and the U.S. Department of Health and Human Services).

1.1.3 Coronary Artery Disease Risk Factors

Certain behaviours and medical conditions increase the risk of CAD. These are usually referred to as “risk factors”. There are many risk factors that play a part in developing CAD, such as coronary vasospasm, high blood pressure, high cholesterol and LDL cholesterol levels, diabetes, and obesity (Wilson et al., 1998). All of these medical conditions put substantial pressure on all internal organs including the heart and will

increase the likelihood of developing CHD alone or in combination with other diseases (Wilson et al., 1998).

Lifestyle can also contribute to occurrence of the disease. The lifestyle factors that increase the risks are smoking, high alcohol consumption, lack of exercise and stress. It has been said that family history may also be a predictor of developing CHD (Wilson et al., 1998). Diabetes is one of the major risk factors in developing coronary artery disease (Grundy et al., 1999). However, the relationship between these two disease states is intensely complex and more research is needed to fully understand this aspect. Table 1.1 summarises some examples of medical and life style factors classed as CHD risk factors.

Atherosclerosis is a condition which is known by its characteristic of "clogging" or "hardening" of the arteries and is recognised as the most common, wide spread, well known and potentially the most serious cause of coronary artery disease. In atherosclerosis, the arteries become narrowed or blocked due to the build-up of plaques inside the artery. The build-up of the plaques alters the artery tone and function and reduces the lumen of the artery, hence restricting the flow of blood to the heart muscle. Atherosclerosis is the usual cause of angina, and myocardial infarction, as insufficient supply of blood results in oxygen deprivation to the heart muscles (Ross, 1999). There is always a risk of rupture of the atherosclerotic plaques. Such ruptures have two main consequences. Firstly, they cause the release of contents from within the plaque, which then get circulated in the blood stream and can block downstream vasculature, and within the brain this triggers a stroke (MacMahon et al., 1990). Secondly, the site of the rupture plaque serves as a stimulus to platelet adhesion and thrombus formation, completely blocking the artery. This blockage ultimately leads to a myocardial infarction. The mortality rate of atherosclerosis due to unstable and ruptured plaque is very high (Faxon et al., 2004).

Medical conditions	Risk factors
Obesity	Obesity can cause raised blood pressure and diabetes, which exerts extra pressure on the heart as well as other internal organs
Raised cholesterol levels	Raised levels of cholesterol in the blood results in a build-up of plaque inside the arteries which can lead to atherosclerosis and thrombosis (Brookes, 2007)
Raised blood pressure	Raised blood pressure results in increased pressure on the walls of the arteries, which can become stiff and lead to atherosclerosis
Diabetes	Diabetes puts substantial pressure on all internal organs, including the heart. Diabetes doubles the risks of CHD (Grundy et al., 1999)
Coronary vasospasm	Necrosis and ischemia in tissue as a result of blood vessel spasm which leads to vasoconstriction and an increased risk of CHD
Life style	**Risk factors**
Excessive smoking	People who smoke put their heart under extra pressure because of the toxins that they inhale (Wilson et al., 1998). These have independent effects on the functionality of the heart and can also increase blood pressure which is another

	risk factor for CHD. Smoking more than doubles the risk of a heart attack (Wilson et al., 1998, S. MacMahon et al 1990).
Poor diet	Risk of CHD is increased by consumption of saturated fats which increases cholesterol in the blood, in turn increasing the risk of atherosclerosis.
Lack of exercise	Lack of physical activity contributes to increase in weight which is a risk factor for CHD.
High alcohol consumption	Heavy drinking raises blood pressure and puts pressure on the heart. Binge drinking can increase the risk of CHD by 45% (MacMahon et al 1990)

Table 1.1- Medical conditions and life style choices and their associated risks in developing coronary artery disease (MacMahon et al 1990, Wilson et al., 1998, Grundy et al., 1999, Brookes, 2007)

1.1.4 Coronary Artery Disease Treatment

There are several treatment options available for treating coronary heart disease, each with specific advantages and disadvantages. They can be broadly divided into two main approaches: medical or surgical.

1.1.4.1 Medical Treatment

The first approach used is medical, which is a non-invasive, non-surgical treatment, using specific drugs available for treating or preventing the underlying causes of CAD.

There are many treatment medications available such as Angiotensin-converting enzyme (ACE) inhibitors, Calcium channel blockers, Thiazide diuretics, Statins, and Antiplatelet drugs. These drugs are used to prevent the development and progression of heart disease in certain high risk groups and also used for managing the symptoms of those patients with coronary heart disease, to slow its progression and try to prevent heart failure and myocardial infarction developing. Examples include the use of ACE inhibitors for lowering blood pressure or statins for reducing cholesterol levels (Fogoros, 2011). The decision to prescribe a particular medication treatment for patients is dependent on a variety of factors, such as severity of condition, location of stenosis and the presence of other co-morbidities. Education on the topics of control of diabetes, diet, exercise, smoking cessation, along with the medical therapy can help the management of symptoms, including reducing the occurrence and severity of periods of angina (Wilson et al.,1998).

1.1.4.2 Surgical Treatment

The second approach for treating an occlusive coronary artery is surgery, so called invasive therapy. In severe cases of multi-vessel disease and in patients with co-morbidities such as diabetes, surgery is usually the preferred surgical method of treatment. The standard procedure is called Coronary Artery Bypass Grafting (CABG), commonly referred to as bypass surgery, which involves bypassing the blockage in the coronary artery with a blood vessel taken from elsewhere in the patient's body. The donor vessel is grafted to the coronary arteries to bypass the narrowed section of the existing coronary artery. The aim of this procedure is to bypass the blockage, thus restoring the blood flow (Fitzgibbon et al., 1996). The first successful CABG was performed in the late 1960s by an American surgeon Robert Goetz and his team (Fayalora, 1998). Since then the surgical techniques have developed considerably. New techniques such as Minimally Invasive Coronary Artery Bypass (MICAB) have been developed which can be beneficial in patients with occluded arteries (Acuff et al.,1996).

In comparison with CABG, this procedure is done through a limited number of small incisions to access the coronary arteries, rather than open chest heart surgery, and allows the heart to continue to beat during the surgery. It is therefore said to have a reduced chance of complications such as infection, bleeding during and after the surgery and the procedure is shorter with a quicker recovery period and less pain (Sivasubramanian, 1998).

While MICAB is easier for the patient there are substantial disadvantages compared with the traditional CABG. It is only possible for patients with one or two occluded arteries unlike CABG where more arteries can be operated on. Operating on a beating heart makes the performance of the anastomosis more difficult and there is a significant risk of ischemia during the procedure (Dijk et al., 2001). MICAB comprises 10% of total heart surgeries and long term follow up is required (Dijk et al., 2001).

Coronary artery bypass grafting (CABG) intervention is a major surgery that can significantly improve the patient's symptoms and lowers the chance of heart attack in 90% of cases (Eagle et al., 1999). However it is important to realise that CABG is not the cure for CAD itself, it merely alleviates the symptoms and reduces the risks of subsequent myocardial infarction. In addition, in 5-10% of the patients the bypassed artery fails within one year and in 40% of patients, angina recurs after 10 years (Eagle et al., 1999). Repeat bypass surgery may be necessary in these patients but the success rate can often be less than in the first surgery.

Coronary angioplasty, also called Percutaneous Transluminal Coronary Angioplasty (PTCA) or Percutaneous Coronary Intervention (PCI) is another procedure aiming to reduce stenosis produced by atherosclerosis within the arteries (Fogoros, 2011). Angioplasty was first described in 1964 by the interventional radiologist Charles Dotter who received the Nobel Prize in medicine in 1978 (Josef et al., 2003). Following in Dotters footsteps, Coronary angioplasty was pioneered in 1977 and the first successful

procedure was performed in September 1977 by Andreas Gruentzig (Josef et al., 2003). During the procedure a long, thin, hollow, flexible catheter, with a deflated balloon attached to it, is delivered to the site of vessel narrowing through the skin (percutaneous), by puncturing the femoral artery in the groin or brachial artery in the arm. The catheter is passed through the arterial circulation to the diseased coronary artery within the heart using a guide wire delivered under x-ray aided guidance. The balloon is then inflated, compressing the plaque that is causing the blockage, thus expanding the vessel and restoring the lumen of the vessel for a better flow. Furthermore, the balloon is deflated and removed. This is called Balloon angioplasty. This technique for restoring the lumen size is effective in short term, however in the longer term (within 6 months after balloon angioplasty) complications are found to occur in 30-40% of the patients (Shedden et al., 2009). The most common complication after this procedure is restenosis. Restenosis is the remodelling of the artery, which results in renarrowing of the luminal diameter. This can occur immediately or up to several months after the procedure, which may lead to acute or delayed restenosis of the artery and a return of symptoms and a consequent risk of cardiac emergencies (Fogoros, 2011).

An approach for decreasing restenosis after coronary angioplasty is implantation of coronary stents. Coronary stenting is now the most common medical interventions now used to reopen an occluded artery (Butany et al., 2005). It has revolutionised current coronary artery disease treatment. Stents are tiny metal mesh tubes, commonly made of cobalt chromium or stainless steel, which are crimped onto a balloon at the end of a delivery catheter. Once inside the occluded artery, the balloon and the stent expand, compressing the plaque, resulting in a wider lumen and a restored blood flow. After deployment, the balloon catheter is extracted from the vessel, leaving the stent in place. The permanent placement of the stent in the artery gives mechanical and structural support to the wall of the stenosed artery and reduces restenosis (Shedden et al., 2009).

The first successful stent implantation was performed by Ulrich Sigwart in1987 (Sigwart et al., 1987). Bare-metal stents (BMS) were used for the procedure and showed promising results by significantly reducing revascularisations in the target vessel compared to balloon angioplasty. However, long term success of this procedure remains limited as in around 30% of the patients in-stent restenosis (ISR) occurs (Shedden et al., 2009).

1.2 Challenges of implanting coronary stents

1.2.1 In-stent restenosis (ISR)

ISR is re-narrowing of the stented artery due to inflammation, smooth muscle cell proliferation and extracellular matrix deposition, resulting in neointimal hyperplasia which leads to a return of the CAD symptoms. The development of neointima can be viewed as a distortion in the healing response to stent implantation (Shedden et al., 2009).

During stent implantation the internal layer of the vessel (endothelium) is removed, the internal elastic lamina is fractured and the tunica media is damaged.
The damage to the artery triggers the healing mechanism where it can be viewed as a cascade of inflammatory events consisting of platelets adhesion, release of cytokines and growth factors which stimulates the smooth muscle cells in the tunica media to proliferate and migrate into the lumen in an attempt to repair the damage. Consequently neointimal, or “new intima”, tissue is formed which continues to grow leading to narrowing of the artery, reducing the blood flow. The first event in the cascade is stent thrombosis. The activation and generation of thrombus as a result of increased platelet aggregation due to lack of the endothelium can also lead to the stent occlusion by blood clots (Shedden et al., 2009). Figure 1.3 is a schematic illustration of events involved in development of ISR. While stent thrombosis can be controlled by prescribing anti-platelet therapies, ISR remained a significant problem with BMS procedures.

The development of ISR is not a one event mechanism but a cascade of events and several predictive factors are involved. The next section gives a detailed explanation of these events and treatments currently available.

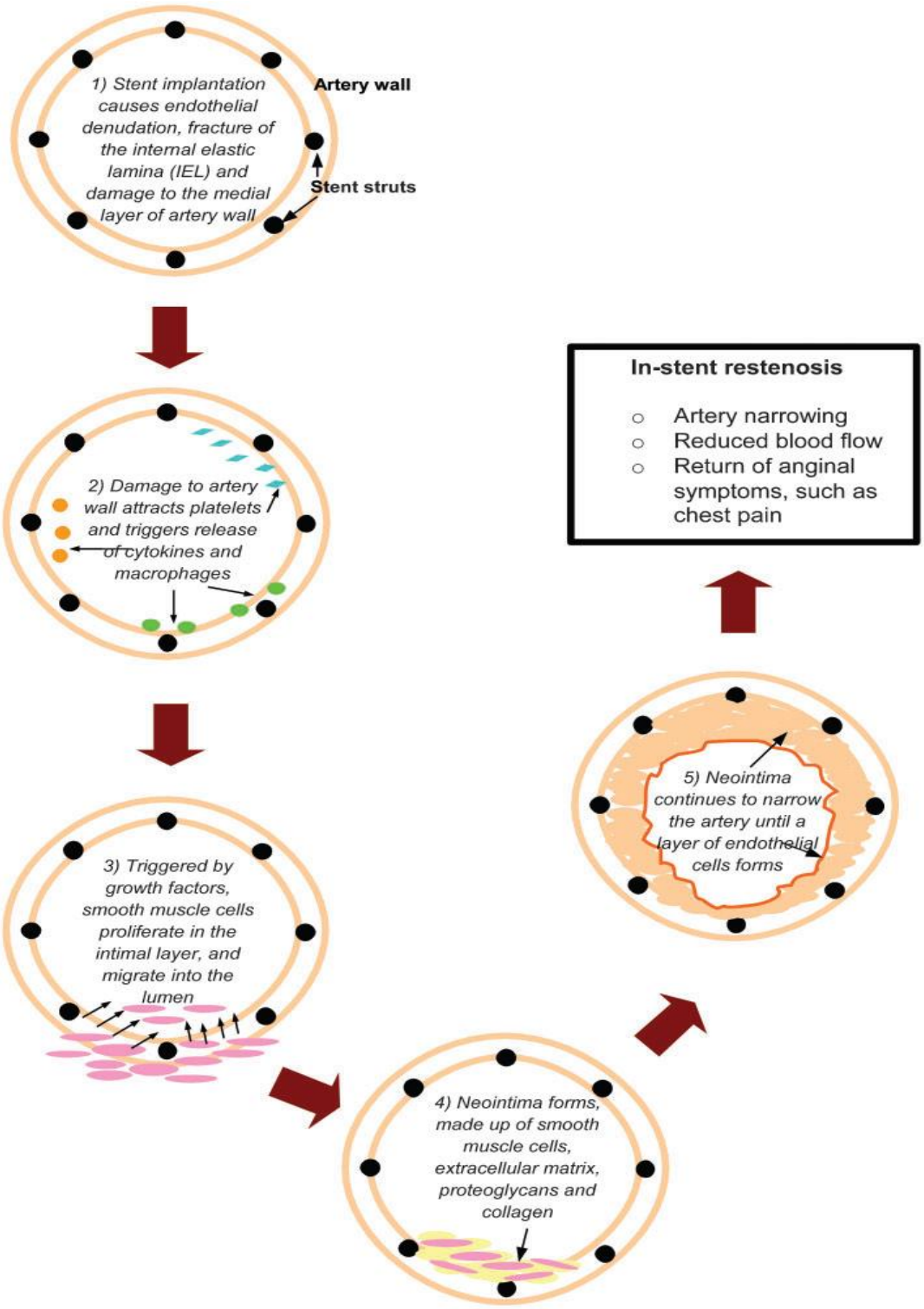

Figure 1.3 Stages of development of in-stent restenosis (Picture extracted, with permission from Sheddon et al., 2009)

1.2.2 Mechanisms involved in development of in-stent restenosis

1.2.2.1 Platelet response

Platelets play a substantial role in the vascular response to injury. Platelets are a small percentage (less than 0.1%) of the formed elements of the blood. They are flattened disks released from megakaryocytes in to the circulation and circulate for 9-12 days before being removed by phagocytes. The rate of platelet formation is dependent on factors such as thrombopoietin, thrombocyte-stimulating factors and interleukin-6 (Kottke-Marchant, 2009).

The function of platelets is very important following any injuries. They are involved in transporting chemicals such as growth factors (thromboxane A_2, ADP, ca^{2+} and platelet derived growth factors (PDGF)), which can directly lead to a temporary clot in walls of damaged blood vessels and a consequent reduction in the blood loss.

Despite the important function of platelet adhesion and aggregation after injuries, the platelet response after stent implantation has long been recognised as an early event leading to in-stent restenosis, occurring within the first 24 hours (Kottke-Marchant, 2009). Platelets exist in a non-activated state. Following a vascular injury, they are recruited at the site of injury and activation is triggered via the release of thrombin following initiation of the coagulation cascade. They adhere to the exposed von Willebrand factor (vWF) and are then engaged with receptors for proteins such as collagen and glycoproteinVI, leading to platelet activation and aggregation (Kottke-Marchant, 2009).

Subsequently, intracellular cell signalling and platelet activation results in adhesion to fibrinogen receptor GPIIb/IIIa leading to thrombus formation. A number of experimental and clinical studies have shown that thrombus formation occurs in the following 3-4 days after stent deployment (Kottke-Marchant, 2009). Platelet activation together with thrombus formation is directly involved in smooth muscle cell proliferation and therefore neointima formation and in-stent restenosis.

1.2.2.2 Smooth Muscle cell proliferation and migration

In addition to platelet aggregation and inflammatory cell infiltration, in-stent restenosis is also characterised by smooth muscle cell proliferation and migration. Following stenting there is an increased proliferative response and neointima formation as a repair mechanism in an attempt to remodel the vascular wall. Vascular smooth muscle progenitor cells are present in the bone marrow, in the circulation, in the vessel wall and in the extravascular sites (Marx et al., 2011). They normally exist as quiescent smooth muscle cells maintained in a non-proliferative phase. After vessel injury following stenting, non-proliferative smooth muscle cells are activated to proliferate and migrate as a result of increased release of various regulatory factors such as growth factors, cytokines, interleukin-1 and interleukin-6.

Destruction of endothelium also plays a part in smooth muscle cell proliferation as it leads to reduced levels of endothelium-derived inhibitory factors including nitric oxide and heparin sulphate proteoglycans which are necessary mediators for maintaining vascular homeostasis and smooth muscle cell quiescence. During normal conditions, the vascular smooth muscle cells are non-migratory and they remain in their quiescent form. There are various antimigratory molecules. Examples are biogenic amines, peptide growth factors and extracellular matrix components (Marx et al., 2011). After vessel injury, vascular smooth muscle cell migration is linked with alteration in adhesiveness of smooth muscle cells to the matrix and activation of various signal transduction pathways. Mechanical factors such as blood flow, sheer stress, and matrix stiffness can also affect the migration of vascular smooth muscle cells. One study suggests that specific mitogens, including basic fibroblast growth factor (bFGF), released from dead cells, stimulate smooth muscle cell proliferation (Lindner et al., 1991). This hypothesis is still not yet fully understood.

Generally, the combined release of the regulatory factors and reduction of endothelial inhibitory factors stimulates smooth muscle cell proliferation and migration with consequent neointima formation migrating into the lumen leading to ISR.

1.2.2.3 Predictive factors of in-stent restenosis

The underlying mechanism for development of ISR is not completely understood at present. Meanwhile, many predictive factors exist that correlate to the initiation and progression of thrombus formation and ultimately restenosis.
Risk factors for ISR were reviewed by Kuntz et al., 1992 which indicated that co-morbidities, such as diabetes mellitus and renal diseases increase the risk of development of ISR and stent thrombosis. Age has also been perceived as a predictor factor by this review. It has shown that there is an increased restenosis rate in elderly patients over 75 years old (Kuntz et al., 1992).

Luminal diameter and lesion size are also important predictor factors in development of ISR. Angiographic observations have shown a correlation between the luminal diameter immediately after the procedure and the rate of ISR after six months (Kuntz et al., 1992). Small vessels with minimal luminal diameter and long lesion length have been associated with increased incidence of ISR.

1.2.3 Strategies to overcome of in-stent restenosis

Considerable research has been aimed to address this problem. Extensive research has been undertaken to inhibit neointima formation using a variety of pharmacological therapies ranging from anti-platelets, anti-thrombotic, anti-inflammatries to ACE inhibitors and anti-proliferative drugs. Despite showing beneficial effects in animals,

they have failed to demonstrate beneficial results in humans. This failure to replicate the positive animal trial data is generally thought to be due to the fact that the high doses delivered systemically could not be safely used in humans (Scarpioni et al., 2005). Consequently, research has focused on development of direct local drug delivery to the target lesion via drug-eluting stents (DES), which can achieve high local concentrations with minimal systemic toxicity.

There is a wide range of research studies concentrating on strategies to overcome ISR by inhibiting the processes detailed above. A brief overview of novel strategies (mechanical and pharmacological) for treatment and prevention of ISR is outlined below.

1.2.3.1 Conventional drugs

ISR is a multifactorial process, hence a large number of conventional drugs have been researched for preventing ISR, each with specific mechanism(s) regulating one or many of the processes outlined in section 1.2.

A large variety of pharmacological therapies have been attempted to reduce neointima formation and also smooth muscle cell proliferation and migration which principally comprise ISR. These range from anti-platelets, anti-thrombotics, anti-neoplastics, lipid lowering statins, anti-inflammatory to ACE inhibitors, migration inhibitors and anti-proliferative drugs (Lowe et al., 2002). The table below contains examples of each group of drugs.

Anti-neoplastics	Anti-proliferative	Migration inhibitors	Enhanced healing factors	Anti-platelets
Sirolimus	Taxol (paclitaxel)	Batimistat	BCP671	Cilostazol
Tacrolimus	Actinomycin	ProlylHydrosylase inhibitors	VEGF	Asprin
Everolimus	Methotraxate	Halofunginone	Estradiols	Ticlopidine
Leflunomide	Angiopeptin	C-preteinase inhibitors	EPC antibodies	Clopidogrel
Dexamethasone	Vincristine	Statins		

Table 1.2 Examples of conventional drugs used to reduce neointima formation and smooth muscle cell proliferation in order to reduce the occurrence of ISR (Htay et al.,2005).

Despite promising results of pre-clinical studies, using systemic pharmacological approaches to prevent ISR has many therapeutic limitations and disappointing clinical results were obtained. Examples of adverse effects from these drugs include delayed re-endothelialisation caused by anti-proliferative drugs, increased chance of internal bleeding and restrictions on any other surgical procedures due to the presence of anti-platelet drugs in the circulation (Scarpioni et al., 2005). However, a number of studies demonstrated the benefits of pharmacological agents on reduction of ISR and therefore research continues on evaluation of potentially beneficial systemic therapies (Scarpioni et al., 2005).

1.2.3.2 Radiation therapy

Radiation therapy, also known as brachytherapy, is a proposed therapy which involves using intravascular γ and β–radiation to inhibit restenosis following percutaneous coronary intervention. Research by Lowe et al., 2002 demonstrated that intravascular radiation targets cellular deoxyribonucleic acid (DNA) and actively disturbs its differentiation thereby halting intimal and medial cells division.

The two main radiation sources tested for brachytherapy are γ and β–radiation. The source of γ radiation is ^{132}Iridium (Ir) generating photons capable of penetrating beyond the vessel wall. β radiation sources used include ^{32}Phosphorus (P) and ^{90}Strontium (Sr) which generate energy in form of electrons with a limited penetration. Since there is an increased activity of smooth muscle cell proliferation during restenosis, this approach has been assessed for inhibiting proliferation of smooth muscle cells.

Brachytherapy initially showed reductions in restenosis rates and the Food and Drug Administration (FDA) approved two intravascular radiation sources but a number of concerns remain. Most of the clinical trials have been conducted on pigs, therefore optimal dosage for human use in not known. There is also evidence of tissue necrosis, delayed vessel healing and late thrombosis which means that, despite the early benefits of using intravascular brachyotherary for ISR, it is no longer a preferred technique since the long term benefits and associated risk remains unclear (Lowe et al., 2002).

1.3 Drug-eluting stents (DES)

Drug-eluting stents (DES) were introduced in 2002. These stents are coated with bioactive drugs (anti proliferative drugs most commonly) which are gradually released over periods of several weeks. These devices therefore provide local, site-specific, controlled drug release that can inhibit smooth muscle cell proliferation and neointima

formation and therefore prevent the need for subsequent intervention (Puranik et al, 2012, Khan et al, 2012).

DESs are classified as a medical device by the Medicine and Healthcare products Regulatory Agency (MHRA) and were approved by Food and Drug Administration (FDA) after a large trial in 2003. They are a combination product made of three individually important components:

- Stent platform – the metal mesh strut
- Stent Coating – polymer coatings and surface modifications have been used to control drug release rates
- Drug - antiplatelet, anticoagulant, anti-inflammatory, which can be entrapped directly on the stent strut or embedded in the biocompatible polymer.

The finished product is called a Drug Eluting stent (DES), developed to provide local, site-specific, sustained release of the drug to inhibit neointima formation in the artery.

Desirable stent characteristics
High flexibility
Host biocompatibility
Low metallic surface area
Good trackability
Thromboresistant
Radio-opaque , radiographic properties
Reliably expandable
High radial strength
Hydrodynamic compatible

Table 1.3 General and desirable characteristics of a stent (Petal et al, 2012, Butany et al, 2005)

The intense work on stent development has successfully led to the introduction of drug eluting stents (DES). The two drug eluting stents which became the recognised market leading first generation stents were the Cypher stent (Cordis Corp) and Taxus stents (Boston Scientific).

Cypher stents are coated with two non-degradable polymers polyethylene-co-vinyl acetate (PEVA) and poly n-butyl methacrylate (PBMA) and sirolimus (also known as Rapamycin). Sirolimus' primary mechanism of action is believed to be its inhibitory effects on smooth muscle cell proliferation (Mark, 2004). Meanwhile, Taxus stents are covered with Translute polymer, poly(styrene-b-isobutylene-b-styrene), which is similar to the PEVA/PBMA copolymer used in Cypher stents (Mark, 2004). Taxus stents are coated with another pharmacological agent called Paclitaxel. Paclitaxel is a broad-spectrum antimitotic agent that inhibits cell division, leading to inhibition of proliferation of smooth muscle cell (Khan et al, 2012).

In order to improve DES, many clinical trials and ex- vivo experiments have been carried out on different types of bioactive, antipoliferative agents. It has been proved that hydrophobic agents (usually taxus-based) have better drug release profiles compared to hydrophilic agents (usually heparin-based) facilitating a more effective drug delivery and distribution (Vairo et al, 2010). Coating the stents with first generation drugs (Sirolimus and Paclitaxel) produced effective results in inhibiting neointima formation compared to BMS (Lüscher et al., 2007).

Although the early successes in the treatment of coronary artery disease with drug-eluting stents resulted in rapid growth in their use, late clinical adverse effects such as late stent thrombosis (LST) were also reported in a small numbers of patients (Lüscher et al., 2007). Given the high fatality rate associated with stent thrombosis, this raised serious concerns and uncertainties about the overall safety of DES. The precise causes of LST are incompletely understood at present. However, studies have suggested that

there is impairment in endothelial regeneration, delayed vessel healing and incomplete endothelialisation, which together may lead to LST (Fujii et al., 2010).Thus, the need for development of the next generation of DES was obvious. The main aim for development of new devices was to achieve a device that was compatible for use in wider range of patients to inhibit neointima formation, without the adverse effects on vessel healing outlined above.

The second generation DES largely incorporated sirolimus analogues, which it is argued had more favourable biochemical and physicochemical characteristics. Table 1.4 below contains some examples of sirolimus analogues and their related DES clinical trials.

Sirolimus derivatives	**Corporation**	**Clinical trials**
Sirolimus	Cordis	Ravel/Sirius
Tacrolimus	Guidant	Jomed
Everolimus	Medtronic/Abbott	Future I/II/III
Zotarolimus	Medtronic	Endeavor

Table 1.4 Examples of sirolimus derivatives and their related corporation, drug eluting stents clinical trials and date of FDA approval (Htay et al., 2005).

Everolimus, an immunosuppressive agent that is closely related to sirolimus, was introduced in 2008 by Abbot Inc in the XCIENCE V stent. Two polymers, an acrylic polymer and a fluoro polymer (Sheiban et al., 2008), are used to control the release of Everolimus from this stent. This was followed by introduction of the Endeavour Zotarolimus stents by Medtronic Inc, which is coated with phosphorylcholine polymer and a newer version of Endeavour stents with a revised design, Endeavour RESOLUTE^{TM}, comprising a new biocompatible polymer called BioLinx™. This polymer is made of three different polymers, a hydrophobic C10 polymer, a hydrophilic C19 polymer, and a hydrophilic polyvinyl-pyrrolidinone. The improved design of the Endeavour Resolute is thought to enhance polymer biocompatibility. There is an

overall reduction in the risk of delayed healing and late stent thrombosis (Tada et al., 2013). Endeavour Resolute compared to the other stents has an improved drug release profile in the vessel, such that ~50% of the zotarolimus is released within the first week, with the remaining drug released beyond 31 days (D. Capodanno et al., 2011). Clinical trials on XCIENCE V and Endeavor zotarolimus were carried out and they were subsequently associated with lower thrombosis rates than first generation DES (Khan et al, 2012). Both stents employ advanced coating technologies and new stent platform designs with varying rates of drug elution, depending on the presence or absence of additional polymer coatings on the stent.

The second generation DES have already demonstrated impressive safety results at medium term follow up, but the specific improvements over CABG are not yet clear for high risk patient groups. In comparison to CABG in high risk patients, patients with diabetes and multi-vessel disease, even the newer stents perform poorly (Serruys et al., 2009). They have also not completely eliminated LST and patients still require prolonged antiplatelet therapy. Therefore there is on-going research on the development of improved drugs and coating technologies for possible use in next generation DES, with the aim to inhibit in-stent restenosis in a wider range of lesion types, across a wider patient group, whilst lowering the risk of late-stent thrombosis.

DES have been referred to as “An effective tool to analyse drug dynamics and to assess dominant effects” (Vairo et al, 2010). Newly introduced DES are still evolving. Despite the great advantages and low adverse effects compared to bare metal stents there are still weaknesses in existing DES. The following section of this research will examine in detail, areas in stent design which require further innovation and improvement.

1.3.1 Challenges with Drug Eluting Stents

1.3.1.1 Delayed healing of endothelium

The layer of endothelial cells that lines the inside of the blood vessels make up the endothelium. The endothelium has many important functions. It is a selectively permeable membrane which controls the movement of molecules and cells in and out of the bloodstream. It is a key regulator of the inflammatory response. It participates in resolution of the inflammatory response which is required for successful tissue healing and regeneration. The endothelium layer provides a non- thrombogenic surface and therefore prevents blood clotting. It is also involved in control of vasoconstriction and vasodilatation of the vessel which helps control blood pressure (Lerman et al., 2005). Following the catheterisation procedure to implant the stent, vascular and endothelial irritation and damage can be observed, which to some extent correlates with an enhanced inflammatory response and to increased neointima formation (Shedden et al., 2009). Re-endothelialisation (growth of new endothelium) is critical after stent implantation to resolve the inflammatory responses, as it may help limit neointima growth and is essential for protecting against stent thrombosis (Shedden et al., 2009). Meta-analyses have indicated that implantation of DES is associated with delayed endothelium healing and hence increased risk of in-stent restenosis (ISR) (Ertas et al., 2009).The presence of the stent's metal matrix and a nonerodable polymer in the coronary artery after its useful function has been served causes persistent arterial wall inflammation, and delayed vascular healing which lead to thrombosis and delayed SR (Beusekom et al, 2010).

1.3.1.2 Hypersensivity responses

Hypersensivity responses to the stent, drug or the coating polymer can occer in patient with implanted bare metal stents or DES. This phenomenon has been explained as a series of inflammatory defence mechanism in response to implantation of a stent in the coronary artery. Hypersensitivity reactions have been associated with ISR and death. The symptoms including rash, itching, chest pain, fever began within 1 day of stent implantation or after a period of up to two weeks (Nebeker Et al., 2006). Research has suggested that DES can cause systemic hypersensivity reactions which may lead to late thrombosis, cardiac arrest and death. Based on the FDA's Manufacturer and User Device Experience Centre (MAUDE), hypersensivity to DES has been classified as a serious complication after stent implantation (Kounis et al., 2007).

1.3.1.3 Sub-optimal drug release profiles

One crucial aspect of optimising DES design is generating an improved drug release profile. This has been looked in to from various aspects but the main challenge still remaining is production of stents coated with the bioactive drugs capable of local delivery at low, constant rate over the desired period of time. There are many research studies and clinical trials taking place to evaluate methods of coating stents with the associated drugs. The most promising of these are described in section 1.3.2, although the extent of research in this area means that a comprehensive description of each developing technology is beyond the scope of this document. However, the reader is referred to an excellent review by A.S. Puranik et al, 2012 for further information.

Another outstanding challenge with existing DES is the fact that they are not suitable for all artery types. As each individual's anatomy is different and the underlying cause

of CAD varies, although in general, little consideration of such differences is factored into DES selection for individual patients.

1.3.2 Latest Developments in Stent Platforms

Since the pharmacological approach to prevent in-stent restenosis has failed in early stages of clinical trials, current research concentrates on development of direct local drug delivery at the site of injury via DES.

Recently, the clinical performance of DES has been associated with the stent design, composition and deployment methods. A diverse number of stents with different designs has been developed in the past decade. It is beyond the scope of this research to provide detail on each development, however, a brief discussion on the critical areas of stent design which has shown improvement in recent developments is included.

Ideal stent characteristics were mentioned previously in table 1.3. Sufficient radial strength is required to hold the vessel open and to prevent vascular recoil, meanwhile, the stent needs to be flexible enough to be delivered percutaneously through vasculature into vessels that are small and can be of complex geometry. Various stents with different geometries have been developed. Meshwire or coil stents were the traditional stents with high flexibility but low radial strength which were largely replaced by slotted tube stents with improved radial strength. More recent stent designs are modular, sinusoidal designs, which are favourable to slotted tube with maximised radial strength and flexibility (Shedden et al., 2009).

Collectively, strategies for an optimal stent generally aim to develop stent platforms with new materials and state-of-the-art designs to deliver outstanding performances. The new technologies used in improving stent designs concentrate on developing the thinnest, smallest stent struts with all the desirable characteristics (table 1.3) using super

alloys such as cobalt chromium (Nevo Stents), magnesium alloy (Biotronik Stent), platinum chromium (Promus Element Stents) each incorporating a first or a second generation drug to serve as a functional DES (Abizaid et al., 2010).

Flexibility and strength of stents comes from the structure design and the material used to make the coronary stent. Mechanical properties of stents have been examined by using different materials. The most commonly used stent material for many years was 316L stainless steel. It offers good mechanical properties and it is corrosion resistant but the downsides of using stainless steel stents are the poor visibility during angiography, and there is the possibility of stent migration at MRI field strengths higher than 3-Tesla (Levine et al., 2007). It may also cause allergic reactions to alloy components (nickel in particular) which increase the immune response leading to in-stent restenosis (Shedden et al., 2009).

Having discovered the disadvantages of using 316L stainless steel as stent material, cobalt-chromium alloy emerged with improved radial strength. In addition, it was radiopaque and MRI compatible (Hagemeister et al., 2005). Medtronics stent platform is an alloy of cobalt- chromium. Similarly Multi-Link stent by Guidant Corp is made of cobalt-chromium and received Food and Drugs Administration approval in United States in July 2003. Cobalt-chromium alloy is now the most commonly used stent material at present.

Extensive research is taking place on other potentially useful materials, such as Nitinol which has shown shape memory and self-expanding characteristics, Magnesium alloy which is biodegradable and platinum for improved deliverability, radial strength and radiopacity (Lévesque et al., 2004).

Strut thickness is a critical factor in stent structure design. It has been shown that stent strut thickness correlates with the degree of vascular injury (Lawe et al., 2002). Thicker struts provide more support but with increased damage to the vessel and related neointima formation. Thinner struts have been used which are less damaging to the

artery wall, with increased flexibility and deliverability and therefore have less chance of development of ISR.

Material science research has indicated materials such as cobalt-chromium and platinum provide ultra-thin stent struts without compromising vascular support (Lévesque et al., 2004).

Another aspect of stent design is the surface characteristics of the coatings, which is an important parameter determining the vessel reaction to the presence of the stent. Properties such as texture and electrical charge are recognised as predictors of thrombus formation and are widely studied.

1.3.3 Drug Release Profiles from Drug-eluting Stents

The drug release profile of drug eluting stents is a critical factor in the success of inhibition of neointima formation and ISR. Hence many research studies have been conducted in an attempt to achieve a desired release profile. A variety of coating technologies have been investigated to control the rate of drug release from the drug-eluting stents.

There are different methods of coating stents with bioactive drugs. Binding the drug to the coronary stent surface is broadly classified into three main types: Polymer-free coating (on-strut), chemical binding and polymer coating.

1.3.4 On-strut binding

On-strut is when drugs can be linked or entrapped directly onto metallic surfaces of the stent and released by diffusion or dissolution. In this approach the drug can be loaded on to the stent by dipping or spraying the bioactive agents on the stents surface or it can be loaded in stent reservoirs. There are several novel and promising approaches for development of polymer-free stent coatings, including employing microporous structures with increased total surface area for drug loading, nanotechnology such as coating the stent with nanomaterials or modifying the stent surface in nanoscales in order to improve the adhesion characteristics. These new perceptions may have the potential to improve stent coatings in the near future (Xiaodong etal., 2012).

1.3.5 Chemical binding

Chemical binding is another approach incorporating the presence of a specific binding site of action where the drug binds to the stent strut via reversible chemical reactions. This method of stent coating is highly dependent on the chemical composition of the drug resulting in a prolonged drug release into the vascular tissue (Borghi et al., 2008). Despite the improvements in the mentioned stent coating methods, there are still many on-going research studies taking place on finding the most efficient coating strategy to produce an optimal drug release profile from DES. The most recently developed and the most favoured method is polymer coating. Consequently, the following discussion concentrates on polymer coating of DES for controlled drug release profiles.

1.3.6 Polymer based local drug delivery

Polymer coating involves embedding the bioactive agents within a biocompatible polymer for a prolonged controlled drug release. The coating polymer often acts as a "biologically inert barrier" to ensure drug retention and elution (Petal et al, 2012).

There are numerous polymers used in DES coatings for durable drug release profiles. The polymers are classed into different categories in relation to their mechanism of drug release.

1.3.6.1 Non-degradable polymers

Non-degradable polymers are a class of polymers frequently used for coating DES. The mechanism of drug release from non-erodible polymers is via diffusion through the matrix, where the rate of drug release is viewed as the function of polymer porosity (Ivan et al., 1995). These stents design are utilizing new polymer technologies, antiproliferative drugs and metal stent platforms to improve clinical outcomes and safety. The various polymers used can be categorized by their mechanism of drug release.

There are various non-erodible polymers that have shown potentially beneficial characteristics for stent coating including silicone, polyurethane, ethylene-vinyl acetate copolymers and other copolymers (Ivan et al., 1995). The commercially available DES based on a non-erodible polymers are Taxus stents which release paclitaxel via the copolymer poly(styrene-B-isobutylene-B-styrene) and Cypher stents coated with two copolymers polyethylene-co-vinyl acetate and poly-n-butyl methylacylate (Ivan et al., 1995). The specific drug is mixed with polymers and applied to the stainless steel 316L stents for the controlled prolonged drug release. It is important to note, in both erodible

and non-erodible polymers, the hydrophilic or lipophilic nature of the polymers can determine the release profile achieved (Fischell,1996).

1.3.6.2 Biodegradable polymers

Hypersensivity reactions to the polymer coated stents occur occasionally in patients following DES implantation or after a prolonged exposure (Morse et al., 2006). As mentioned in section 1.2.6 hypersensivity reactions can lead to ISR if untreated. Hence, a considerable amount of research has been conducted on biodegradable polymers to optimise factors such as the chemistry of the polymer, degradation and degradation time and also the physical, biological and environmental factors (Garg et al, 2010). There are many biodegradable polymers but amongst all the biomaterials and polymers, the most widely use are PLLA (Poly-l-lactic acid) and PLGA (Poly lactic-co-glycolic acid) (Ivan et al., 1995). The stainless steel matrix of the stent is coated with these polymers in order to enhance the release pharmacokinetics of drugs from the stent in the target area over a desired period of time to reduce neointima formation. In degradable polymers, the drug released is affected by degradation of the polymer matrix, hence the rate of drug release can be modified by the polymer degradation rate. PLGA is a suitable polymer for the release of paclitaxel and PLLA has been used to release paclitaxel or sirolimus (Makadia et al, 2011). They are highly biocompatible, biodegradable and toxicologically safe, exhibiting sustained drug delivery and with favourable overall performance and degradation characteristics (Makadia et al, 2011).

Commercially available stents coated with biodegradable polymers are Nobori® stents, manufactured by Terumo International. The abluminal side of the stent is coated with a biodegradable polymer and releases biolimus A9 drug. Biomime is another example, manufactured by Meril Life sciences which release sirolimus from its biodegradable polymer biopoly^{TM} (PLGA + PLLA) (Smith et al., 2013). They have shown

considerable improvement in luminal diameter and reduction in in-stent restenosis for treatment of coronary artery lesions (Danzi et al.,2012).

Biodegradable stents have become the focus of recent interest because theoretically they offered support to the artery during the period of highest risk of recoil and ISR, without the long term presence of the stent. There are several undergoing concurrent clinical trials but there are many challenges remaining for these new stents including biocompatibility, degradation time, choice of material and many more (Smith et al., 2013). There are considerable doubts about the pharmacokinetics of the chemicals released from degradation of the stent which can be associated with significant inflammatory reactions leading to ISR, which may therefore cancel out the main beneficial aims of developing such bioabsorbable stents. Therefore there is a need for large scale clinical trials with long term follow up to determine whether the concept of biodegradable polymer stents is safe.

1.3.6.3 Polymer free stent

One step further from original DES with polymers are non-polymeric DES. They are completely polymer free. Current clinical studies on polymer free stents are limited but the benefits reported so far includes, improved healing with a possibility of shortened duration of dual antiplatelet therapy. As there is no polymer present, the risk of hypersensivity and inflammatory reactions are thought to be reduced (Garg et al, 2010). To serve the main aim of a DES, the polymer free stent needs to be able to elute the anti-proliferative drugs in a controlled manner. There are various surface modification techniques available to achieve optimal drug release including embedding anti-proliferative agents into non-polymeric biodegradable carriers on the stents surface, impregnating the anti-proliferative drugs onto microporous surface of the stent. The long-term outcome using this method of drug delivery is still unknown.

1.3.6.4 Next generation of DES coatings

There are a number of coating methods being evaluated at present for potential use in the next generation DES. Examples of new methods are a pro-healing approach which has shown some interesting results by enhancing vessel healing and reducing ISR. Examples include the Genous stent, a bare metal stent coated with anti-CD34 antibody. This stent captures endothelial progenitor cells and promotes rapid re-endothelialisation (Yang et al., 2012). It is too early to decide whether stents with the absence of anti-proliferative agents coating will be beneficial in the long term compared to DES currently in use. Also, Biotronik MgO_2 stent and Abbott absorb polymer stent have the ability to degrade completely after they have served their function (Moravej et al.,2011). Most studies undertaken so far are on animals and therefore, there is limited data available on these stents but they may have benefits in long term health of the vessel.

1.4 Conducting polymers

1.4.1 Conducting polymers for coronary stent coating

Considering all of the disadvantages of stent coatings mentioned in the previous section, there is an obvious need for an improved coating strategy to achieve a heamo- and tissue compatible drug-eluting stent. Many researchers have been focusing on creating a uniformly coated stent by a “simple, reproducible, one step coating process” (Okner et al., 2007).

Conducting polymers (CP) were discovered in the late seventies (1977) by Alan Heeger and co-workers (Shirakawa et al., 1977). Since then this class of polymers have received special attention in many applications, especially in biomedical applications.

Recently an interest has been raised in using conductive polymers for developing stent coatings in order to prevent restenosis, by incorporating the drug directly bound to the monomer precursor (Okner et al, 2007) or by encapsulation in a carrier during electropolymerisation (Arbizzani et al., 2007). The appealing characteristics of conducting polymers for stent coatings are their stability, conductivity, ease of synthesis and processing.

In the past few years conducting polymers have been recognised as a suitable candidate for various biomedical applications such as developing biosensors, optoelectronic devices, electrocatalysts and indeed stent coatings (Guimard et al, 2007). Examples of representative conducting polymers are poly (p-phenylene vinylene), polythiophene, polypyrrole, and polyaniline. They are polyconjugated polymers with conductive, magnetic and mechanical properties typical of metals and hence are also called synthetic metals. These are highly conductive polymers when they are doped with a small concentration of a dopant ion, having conductivity ranging from 1 to 105 S cm^{-1}, which is similar to metals and semiconductors (Vernitskaya, Efimov, 1997). The process of doping can be performed in two ways, by chemical or electrochemical means. During chemical doping large quantities of the monomer are mixed with an oxidising agent, usually $FeCl_3$. In contrast, electrochemical synthesis is the most widely used method as it is a simpler technique with better control over the thickness and geometry of the material and also the extensive availability of dopants (Ateh et al., 2006). Electrochemical synthesis is achieved by the means of electrochemical oxidation or reduction, which produces positive or negative polymeric chains, respectively (Vernitskaya, Efimov, 1997).

1.4.2 Polypyrrole

The most interesting characteristics of polypyrrole as a conducting polymer includes its increased stability, ease of synthesis through electrochemical polymerisation in aqueous

medium, good conductivity and its distinct redox properties (Vernitskaya, Efimov, 1997). Another acknowledged property of polypyrrole is its biocompatibility which is a critical parameter for its use in biomedical applications (Arbizzani et al., 2007). Collectively, all these criteria influence the polymer-drug interaction which is an important factor in any drug delivery system (Okner et al., 2007). With all the mentioned desired characteristics, polypyrrole has a great opportunity to be used as DES polymer coating.

Among conducting polymers, polypyrrole is the most studied due to its unique chemical and physical properties and morphology, but only a limited number of studies have investigated the potential use of polypyrrole for stent coatings (Arbizzani et al., 2007, Okner et al., 2007, Svirskis et al., 2011, Sirivisoot et al., 2011). In the study conducted by Arbizzani et al, platinum wires were coated with polypyrrole and sodium salicylate through potentiostatic electropolymerisation.

During the electropolymerisation of pyrrole, additives such as sodium salicylate and tirons are added which have shown improvements in the polymer synthesis. Using sodium salicylate as an additive, produced a strong adherent polypyrrole film on the stainless steel surface (Shi et al., 2010). It has been said that sodium salicylate organises the ions on the materials surface to achieve a complex which prevents the dissolution of metallic substrates. However this reorganisation does not inhibit pyrrole electropolymerisation (Shi et al., 2010).

In the study by Okner et al, another electropolymerisation method, known as cyclic voltammetry was used (Okner et al., 2007). In those studies which have examined polypyrrole as potential stent coatings, a number of limitations were present. Arbizzani et al measured the release of salicylate from coated platinum wires with polypyrrole, which does not correlate with the stents which are commonly made of stainless steel or other metal alloys. The experiment was performed at room temperature, which does not mimic the body temperature. There was no comparison with other coating methods made in order to find out the method with the best results (Arbizzani at al., 2007). The

same limitations were reported in the Okner et al study. There have been no studies attempting to coat sirolimus and measure its release from conducting polymer coatings. Considering the existing good results with sirolimus on Cypher stents, it would be worthwhile to investigate the positive effects of conducing polymers as stent coatings to improve on current DES release profiles.

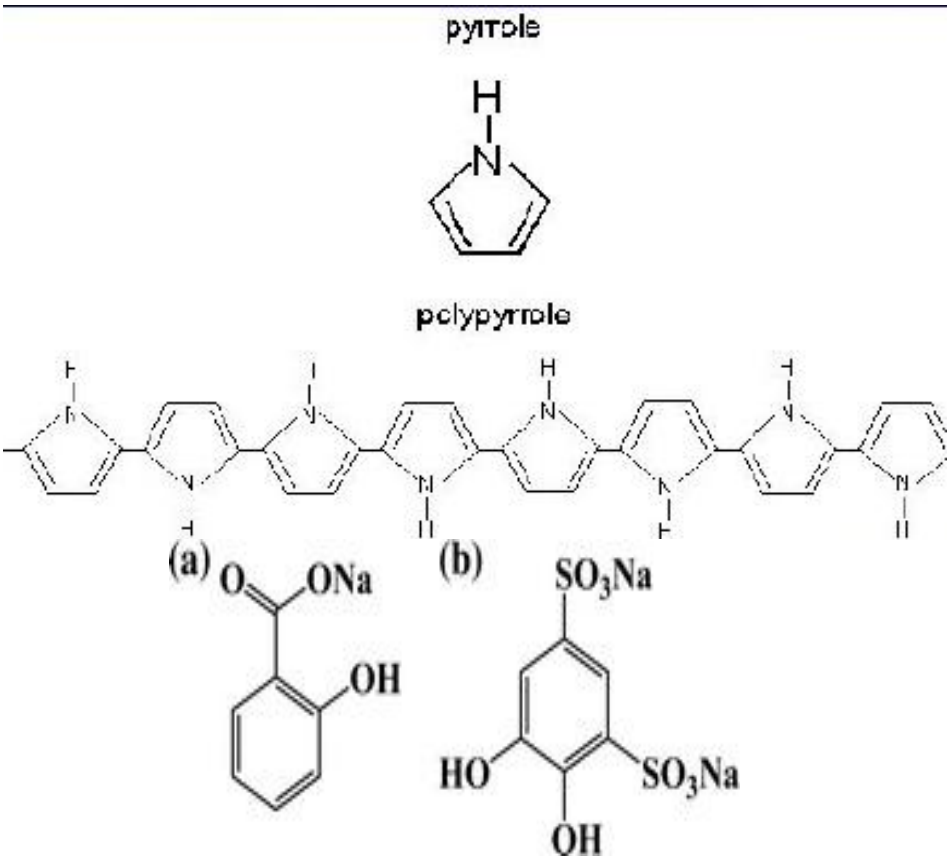

Figure 1.4 Chemical Structure of pyrrole monomer and the polypyrrole chain (picture extracted from Singh et al., 2010) a- sodium salicylate and b- Tiron (Shi et al., 2010). Pictures were extracted from the articles with author's permission.

1.5 Project hypothesis and experimental aim

The introduction of DES revolutionised treatment of coronary artery disease and led to significant improvements in reduction of in stent restenosis.

However, several limitations are present for the use of current DES. The most critical limitations are poor drug release profiles, hyperesensivity reactions and delayed endothelium healing which can lead to fatal thrombotic events. Within this context, the overall aim of this study was to develop a novel drug-eluting stent coating with an improved drug release profile using conducting polymers.

There are few studies which have investigated the potential use of conducting polymers for this application (Arbizzani et al., 2007, Okner et al., 2007). Reviewing the following studies, it was concluded that conducting polymers with their distinct chemical/physical characteristics are suitable candidates for stent coating. Therefore this project aimed to investigate the possibility of coating the stents with the conducting polymer, polypyrrole, through electropolymerisation, to produce a coating with the ability to release sirolimus over a therapeutically relevant time period. Stainless steel wires were coated with polypyrrole in order to mimic the stainless steel stent strut. To achieve the proposed aim of the project the following specific steps were taken:

- An investigation of the effects on the characteristics of drug release of varying the voltages used during electropolymerisation of polypyrrole coatings.
- An investigation of the effects of sirolimus coating concentration on drug uptake, and drug release.

The study hypothesised that changing the concentration of sirolimus within the coating solution would affect the drug release profile. Another hypothesis was that varying voltage during polymer coating would impact the drug release profile. The assumptions were that higher concentrations of sirolimus on a stent coated with higher voltage would

lead to increased drug release. The following chapter will detail the experimental procedures conducted to achieve the ultimate aim of this project.

CHAPTER TWO
METHODOLOGY

2.1 Introduction

Following from the previous chapter, which highlighted some limitations in the current research work in this field, it is clear that improvements are required in DES technologies. Focusing on the potentially sub-optimal drug release profiles from existing DES, one of the main objectives of this project included investigating conducting polymers for use as stent coatings to improve the release of specific drugs from drug-eluting stents. As mentioned before, a limited number of studies have investigated the effectiveness of conducting polymers in stent coatings and drug release profiles (Okner et al., 2007, Arbizzanni et al., 2007, Svirskis et al., 2011, Sirivisoot et al., 2011). Whilst demonstrating the potential of conducting polymers, these studies also highlighted various unresolved problems and further investigation is therefore required.

In the first part of this chapter the process of electrochemical polymerisation and the method employed to measure drug release are explained in detail. This provides essential background information for understanding the second part of this chapter, which covers the specific methodology, materials and equipment used to achieve the proposed aims of the project.

2.2 Electrochemical polymerisation

Conducting polymers such as polypyrrole are attractive candidates for this project for their ease of synthesis, flexibility in processing and biocompatibility (refer to section 1.4). Conducting polymers are synthesised either by chemical or electrochemical reactions. Each method of synthesis has its advantages and disadvantages. Chemical synthesis is via chemical reactions using a chemical oxidant. This method is classified as condensation polymerisation and is achieved by the loss of small molecules such as water from the monomer or additional polymerisation, involving activation of the

radical cation $C_4H_4NH^+$, which attacks the carbon backbone of unoxidised pyrrole molecules, resulting in formation of dimeric radicals $(C_4H_4NH)_2^+$ (Guimard et al., 2007). Chemical synthesis of conducting polymers allows the synthesis of various conducting polymers in large scale productions, which is a key advantage over electrochemical synthesis. But there are down sides in using chemical synthesis, such as the complexity of the process leading to limited control over immobilisation which can denature entrapped proteins. In addition, rapid release and thin film coatings cannot be achieved (Guimard et al., 2007).

Electrochemical polymerisation is a simple method of producing conducting polymers which is commonly used. This method of synthesis provides polymer on a conducting surface via oxidative coupling of the monomer (Okner et al., 2007). There are several advantages of electropolymerisation, including formation of thin film coatings with the ability to entrap molecules in the conducting polymer. One of the most significant advantages of electrochemical polymerisation compared to chemical synthesis is the ease of synthesis and the relatively simple procedure used in the process. This makes it the most appealing method for synthesising conducting polymers for certain biomedical coating applications, such as those examined in the present study (Guimard et al., 2007).

2.2.1 Justification and modification to achieve optimised coating

To achieve a homogeneous polymer layer, optimal magnitude and duration of the applied charge during the electropolymerisation is required. Obtaining these parameters is one of the challenges of this process. In the present study, several trial experiments had to be carried out during a period of preliminary laboratory work, in order to find out the required voltage and time taken to achieve a uniform coating on the stainless steel wires. In previously published work, parametric studies have shown that there is a proportional relationship between the magnitude and duration of applied charged and

the extent of polymer formation and deposition of the polymer coating. Increasing the applied charge will enhance the deposition of the polymer on the surface (Ba-Shammakh et al., 2002). There is a minimum potential required for the polymerisation to occur which gives a very thin, smooth and uniform polymer coating on the surface and below this potential no synthesis can occur (Boyle et al, 1990) whereas at higher potentials, a thicker, smooth but less regular surface coating is achieved. At below minimum or above maximum optimal experimental parameters, there is loss of mechanical and chemical properties of the polymer (Boyle et al, 1990, French etal., 2001). At higher voltage, there is a decrease in the time needed for the polymer deposition on the surface (Ba-Shammakh et al., 2002).

Currently, electrochemical polymerisation of conducting polymers is most commonly performed using a three electrode configuration, consisting of a working electrode, a counter electrode and a reference electrode immersed in a solution of the monomer, appropriate solvent and specific dopant ions (figure 2.1) (Guimard et al., 2007).

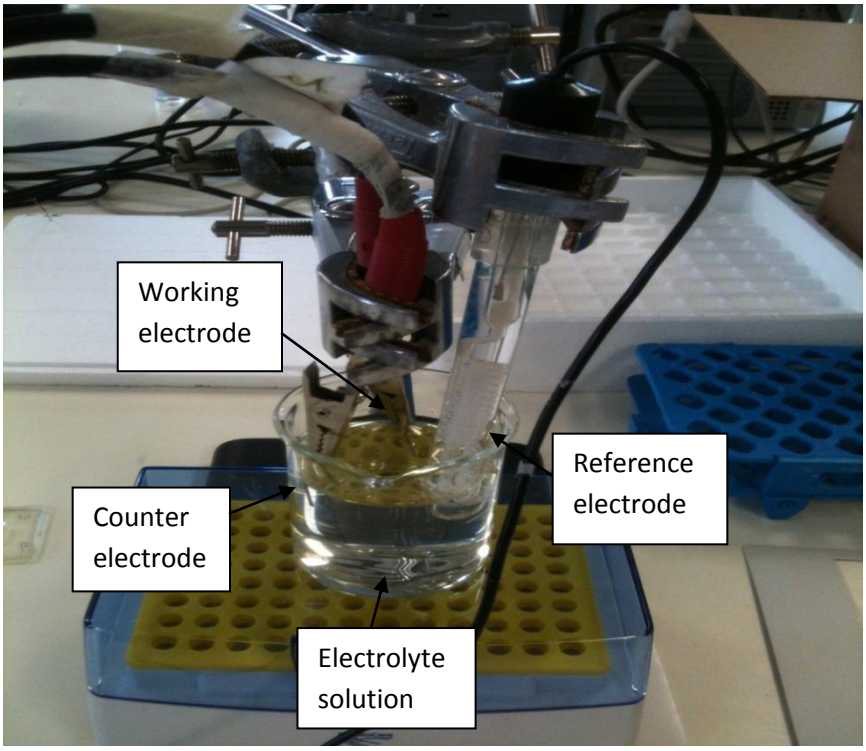

Figure 2.1 Three electrode configuration used for electrochemical polymerisation of conducting polymer. Showing the three electrodes (working, counter and reference) immersed in electrolyte solution contained in the beaker.

In the current project procedure, the counter electrode was a platinum wire, a stainless steel wire (which represents stent strut) was used as the working electrode, where the polymer deposition occurs, and KR5 glass electrode acted as the reference electrode. All the electrodes were connected to a potentiostat/galvanostat which provided the necessary voltage for the deposition of the polymer to occur. The current runs through the electrolyte solution and the working electrode become positively charged (anode) which leads to electrodeposition of the polymer on the working electrode surface. The electrodeposition occurs due to oxidation of the monomers on the surface of the working electrode forming cations that can react with other cations or monomers in the electrolyte solution (Guimard et al., 2007).

The figure 2.2 illustrates the different stages of electrochemical polymerisation of the polypyrrole monomer, which involves oxidation of the monomer at the working electrodes to produce cations that can react with other cations/monomers for polymer deposition.

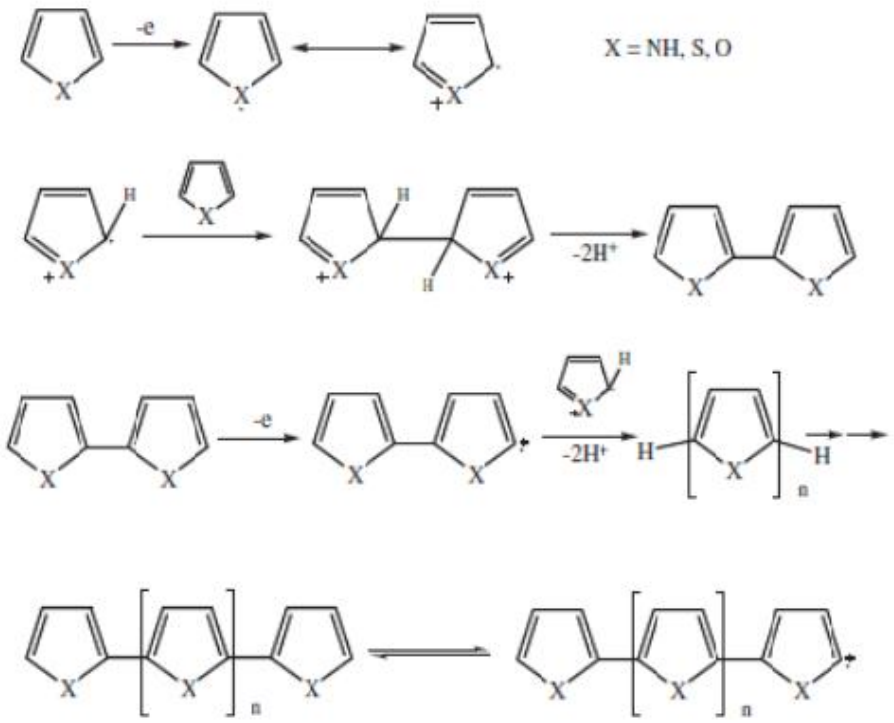

Figure 2.2 Electrochemical polymerisation of polypyrrole (Guimard et al., 2007)

The potentiostat/galvanostat can perform electrochemical polymerisation through different methods. Examples are potentiostatic, where a constant, time dependent potential is transferred via an internal voltage scanner which controls the current through the counter electrode to maintain a potential difference between the working electrode and reference electrode. Another method used is potentiodynamic, also known as cyclic voltammetry. During this method the voltage is increased in steps from a minimum to a maximum, then decreased from the maximum to the minimum which

represents one voltage cycle and this can be repeated several times (cycles) (Silverman 1998).

In galvanostatic mode, the current flow between the reference electrode and the working electrode is controlled and continuously monitored to correspond to the value defined by the user. This process works by maintaining a constant current throughout the polymerisation process, with the potentiostat/galvanostat controlling the potential difference between the working electrode and reference electrode against the counter electrode (Brad et al., 2011).

2.3 Analysis of drug release from the DES

Measuring drug release profiles from the DES provides valuable information in the DES design process, indicating the extent of release of the drug from the stent at various time points. An understanding of this profile helps improve the overall understanding of the clinical safety performance and device efficacy of DES.

In this project the drug release profiles from stainless steel wires coated with a conducting polymer polypyrrole were to be investigated.

There are various ways of measuring drug release from DES, including in-vivo, in-vitro and computerised simulations. Ideally the most accurate measurement is in-vivo testing, where the stent is implanted into a diseased artery and the drug release into the circulation can be determined by the presence of radio- or fluorescent- labels compounds in the serum or drug can be detected in the urine by the first pass effect or by blood sampling at the immediate implant site (Schwartz et al., 2002). However, the most important piece of information provided from these in-vivo studies is the drug remaining on the stent and in the artery tissue at different time points. Crucially, during in-vivo studies, the animal can be sacrificed and the stent removed from the harvested artery after specific time points to determine the residual drug on the stent and in-tissue

concentrations (Schwartz et al., 2002, Ma et al, 2011). For in-vivo studies, DES can be implanted into several different arteries from several alternative animal species, including the rabbit iliac artery, rat aorta and pig coronary artery. Of these, the most recommended animal model is the pig coronary artery, due to physiological and anatomical similarities to the human heart and its response to stent injury (Schwartz et al, 2002).

As part of the development and optimisation of new DES polymer coatings, in-vitro analysis has been a useful quantitative tool for measuring drug release from drug eluting stents (Yair Levy et al., 2008). For in-vitro analysis, coated DES are immersed in to a physiological release medium, such as phosphate buffered saline or other alternative release media, which can be tailored to the physico-chemical properties of the drug, for example with the inclusion of solubilising agents for poorly water soluble drugs. Samples are taken at specific time points and drug release concentrations are measured by a number of analytical methods such as UV/VIS spectroscopy and high-performance liquid chromatography (HPLC). Ideally, the stents are immersed in release medium of neutral pH at 37°C and rotated for 100 rpm to stimulate normal physiological conditions of the body (Yair Levy et al., 2008).

2.4 Polypyrrole electropolymerisation

2.4.1 Equipment

Electrochemical polymerisation of pyrrole was performed using a Solartron SI 1287 Electrochemical Interface (Solartron Analytical, Hampshire, UK). As mentioned before, a three electrode configuration was used in this experimental work. Each electrode was labelled to ensure the electrochemical interface was configured correctly for each repeat experiment. The working electrode was a stainless steel wire (SS316L) stimulating the stent in the experiment and a platinum wire as the counter electrode. Platinum and stainless steel wire were purchased from Goodfellow Cambridge Ltd, Huntingdon, UK. The approximate dimensions of stainless steel and platinum wires used were 1mm

diameter and 30mm in length. The reference electrode was a KR5 glass electrode, purchased from ThermoScientific UK Ltd, Leicestershire, England.

The Solartron SI 1287 was connected to a desktop computer with the CorrWare 2.0 software, which sets up and controls the electrochemical polymerisation according to user selected voltage, time and method of polymerisation. In addition, Corrview 2.0 software, was used along with Corrware for plotting the graphs and carrying out preliminary experimental data analysis.

2.4.2 Materials

Pyrrole (C_4H_5N, 98% purity, Molecular weight = 67.09g/mol), Sodium salicylate (HOC_6H_4COONa, ≥99.5% purity, Molecular weight=, 160.10g/mol), Ethanol (CH_3CH_2OH, Molecular weight = 46.07 g/mol,), were purchased from Sigma-Aldrich (Poole, UK). 1mm in diameter stainless steel wire (SS316L) was cut in to pieces of approximately 3 cm in length.

2.4.3 Methods

The electrochemical polymerisation technique was carried out in aqueous solution made from pyrrole monomers and sodium salicylate at room temperature. A 500 ml volume of 0.1 M Pyrrole monomer (Py), 0.1M sodium salicylate (NaSa) was prepared in distilled water.

The three electrode configuration was set up and connected to the Solartron SI 1287 (see figure 2.1). Before running the electropolymerisation for each experiment, the platinum wire and the stainless wire were washed with ethanol to remove any contamination or debris and left to dry. The platinum wire was also sanded down after each experiment to remove the depositions from the previous run, which have the potential to reduce the efficiency of the electropolymerisation. The reference electrode

was kept in concentrated potassium chloride (KCl) solution between experiments. Therefore, before and between each experiment the electrode was rinsed with distilled water. 80ml of the coating solution (0.1M Py, 0.1M NaSa) was poured into a clean beaker and the electrodes were immersed to a consistent depth of approximately 25 mm. Before starting the experiment, the optimal voltage and time period required to provide a uniform polypyrrole coating with sufficient thickness on the surface of the working electrode was selected, based on existing literature and several trial runs that were performed in the preliminary stages of the project. For potentiostatic synthesis of Py-Sa, a constant potential of 1.3V for 15 minutes was selected to produce adequate electrodeposition. To compare the drug release from coatings of different voltage levels, another electropolymerisation setting was selected to provide thicker polypyrrole coatings on the working electrode. A constant potential of 1.5 V was used for a period of 15 minutes for this synthesis process. Several wires were coated using this method and 3 wires from each voltage group displaying the most uniform surface coatings were used in subsequent drug release experiments. Following the electropolymerisation process, the coated stainless steel wires were removed and left to dry overnight at room temperature prior to being used in the drug loading step of the procedure.

2.5 Drug loading

2.5.1 Material

Sirolimus ($C_{51}H_{79}NO_{13}$, 99% purity, Molecular weight= 914.2g/mol) was purchased in its solid form from Cfm Oskar Tropitzsch, Germany.

2.5.2 Methods

Following the electrochemical polymerisation experiment, the coated stainless steel wires after drying were immersed into one of two different concentrations (1% and 2% w/v) of the selected drug sirolimus, to study the uptake of different concentrations onto polyprrole surfaces of varying polymer thicknesses. 20mg/ml of the drug was dissolved

in pure ethanol to provide a 2% (w/v) solution. This 2% solution was then used as a stock solution, from which dilutions in ethanol were made to yield a 1% (w/v) solution.

The polypyrrole coated wires were immersed into 500 µl of 1% or 2% concentrations of sirolimus solution for 30 minutes and allowed to air dry for a minimum of 24 hours.

The table 2.1 below summarises the different polymer and drug coatings applied to each of the wires used in the study.

Wire	Coating solution	Coating Method (voltage/duration)	Drug Solution concentration
1	0.1M NaSa 0.1M Py	Potentiostatic 1.3V 15 min	1% Sirolimus
2	0.1M NaSa 0.1M Py	Potentiostatic 1.3V 15 min	1% Sirolimus
3	0.1M NaSa 0.1M Py	Potentiostatic 1.3V 15 min	1% Sirolimus
4	0.1M NaSa 0.1M Py	Potentiostatic 1.3V 15 min	2% Sirolimus
5	0.1M NaSa 0.1M Py	Potentiostatic 1.3V 15 min	2% Sirolimus
6	0.1M NaSa 0.1M Py	Potentiostatic 1.3V 15 min	2% Sirolimus
7	0.1M NaSa 0.1M Py	Potentiostatic 1.5V 15 min	2% Sirolimus
8	0.1M NaSa 0.1M Py	Potentiostatic 1.5V 15 min	2% Sirolimus
9	0.1M NaSa 0.1M Py	Potentiostatic 1.5V 15 min	2% Sirolimus

Table 2.1 Coating solution, coating method and drug solution concentrations of the wires used in the experiment.

2.5.3 Salicylate release experiment

For further analysis of drug release profiles from the wires, nine more stainless steel wires were coated with 0.1M pyrrole monomer and 0.1M Sodium Salicylate, following the exact electropolymerisation techniques mentioned above.

The coated wires were immersed in the release medium, without any prior immersion in sirolimus, to measure the release of sodium salicylate alone from the wires. Three of the electropolymerised coated wires were immersed in ethanol prior to immersion in the release medium, to investigate the rate of release of salicylate during the course of the experiments.

Since the polypyrrole coated wires in the first set of experiments (2.5.2) were immersed in sirolimus solution, there is a possibility that salicylate may have been released into the ethanol solution before immersion in PBS:ethanol. The data from polypyrrole coated wires immersed in ethanol alone therefore helps explain any sodium salicylate that may have been released in the sirolimus/ethanol solution before the subsequent immersion in PBS: ethanol.

2.5.4 Coated Wires Immersion and Sampling

The release medium solution was prepared by fully dissolving a PBS tablet in 200mls of distilled water. Then 180ml of the PBS solution was added to 20mls of ethanol to make a ratio of 9:1. The polymer-drug coated wires were immersed in to vials containing 1ml of the release medium. The wires immersed in the solution were kept in the oven at 37°C to stimulate body temperature.

At various time points during the experiment, the wires were transferred into another glass vial containing fresh PBS with the same pH and temperature. The new vial was then stored in the oven once again and the old sample vial was stored in the freezer (-20°C) for subsequent UV-spectroscopy analysis. The time points selected to measure the drug release were as follows: 10 minutes, 1 hour, 6 hours, 1 day, 3 days, 7 days, 14 days and 28 days.

2.6 Drug release measurements

2.6.1 Equipment

The release of drug into the release medium was measured using a UV 2401 PC UV/VIS Recording Spectrophotometer with the UV Probe 2.21 software installed on the computer which provided the resultant graphs. UV-Cuvette UV-Transparent Spectrophotometry Cuvettes were purchased from BrandTech Scientific, inc (Essex,CT). A standard oven was used to store the wires in the solution at 37°C for the period of the experiment.

Figure 2.3 The UV 2401 PC UV/VIS Recording Spectrophotometer

2.6.2 Materials

Phosphate Buffer Saline (PBS) tablets were purchased from Sigma-Aldrich, UK. Ethanol (CH3CH2OH, Molecular weight = 46.07 g/mol,) was also purchased from Sigma-Aldrich, UK. Distilled water was used to prepare the solutions.

2.6.3 Methods

2.6.3.1 Calibration curves

2.6.3.1.1 Sirolimus calibration curve

Calibration standards were prepared by diluting a stock ethanol solution containing $1x10^{-3}$M sirolimus in PBS:ethanol (90:10). This was used to form standard solution samples to generate the calibration curve. The calibration curve was based on seven sirolimus concentrations (M): $1x10^{-4}$, $3x10^{-5}$, $1x10^{-5}$, $3x10^{-6}$, $1x10^{-6}$, $3x10^{-7}$, $1x10^{-7}$.

A new calibration curve was produced for each day that sample analyses were performed.

2.6.3.1.2 Sodium salicylate calibration curve

Calibration samples of different concentrations were made by diluting $1x10^{-2}$ M stock solution, which was made by dissolving 160.10 mg of sodium salicylate in 100 ml of 0.1 M PBS:ethanol. The dilution process was repeated until a calibration curve was achieved with concentrations ranging from $1x10^{-2}$ M to $3x10^{-7}$ M. The table below shows how these dilutions were made.

A new calibration curve was made on each day when sample analyses were performed.

Sodium salicylate release experiments and measurement were carried out exactly as previously mentioned in section 2.5.4, with the only difference that the sodium salicylate concentration has been measured at 296 nm wavelength of light absorption (Galluzzo, 2012) in the 200-350 nm range using UV 2401 PC UV VIS Recording Spectrophotometer.

2.6.4 Drug release measurement using UV spectroscopy

After completing the 28 day sampling period measurements, the samples were taken out of the freezer and allowed time to reach room temperature. After generating the calibration curve, 600µl of each sample was transferred to the transparent spectroscopy cuvette. Another cuvette containing 600µls of blank PBS:ethanol solution was used to provide a baseline for the experiment. This method is precise and accurate at low concentrations; hence it is commonly performed for determination of drug concentration released from drug-material combination products (French et al., 2001).

Sirolimus was measured as a peak occurring at 278 nm wavelength of light absorption in the 200-400nm range using a UV 2401 PC UV VIS Recording Spectrophotometer. The UV absorption wavelength was selected by reviewing the available literature (French et al., 2001).

CHAPTER THREE

RESULTS

3.1 Introduction

Following on from the previous two chapters, where chapter one examined the limitations of existing drug-eluting stents (DES) and chapter two outlined the methodology adopted to investigate the development of novel conducting polymer coatings to improve drug release profiles, this chapter presents the results from a series of polypyrrole coated wires, produced by electropolymerisation under different experimental conditions, and examines the effect of various experimental parameters on drug loading and drug release.

3.2 Polypyrrole electropolymerisation

3.2.1 Low voltage coatings

In order to produce a uniform coating with appropriate thickness, all of the experiments were carried out using potentiostatic electropolymerisation at a fixed potential of 1.3 V for 15 minutes duration. Following the electropolymerisation, the wires showed a light black film coated on the surface of the stainless steel wires surface (figure 3.1). The electrolyte solution was prepared fresh on the day of the experiment and was used for coating 6 stainless wires at room temperature. This improved the consistency of the coating as the electrolyte solution had the same pH and similar temperature for all experiments.

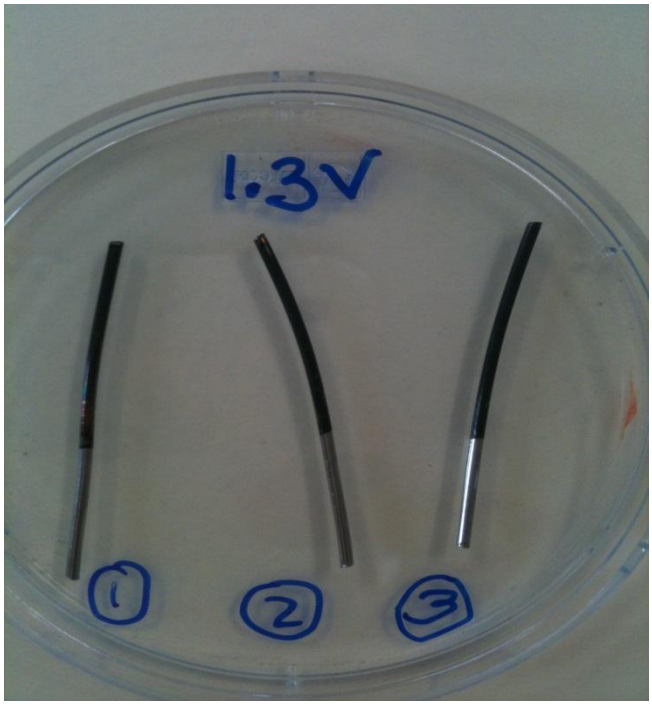

Figure 3.1 Stainless steel wires coated with polypyrrole polymer during potentiostatic electropolymerisation using 1.3V potential (n=3)

3.2.2 High voltage coatings

To investigate the effects of varying voltage during electropolymerisation on the drug loading and drug delivery, three more stainless steel wires were coated potentiostatically, with the potential for the electropolymerisation being increased to 1.5 V for the same duration of 15 minutes. The wires showed darker black and a more uniformly deposited coating by visual inspection (figure 3.2). To enable accurate comparisons between experimental groups, all the experimental conditions remained the same, with only the potential difference being varied.

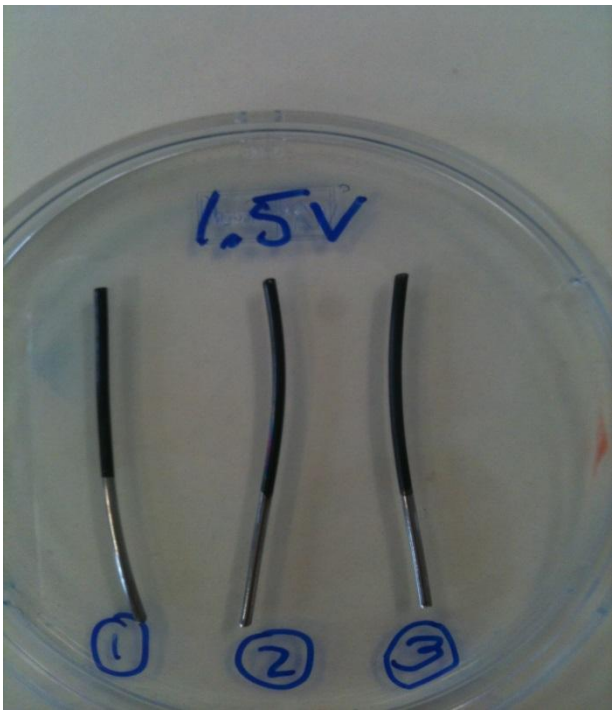

Figure 3.2 Stainless steel wires coated with polypyrrole polymer during potentiostatic electropolymerisation using 1.3V potential (n=3)

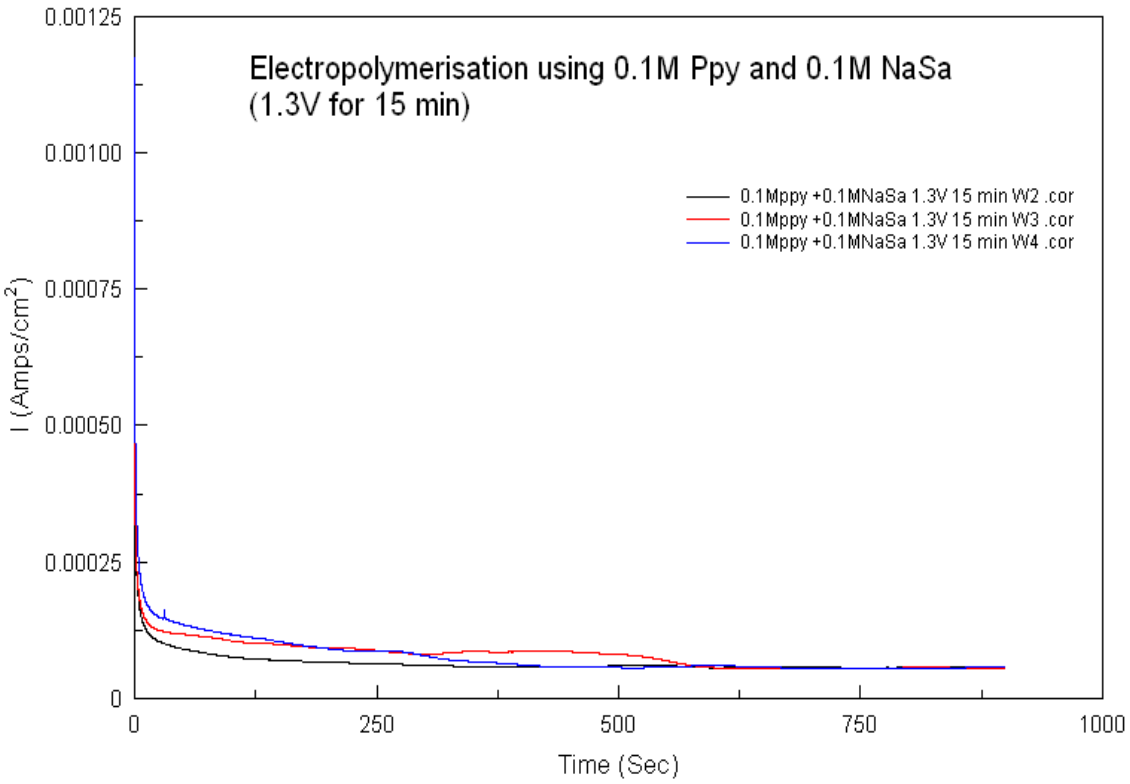

Figure 3.3 Current Density (Amp/ cm^2) versus Time (Sec) graph of three separate SS wire coated by electropolymerisation in 0.1M NaSa 0.1M Py solution at 1.3V for 15 minutes. Graph produced with CorrWare.

During the electropolymerisation, the CorrWare software created graphs of current density (Amps/cm^2) versus time (sec) for each experiment. Figures 3.3 and 3.4 show examples of graphs obtained from the electropolymerisation at the two voltage settings (1.3V and 1.5V). Although the procedure and the solution were the same for every coating within its selected setting (either 1.3V or 1.5V), the current-time profiles observed for each coating can be seen to be slightly different to each other. This is particularly clear for those coatings produced at 1.5V (Figure 3.4).

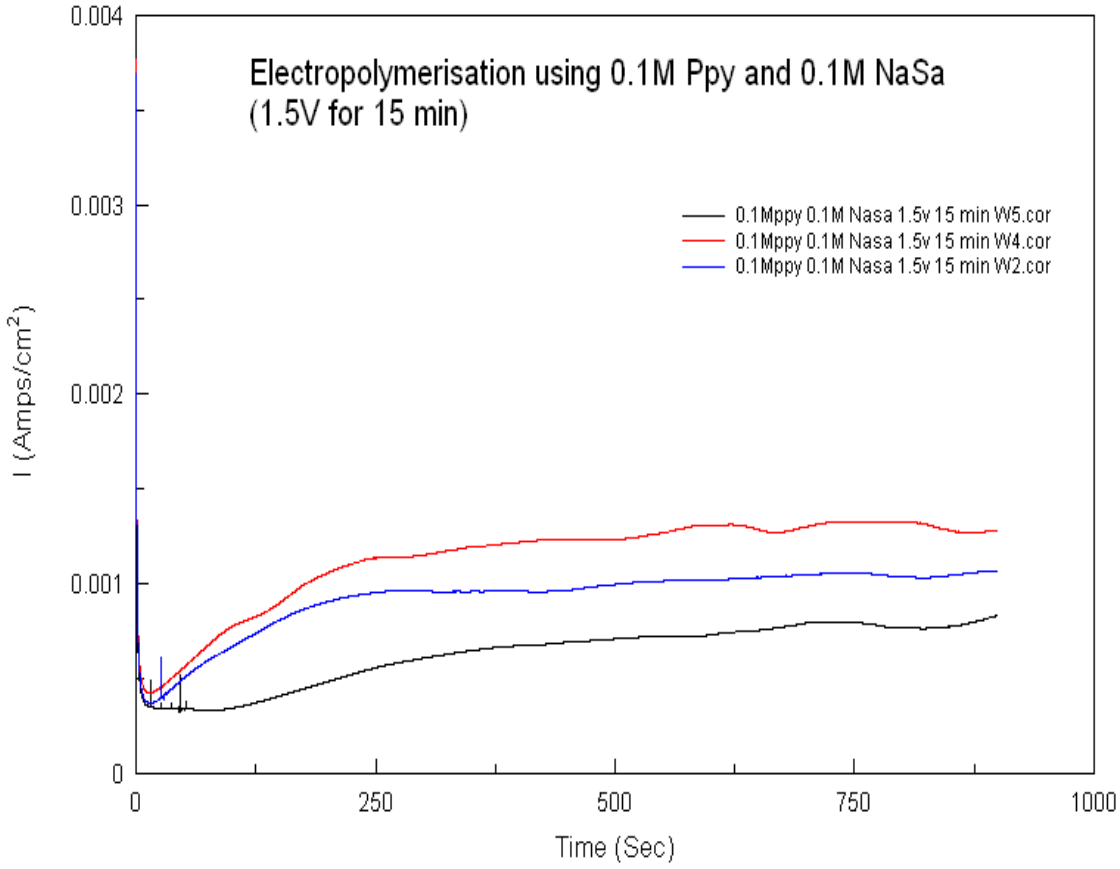

Figure 3.4 Current Density (Amp/ cm^2) versus Time (Sec) graph of 3 separate SS wires coated by electropolymerisation in 0.1M NaSa 0.1M Py solution at 1.5V for 15 minutes. Graph produced with CorrWare.

Table 3.1 below summerises the level of error and repeatability of the electropolymerisation coating process within each potential level examined.

Electropolymerisation potential	Average stabilized current density with standard deviation (amps/cm^2)
1.3V	0.0001 +/- 0.0000 stdev
1.5V	0.001067 +/- 0.0003 stdev

Table 3.1 Table of errors and standard deviation for each group of electropolymerisation coatings (1.3V and 1.5V) (n=3)

3.2.3 Low voltage versus high voltage coating

To gain a better understanding of the differences in the electropolymerisation coating process at the two different potentials, representative current density (Amp/ cm^2) versus Time (Sec) profiles for SS wires coated at 1.3V and 1.5V for 15 minutes have been plotted on the same chart (see figure 3.5). In both profiles, there is a decrease in current in the first thirty seconds. In the coating produced at 1.3 V (thin coating), the current decreases until it stabilises after reaching a current value of approximately 0.0001+/- 0.0000 amp/cm^2. This behavior is in contrast with the electropolymerisation at 1.5 V (high voltage coating) where after the initial decrease in current, there is an increase in the current density over a period of 200 seconds before it reaches its stabilised current value of around 0.001 +/- 0.0003 amps/cm^2. This evident difference between the profiles produced at different potentials can be seen clearly from figure 3.5.

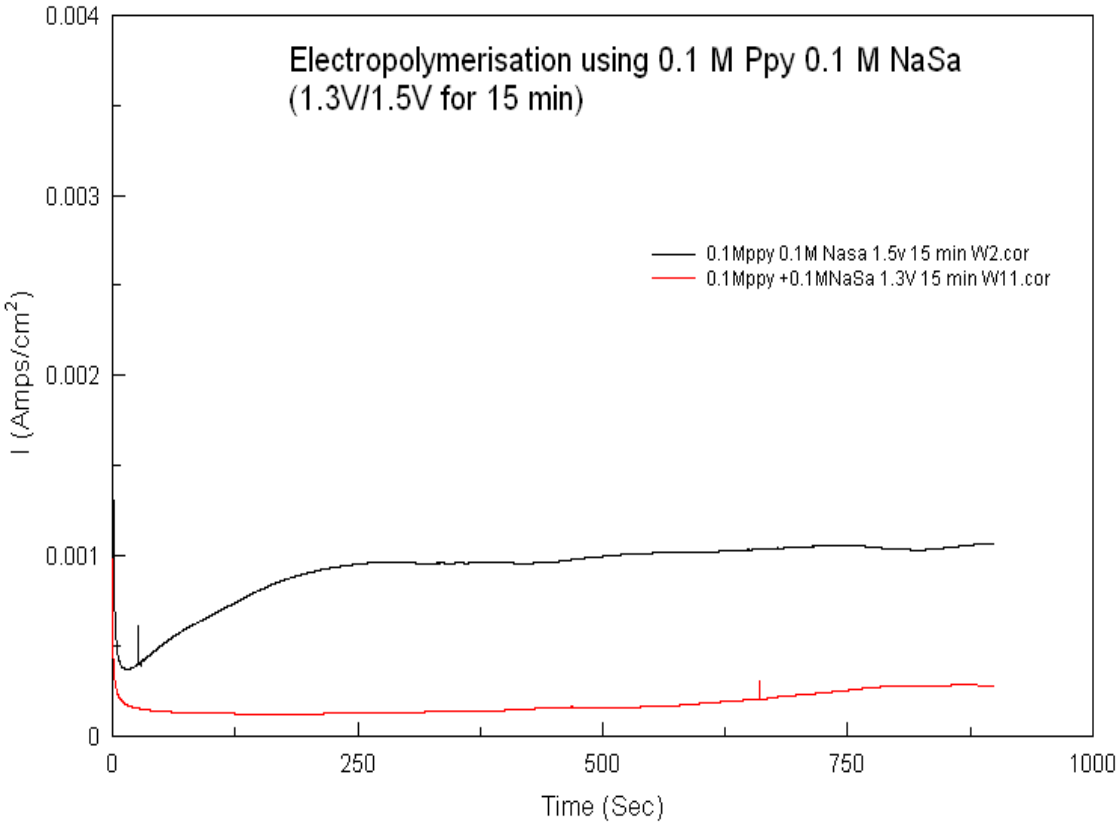

Figure 3.5 Comparison of potentiostatic polypyrrole electropolymersiation - Current Density (Amp/ cm^2) versus Time (Sec) graph of SS wire in 0.1M NaSa 0.1M Py solution at 1.3V and 1.5V for 15 minutes. Graph produced with CorrWare. The two traces shown are representative from 3 replicate experiments performed at each voltage.

3.3 Drug loading

The table below summarises the experimental conditions used for the electropolymerisation of the coatings and the drug concentrations used in the drug loading immersion step. As previously mentioned in section 2.5, to investigate the effects of conducting polymers as a novel stent coating in order to improve drug release profile from DES, stainless steel wires coated at two different voltages were immersed

in varying concentrations of drug and the drug released from the wire measured over a period of 28 days. In the first set of experiments, the stainless steel wires produced at 1.3V and 1.5V were immersed in 1% or 2% sirolimus in ethanol solutions before being immersed in PBS:ethanol to determine the sirolimus release characteristics. For the second set of experiments, the stainless steel wires coated with the conducting polymer via the same process of electropolymerisation, were immersed in release media without any contact with the sirolimus drug. This was carried out to measure the release of sodium salicylate from the wire. This was repeated for both Low and High voltage coatings to gain a better understanding of the effects of polymer thickness on the salicylate release profiles. A further three coated stainless steel wires were immersed in pure ethanol before immersion in the release media to find out if the immersion in ethanol would lead to rapid release of sodium salicylate from the Polypyrrole coatings. This was carried out to enable an effective comparison between the salicylate release profiles from the first set of experiments (containing sirolimus) and in this final set of experiments (where no sirolimus was present).

Number of wires	Electropolymerisation method	Electrpolymerisation solution	Elelcropolymisation voltage/ duration	Drug loaded
3	Potentiostatic	0.1M NaSa + 0.1M Py	1.3V / 15 minutes	1% Sirolimus
3	Potentiostatic	0.1M NaSa + 0.1M Py	1.3V / 15 minutes	2% Sirolimus
3	Potentiostatic	0.1M NaSa + 0.1M Py	1.5V / 15 minutes	2% Sirolimus
3	Potentiostatic	0.1M NaSa + 0.1M Py	1.3V / 15 minutes	-
3	Potentiostatic	0.1M NaSa + 0.1M Py	1.5V / 15 minutes	-
3	Potentiostatic	0.1M NaSa + 0.1M Py	1.5V / 15 minutes	Ethanol

Table 3.2 Summary of all the experiments and experimental conditions

3.4 Drug release measurement

The following results section consists of two parts. The first part is analysis of sirolimus release from the polymer coated wires. The second part is analysis of salicylate release from the coated wire. Before starting each of these experiments a calibration curve was produced by diluting a series of standard concentrations of each drug. It can be seen that over the concentration range examined in this study, that the calibration curves for both drugs were linear with R^2 values close to 1.

Several calibration curves were produced over the period of analysis and overlapping curves indicated that the results from the experiments are reliable and the instruments used produced repeatable measurements (Figure 3.8 and 3.9).

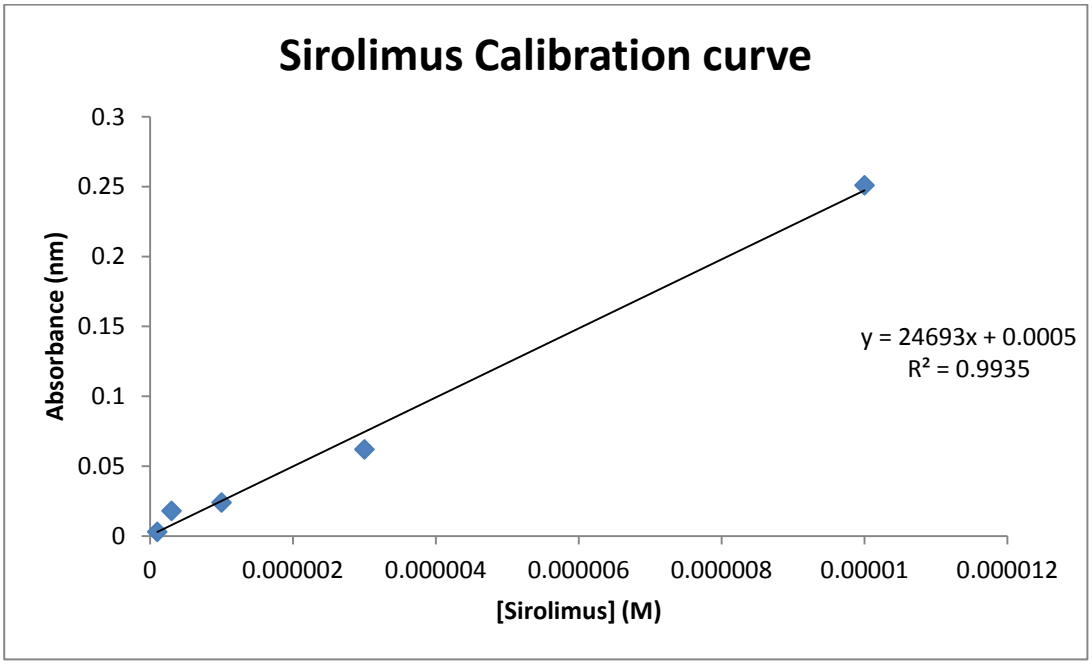

Figure 3.6 Calibration curve produced using sirolimus standard concentration range of $1x10^{-4}$ to $3x10^{-7}$ M

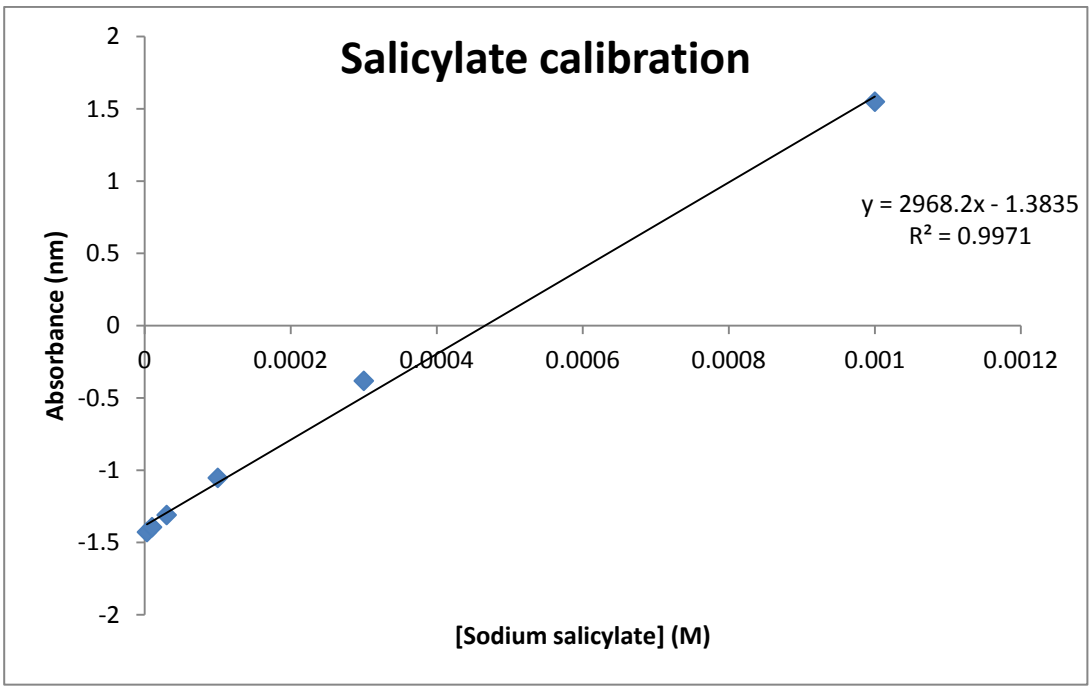

Figure 3.7 Calibration curve produced using sodium Salicylate standard concentration range of $1x10^{-3}$ to $1x10^{-7}$ M

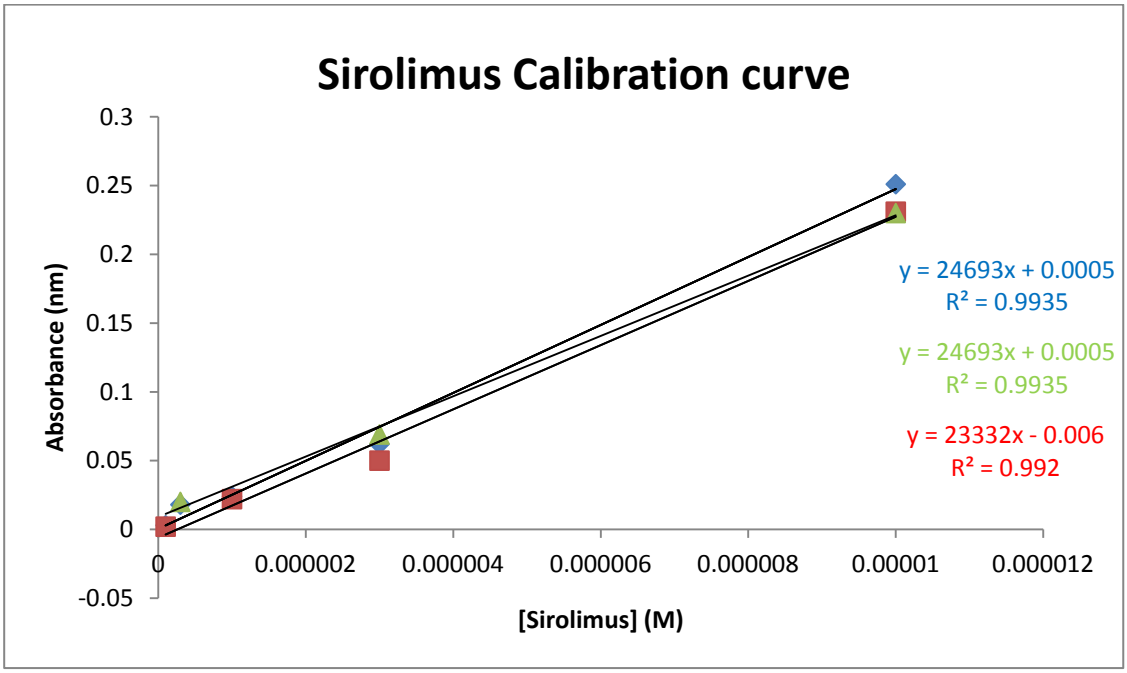

Figure 3.8 Comparison of three calibration curves produced on different days using the dilutions of sirolimus ranging from $1x10^{-4}$ to $3x10^{-7}$

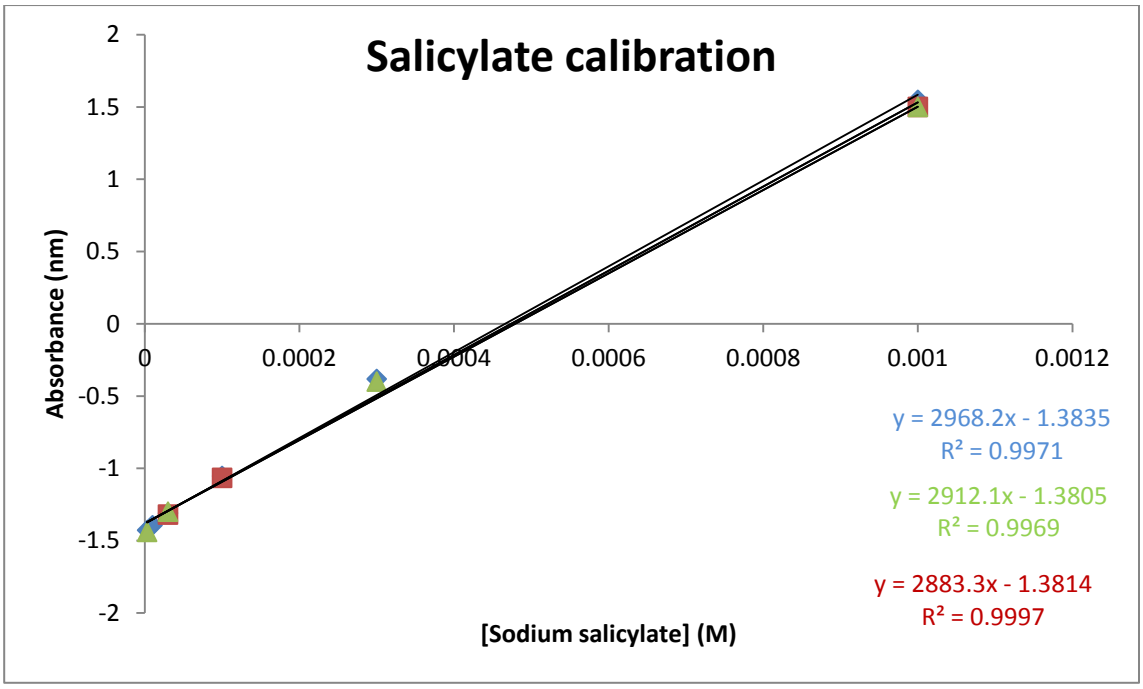

Figure 3.9 Comparison of three calibration curves produced on different days using the dilutions of sodium salicylate ranging from $1x10^{-3}$ to $1x10^{-7}$.

3.4.1 Sirolimus release

The sirolimus release profiles generated for each of the different wire coatings are shown below in figure 3.10.

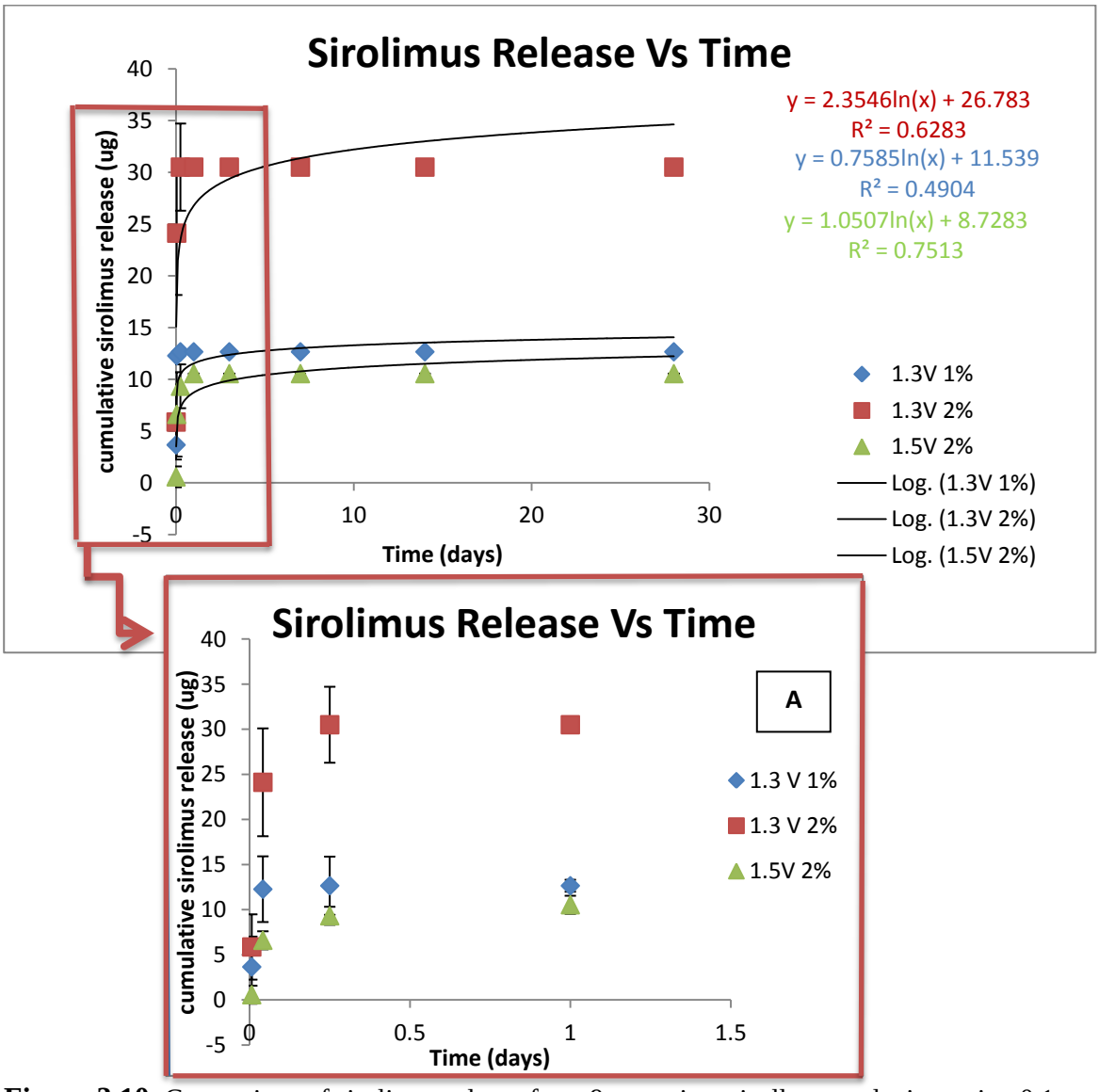

Figure 3.10 Comparison of sirolimus release from 9 potentiostatically coated wires using 0.1 M Py and 0.1 M NaSa for the period of 28 days immersed in PBS:ethanol. Three wires were coated at 1.3V and immersed in 1% sirolimus, three wires were coated at 1.3V, immersed in 2% sirolimus and three wires coated at 1.5V, immersed in 2% sirolimus. Figure 3.10A magnifies the sirolimus release profiles during the early stages of the drug release period.

It can be seen that there is an initial burst of release within the first 24hrs of the experiment and after that, for the remaining period of the 28 days, there is a plateau in the release curves indicating no further drug release. This suggests that most of the drug has been released in the early stages of the experiment. Figure 3.10A, magnifies the period of the experiment were the maximal drug release was recorded. Looking at this figure, it is clear that drug releases from the three groups has completed within the first day after immersion in PBS:ethanol.

Comparing the drug release profiles for three groups, in the first group (1.3V, 1% sirolimus, n=3) after 28 days approximately 13 µg of sirolimus release was recorded. This compares to 30 µg sirolimus release from the second group of wires (1.3V, 2% sirolimus, n=3).

In the third group (1.5V, 2% sirolimus n=3), where a thicker coating was observed by visual inspection, approximately 10 µg sirolimus was released during the 28 days of the experiment.

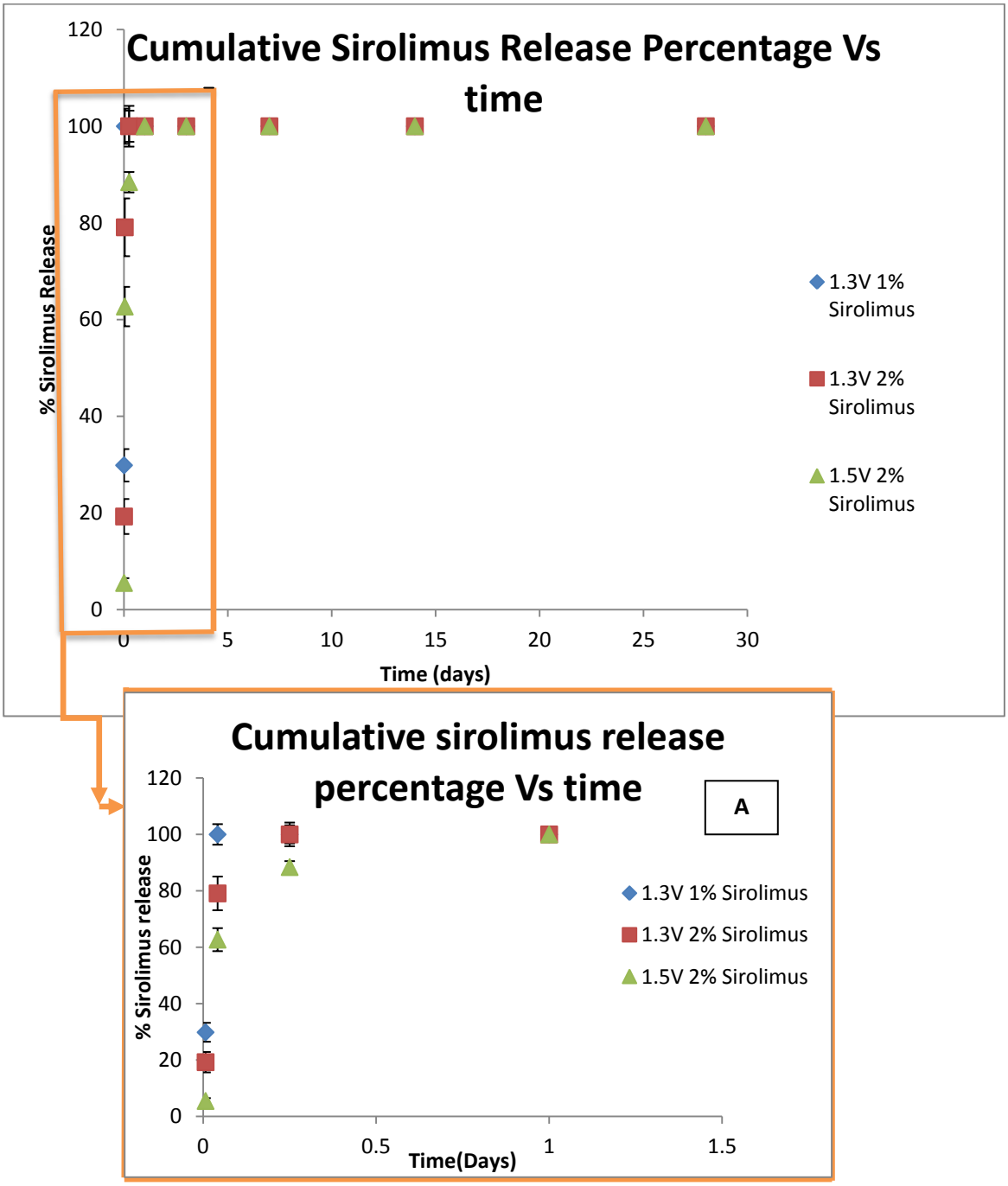

Figure 3.11 Comparison of sirolimus release in PBS percentage versus Time profiles of the three groups of wires coated through potentiostatic method in 0.1M NaSa 0.1M Py using 1.3V or 1.5V and immersed in 1% or 2% sirolimus. Figure 3.11A magnifies the cumulative sirolimus release percentage profiles during the early stages of the drug release period.

In Figure 3.11, the drug release percentage trend lines of all coatings are compared. This graph was produced by dividing the amount of drug released at a given time by the total drug released at the time 28 days. Regardless of amounts of drug released in each group the trends of the percentage release profiles remained the same in all three coatings.

3.4.2 Salicylate release

From the previous experiment, an interesting finding was that there was no evidence of salicylate release in the release medium following the immersion in sirolimus. Hence, a further three wires were coated and immersed in ethanol alone in order to investigate salicylate release profile and the underlying reason for the absence of salicylate release in the first experiment.

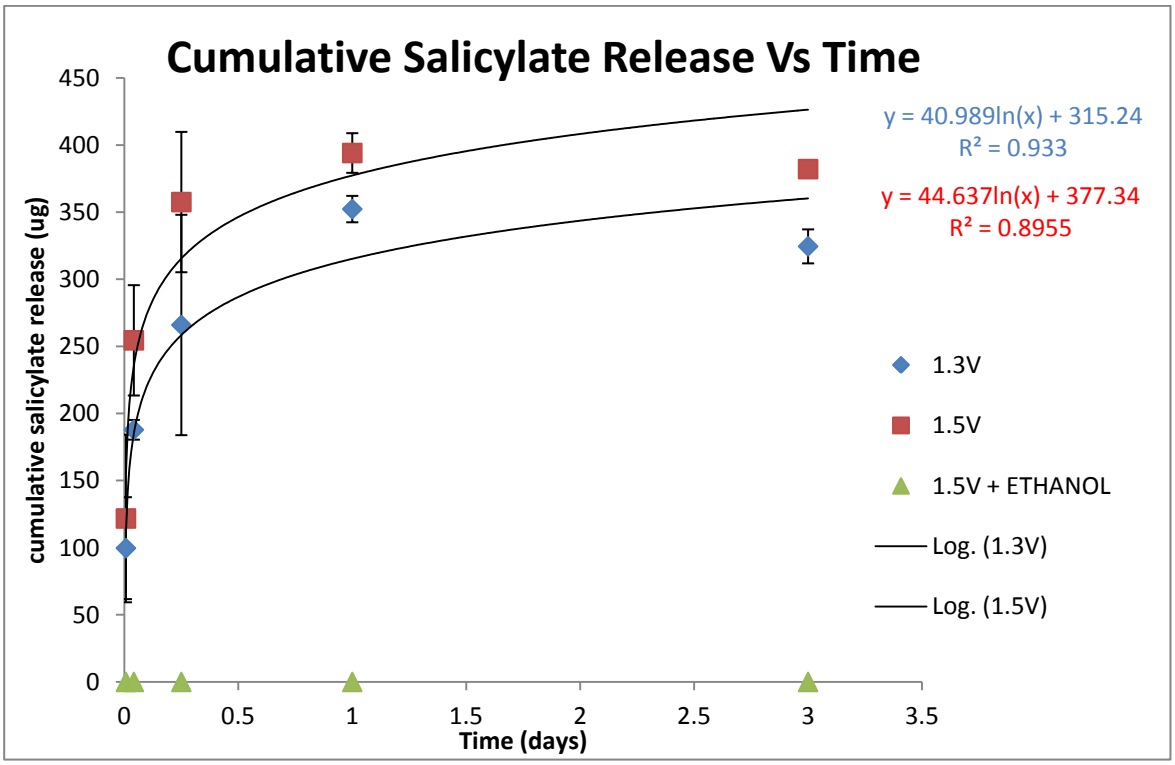

Figure 3.12- Comparison of sodium salicylate release from 9 potentiostatically coated wires using 0.1 M Py and 0.1 M NaSa for the period of 28 days immersed in PBS. Three wires coated by 1.3V potential, three wires coated by 1.5V and three wires coated by 1.5V, immersed in pure ethanol. N=3 for all groups.

Figure 3.12 illustrates the cumulative sodium salicylate release trend lines of the three different coating types. Looking at the graph, it is clear that the wires coated using 1.3 V and 1.5 V electropolymerisation have similar release profiles. It takes approximately one day for almost all of the drug to be released from the 1.3V coating, reaching a maximum of 352 µg at this point, which corresponds to 100% drug release over the 28 days period.

Similarly, the release curve for the coated wires at 1.5 V, follows the same trend line with an increase in drug release in the first hours, with the maximum salicylate release of 394 µg being released after one day, which again corresponds to 100% release over the 28 days period.

It also can be seen that the release profile for the wires immersed in ethanol prior to immersion in PBS:ethanol, is different to the two other release profiles. There is an absolute absence of salicylate released over the period of 28 days. Figure 3.13 below, shows the salicylate release percentage over 28 days period of the experiment which highlights the drug release profiles of each group.

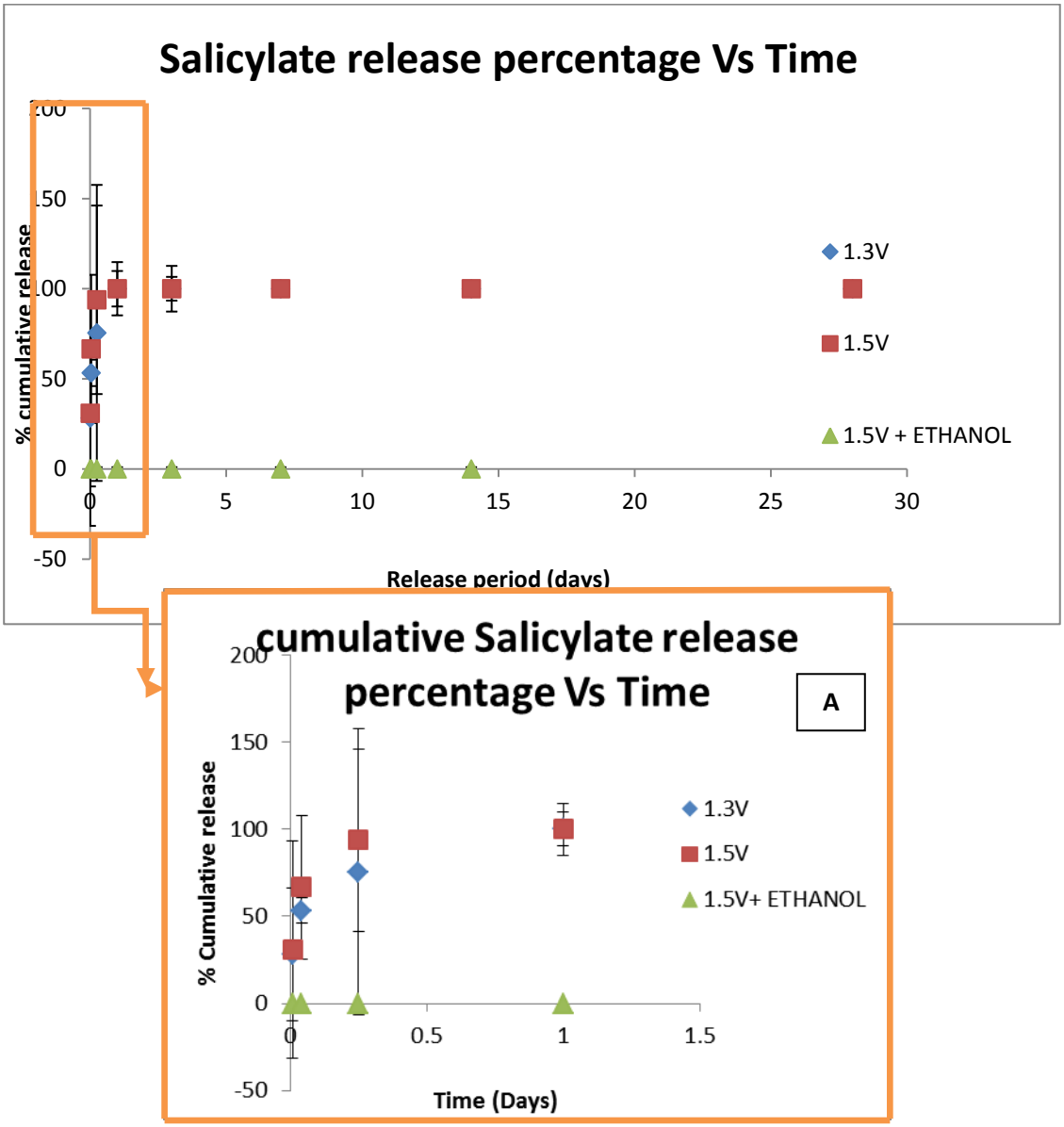

Figure 3.13- Comparison of sodium salicylate release in PBS percentage versus Time profiles of the three groups of wires coated through potentiostatic method in 0.1M NaSa 0.1M Py using 1.3V or 1.5V and immersed in ethanol. N=3 for all groups. Figure 3.13A magnifies the cumulative salicylate release percentage profiles during the early stages of the drug release period.

Similar to figure 3.11, the trends of cumulative percentage salicylate release profiles for two different groups of coatings are similar. This is clearer in figure 3.13A.

CHAPER FOUR

DISCUSSION

4.1 Introduction

Since the introduction of coronary stents, mortality rates of coronary heart disease (CHD) have been decreasing but CHD remains the most common cause of mortality in the UK (NICE, 2007).

In chapter one, various treatment methods to treat CHD were discussed. These treatment options have their specific benefits and disadvantages. Currently drug-eluting stents (DES) are thought to be the most suitable treatment option for the majority of patients (Butany et al., 2005). These coronary stents are coated with an active agent, usually an anti-proliferative drug, which elutes slowly in to the vessel in order to inhibit smooth muscle cell proliferation and in-stent restenosis (Acharaya et al., 2006).

Unfortunately, there are still several disadvantages of using DES, including late stent thrombosis, delayed endothelial healing and potentially sub-optimal drug release profiles (Garg et al., 2010). These disadvantages were explained in detail in section 1.3.1.

To overcome these limitations, stent technology has been rapidly evolving in recent years, with modifications being made to the stent platform, shape, strut thickness and alloys used. Novel polymers and coating methodologies have been investigated to improve the biocompatibility and drug release profiles. The ultimate aim of this work is to develop DESs which provide an optimal drug dose to the artery wall and which are suitable for long term implantation with the ability to inhibit in-stent restenosis and thrombosis.

One of the most recent developments in DES is the use of drug-polymer coatings in order to improve drug release profiles and hence improve clinical outcomes (NICE technology appraisal guidance 71, Garg et al., 2010). As explained previously in section 1.4, conducting polymers, such as polypyrrole have very interesting characteristics, including biocompatibility, ease of synthesis, and conductivity which

make them promising candidates for incorporating into stent coatings for use in next generation DES (Guimard et al., 2007). The potential use of these polymers for new stent coatings has recently been investigated in a small number of studies (Okner et al,. 2007, Arbizzani et al., 2007, Svirskis et al., 2011, Sirivisoot et al., 2011).

Arbizzani et al, demonstrated the release of a variety of molecules from polypyrrole coatings, although the extent to which these molecules would be effective restenosis inhibitors remains in doubt. In contrast, Okner et al, demonstrated the release of paclitaxel, which has proven clinical efficacy within various DES. Despite the wealth of clinical data supporting the use of sirolimus within DES, no study has examined its incorporation or release from conducting polymers. This project therefore set out to investigate the possibility of developing a novel DES coating with a therapeutic drug release profile using polyprrole and sirolimus.

To accomplish the overall objectives of this project, a series of conductive polypyrrole coated stainless steel wires, which represent the stent struts, were produced using a potentiostatic method of electropolymerisation. In order to develop a number of distinct sirolimus release profiles, different potentials were used during the electroplymerisations and their effects on the dug release were investigated. The polypyrrole coated wires were then dip-coated in a solution of sirolimus. Different concentrations of the drug were used to explore the relationship between the drug release profiles of the polypyrrole coated stents and varying concentrations of sirolimus.

4.2 Experimental results

4.2.1 Sirolimus release profiles from polyprrole coating

4.2.1.1 Effect of Voltage Level on Coating Characteristics

All the coatings were produced through potentiostatic electropolymerisation using 0.1 M Py 0.1 M NaSa solution. To investigate the effects of different surface characteristics on the release profiles, low (1.3 V) and high (1.5 V) potentials were used during the electropolymerisation, which gave rise to different thicknesses of polyprrole coatings. On visual inspection, the surface of the wire coated with 1.5V appeared to be darker and thicker than the 1.3V coatings (figures 3.1 and 3.2).

4.2.1.2 Effect of Voltage Level on Sirolimus Uptake and Release

A comparison was made between the drug release profiles from the two voltage groups. It was hypothesised that the higher voltage used during the electropolymerisation would lead to a darker, more uniform polymer coating available for sirolimus uptake, and that this would therefore lead to a greater mass of subsequent drug release. However, it was found that the group of wires coated with the higher voltage released less sirolimus (Figure 3.10). This suggests that the presence of a greater density of polymer coating at 1.5V was associated with a reduced sirolimus uptake and release. As mentioned before (section 3.2.3), it was not possible to measure the mass differences between the wires electropolymerised within two voltage groups, since mass changes were below the detection limit of the balance. This is a limitation of this procedure.

No previous study has investigated the release profiles of sirolimus from polypyrrole coating stents. The findings from this experiment showed that sirolimus was released

from the polypyrrole coated wires very rapidly during the first 24 hours of immersion in release medium. This result was surprising, given the previous results reported by Okner et al., 2007, which demonstrated that paclitaxel could be adhered and then released from polypyrrole coated stents over a period of days. Their results showed a rapid release of the absorbed drug from the polypyrrole coating, with up to 60% release in 1 day followed by a more gradual release of the remaining 40% over 30 days (R.Okner et al., 2007). This is an example of the optimal drug release profile we were targeting in the current study of sirolimus-polypyrrole coated wires. In understanding these contrasting results, it is important to consider the differences in the methodology used by Okner et al.,. The electropolymerisation used in the Okner et al study was cyclic voltammery technique. For this project the wires were coated potentiostatically, which may have led to differences in the coating structure and hence its capacity to absorb drug. In addition, paclitaxel was the drug examined by Okner et al, whereas in the present study, sirolimus was examined, and although both drugs have similar physical properties, their uptake characteristics may be quite different.

It was anticipated that the drug release profiles from polypyrrole coated wires would vary in relation to the concentration of sirolimus used in the coating solution. The results show that the drug release from the first group of wires, coated at 1.3V and immersed in 1% sirolimus solution, after 28 days was approximately 13 µg. This compares to a higher level of drug release from the second group, where the wires were coated at 1.3V then immersed in 2% sirolimus solution, which released 30 µg of sirolimus after 28 days (figure 3.10). This result was expected and appears to suggest that the higher concentration of the drug in the coating solution will lead to a greater drug uptake on the polypyrrole coating, which in turn gives a higher release concentration over time. This therefore represents a means of achieving controlled drug dose delivery from stents in a similar manner to some well established drug delivery stent platforms (Acharya et al.,2006). It is interesting to compare the sirolimus release profiles from existing DES like Cypher (Acharya et al.,2006) to the release profiles produced for this project. Cypher stents, of comparable length to the wires used in this

study, release considerably higher concentrations of the drug (~200 µg), with most of the drug being released within 30 days (Vankatraman et al., 2007). This compares to around 20 µg of sirolimus release within the first day observed in this experiment. This is significantly lower and it could be said that it may not be enough to have positive effects on the vessel wall. However, it is known that high local concentrations of sirolimus could be toxic (Desai et al., 2003), and since sirolimus retention time in the artery walls is very high (Desai et al., 2003) it could be suggested that the release profiles obtained in the present study, consisting of an initial burst of the drug with no further release, may be beneficial. However the correct dose remains the subject of considerable debate, and further studies would have to be carried out to confirm this hypothesis.

The most unexpected result from the above data is the rapid release of the drug during the first 24 hours from the polymer coated wires, since this is in contrast to data previously reported by Okner et al, for paclitaxel. This effect was observed irrespective of voltage or sirolimus concentration used. It may be due to a variety of factors, such as polypyrrole surface characteristics, electropolymerisation methods and drug concentrations used. This needs to be further investigated, since there has been no comparable study on the drug release profiles of sirolimus from polypyrrole coated stents.

4.2.2 Salicylate release profiles from polypyrrole coatings

Following from the experiments outlined above, release of salicylate was also measured from the two groups of coatings produced at different potentials (1.3V and 1.5V). In a final study group, the salicylate release profiles were measured after an initial immersion of the coated wire in ethanol, in order to examine the effect of release medium on the drug release.

4.2.2.1 Effect of voltage on salicylate release

It was seen from figure 3.12 that the group of wires coated using 0.1 M Py 0.1M NaSa at 1.5V released more salicylate in comparison with the 1.3V group. The electropolymerisation process proceeds by the uptake of negatively charged dopant ions (salicylate) into the polymer structure, meaning that an increased current density would indicate increased uptake of salicylate. The greater release of salicylate observed from those coatings produced at higher voltages and current densities are therefore an expected finding from the present study. These findings were also demonstrated in the work of Arbizzanni et al., 2007, which investigated incorporating anionic dopants such as salicylate and naproxene in polypyrrole electropolymerisation and measured their discharge, in order to estimate the drug mass entrapped and the amount eluted (Arbizzani et al., 2007). Similarly, they came to the conclusion that the incorporation of "molecules of pharmacological interest with a complex structure" such as salicylate will indeed affect the electropolymerisation process and the release profiles could be controlled by the amount of the dopant and the charge involve in the electropolymerisation process (Arbizzani et al., 2007).

The interesting results from the first experiment, where the polypyrrole coated wires were first immersed in sirolimus before drug release was measured, showed no evidence of salicylate release when immersed in PBS:ethanol for the period of 28 days. One possible explanation for this unexpected finding is that all of the salicylate taken up into the polymer during electropolymerisation, is released in the ethanol solution. Looking at the data from polypyrrole coated wires, immersed in ethanol alone, helps explain this phenomenon. It can clearly be seen from the figure 3.12 that no further salicylate has been released from those wires which had been previously immersed in ethanol solution before the subsequent immersion in PBS: ethanol. These results, together with the absence of salicylate release in the first series of experiments (4.2.1.2) suggest that salicylate is released in ethanol very rapidly. This highlights the

importance of selecting the correct release medium, which most accurately mimics the in vivo environment. In addition, given the potential use of conducting polymer surfaces in biosensing and implantable applications, the ability to rapidly remove potentially interfering dopant ions by a simple ethanol wash, as demonstrated here, may have interesting applications beyond the initial scope of this study.

4.3 Summary of Key Findings

It was hypothesised that changing the concentration of sirolimus will affect the drug release profile. With the experiment completed and preliminary results collected, evidence was found that higher concentrations of sirolimus provide higher concentrations of drug release during the therapeutic elution period and this therefore provides one method of improving drug release profiles. The results of this aspect of the study support the original hypothesis.

Following onto the second part of the study, it was hypothesised that varying the voltage during the electropolymerisation of the conducting polymer would have an effect on the therapeutic drug release profiles. It was found that the higher voltage during electropolymerisation of polypyrrole did not increase the sirolimus uptake and release. Therefore the assumptions made were not supported by the experimental data generated.

4.4 Study limitations and future work

There are a number of limitations of this work, which can affect the morphology of the polymer coating and the drug release profiles. It is beyond the scope of this study to detail all the possible factors. However, key potential limiting factors include the temperature and pH of the electrolyte solution. A study by Svirskis et al., 2010, has

closely looked at these parameters and concluded that the pH of the electrolyte solution during electropolymerisation influences the rate of the synthesis. In acidic conditions, fast synthesis occurs compared to slower synthesis in neutral conditions. In basic electrolyte solutions, no polymerisation happens.

Since this experiment was carried out at room temperature there would likely have been variations in temperature each day. The temperature of solutions such as ethanol and the drug solution were also at room temperature. These variations in temperature were neglected, which may influence the rate of the polymerisation and the morphology of polymer as well as the drug uptake characteristics. It is highly recommended to perform all the experiments in a temperature controlled environment in order to minimise such variations.

Another factor worth mentioning is the purity of the pyrrole. The purity of the pyrrole will decrease over time due to the oxidation of the monomer. Although the pyrrole for this experiment was bought fresh prior to the experiment, no distillation of the pyrrole was carried out, and some level of degradation during storage cannot be ruled out.

Earlier in this chapter (section 4.2.1) it was mentioned that the sirolimus release profiles were not as expected and all of drug was released within the first 24 hours after immersion. The precise reason for this result is unknown and requires further investigation. One potential modification in the methodology which may increase the efficiency of the drug loading process could be incorporation of the sirolimus drug within the electrolyte solution used during the electropolymerisation process. In this way, it may be possible to coat the stainless steel wires with polypyrrole and sirolimus simultaneously. This may have an impact on the uptake of the drug on the polymer and hence improve the drug release profile over a therapeutically relevant period of time.

Another modification to this experiment which may influence the quality and uniformity of the coating is using a stirrer or rotating electrode, which may result in the

formation of a more homogenous coating over the chosen surface. This wasn't available for this experiment but it is recommended for future improvements.

In this study, the drug release was measured in a static environment of a glass vial. Whilst the vial contained a physiological release medium maintained at 37 C, this does not fully mimic the in vivo environment. The stent inside a coronary artery is exposed to a constant blood flow. This was also a limitation of this experiment and for future drug release experiments, the introduction of flow via a perfusion system could be used to improve the experimental results.

It can be assumed that surface area and porosity of the polymer coating may also influence the drug release profiles. In terms of surface roughness and surface area, it could be said that those polymer coatings with a greater porosity provide greater surface area for the drug to bind to and therefore be eluted in the later stages. The surface characteristics of the polypyrrole coating on the stainless steel wires were not measured following electropolymerisation in this study. This is a source of variability in this experiment, as the precise thickness and porosities of the coating were not measured. In future work, SEM imaging and weight measurements should be obtained to establish a relationship between surface area, surface characteristics and drug release profiles. This may provide interesting insights and represent a worthwhile avenue for investigation for improving DES release profiles.

In the present study, stainless steel wires were used to represent stent struts, which do not ultimately a stent. DES are crimped onto the guide wire and during implantation, they are expanded inside the target artery. Therefore, in future development of such coatings, it will be important to investigate the effect of stresses and strains on the stent strut coatings during placement. It will therefore be necessary to carry out the experiment on stainless steel stents in order to be able to determine that the experimental findings demonstrated here are valid when extended to DES.

There are limitations regarding data analysis in this project. One of these is the relatively small number of samples used. Three samples for each group is an insufficiently high number of replicates to enable powerful statistical analyses to be carried out. Therefore, for future improvements to obtain more statistically accurate and repeatable results it is necessary to increase the number of experimental samples. Another short coming was in the cumulative percentage release profiles calculations (figure 3.11 and 3.13). This was calculated without knowing the initial amount of the drug coated on to the stent. This is also a limitation of this project. For future improvements, one way of obtaining accurate percentage release, is to immerse the coated wires in a suitable solvent that will strip out all the drug very rapidly and this will allow measurement of total drug loaded onto the stent.

4.5 Conclusion

The novel aim of this project was to investigate the properties of the conducting polymer, polypyrrole for use as a stent coating to provide sustained sirolimus and salicylate release profiles. Varying the parameters involved in electropolymerisation process, including voltage and duration, were investigated to determine if the resultant changes in the polymer coating influences the drug release from the device. Furthermore, the effect of changing the sirolimus concentration on the release profiles was also investigated. This was carried out to determine a relationship between the varying concentrations of the drug on the drug release profiles.

In this project it was found, that the increasing the voltage does not necessarily lead to increased drug release. This novel finding remains preliminary in nature, and more in depth research is required to understand the underlying reasons for this finding.
In relation to the use of higher concentrations of sirolimus drug on the coated wires, this project has shown that there is a relationship between the drug concentration used in the coating solutions and the drug release profile.

One of many ideal characteristics of DES is having an optimal drug release profile, with the capacity to deliver therapeutic treatments over a long period of time. This preliminary study demonstrated the potential beneficial properties of conducting polymers, specifically polypyrrole as a stent coating on improving performance of existing drug eluting stents, and therefore represents a step forward in the right direction for development of new DES with optimal drug release profiles.

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