PRODIGIOSIN ENCAPSULATED POLY LACTIDE-CO-GLYCOLIDE (PLGA)-COATED STENT FOR CORONARY CARDIOVASCULAR INTERVENTIONS

A

THESIS

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NUWOE KELLEN

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ABSTRACT

This research focuses on the design of a robust but flexible prodigiosin eluting stent coating for possible coronary cardiovascular implant. When coated with the drug embedded polymer matrix, the stent would be expected to dilate the vessel around the fatty blockages while the drug eluting polymer membrane delivers anti-proliferative drugs over a period of time to prevent the restenosis that otherwise would occur. The goal of this work is to incorporate anti-cancerous drug prodigiosin in the PLGA polymer matrix and then ascertain its release kinetics. In this research, Poly vinyl Pyrrolidone was used as a binder and cross-linker to create adhesion between the metallic stent strut and the drug encapsulated polymer matrix as well as between the polymer and the drug. This work also explores diffusion and degradation phenomena to explain the transport, dissemination, dispersion and absorption of drugs at the interface between the stent and the vessel wall. The expected results will then be discussed for potential applications via the incorporation of these prodigiosin-eluting stents for the treatment of coronary cardiovascular diseases.

Key words: stent, degradable stent coating, controlled release, coronary disease, poly(lactide-co-glycolide), Poly (vinyl pyrolidone), prodigiosin, diffusion and degradation mechanism.

DEDICATION

To the God of peace, from whom every good thing comes, I am more than grateful for Your favor, all of which I do not deserve-Your mercy endures forever.

To my irreplaceable mom and dad, Mr & Mrs Kellen, I certainly would not have achieved this without your uncompromising love and support. I wish it was ever possible to repay you.

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To my friends and colleagues, having you has been fun all through, the courage I derived from you couldn't possibly be got from somewhere else. You all have driven this success from the very beginning, thumbs up! I am more than grateful.

With excruciating sorry, I present this work in loving memory of the thousands of people who recently lost their lives in the fight against the ebloa virus disease in West Africa. May God almighty heal our land and restore us all to his divine favor.

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CHAPTER ONE

1.0 BACKGROUND AND INTRODUCTION

Cardiovascular disease is currently the leading cause of death in Africa and the world at large, accounting for at least 30% of all death. One of the major causes of cardiovascular disease is arteriosclerosis. It is as a consequence of the build-up of fatty cells and tissues within blood vessels. In most cases, this built-up is not detected until complete blockage which prevents the flow of blood and subsequently leads to heart attack, stroke or death. The objective is to develop prodigiosin embedded polymer-coated cardiovascular stents that will successfully deliver drugs and bioactive agents to blood vessels and treat coronary cardiovascular diseases while at the same time preventing restenosis-the narrowing of the blood vessel after angioplastic procedure. The polymer earmarked for these biodegradable coating is poly lactic-co-glycolic acid (PLGA) because of it biocompatibility and high rate of biodegradation. Over the past decade, drug eluting stents have been used to treat arteriosclerosis. Essentially, the stent dilate the vessel around the fatty blockages while the drug eluting coatings deliver anti-cancer drugs to prevent the restenosis that otherwise would occur. However, the stents remain permanently in the blood vessels after the duration of drug elution. Furthermore, vessel irritation, endothelial dysfunction, vessel hypersensitivity and chronic inflammation at the site of implantation are critical parameters that have attracted serious attention. There is therefore a need for multi-component, multifunctional materials for novel cardiovascular stents whereby, one important function should be degradability of the polymer so that after degradation, a functional vessel wall is regenerated [1].

There is also an equal need for reasonable stent coatings that can degrade gradually and be absorbed slowly by the body without creating the afore-mentioned adverse side effects.

1.1 STATEMENT OF THE PROBLEM

Cardiovascular disease (CVD) is a result of the disorder of the heart and blood vessels. Atherosclerosis, the main cause of coronary artery disease (CAD), is an inflammatory disease in which immune mechanisms interact with metabolic risk factors to initiate, propagate and activate lesions in the arterial wall [2]. About two decades ago, it was widely expected that the treatment of hypercholesterolemia and hypertension would eliminate CADs by the end of the 20^{th} century but this has not been the case. CVDs include coronary heart disease (heart attacks), cerebrovascular disease (stroke), high blood pressure (hypertension), peripheral artery disease, rheumatic heart disease, congenital heart disease, and heart failures [3]. The first two are the foremost causes of death in Africa and the world over. In 2008, about 17.3 million deaths were as a result of cardiovascular diseases. These represented 30% of the global death toll in 2008. Out of this figure, 7.3 million were due to coronary artery disease (Atherosclerosis), 6.2 million were due to stroke and 5.8 million were jointly caused by hypertension, high blood pressure, diabetes, and heart failure. Moreover, Coronary Artery Disease (CAD) is the principal cause of death in both males and females in the low-income and high-income countries. There is, therefore, an urgent need for improved approaches for the treatment of CAD in both developed developing and underdeveloped countries. People in low and middle-income countries are usually more exposed to risk factors of CVDs and do not often benefit from prevention programs as do people in high-income countries. As a result, over 80% of all deaths due to CVDs occur in low and middle-income countries. This huge statistical disproportion in low and middle income

countries is also due to the lack of effective and affordable health care services including early detection and treatment of the disease. This leads to the early death of people from the disease in these regions, often in their most productive years. It has been estimated that the number of people who die of CVDs will increase to 23.3 million by 2030 and is likely to remain the largest killer of people [4].

1.2 CORONARY ARTERY DISEASE (ATHEROSCLEROSIS)

Coronary artery disease simply refers to the buildup of fatty cells in the coronary artery. As a consequence, the regular flow of blood is retarded due to the narrowing of the arterial vessel. This accumulated reduction in blood flow eventually lead to ischemia and myocardial infraction-conditions that result from the damage of the heart tissues. Any arteries in the body, including arteries in the heart, brain, arms, legs, and pelvis, can be clotted by plaques. As a result, different diseases may develop based on which arteries are affected. Factors such as high blood pressure, high cholesterol levels, and low fatty acid metabolism precede the onset of coronary artery disease. Essentially, the most famous approach available for treating atherosclerosis is the insertion of a stent in the arterial wall which then prop opens the vessel to allow the flow of blood. Current drug-eluting stents are achieved by combining different materials: metals for mechanical strength, polymer coating for hemocompability, and drug to be release for prevention of restenosis, the recurrence of the narrowing of the blood vessel (e.g., by ingrowth of smooth muscle cells into the arterial wall leading to restricted blood flow) [1].

1.2.1 Mechanism of Atherosclerosis

Coronary arteries are hollow tube-like blood vessels through which oxygen-rich blood flows to the heart muscles. The muscular walls of the coronary arteries are normally smooth and elastic. Lining the walls are layers of cells called the endothelium. The endothelium provides a physical barrier (protective layer) between the blood stream and the coronary artery walls, while regulating the function of the artery by releasing chemical signals in response to various stimuli.

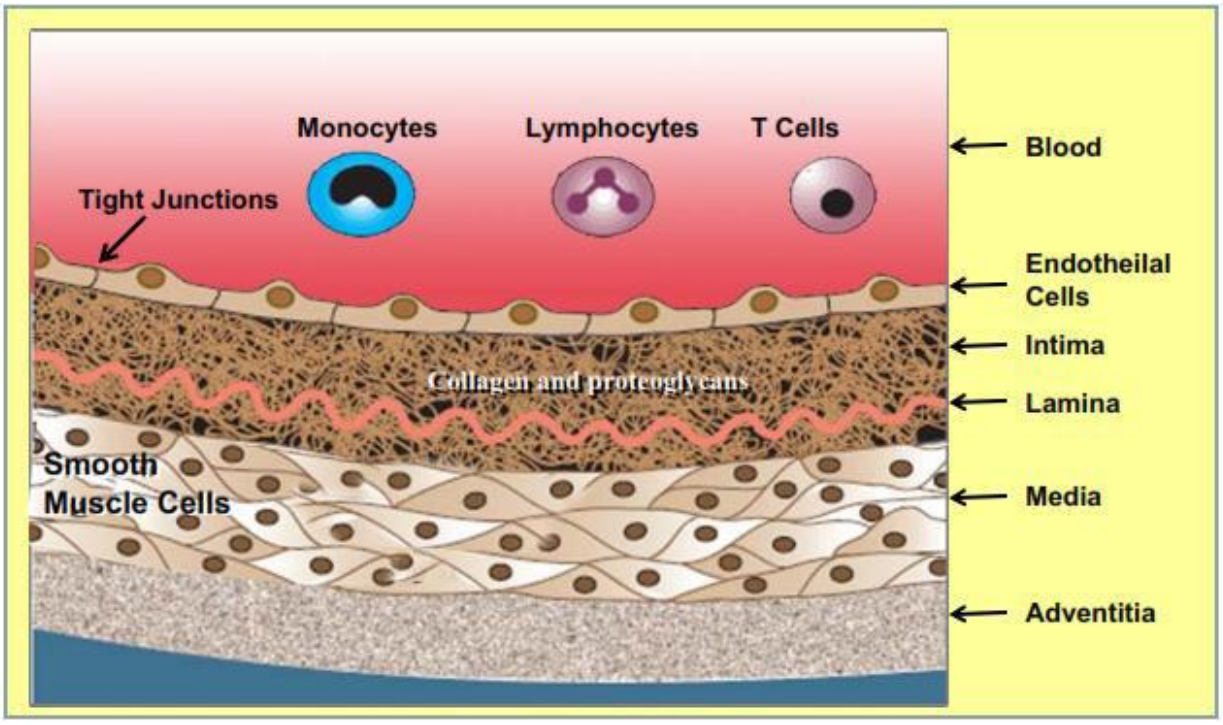
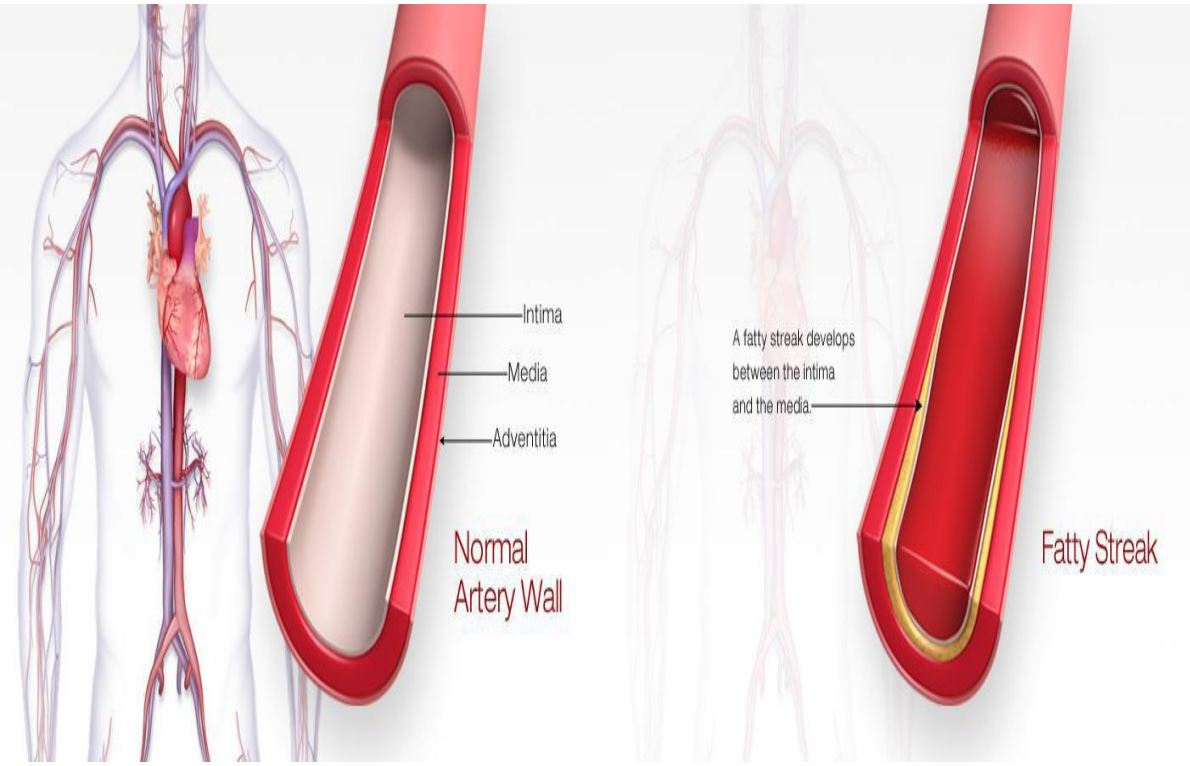


Figure 1.1 Structural arrangement of the coronary artery

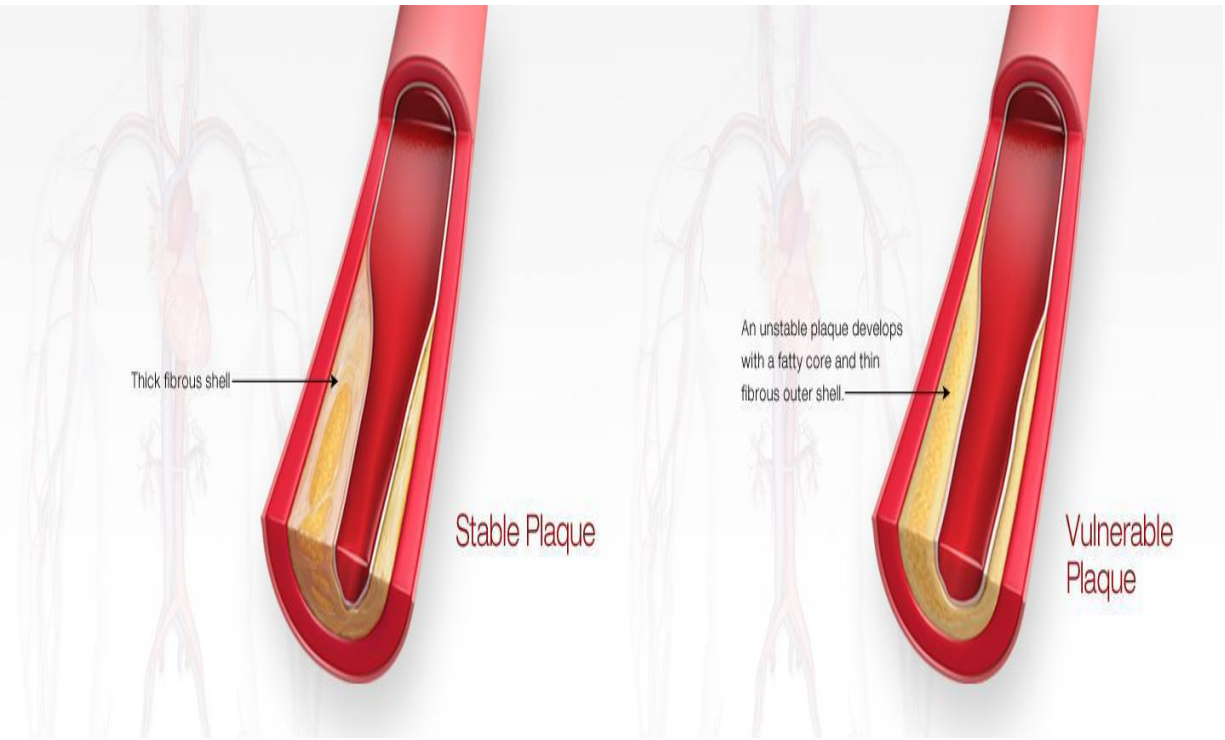
A fully developed artery should consist of three morphologically distinct layers. The intima, the innermost layer, is bounded by a monolayer of endothelial cells on the luminal side and a sheet of elastic fibers, the internal elastic lamina, on the peripheral side. The normal intima is a very

thin region (size exaggerated in this figure) and consists of extracellular connective tissue matrix, primarily proteoglycans and collagen. The media, the middle layer, consists of smooth muscle cells (SMCs). The adventitia, the outer layer, consists of connective tissues with interspersed fibroblasts and SMCs [5]. The pathogenesis of coronary artery disease (Atherosclerosis) is associated with the buildup or accumulation of cholesterol (low-density lipoprotein), fatty cells, calcium and other blood nutrients on the walls of coronary artery. Atherosclerosis starts when the endothelium becomes damaged, allowing low-density lipoprotein cholesterol to accumulate in the artery wall. The body instructs macrophage white blood cells to clean up the cholesterol. In the event of doing so, they sometimes get stuck at the affected site. Over time this results in plaque built-up, consisting of bad cholesterol (LDL cholesterol) and macrophage white blood cells [6]. The early stage of Atherosclerosis; Fatty streak, which is common in infants and young children, is a pure inflammatory lesion consisting of monocytes-derived macrophages and T-lymphocytes [7] (Figure 1.2 (a) [6]. As one advances in age, the fat keeps building up and damages the protective layer of the wall; the endothelium. This is termed endothelial dysfunction. Consequently, some of the fat, calcium and other nutrients within the blood escape in to the smooth muscles (Figure 1.2(b)) and affects the dilation and contraction of the smooth muscles which controls the flow of blood within the coronary artery. Over time, the inside of the arteries develop plaques of different sizes (c). Several of the plaque deposits are soft on the inside with a hard fibrous “cap” covering the outside. If the hard surface cracks or tears, the soft, fatty inside is exposed (d). Platelets (disc-shaped particles in the blood that patronize clotting) come to the area, and blood clots form around the plaque (e-f). The endothelium can also become irritated and fail to function properly, causing the muscular artery to squeeze at inappropriate

times. This may cause the artery to narrow even more. Sometimes, the blood clot breaks apart, and blood supply is restored. In other cases, the blood clot (coronary thrombus (g)) may swiftly block the blood supply to the heart muscle (coronary occlusion (h)), causing one of three serious conditions called acute coronary syndromes [8].

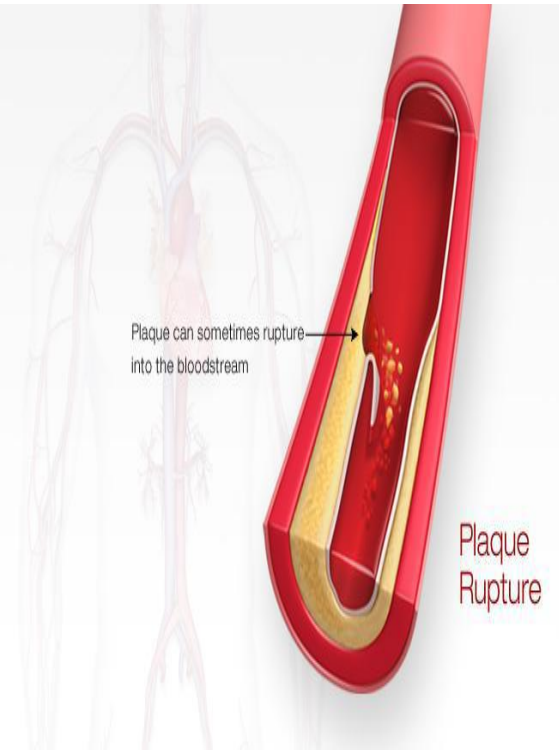


(a) Normal artery (b) Fatty streak

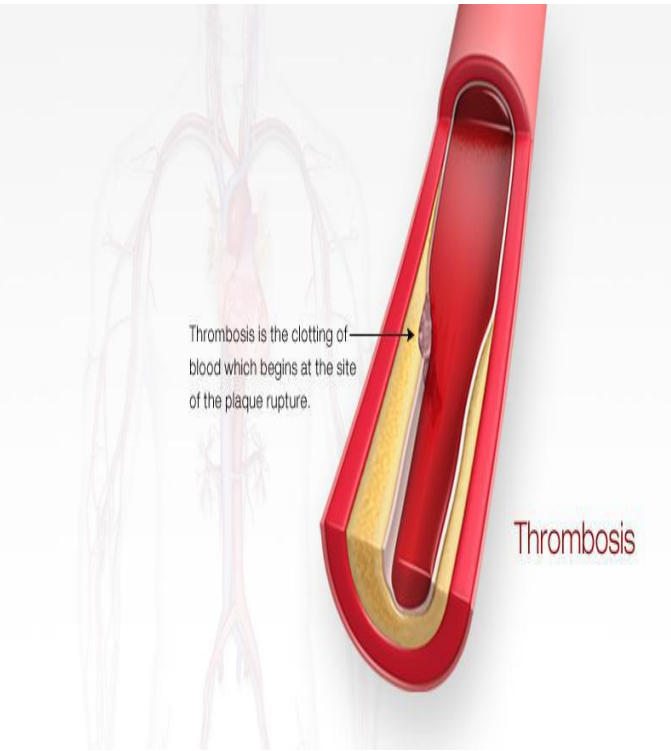


(c) Stable plaque growth

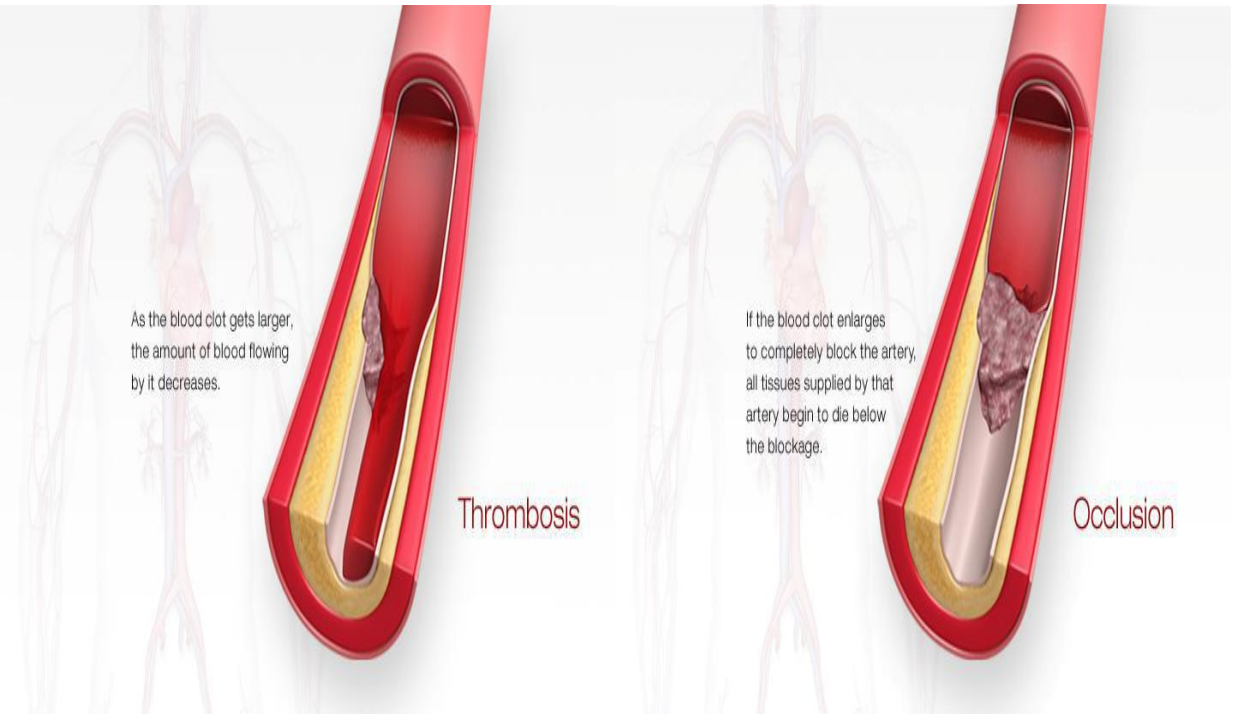
(d) Unstable plaque growth



(e) Rupture of plaque



(f) Thrombosis



(g) Thrombosis grows (h) Occlusion

Figure 1.2 Progression of Atherosclerotic plaque

1.2.2 Treatment Trends

A number of treatment options are available for the treatment of coronary Heart disease. They include but are not limited to angioplasty, bypass surgery, and medication. Why not a coronary bypass surgery or just angioplasty? A coronary bypass surgery is usually done by creating an alternative pathway through which the heart muscles can be fed with blood nutrients and other essentials. However, a lot more time will be required for wound healing than with the case of

stent insertion. Moreover, there could be a recurrence of the narrowing of the vessel after a few months. A mechanically robust, copolymer stent that releases anti-cancerous drug will therefore, more often than not, prevent thrombosis and restenosis after stent insertion. The first of such procedure for treating the disease was performed by Dr. Andreas Gruentzig on 14th September 1977 in Zurich, Switzerland on a 38 year old patient [9, 11]. The procedure was called Percutaneous Transluminal Coronary Angioplasty (PTCA).

Transluminal Coronary Angioplasty (PTCA) otherwise known as balloon procedure [5]. Furthermore, the establishment of a comprehensive knowledge and understanding that atherosclerosis is an inflammatory disease, offers novel opportunities for prevention and treatment of CAD. The use of efficient immunosuppressant or anti-inflammatory agents would serve to provide attractive treatments for acute coronary syndromes [2]. Cyclosporine, sirolimus and paclitaxel are just a few of the immunosuppressive drugs that inhabit the activation of T cells. At relatively high concentrations, they also prevent the proliferation of smooth-muscles cells by inhabiting intimal lesion. Previous generations of drug eluting stents have employed the use of sirolimus and paclitaxel polymers coatings for the prevention of restenosis after angioplasty [2].

1.3 UNRESOLVED ISSUES

There has been a considerable shift from the use of bare metal stent to the implantation of first and second generation stent primarily because of the early restenosis associated with its implantation. Additionally, first and second generation stents have been quite effective in the treatment of early restenosis but poses a high risk of late restenosis and thrombosis. These have been attributed to the Presence of durable polymer that induces inflammation and local drug

toxicity [15]. Also, preclinical analysis confirmed that these durable polymers were associated with delamination and webbed polymer surface subsequently leading to stent expansion followed by plastic deformation. Hence, the focus is now on the development of novel DESs with biodegradable drug carriers from which drug can be dispensed in a modulated manner.

Shown in figure 1.3 are cross-sections and platforms of a bare-metal stent (Section A) and a drug-eluting stent (Section B). Stent implantation causes an arterial injury that activates vascular smooth-muscle cells and drives their migration and proliferation, with extracellular-matrix formation resulting in the production of neointimal tissue. Extreme neointimal hyperplasia leads to restenosis within the treated segment, with ischemia requiring repeat revascularization [9]. DESs provide site-specific, controlled release of anti-proliferative agents targeting the suppression of neointimal hyperplasia. Anti-proliferative drugs used in in first and second generation DESs and their mechanisms of action are shown in Section C.

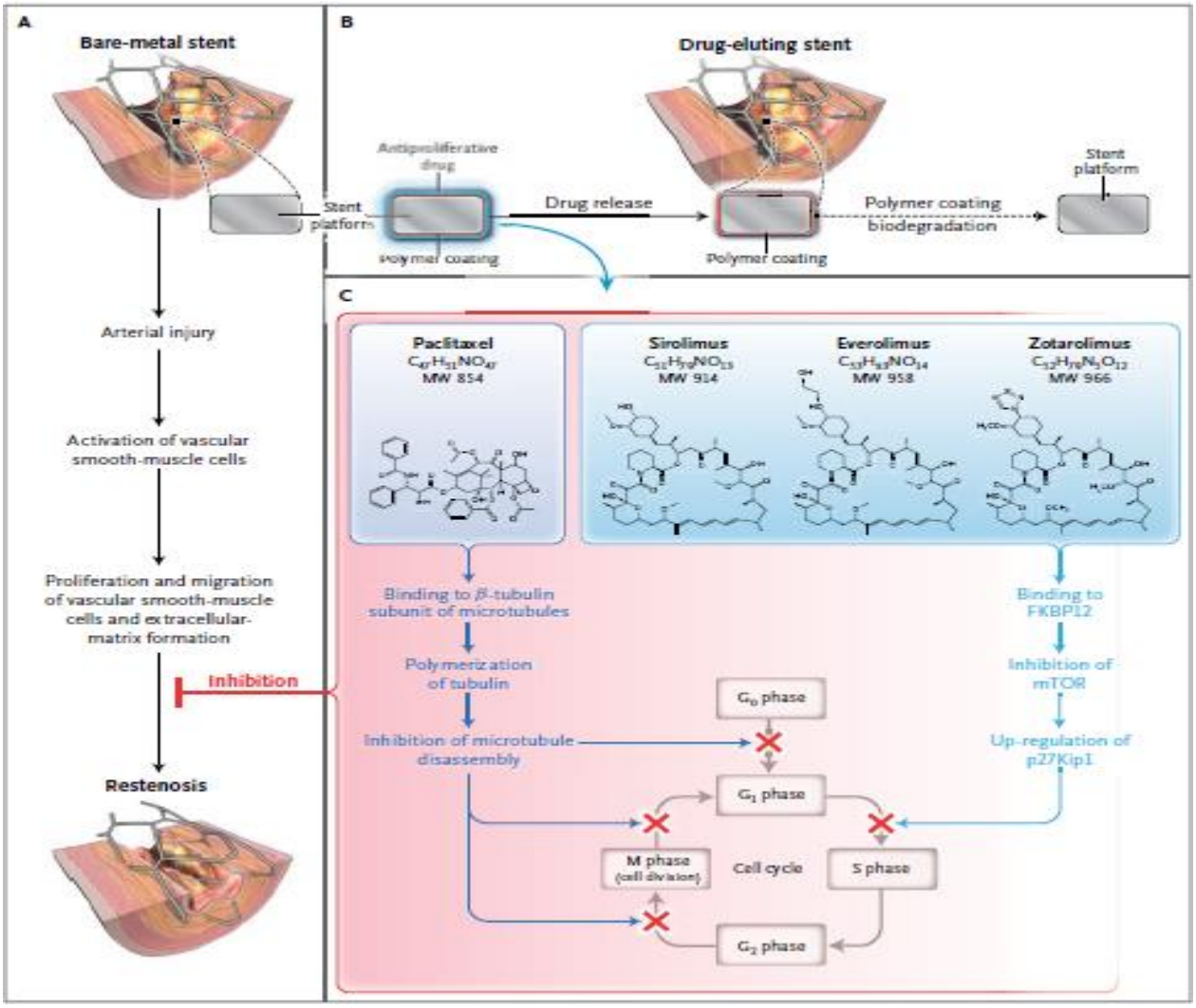


Figure 1.3 Mechanism of Stent Thrombosis [9, 10] and nejm.org, Retrieved, October 2014

These agents bind to the intracellular receptor FKBP12, impeding the mammalian target of rapamycin (mTOR), which results in up-regulation of cyclin-dependent kinase inhibitor p27Kip1. This blocks the proliferation of smooth-muscle cells in the gap 1 (G1) phase of the cell cycle [9]. On the other hand, paclitaxel, used in first generation DESs, binds to the β-tubulin subunit of microtubules, inhibiting the disassembly of microtubules and thereby arresting cell replication in the G0–G1 and mitotic phases of the cycle of smooth-muscle cells [9].

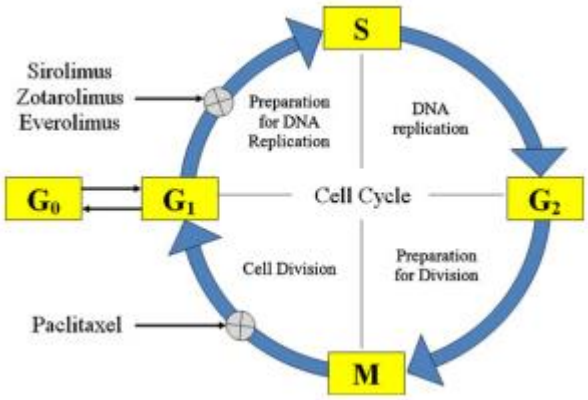


Figure 1.4 Cell-cycle and mechanism of action of sirolimus, zotarolimus, everolimus & paclitaxel.

In spite of all these precautions, stent thrombosis remains a critical issue to consider. Stent thrombosis, a rare but problematical phenomenon arising from the treatment of coronary heart disease, has been attributed to procedural factors and inadequate platelet inhibition during post implantation as well as to chronic inflammation and delayed arterial healing during late follow-up [9, 10]. Numerous definitions for stent thrombosis have been proposed by a number of researchers thus, creating lots of confusion. However, the Academic Research Consortium provides a standardized time-regulated definition (i.e., early, ≤1 month; late, >1 month to ≤1 year; or very late, >1 year) and the definite, probable or possible degree of certainty in diagnosis [9, 12].

Studies conducted by [13, 14] revealed that sirolimus and paclitaxel-eluting stents induced higher risk of very late stent thrombosis in comparison to bare metal stents.

Since the development and subsequent implantation of coronary stents in humans, concerns about their long term safety have frequently been raise. The need to exploit the safety of the bare metal stent combine with the efficacy of a durable biodegradable polymer in the formulation of a novel DES has ever been of paramount concern. The localized elution of the anti-proliferative drug from the proposed novel DES is expected to drastically inhabit restenosis as well as reduced thrombosis and target lesion revascularization rates.

1.4 SCOPE OF WORK

This work highlights the use of prodigiosin loaded PLGA polymer for cardiovascular stent coating. The mechanism of drug release from the polymer matrix, aimed at preventing restenosis, thrombosis and inflammation will be comprehensively explained. To this effect, the drug loading efficiency which corresponds to optimum therapeutic effect will be established. Diffusion and degradation release processes and their influences on the release of drug from the stent strut will be extensively elucidated. By measuring the change in drug concentration with respect to time, one can develop a model to fully explain the drug release profile. The Implication of the results thereof will be discussed for the design of drug eluting biodegradable, polymer-coated stents for cardiovascular interventions.

References

[1] Jung F., Wischke C., Lendlein A., Degradable, Multifunctional Cardiovascular Implants: Challenges and Hurdles, MRS Bulletin 607-610 (2010)

[2] G. K. Hansson; Inflammatory Atherosclerosis and Coronary Artery Disease, New England Journal of Medicine, 2005 10.1056/NEJM 200101113440217

[3] World Health Organization Statistics Atlas for Cardiovascular Disease, 2011

[4] Mathers C.D, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*, 2006, 3(11):e442.

[5] PTCA /stent procedure/ www.stormontvail.org

[6] Russell Ross: Atherosclerosis Inflammatory Disease, the New England Journal of Medicine, 005, 340

[7] David Martin et al.: Drug eluting stents for coronary artery disease (review), Med Eng Phys, 2010

[8] Sydell and Arnold Miller: Coronary Artery Disease Treatment Guide, Heart & Vascular Institute, Cleveland Clinic

[9] Stefanini, G. G., and Holmes, D. R., Drug-Eluting Coronary-Artery Stents, New England Journal of Medicine, 2013;368:254-65, copyright @ 2013, Massachusetts Medical Society

[10] Holmes D.R., Kereiakes, D.J., Garg, S., et al. Stent thrombosis. J Am Coll Cardiol 2010;56:1357-65.

[11] Holmes Dr. et al : Restenosis After Percutaneous Transluminal Coronary Angioplasty (PTCA), Am J Cardiol 1984:53 (Suppl C), 77-81

[12] Cutlip D.E., Windecker S., Mehran R., et al. Clinical end points in coronary stent trials: a case for standardized definitions. Circulation 2007;115:2344-51.

[13] Stone GW, Moses JW, Ellis SG, et al. Safety and efficacy of sirolimus- and paclitaxel-eluting Coronary Stents N Engl J Med 2007;356:998-1008.

[14] Räber L, Magro M, Stefanini GG, et al. Very late coronary stent thrombosis of a newer-generation everolimus-eluting stent compared with early-generation drug-eluting stents: a prospective cohort study. Circulation 2012;125:1110-21.

[15] Stettler C, Wandel S, Allemann S, et al. Outcomes associated with drug-eluting and bare-metal stents: a collaborative network meta-analysis. Lancet 2007;370: 937-48.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DRUG ELUTING STENTS (DESs)

A stent is a cylindrical, mesh-like structure designed for mechanical flexibility and long-term durability when implanted in an arterial vessel. They are often laser-cut from bulk metallic structures such as stainless steels or metallic alloys such as Co-Cr and Ni-Ti [1]. Their sizes are of the order of units of mm in diameters and tens of mm in length depending on where it is to be used. For instance, stents of the brain are much small while those of the legs and arms are longer. Polymeric stents with surfaces in direct contact with blood flow can initiate the secretion, activation, adherence and aggregation of platelets and trigger subsequent plasmatic coagulation and immunological responses, depending on the stent's hemocompatability. The sensitivity of the polymer to various environmental conditions as well as its degradability is a pertinent parameter to consider. Obtaining sophisticated and improved biocompatibility will be a major step going forward. The first version of coronary stents developed for percutaneous transluminal coronary angioplasty (PTCA) were bare metal stents and are typically made of stainless steel or cobalt-chromium alloy [1]. The risk of restenosis is quite high with bare metal stents since there is no elution of any anti-proliferative agent to inhabit the ingrowth of smooth muscle cells.

Essentially, drug eluting stents are formulated by integrating a polymer structure and a metal or composite material for control release of drug in an effort to prevent restenosis, thrombosis, target

revascularization (TVR) and other side effects. Usually the drug is encapsulated within the polymer and a release profile is carefully determined.

2.1.1 First Generation Drug Eluting Stents

Essentially, first generation stents include Taxus paclitaxel-eluting stent (PES) manufactured by Boston Scientific in Massachusetts, the US and cyphers sirolimus stent (SES) fabricated by both Cordis and Johnson & Johnson in New Jersey, the US. These stents were associated with very late stent thrombosis and an increase in restenosis rate as late as twelve months after implantation. These complications were, in part, due to the toxic nature of the drugs eluted. Sirolimus, for instance, has been reported to inhabit not only the proliferation of smooth muscles cells but also endothelial regeneration in vivo (Jeanmart et. Al, 3). They observed severe impairment of relaxant responses to serotonin and bradykinin in epicardial arteries exposed to sirolimus, suggesting a direct adverse effect of sirolimus on endothelial function. Furthermore, more recent data have reveal that sirolimus may also affect the growth and differentiation of progenitor cells [3]. As for the case of paclitaxel, it has been demonstrated that high-dose Taxol potentially inhabits not only smooth muscles cells but also endothelial cell proliferation and migration as well as vessel wall toxicity (Axel et al. and Farb et al.).

To achieve a sustained elution of the anti-proliferation drug, the Taxus^{TM} stent incorporates poly (styrene-isobutylene-b-styrene) triblock copolymer blend thus, creating a monolithic system where the release of drug to the physiological medium is by diffusion through the polymer matrix (23, 29., 2006; Ranade et al., 2005).

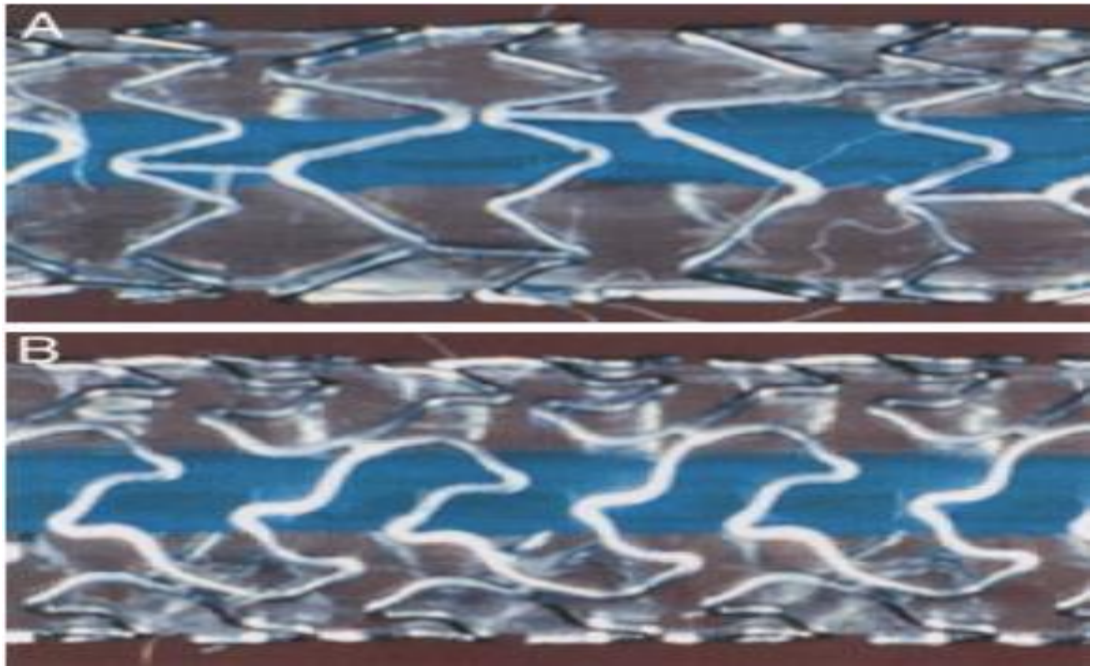


Figure 52.1.1 (a) Taxus Express2 PES and (b) Taxus Liberté PES

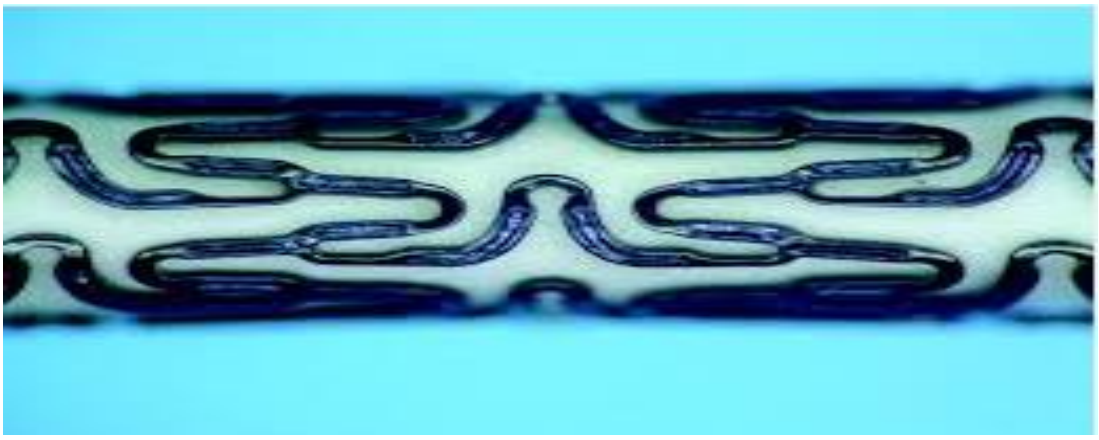


Figure 62.1.2 Cypher – Sirolimus Drug Eluting Stents

2.1.2 Current Generation Drug Eluting Stents

Current or second generation stents include the Endeavor Zotarolimus-eluting stent (ZES) developed by Boston scientific and Medtronic Vascular, CA, US as well as the Xience-V everolimus-eluting stent (EES) produced by Abbott Vascular, CA, US.

Current generation DESs are quite more superior to first generation DESs largely because of the incorporation of durable and reliable polymer structure for drug elution. A study by [9] compared first-generation stents to their second-generation counterparts. The results showed that second-generation drug-eluting stents have been associated with better clinical outcomes in randomized clinical trials, specifically by reducing the rates of stent thrombosis and early restenosis. A number of current generation stents have been available during the last decade and have solely been used for coronary cardiovascular procedures. Comparatively, the cobalt-chromium everolimus-eluting stent has been observed to be the safest and most efficient. Other current generation DESs include Magnesium composite stent, zotarolimus-eluting stents and platinum-chromium everolimus-eluting stent. The platinum-chromium everolimus-eluting stent has been observed to have the lowest rates of TVR.

Figure 72.1.3 ZotarolimusDrugElutingStents

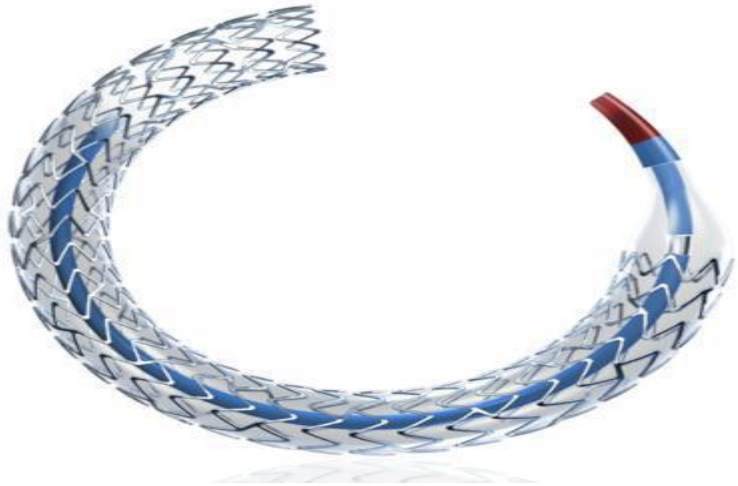


Figure 82.1.4 Xience – V EES drug eluting stent

2.2 POLY (LACTIC-CO-GLYCOLIC ACID) PLGA FOR STENT COATING

Polyglycolide (PGA), polylactide (PLA) and theirs co-polymer are members of the class of thermoplastic aliphatic poly (esters). In relation to design and performance, poly lactic-co-glycolic acid PLGA is probably the best known biomaterial for drug delivery systems. It can be fabricated into virtually any dimensional configuration and can encapsulate practically drugs of any size and shape [12]. For at least two decades now, PLGA has been among the most attractive polymeric candidates used to fabricate devices for drug delivery and tissue engineering applications. The use of PLGA as a drug delivery vehicle has yielded lots of dividend over the past years. PLGA is biocompatible and biodegradable, exhibits a wide range of erosion times, has tunable mechanical properties, has immense potential for sustained drug delivery and causes no inflammatory ramifications after its application or insertion in physiological environments.

Importantly, it is amongst the class of FDA approved polymers for the delivery of drugs, protein and various macromolecular components such as nucleic acids and peptides [12]. In particular, PLGA has been extensively studied for the development of devices for controlled delivery of small molecule drugs, proteins and other macromolecules in commercial use and in research. PLGA degradation products are non-mutagenic and non-cytotoxic, it metabolizes in the body and is excreted through the normal biological pathways and its aerobic degradation end products are carbon dioxide, water and minerals [10]. The commercial availability of PLGA with different molecular weights, co-polymer ratios and various chemical end groups provide a wide range of options for its pharmaceutical applications. Recent studies have shown that the drug release capabilities of PLGA can be manipulated by altering and subsequently controlling important physical parameters such as molecular weight of the co-polymer, the ratio of lactic acid to glycolic acid and the polymer-matrix drug concentration to achieve the desire release profile of the particular drug under consideration.

Polymer lactic acid possesses an asymmetric α-carbon which is stereochemically designated as the D or L form. Therefore, poly D- lactic acid (PDLA) and poly L-lactic acid (PLLA) are enantiomers of PLA which are generally branded PLGA when there are equal amounts of D and L lactic acid [12]. From a general perspective, PLLA is the crystalline constituent of PLA while PDLA is completely amorphous due to disordered polymer chain [12]. In a related case, poly glycolide is highly crystalline due to the lack of methyl side groups in the structure in comparison to PLA [10, 12]. The presence of methyl side groups in the structure of PLA ensures greater hydrophobicity, less water absorption and slower degradation rate. The degradation rate of drug entrenched PLGA polymer can therefore, be altered by varying its co-polymer ratio. An

increase in the PLA content in the PLGA matrix decelerates the degradation and hence, the molecular weight loss of the PLGA. For this reason a PLGA with equal ratios of PLA and PGA will degrade faster than that with higher ratio of lactide to glycolide.

PLGA 50:50 (PLA/PGA) exhibited a faster degradation than PLGA 65:35 due to preferential degradation of glycolic acid proportion assigned by higher hydrophilicity. Subsequently PLGA 65:35 shows faster degradation than PLGA 75:25 and PLGA 75:25 than PLGA 85:15 [12; 13]. PLGA has a Tg of 45-50 ^{0}C and an inherent viscosity of 0.5-0.8 mPa [14]. Consequently, the Tgs of the co-polymers of PLGA are well above the normal body temperature (37 ^{0}C) and as such, they are normally glassy in biological environments. This further gives them slightly rigid chain structures, and hence, accounts for the significant mechanical stability they possess to be fabricated as implantable biodegradable devices. Reports have claimed that as the lactide content in the co-polymer composition decreases, the Tg of the PLGA blend decreases as well with decreasing molecular weight [14].

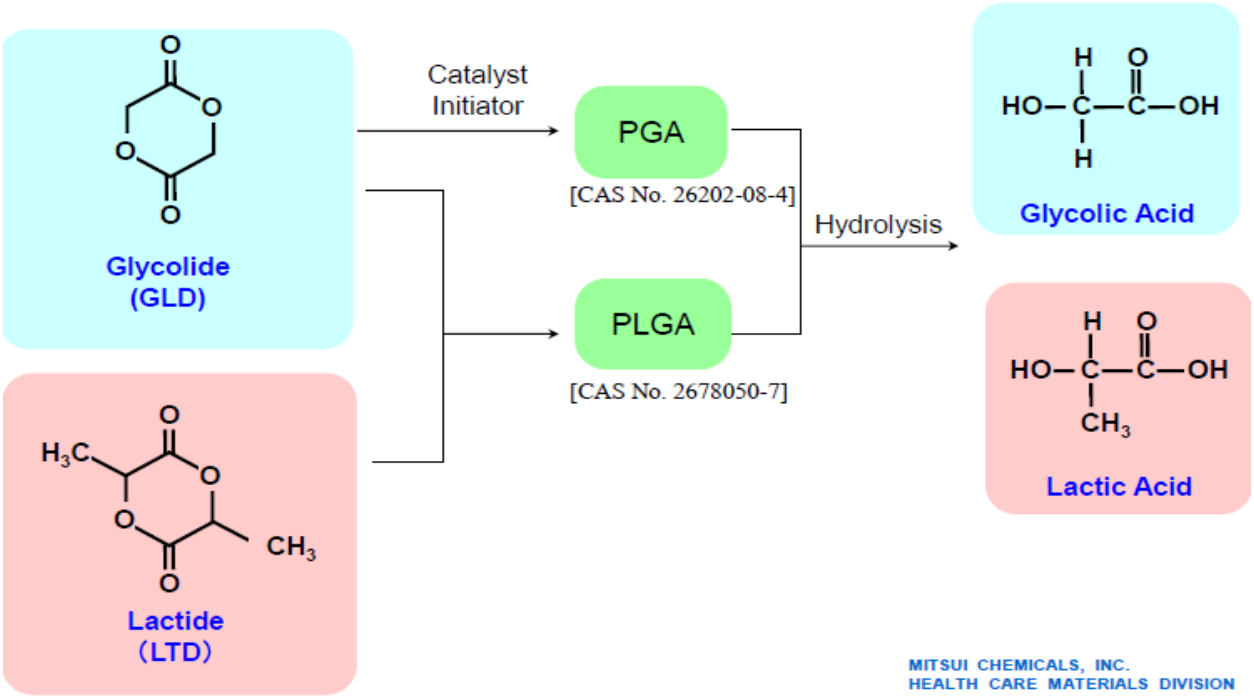


Figure 912.2. Ring Opening Polymerization of PLGA or PLA

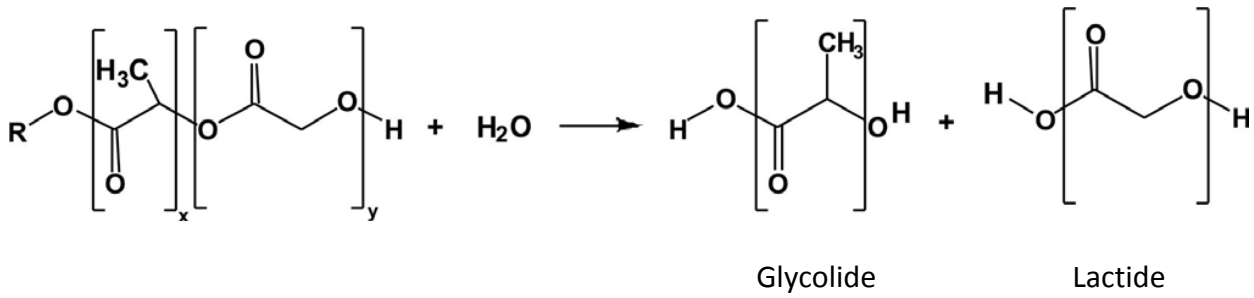


Figure 102.2.2 Hydrolysis of Poly (lactide-co-glycolide) PLGA

2.3 PRODIGIOSIN

Prodigiosin is a family of natural tripyrrole red pigments characterized by a common pyrrolylpyrromethane (4-methoxy, 2-2 bipyrrole) skeleton ring system and are produced by various bacteria that are first characterized from *Serratia marcescens* [4, 15]. Numerous studies have suggested that this pigment possesses antifungal, anti-cancerous, anti-proliferative, anti-malaria and immunosuppressive characteristics thus, making prodigiosin a hugely promising drug with commercial significance. The biosynthesis of the pigment is a bifurcated process in which mono and bipyrrole precursors are synthesized separately and then assembled to form prodigiosin [16]. Maheswarappa et al. reported that prodigiosin induces apoptosis in human cancer cell line. They used the pigment to treat three different cell lines: Vero cell line (African green monkey's kidney epithelial cells) for evaluating apoptotic property, HeLa cells (Human cervical cancer cell line) and Hep2 cell line (Human laryngial cancer cell line) for the assessment of anti-cancer property of prodigiosin pigment [15]. They also reported a dose dependent cytotoxic and apoptotic behavior of the prodigiosin pigment. Their results also showed a dose dependent antineoplastic activity of prodigiosin pigment. This antineoplastic activity was different for different cancer types and more effective for Hep2 cells than HeLa cells [15]. In another study, Zhang J et al. [17] demonstrated that prodigiosin effectively inhibit tumor metastasis in vitro and in vivo. Metastasis forms as a result of series of interactions between tumor cells and the physiological environment hosting them. During metastasis, there is initially detachment of the malignant cells from the primary tumor and subsequent evasion into surrounding tissue [18]. Greater interaction, optimum attachment and little or no migration is expected amongst cells of a normal tissue. This is actually not the case with malignant cells

which are generally loose and can detach from primary tumor and drift outward [19]. Zhang J et al. observed that prodigiosin could effectively inhabit the migration of cancer cells in vivo with EC_{50} less than 5μM. They also reported that prodigiosin can facilitate the adhesion of cancer cells to each other in an aggregated fashion thus, preventing the initial spread of tumor cells. The attachment of cancer cells to the extracellular matrix (EM) is very key to the spread of the tumor cells and critical to the initiation of metastasis. The adhesive ability of cancer cells can be reduced by half if bombarded with 2.5 mM prodigiosin or higher [17]. Prodigiosin's ability to inhabit cell adhesion is likely to contribute to the inhibition of metastasis. An investigation carried out by Pandi-Suba, K. et al [21] revealed that prodigiosin possesses sufficiently reasonable apoptotic efficacy as it effectively induces caspases-3 and 9 but inhibit TNF-α either directly or through other mechanisms. They further observed that prodigiosin induces apoptosis in human oral cancer HSC-2 cells and can therefore be used as potent anti-cancer agent [21].

2.4 COATING OF STENTS

Traditionally used industrial coating techniques such as ion beam deposition, chemical vapor deposition, plasma vacuum technology, etc. are incompatible with the coating of DESs [1].The primary technique for applying coatings to stents is called spray nozzles although, other deposition techniques are currently being evaluated. When applying coating to a stent, the uniformity of the coating (both internally and externally), its lack of hole and other defects and its reproducibility, with little change in weight from a stent to the next, must be high on the agenda [1]. Lots of those challenges are, however, encountered when trying to properly coat a stent. Webbing, irregular surface texture and rough surface are all morphological incoherencies that degenerate into potential problems after a stent has been coated.

2.4.1 Hot-Melt Extrusion

Hot-melt extrusion was historically used for the production of plastics before gaining widespread acceptance in fields of the pharmaceutical industry [6,7]. This study employs a method similar to that of the hot-melt extrusion process for the encapsulation of the anti-proliferative drug prodigiosin. Hot-melt extrusion is considered quite a proficient method for the preparation of biodegradable implants for sustained drug delivery. The sole purpose is to have uniform drug dispersion throughout the polymer matrix. As opposed to couple of other methods, this method does not require the use of biocompatible solvents for dissolution of the polymer. Hot-melt extrusion or simply melt extrusion involves the melting, mixing, and forcing of the mixture through a small opening called a die. Heating of the polymer to an elevated temperature preferably 15-60 ^{0}C above its T_g provides the required viscosity needed for the incorporation of the drug [6]. However, cognizance must be taken against the use of very high temperature since this can thermally degrade the drug thus, causing it to lose its potency. A number of previous works have recommended that the maximum extrusion temperature be with the range of 50-100 ^{o}C. The variation in temperature is dependent on factors such as drug loading, molecular weight of the polymer, and the type of the hot-melt extruder used [8,5]. Drug embedded polymer matrix prepared via this method is basically a matrix system. In some instances, a homogeneous extrudate, resulting from a blend of the polymer and the drug is achieved by premixing. Henceforth, the PLGA polymer carrier serves to act as a thermal binding, holding firm the drug particles in its matrix [6]. However, under physiological conditions when hydrolysis of the polymer backbone is prevalent, this binding strength relaxes and the active agent is released by means of diffusion. In fact, a study conducted by Schwach et al. showed that a drug, protein or

peptide, which is sensitive to organic solvents or water can still be incorporated into the polymer matrix via the hot-melt extrusion process at even higher efficiency. For instance, they showed that degarelix had greater stability when the drug delivery system was prepared by means of hot-melt extrusion process compared to solvent associated methods such as double-emulsion solvent evaporation and spray drying [11]. The result of their experiment was explained in terms of shielding effect. A higher dispersion efficiency of peptide in the PLGA matrix ensured greater shielding effect in the hot-melt extrusion process [11]. In other studies, the peptides nafarelin and melanotan-I were accessed to ascertain their cytological proficiency after encapsulation by various solvent related methods and hot-melt extrusion. A negligible loss of biological activities was reported using hot-melt extrusion processing [52]. Antipsychotic, anti-inflammatory drugs, for instance, haloperidol and diclofenac sodium, have been loaded into biodegradable implants via hot-melt extrusion [53]. The drug loading efficiency could reach 40% by weight and sustained releases can be observed. A two or three phase release profile was observed as in implants prepared by dissimilar methods. The initial phase of the release profile is, however, different. If the matrix is dense enough, less burst effect could be realized [5, 6].

2.4.2 Spraying of Stent

Industrially, two types of spray nozzles are currently being considered: twin nozzle and ultrasonic nozzle [1]. The technology incorporating the use of ultrasonic nozzle is perceived as the best spray method for attaining the wanted results. In this application, the flow rate requirement is in the range of 5-100 µl/min and the spray diameter is expected to be of the order of 0.5-2 nm [1]. Ultrasonic nozzle, which is mounted above the stent, is sophisticated enough to fulfill all these requirements. In this technique, the stent is allow to rest on a mandrel that is in

turn attached to a rotating shaft that also translates so that the stent is sprayed along its entire length. A number of traverses would be needed to attain the desired weight of the coating. Essentially, the sprayed medium is composed of the polymer and the drug dissolved in an appropriate high vapor organic solvent to propel the rate of drying. The best coatings and optimum material transfer efficiency can be achieved by repeated traverses coupled with low flow rate. Varying rotational speed, distance of spray from the stent, and the number of traverses would be added advantages in an effort to optimize the process. These processes are usually carried out in an environment containing adequate nitrogen since nitrogen promotes better liquid flow characteristics, by lowering the surface tension of the liquid as it contacts the stent surface [1].

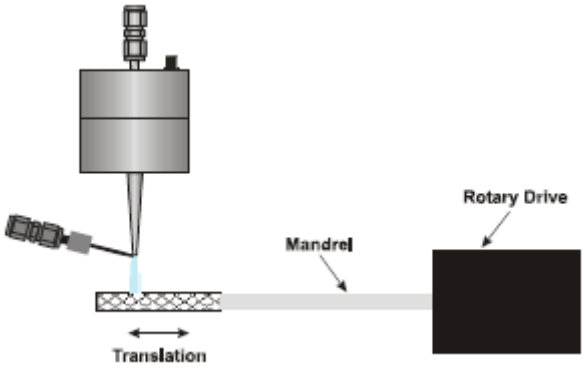


Figure 11 2.4.1 Ultrasonic Nozzle Spray System [1]

2.4.3 Ultrasonic Nozzles for Stent Coating

Principally, there exist two ultrasonic nozzle designs that are commonly used in coating of stents. Ideally, both architectures rely on the use of a gas stream with low-pressure to shape the slow-moving drops into a narrow spray beam. The two designs operate at a frequency of 120 kHz. The first of the two designs (nozzle A) is represented in Figure 2.4.2 (a). Compressed gas, typically at 1 psi, is infused into the diffusion chamber of the gas shroud, which produces a uniformly distributed flow of air around the nozzle stem**.** An adjustable focusing mechanism on the gas covering allows complete control of spray width. The spray envelope is conical. Moving the focus-adjust mechanism in and out controls the width of the bow. The distance between nozzle and substrate can be varied from near contact to approximately two inches. The narrowest beam diameter achievable at the focal plane is of the order of mm [1].

The second design (nozzle B) is illustrated in Figure 2.4.2 (b). It supports the feeding of liquid to the surface of the atomizer through an externally mounted cannula. The gas is fed through the opening of the nozzle. The gas stream draws the spray to it thus, creating an extremely narrow spray beam, as small as 0.5 mm [1]. The distinguishing feature of this technique, of course is that, the liquid feed is external. In other words, the liquid is completely isolated from nozzle vibrations up to the time that atomization occurs. This translates into a high degree of spray stability and a greater degree of reproducibility, from one spray cycle to the next.

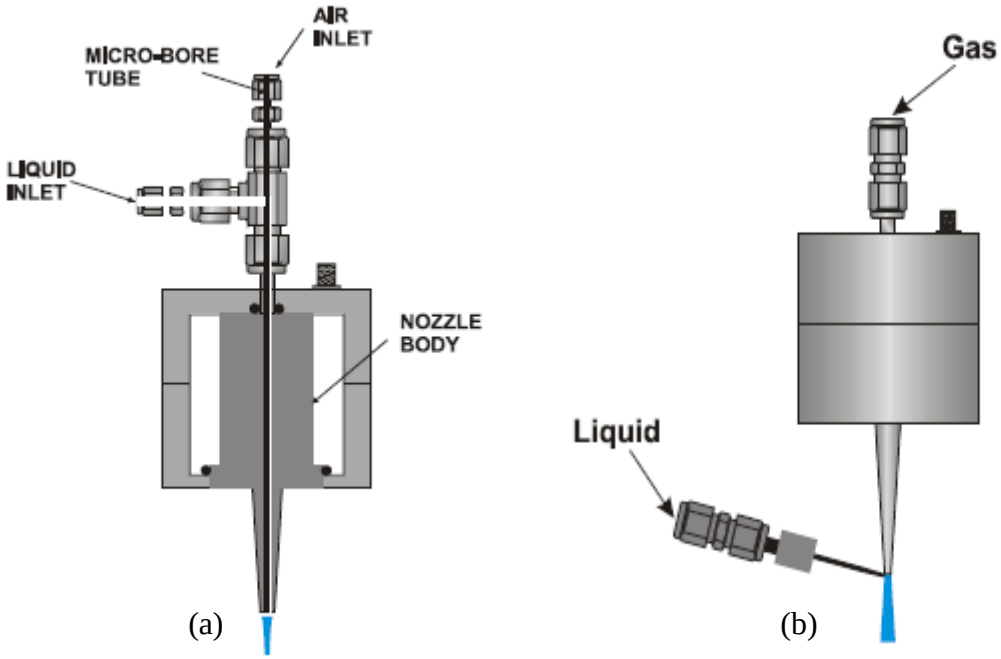


Figure 12 2.4.2 (a) Ultrasonic Nozzle with Internal Liquid Feed (b) Ultrasonic Nozzle with External Liquid Feed

The following are advantages of ultrasonic spray for stent coatings:

a) Highly controllable and repeatable spray

b) Non-clogging ultrasonic technology

c) Ability to spray at flow rates as low as 5 microliters per minute

d) Droplet sizes as small as 13 microns (with water) with very tight drop distributions

e) Low velocity spray adheres to stents without bounce-back or overspray

f) Proven process for coating implantable stents & other precision medical devices

g) Deposits highly durable coatings which do not flake or peel

h) Over a decade of experience, coating with hundreds of systems in operation

2.4.4 Electronanospray

Electrospraying has been traditionally used for applications such as surface coatings, agricultural treatment, emulsion, fuel spraying, micro-encapsulation, ink-jet printers and colloid micro-thrusters. The electronanospray mode can be used to produce airborne nanoparticles from a solution of colloidal suspension of desire solute materials, typically in a size range of 2–100 nm, hence, the term 'Electronanospray' was coined [20]. In its simplest Set-up, a syringe pump is utilized in delivering drug-polymer solution or suspension into a capillary tube. As the liquid exits the capillary tube, it is exposed to a high electric field established between the tips of the capillary, to which a high voltage is applied, and a target that is grounded or oppositely charged. This non-uniform electric field causes the liquid meniscus to assume a conical shape (Figure 2.4.3), established by the balance between the surface tension force and the electrical force on the cone. The liquid is then distributed through the cone tip in the form of a liquid jet of nanometer diameter. The liquid jet becomes highly unstable and subsequently splits up into nanoparticles, each carrying a very high charge level [20]. The particles repel each other in the gas phase due to their positive charge characteristics thus, allowing them to solidify as independent nanometer-sized objects. Flow rate and conductivity of the solution are the key variables that govern particle size when operating the electrospray in the cone jet mode [20].

Other factors, such as the diameter of the feed capillary, viscosity of the fluid and volatility of the carrier solvent influence particle size as well. The use of a spray nozzle comprised of coaxial capillaries will produce highly uniform particles and provide substantial process flexibility. In a study carried out by [20], on the release profiles of smooth and particulate films over a period of 28 days, it was revealed that both films displayed gradual release, but the rate was much slower for the continuous film. The cumulative release was ~14% for the smooth film and 25% for the particulate film in 28 days [20].

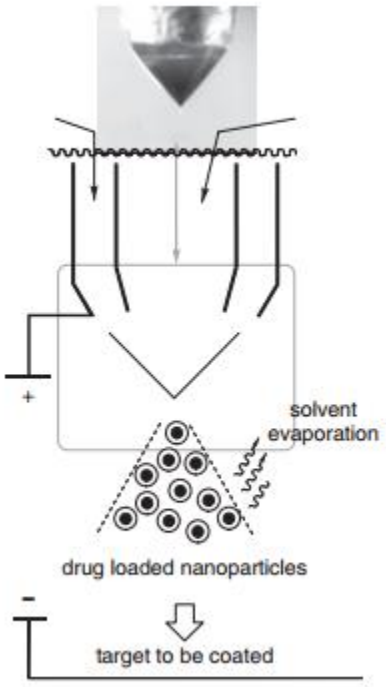


Figure 13 2.4.3 Schematics of Electronanospray Architecture

J. E. Puskas et al., Drug-eluting Stent Coatings, John Wiley & Son s, Inc., 2009

2.5 CONTROL DELIVERY OF DRUG

Conventionally, drug delivery has most notably been via oral and intravenous administration. High concentration of drug in the plasma resulting in growing side effect, as well as long and frequent administration to compensate for the short duration of action have been some of the drawbacks of the tradition system (Edlund and Albertsson 2002). The physiological significance of drug release in a modulated fashion cannot be overemphasized. Exploits of the parental systems for drug release are proving much greater efficacy since they provide consistent, predictable, desirable and reliable drug release profiles. Maintenance of drug concentration within the therapeutic window, enhancement of patients response by decreasing invasive administration and dosing frequencies, increasing specificity and reducing systemic adverse effects for targeted delivery, the prospect of achieving a precise drug release rate and achievement of long drug half-life especially when peptides and proteins are used are all essential advantages offered by the parental system [10].

For the case of drug eluting stents (DESs), control delivery of drugs is achieved firstly by encapsulating a solution or powdered form of the drug within the micro-porous polymer structure [22, 23]. There are a number of drug release methods, however, owing to the highly demanding nature of an outstanding cardiovascular stents, only a few of these methods are suitable for application. An engineer designing a drug delivery stent system must have a clear and comprehensive understanding of the physiology of the arterial vessel and the mechanisms by which restenosis and thrombosis occur after stent insertion procedures. Environmental influences such as flow rate, temperature, pressure and humidity will usually have their respective contributions to the elution of the drug via a specified elution profile. This controlled released

process should also ensure that the drug is eluted at sufficient and relatively constant concentration with no cytotoxicity. In other words, the method chosen must be within the save and effective dosage regime of the drug under consideration [23]. There must not be a huge variation in the amount of drug exposed to the procedural site at any given time so as to avoid complications such as slow healing, inflammation and thrombosis. It is therefore quite important to scrupulously study the release kinetics and arterial dosage of the drug to avoid any medical complications. Importantly, the drug eluted is to ensure anti-restenosis, anti-thrombosis, anti-inflammation and must serve to heal the site from which the plague has been expelled. One important objective is to inhabit the formation of scar cells and the proliferation of smooth muscles cells without influencing the endotheliszation process of the endothelial walls [23, 24].

The influences of diffusion and convection are very essential in determining the transport, dissemination, dispersion, and maintenance of the drug in the vessel wall [23, 25, 26].

The water repellent or water attracting ability of a drug, its pharmacological nature and the structural nature of the artery all have significant influences on the release kinetics of the drug and its absorption at the interface between the stent and the artery. [23, 27, 28]. the drug release rate is a function of several factors including physiological transport parameters, the nature of the polymer encapsulating the drug, the amount of the drug to be released, the determination of the release kinetics and all are very crucial in exploring the efficacy of the coated stent structure in the prevention of restenosis, thrombosis and other adverse effects.

2.5.1 Drug Release Mechanisms

A number of mechanisms have been proposed for drug release in many different applications. Intuitively, a stent system should comprise a metal with sufficient mechanical strength and stiffness since it remains in the arterial wall permanently. The drug is encapsulated in a bioabsorbable, micro-porous polymer from which it is released at a control rate and concentration. The outer section of the system could be covered with a thin, drug-free sheet at the top of the polymeric layer for the sole purpose of further modulating the release rate. The release mechanism need be clearly defined by an empirical relation that accounts for the effect of all the major contributing factors. To this end, a diffusion controlled process will provide a lot of insight as to how the release proceeds with respect to time. Two schemes are used to classify current stent systems: 1) Monolithic structure in which the drug is disseminated in a polymer matrix to be release by controlled diffusion through the matrix; and 2) Reservoir system in which the drug is enclosed in an inner compartment which is in turn surrounded by a thin polymer membrane and the drug diffuses to the surrounding through the thin, rate-modulating membrane. The two schemes have been of practical success and DESs incorporating this scheme have proven safety and proficiency in clinical trials [23, 29; 30].

A research conducted by Kothwala et al. [31], using a biodegradable polymer blend of polylactides and co-polymers of polylactide-co-glycolide revealed an initially high drug release rate in simulated physiological fluids and then a drop in release rate in latter stages. Consequently, they attributed this initial burst followed by slow release to the incorporation of amorphous polymer in the upper layer and the subsequent insertion of highly hydrophobic and semi-crystalline polymer in the base layer respectively. Furthermore, an investigation also

revealed that after incubation, coated thin films were observed to be porous and there were glimpses of swelling. This indicated an initial water absorption leading to an early burst due to surface dissolution followed by polymer matrix degradation and slow drug release rate. What this means is that, a stent system incorporating a biodegradable polymer and/or a biodegradable copolymer blend will patronize drug release controlled by diffusion, dissolution and swelling, to be followed by degradation of the drug encapsulated polymer matrix.

Raval et al. [32] observed that by coating a stent, in multiple layers, using similar polymers, they could attain adequate control over paclitaxel drug release. They recorded different release rates depending on the compositions of the respective layers and the inherent structural and chemical properties of the biodegradable polymers such as crystallinity, hydrophilicity and hydrophobicity, molecular weight and co-polymer stereochemistry.

Further experiments by Alexse et al [33] with the biodegradable stent (made up of poly-DL-lactide and poly-DL-lactide-co-glycolide) prototypes were conducted. The anti-proliferative drug paclitaxel and the immunosuppressive drug rapamycin were incorporated separately. The result revealed that the drug release phenomenon showed a slow diffusion controlled phase followed by a rapid degradation controlled phase. Controlled elution kinetics were achieved by careful selection of the drug-embedded polymer blends and using suitable formulation of the matrix, while no burst effects were observed.

In another work done by Wang et al. [34], a multilayer sirolimus-eluting, completely biodegradable polylactide-co-glycolide (PLGA) stent, prepared with multiple layers was investigated. The release kinetics of sirolimus form the bilayer Poly-L-Lactide and PLGA

coating was found to depend on factors such as different drug loadings, effect of plasticizers in the formulation and the presence of drug-free top layer for retarding the release rate.

2.5.2 Factors Influencing Drug Release

Basically, the two controlled release systems used for DESs have been identified. These assemblies, either as a drug-embedded polymer matrix or reservoir archetecture are relatively easy to design but yet, they have proficient, controllable and desirable drug release rate [23]. Their release kinetics are significantly influenced by all contributions leading to the formulation of the DES and the principal processing parameters under which it is coated. It is therefore, important to thoroughly understand how each of these constraints influences the release process since any one of them can have a great effect on the release kinetics and they can also affect the process mutually [35]. In addition, a number of other biochemical phenomena should be investigated in parallel. Diffusion-convection drug transport, physiological properties of the tissues, ultra structure of the artery and hydrodynamic conditions at the site of implantation and the stent design significantly modulate the release rate and final physiological response to DESs [35].

2.5.3 Drug Delivery from stents

The need to develop appropriate and efficient coating strategy aimed at preventing late stent thrombosis and hypersensitivity, incomplete drug elution, and a suboptimal drug elution rate is crucial and cannot be over emphasized [23]. The laser drilled struts of a Co-Cr stent developed by (Conor Medsytems) contained holes that functioned as drug-polymer embedded reservoirs to

achieve control release kinetics to cope with restenosis [36]. Control paclitaxel elution was attained by full erosion of PLGA polymer and drug diffusion and in a controlled direction [35]. Over the last two decades, a number of naturally occurring substances have been studied and found to be appropriate vehicles for stent coatings when blended with a biodegradable polymer. They include, but are not limited to, chitosan, phosphorylcholine, hydroxyapatite, collagen, fibrin, antibodies, cells materials like glycocalix, and cellulose [37, 38].

No single mathematical or empirical model has been able to fully explain all drug release phenomena from implantable devices since there are couple of influences associated with the release process. The difficulty in providing appropriate explanation is due to the changes in the physical and chemical properties of the polymer due to swelling and de-swelling associated with diffusion of drug through the micro-porous polymer medium as well as erosion or degradation.

Even though, polymer degradation and erosion are often used interchangeably, there is a fundamental difference between the two. Polymer degradation simply refers to the cleavage of polymer chains into monomers and oligomers. Polymer erosion on the other hand refers to loss of materials from the polymer matrix thus, resulting in a decrease in weight or mass. Degradation usually precedes erosion and results in a decrease in molecular weight [10]. The degradation process is usually viewed as a component of polymer erosion which also involves swelling and mass transfer. In theory, polymer erosion can be categorized as either surface or balk erosion [10]. Surface or heterogeneous erosion is observed when the degradation of the polymer backbone is faster than the diffusion of water through the polymer matrix. This results in polymer-matrix size reduction as a function of time [10]. On the other hand, if the diffusion of

water is faster than the polymer degradation process, a bulk or homogeneous erosion phenomenon is observed. Under this process, the degradation of the polymer is uniform throughout the polymer membrane [10, 39, 40].

Polymer corrosion is often thought of as polymer degradation. Polymer degradation refers to the hydrolytic or enzymatic cleavage of covalent bonds holding in place the polymer structure. It is pH and temperature dependent phenomenon. Plasticizers allow and promote molecular motion.

A number of factors influence the degradation rate of PLGA. They include, but are not limited to, molecular weight, pH, temperature, size, the presence of additives and processing routes [39, 40]. PLA possesses a much higher degree of hydrophobicity than PGA due to the presence of a methyl group in the structure. This high degree of hydrophobicity reduces the intake of water by the polymer backbone. For this reason, the degradation of rate of PLA is much slower than that of PGA [41]. It is then intuitively clear that the degradation of PLGA is significantly affected by the co-polymer ratio. Faster polymer degradation will occur with a higher ratio of glycolide in PLGA if the two constituents have similar molecular weights. Furthermore, the degradation of PLGA is due to the hydrolysis of ester bonds, therefore, easier polymer hydration is attained with the greater hydrophobicity of laclide. This creates the platform for the water to reach into the ester bonds into the backbone of PLGA [10; 42,44].

Drug release from implantable devices is governed either by diffusion phenomena and /or polymer erosion. Ideally, many governing equations have been developed to guide the drug delivery and how the device can be controlled to ensure that the drug concentration remains

within the therapeutic window. Most of these models patronize diffusion controlled release kinetics. Essentially, a diffusion controlled drug release model of this sort was suggested by Siepmanna and Peppas [45]. This model suggests that solute (in this case, the anti-cancer drug) released from the PLGA polymer matrix can be modulated by diffusion and/or viscoelastic relaxation of swelling. According to Siepmanna and Pepass, the power law relation for this model is given by:

$$\frac{W_t}{W_\infty} = 4\left(\frac{Dt}{\delta^2}\right)^n = kt^n \qquad (1.0)$$

where k is a constant relating the structural and geometric characteristics of the controlled release that also incorporates the diffusion coefficient, D; W_t and W_∞ are the weight of the drug released at time t and infinite time respectively, so $\frac{W_t}{W_\infty}$ is the drug release fraction at time t, and n is an exponent that indicates the release mechanism; a value of 0.5 signifies a Fickian diffusion, while a value of 1 suggests a viscoelastic mechanism, and values between them correspond to anomalous (non-Fickian) transport or coupled mechanism. Equation (1.0) can be used to describe the diffusion controlled drug release and viscoelastic swelling of the PLGA membrane [46, 47]. Taking logarithm of both sides of equation (1.0) leads us to deduce an equation for the release of the anti-cancer drug from the polymer matrix [46, 47].

$$\text{In}\left(\frac{W_t}{W_\infty}\right) = \text{In k} + \text{n ln t} \qquad (2.0)$$

By plotting $\ln\left(\frac{W_t}{W_\infty}\right)$ versus ln t, we can obtain the values of n and k.

Also, from equation (1.0), we can obtain the diffusion coefficient, $D = \frac{kt\delta^2}{4}$ (3.0)

where δ is the thickness of the PLGA microparticles.

By determining these quantities (the drug release fraction at any time t, the weight of the drug release at both time t and infinite time, the diffusion coefficient of the released drug and the exponent that qualifies our release mechanism) for our stent device, we can efficiently ensure that the release of the anti-cancer drug remains within the therapeutic window, i.e., not exceeding the toxic, overdose level and not going below the minimum effective level required by the body.

Another essential drug delivery model that could be used in a stent device is the homogenous or 'monolithic model', which involves the dissolution of the therapeutic agent (in this case, the anti-cancer drug) in the PLGA polymer membrane and its release, controlled by diffusion from the polymer matrix. This release rate, as a function of time, may be defined by one of the following equations: the early time approximation shown in equation (4.0), and the late time approximation shown in equation (5.0) [46, 48].

$$\frac{dM_t}{dt} = 2M_x\left[\frac{D}{\pi\delta^2 t}\right]^{1/2} \quad (4.0)$$

$$\frac{dM_t}{dt} = \frac{8DM_x}{\delta^2} exp \frac{\pi^2 Dt}{\delta^2} \quad (5.0)$$

These equations predict rate of drug release from the PLGA matrix of thickness δ, where D is the diffusion coefficient, M_x is the total amount of drug dissolved in the membrane, M_t is the amount of drug released at time t, and $\frac{dM_t}{dt}$ is the drug release rate. According to equation (4.0) which is

valid for about the first 60% of the release time, the rate decreases exponential with the square root of time when plotted as shown below. During the latter 40% of the release time, the rate decays exponentially with time as shown by equation (5.0). Plots of these two approximations are shown below in figure 2.5.

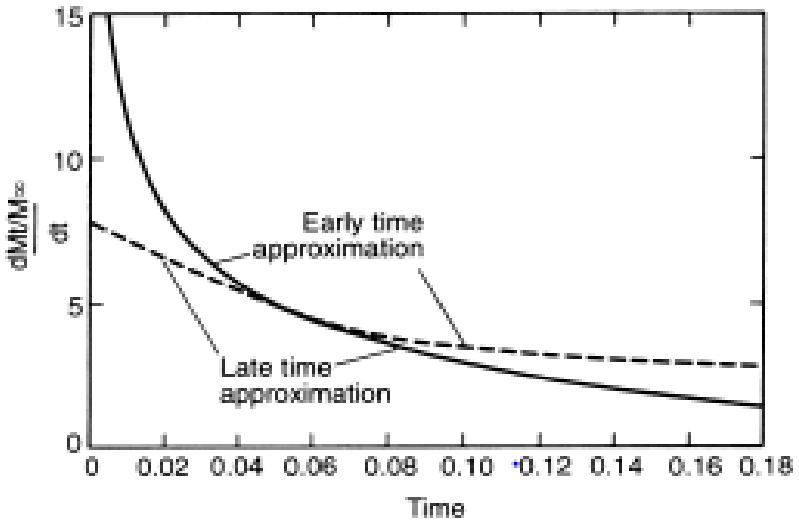


Figure 14 2.5 Release rate of the anti-cancer drug initially dissolved in a PLGA membrane as a function of time. Adapted from [48]

Just like in the previous model, once we are able to determine the drug release rate as well as the total amount of drug released at any given time t, and the early and late time approximations of the drug released from our device, we can efficiently predict the specific amount of drug (within the therapeutic window, i.e., not toxic and not below the minimum level required) that will be used by the patient over a period of ninety days.

A final model to consider that can guide the drug delivery from the polymer-coated stent is the diffusion flow of the drug through the micro-pores (channels) within the PLGA to the surrounding fatty tissue. Diffusion in the PLGA micro-porous membrane occurs principally by diffusion through the drug-filled pores, where the composition of the drug will control the overall transport flux across the membrane. In this system, the flux is described by equation (6.0).

$$J = \frac{\varepsilon D K \Delta C}{\tau l} \qquad (6.0)$$

where ε is the volume fraction porosity, which is normalized as fractional area (area of pores per unit area) of the membrane, D is the diffusion coefficient, K is the partition coefficient, ΔC is the difference in concentration between the solutions on either side of the membrane, τ is the tortuosity (average length of pore channel traversing the membrane), and l is the thickness of the membrane.

One of the most important consequences of the above equation is that the flux J (release rate) will remain constant (within the therapeutic window, in controlled release) provided that the membrane material does not change with time, i.e., provided that D, K , ε, and τ remain constant. This further implies that the concentration of the drug cannot change with time and that the drug released from the device must be rapidly taken up [48]. This serves to ensure that the stent-drug concentration remains within the therapeutic window for a long period of time as the drug gets consumed gradually.

It can also be seen clearly from the above equation that the release rate of the drug remains constant across the membrane with constant concentration difference, ΔC. For an implanted stent, this can cause the concentration of the drug within the device to fall after some time as the drug is released. But if the difference in drug concentration between the fatty tissue and the drug loaded device is sufficiently large, the effect will be minimized and the drug release can remain relatively steady within the therapeutic window for much of the time. [46].

Hence, to ensure that stent device continues to discharge its drug within the therapeutic window, the membrane enclosing the loaded drug (in this case, the PLGA) should be carefully prepared and tested to avoid its untimely rupture that might lead to sudden release of the drug [48].

2.6 DRUG-POLYMER ADHESION

The binding force that ensures the attachment of the drug to the polymer could be either physical or chemical. Essentially, if this link is physical only, the drug release is said to be controlled by diffusion and the release rate is expected to be fast. A slow release rate is achieved if the binding is chemical in nature [54]. Besides, the interaction between the drug and the polymer could also be hydrophobic or electrostatic. This approach is used to prepare homogeneous PLGA microparticles with different dimensions. Ideally, poly vinyl pyrolidone (PVP), a colorless, pale yellow amorphous polymer, has been identified as a suitable material for the preparation of solid drug dosage forms, owing to its ability to serve as binder lubricant and coating agent in wet granulation. This characteristic also makes PVP K-30 useful in the preparation of effervescent tablets and in other pharmaceutical applications as it dissolves rapidly in water to form a clear solution. It is also highly soluble in a number of other organic solvents. Some specific

effervescent vitamins tablets resulting from its use include ascorbic acid tablets and multivitamins as well as diclofenac sodium tablets. Additionally, PVP has had a long history of biocompatibility and non-toxicity and has thus, been used for non-parenteral applications. In about half a century since the invention of PVP, a very extensive volume of toxicological data in animal and humans has been developed. It is perhaps the most intense volume of toxicological information available on any pharmaceutical excipient or food additive in use today. The data supports the conclusions reached by individual workers, as well as several regulatory bodies, that PVP is safe. The acute, subchronic and chronic toxicity of orally administered PVP is extremely low [51]. The currently permitted United Nations' World Health Organization's Food and Agriculture Organizations joint expert committee on food additives established an allowable daily intake of 50mg/kg per day for food use thus, providing an adequate margin of safety. Essentially, there would appear to be no reason to restrict its oral pharmaceutical use in any way. Moreover, there have been no reports of adverse effects following its use intravenously as a plasma expander, even after the administration of very large amounts [51]. Because of its unique chemical nature, PVP would be expected to be biologically inert apart from exerting osmotic activity [52]. A large number of animal and human studies support the metabolic inertness of this polymer and hence the safety of PVP. With normal use, PVP does not modify physiological activity.

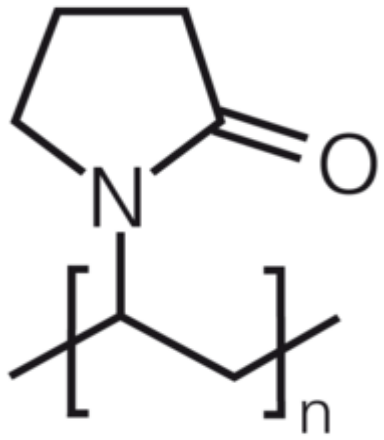


Figure 15 2.6 Structural Configuration of PVP

2.6.1 Polymer-Stent Adhesion

Due to their non-physiological nature, bare metallic stents are attacked by the body's natural immunity upon implantation in the artery. A bulk mass of lymphocytes forms around the implanted stent in response to the body's natural tendency to rid itself of foreign objects. As earlier on discussed, the bulk formation of these cells can lead to thrombosis and reclogging of the artery (re-occlusion) [1]. The latest generation of stents has employed coatings with anti-cancerous drugs to inhabit the formation of these cells. Usually, a biodegradable polymer such as PLGA of a non-biodegradable polyurethane-base elastomer is used to encapsulate the drug [1]. Burger has identified three major characteristics that a stent coating must possess, they include: a) pliability, owing to the flexible nature of the stent, b) the ability to provide smooth and continuous finish and c) adhesion of the polymer to the stent surface [1]. The drug eluted from the polymer matrix, over a period of months, has the propensity to reduce the proliferation of cell

masses, and hence significantly reduces the probability of thrombosis and restenosis to a level of 2-3% compared to 25% for uncoated stents, according to results obtained from clinical trials [1].

References

[1] Berger H.L., Coating Drug-eluting Arterial Stents Using Ultrasonic Spray Nozzles, 19th Annual Conference on Liquid Atomization and Spray Systems, Sono-Tek Corporation Milton, 2006

[2]?

[3] Fukuda et al.; Potent Inhabitory Effect of Sirolimus on the Circulating Vascular Progenitor Cells, Circulation, 2005: 925-928

[4] Khanafari A., Assadi M.M. and Fakhr F.A. Review of Prodigiosin, Pigmentation in *Serratia marcescens,* Online Journal of Biological Sciences, 2006, 6: 1608-4217

[5] Amann, L., M. Gandal, R. Lin, Y. Liang and S. Siegel. In vitro-in vivo correlations of scalable PLGA-Risperidone implants for the treatment of schizophrenia. *Pharm Res,* 2010. 27(8): 1730-1737.

[6] Crowley, M. M., F. Zhang, M. A. Repka, S. Thumma, S. B. Upadhye, S. K. Battu, J. W.

McGinity and C. Martin. Pharmaceutical applications of hot-melt extrusion:

part I,. *Drug Dev Ind Pharm*, 2007. 33: 909-926.

[7] Rosato, D. V. and D. V. Rosato, *Injection molding handbook*.2nd ed. New York: Chapman & Hall, 1995.:

[8] Wang, C.-K., W.-Y. Wang, R. F. Meyer, Y. Liang, K. I. Winey and S. J. Siegel. A rapid method for creating drug implants: Translating laboratory-based methods into a scalable manufacturing process. *J Biomed Mater Res,* 2010 *B* 93B(2): 562-572.

[9] H. Dores et al.; Stent Thrombosis with Second- Versus First-Generation Drug-Eluting Stents in Real-World Percutaneous Coronary Intervention:Journal if Invasive Cardiology

[10] Shuwisitkul D.; Biodegradable Implants with Different Drug Release Profiles, Innaugural-Dissertation, Freie Universitat, Berlin, 2011

[11] Schwach, G., N. Oudry, S. Delhomme, M. Lück, H. Linder and R. Gurny.. Biodegradable microparticles for sustained release of a new GnRH antagonist - part I: screening commercial PLGA and formulation technologies. *Eur J Pharm Biopharm,* 2003 56: 327-336.

[12] Makadia H.K. and Siegel S.J.; Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier, Polymers, 2011

[13] Park, T.G. Degradation of poly(lactic-co-glycolic acid) microspheres: Effect of copolymer composition. Biomaterials 1995, 16, 1123–1130.

[14] Gadad A.P., Vannuruswamy G., Sharath Chandra P, Dandagi P.M and Mastiholimath V.S. Study of Different Properties and Applications of Poly Lactic-Co-Glycolic Acid (PLGA) Nanotechnology, 2012: An Overview

[15] Maheswarappa, G., Kavitha, D., Vijayarani, K and Kumanan, K., Prodigiosin as anticancer drug Produced from bacteria of termite gut, Indian Journal of Basic and Applied Medical Research; 2013

[16] Boger D.L. and Patel M., Total synthesis of prodigiosin, prodigiosene and desmethoxyprodigiosin: Diels-Alder reactions of hetero cyclic azidenes and development of an effective palladium (II)-promoted 2'2'-bipyrrole coupling procedure, 1988. J. Org. Chem., 53: 1405-1415.

[17] Zhang J., Shen Y., Liu J., Wei D., Antimetastatic effect of prodigiosin through inhibition of tumor invasion, Biochemical Pharmacology, 2004

[18] Glinsky V.V, Huflejt M.E, Glinsky G.V, Deutscher S.L, Quinn T.P. Effects of Thomsen–Friedenreich antigen-specific peptide P-30 on b-galactoside-mediated homotypic aggregation and adhesion

[19] Matsuyoshi N., Hamaguchi M., Taniguchi S., Nagafuchi A., Tsukita S., Takeichi M.. Cadherin-mediated cell–cell adhesion is perturbed by v-src tyrosine phosphorylation in metastatic fibroblasts. J Cell Biol 1992;118:703–14

[20] Puskas J.E. et al., Drug-eluting stent coatings, John Wiley & Sons, Inc. *WIREs Nanomed Nanobiotechnol,* Advanced Review, 2009

[21] Pandi Suba, K., Arul, D., Smiline Girija, AS., Hairul Islam, V., Saravanan, S., Valli, G. and Raghuraman, R. Prodigiosin Induced Apoptosis and Inhibited Proliferation in Carcinoma HSC-2 Cells International Journal of Current Research, 2011. 33:151-156

[22] Wise, L. D., Handbook of pharmaceutical controlled release technology. Marcel Dekker, Inc. New York, Basel (2000)

[23] Raval A., Parikh J. and Engineer C., Mechanism of Controlled Release Kinetics from Medical Devices, Brazilian Journal of Chemical Engineering, 2010, 27: 211 - 225,

[24] Deconinck, E., Sohier, J., De Scheerder, I., Van Den Mooter, G. Pharmaceutical Aspects of Drug Eluting Stents. Journal of Pharmaceutical Sciences, 97, No.12, 5047-5060 (2008)

[25] Hwang, C. W., Wu, D., Edelman, E. R., Physiological transport forces govern drug distribution for stent-based delivery. Circulation, 104, 600-605 (2001).

[26] Lovich, M. A., Philbrook, M., Sawyer, S., Weselcouch, E., Edelman, E. R. Arterial heparin deposition: role of diffusion, convection, and extravascular space. American Journal of Physiology, Heart and Circulatory Physiology, 275, 2236-2242 (1998).

[27] Hwang, C. W., Wu, D., Edelman, E. R., Impact of Transport and Drug Properties on the Local Pharmacology of Drug-Eluting Stents. International Journal of Cardiovascular Interventions, 5, 7-12 (2003).

[28] Lovich, M. A., Creel, C. J., Hong, K., Hwang, C. W., Edelman, E. R., Carrier proteins determine local pharmacokinetics and arterial distribution of Paclitaxel. Journal of Pharmaceutical Sciences, 90, No. 9, 1324-1335 (2001).

[29] Kamath, K. R., Barry, J. J., Miller, K. M., The Taxus™ drug-eluting stent: A new paradigm in controlled drug delivery. Advanced Drug Delivery Reviews, 58, 412- 436 (2006).

[30] Leon, M. B., Abizaid, A., Moses, J. W., The CYPHER™ Stent: A New Gold Standard in the Treatment of Coronary Artery Disease, TheCardiovascular Research Foundation, New York, NY (2003)

[31] Kothwala D., Raval. A., Choubey, A., Engineer, C., Kotadia H., Paclitaxel Drug Delivery from Cardiovascular Stent. Trends in Biomaterials & Artificial Organs, 19, No. 2, 88-92 (2006).

[32] Raval, A., Choubey, A., Engineer, C., Kotadia, H., Kothwala, D., Novel Biodegradable Polymeric Matrix Coated Cardiovascular Stent for Controlled Drug Delivery. Trends in Biomaterials & Artificial Organs, 20, No. 2, 101-110 (2007).

[33] Alexis, F., Venkatraman, S., Rath, S. K., Boey, F., In vitro study of release mechanisms of Paclitaxel and Rapamycin from drug-incorporated stent. Journal of Controlled Release, 98, 67-74 (2004).

[34] Wang, X., Venkatraman, S., Boey, F., Loo, J., Tan, L.P., Controlled release of Sirolimus from a multilayered PLGA stent matrix. Biomaterials, 27, 5588-5595 (2006).

[35] Yang, C., Burt, H., Drug-eluting stents: Factors governing local pharmacokinetics. Advanced Drug Delivery Reviews, 58, 402-411 (2006).

[36] Finkelstein, A., McClean, D., Kar, S., Takizawa, K., Varghese, K., Baek, N., Park, K., Fishbein, M. C., Makkar, R., Litvack, F., Eigler, N. L., Local Drug Delivery via a Coronary Stent with Programmable Release Pharmacokinetics. Circulation, 107, 777-784 (2003).

[37] Leon, M. B., Endeavor IV: A Randomized Comparison of a Zotarolimus-Eluting Stent and a Paclitaxel-Eluting Stent in Patients with Coronary Artery Disease. Transcatheter Cardiovascular Therapeutics, Washington D.C. (2007).

[38] Waksman, R., Next Generations Drug Eluting Stents will safety be addressed? ACC 2007, 56th Annual Scientific Session, New Orleans (2007).

[39] Alexis, F., Factors affecting the degradation and drug-release mechanism of poly(lactic acid) and poly[(lactic acid-co-(glycolic acid)], 2005. *Polym Int* 54: 36-46.

[40] Winzenburg, G., C. Schmidt, S. Fuchs and T. Kissel, Biodegradable polymers and their potential use in parenteral veterinary drug delivery system, 2004. *Adv Drug Deliver Rev* 56: 1453-1466.

[41] Engelberg, I. and J. Kohn, Physico-mechanical properties of degradable polymers used in medical applications: a comparative study, 1991. *Biomaterials* 12: 292-304.

[42] Pham, C. V.. *Acid end group poly(D,L-lactide-co-glycolide) copolymers with high glycolide content,* 2004. US patent 6,703,477.

[43] Wu, X. S. and N. Wang, Synthesis, characterization, biodegradation, and drug delivery

application of biodegradable lactic/glycolic acid polymers. Part II: Biodegradation, 2001. *J Biomater Sci Polymer Edn* 12(1): 21-34.

[44] Lu, L., C. A. Garcia and A. G. Mikos, In vitro degradation of thin poly(DL-lactic-coglycolic acid) films, 1999. *J Biomed Mater Res* 46: 236-244.

[45] Siepmanna J., Peppas N.A, "Modeling of Drug Release from Delivery Systems based on Hydroxypropyl methylcellulose (HPMC)", Advanced Drug Delivery Reviews 48(2001):139-157.

[46]. Oni Y.O, "An Implantable Biomedical Device and Nanoparticles for Cancer Drug Release and Hyperthermia", *PhD Thesis, Department of Mechanical and Aerospace Engineering, Princeton University*, USA, Sept 2010, pp 1-160.

[47] Danyuo Y., "Implantable BioMEMS Devices for Localized Breast Cancer Drug Delivery", *MSc Thesis, Department of Materials Science and Engineering, African University of Science and Technology*, Abuja, Nigeria, Dec 2011, pp 1-83.

[48] Ratner D., Hoffman A.S., Schoen F.J., Lemons J. E., "Biomaterials Science: An Introduction to Materials in Medicine (2nd ed), Elsevier Academic Press, 2004, pp137-66, 181-88, 214-5, 218-9, 237, 262, 527-33, 673, 754-64,.

[49] Robinson B.V., Sullivan E.M., Borzelleca J.F., and Schwartz S.L., "PVP-A Critical Review of the Kinetics and Toxicology of Polyvinylpyrrolidone (Povidone)," (Chelsea, MI:CRC Press, 1990).

[50] Nishida K., Ando Y., Toriumi S.,"The Desorbing Action of Polyvinylpyrrolidone,"J. Soc. Dyers Colour, 104:96-9 (1988)

[52] Bhardwaj, R. and Blanchard J. 1997. In vitro evaluation of poly (D,L-lactide-co-glycolide) polymer-based implants containing the a-melanocyte stimulating hormone analog, Melanotan-I. *J Control Release,*. 45: 49-55.

[53] Viitanen, P., E. Suokas, P. Tormala and N. Ashammakhi. 2006. Release of diclofenac sodium from polylactide-co-glycolide 80/20 rods. *J Mater Sci Mater Med* **17**(12): 1267-74.

[54] Singh, S. K., A. F. Jalali and M. Aldén. 1999. Modulated temperature differential scanning calorimetry for examination of tristearin polymorphism: 2 isothermal crystallization

CHAPTER THREE

3.0 METHODOLOGY

3.1 MATERIALS AND METHOD

Ideally, prodigiosin was selected as the anti-cancerous drug of preference since it is said to be antifungal, anti-cancerous, anti-proliferative, antimetastatic and immunosuppressive [1,2,3]. Furthermore, it is less cytotoxic in comparison to paclitaxel (Taxol^{TM}). Cytotoxity of paclitaxel undesirably leads to the death of healthy cells around the site where it is administered. The prodigiosin used was synthesized by scientists at the Sheda Science and Technology Complex, Abuja, and had a purity >92%. Its molecular weight is 323.43g/mol. As was discussed earlier, another advantage that prodigiosin offers is its ability to prevent the attachment of tumor cells to the EMC thereby, inhabiting migration of cancer cells and the initiation of metastasis (Zhang et al, 2004). All PLGA samples used were manufactured by Sigma Aldrich, St Louis, USA. The molecular weight of the PLGA 65:35 as stipulated by the manufacturers is 40, 000 – 75,000. The PLGA serves as the drug delivery medium. The Polyvinyl pyrolidone (PVP), produced by Qualikems New Delhi, India, was used to bind the drug to the PLGA polymer as well as promote adhesion between the coating and the stent. Its molecular weight as stipulated by the producers is $(111.15)_n$ Dichloromethane, DCM, (CH_2Cl_2) was manufactured by Sigma-Aldaich and used to dissolve the PVP. A digital USB proscope HR, produced by BODELIN technologies, Lake Oswego, USA, was used to obtained micrographs of the sample before, during and after the release. To ascertain the the release of drug with respect to time, the absorbance of each sample

was taken at three-day interval. A digital UV-Vis spectrophotometer was used to ascertain the absorbance of the samples.

COATING OF STENTS

Traditionally stent coating techniques have not utilized various deposition techniques since they have proven ineffective in the coating of DESs. Dip coating has been a popular conventional technique used to coat DESs.

Usually, the drug-polymer coating is applied by dipping of solution consisting of drug and polymer, mixed in desired proportion and using ethanol and DCM (evaporative solvent material of relatively high vapor pressure) to produce the desired viscosity and quickly establish coating layer thickness [4].

In this study, two separate coating techniques were employed. In the first case, the polymer was heated at a temperature 10˚C above its Tm and then mixed with a solution of poly (vinyl pyrrolidone (PVP) dissolved in dichloromethane (DCM) and a solution of prodigiosin dissolved in ethanol. In another case, both PLGA and PVP were separately dissolved in (DCM). Stents were coated with two different concentrations of prodigiosin. One of the two Co-Cr stent struts was coated with a prodigiosin-PLGA-Polyvinyl pyrolidone mix having prodigiosin concentration of 7.467mg/ml, while the other was coated with a prodigiosin-PLGA-Polyvinyl pyrolidone mix having prodigiosin concentration of 100mg/ml. In the first case, 0.358 g of 65:35 PLGA was weighed using a weighing balance (OHAUS, Analytical model AR3130, Nanikon, Switzerland) and heated to a temperature of 182 ^{0}C using a hotplate & stirrer (model 1000, voltage 230V, Bibby Scientific Ltd, UK). Also, a 0.043 g of PVP was weighed and dissolved in 1ml of 99.8%. 7.467mg of prodigiosin was then dissolved in 1ml of dichloromethane (DCM). A mixture of the

melted PLGA, and the dissolved PVP was then finally mixed with the solution of the drug to obtain the coating for one of the stents. In another scenario, 100 mg of prodigiosin was dissolved in 1 mL of DCM while the amounts of the other components were retained. The resulting mixture was then used to coat the other Co-Cr stent. The coated stents were then dried in air at a temperature of 28˚C. The mass of each of the bare Co-Cr stent was measured to be 0.093g. The stents were weighed before and after they were coated. The thickness of the coated stents were also found to be 0.15 cm for that prepared using the Hot-melt process and 0.2cm for that obtained via the solvent dissolution method .

In an effort to compare the release profile of paclitaxel to that of prodigiosin, two stents were also coated with paclitaxel-PLGA mix. To this end, 1 g of PLGA 63:35 was dissolved in 1 ml of DCM and then mixed with 1 ml stock solution of PVP. Paclitaxel of concentration 0.01g/ml (11 Mm) was then added to the PLGA-PVP mix, stirred and used to coat both stents. The thicknesses of the coatings were measured to be 1cm and 0.15 cm.

3.2 PREPARATION OF PBS SOLUTION

In order to observe the drug release phenomena, we needed to mimics the body's physiological conditions. This was the driving force for the preparation of phosphate buffer saline (PBS) solution in which the coated stent were eventually placed. A mixture of 98.78% of distilled water, 0.025% 0f KCl, 0.178% Na_2HPO_4, and 0.0296% KH_2PO_4 was used to form the PBS solution. Dilute versions of HCl and NaOH were used to regulate the pH of the solution. Finally, a pH of 7.4 (pH of the blood) was obtained.

For in-vitro prodigiosin release kinetics study, stents were incubated in 10 ml of (PBS) solution at 37°C (Danyuo, Y. et al., and 4], with constant manual shaking. Each of the three stents were removed at 72 hours (3 days) interval from its release medium and analyzed for quantity of prodigiosin release in PBS [4]. A UV-Vis spectrometry was carried out on each of the release media to measure the absorbance of prodigiosin. The amount of prodigiosin released at regular interval and cumulative amount from each stent was calculated.

3.3 PURIFICATION OF PRODIGIOSIN

As a general rule, any material used in the biological system must be void of contaminants as much as possible. Hence, the purity of the active agent, prodigiosin, had to be accessed. As mentioned earlier, the prodigiosin used was synthesized by scientists at the SHESTCO's advanced biotechnology laboratory. Chromatographic technique was subsequently carried out to establish the purity of the drug. Further high purity liquid chromatographic (HPLC) studies confirmed prodigiosin purity > 92%

3.4 COATING METHODS

After coating, the stents were left to dry in air at room temperature (for 6-7 days before being placed in test tubes containing 10 ml PBS solution each. The test tubes were then placed in an Incubator Shaker set at a temperature of 37 ^{0}C and manual periodic shaking to account for the body's revolution per minute (rmp).

References

[1] Maheswarappa, G., Kavitha, D., Vijayarani, K and Kumanan, K., Prodigiosin as anticancer drug Produced from bacteria of termite gut, Indian Journal of Basic and Applied Medical Research; 2013

[2] Zhang J., Shen Y., Liu J., Wei D., Antimetastatic effect of prodigiosin through inhibition of tumor invasion, Biochemical Pharmacology, 2004

[3] Khanafari A., Assadi M. M. and Fakhr F. F.; Review of Prodigiosin, Pigmentation in *Serratia marcescens*,2006

[4] Raval, A. et al., Novel Biodegradable Polymeric Matrix Coated Cardiovascular Stent for Controlled Drug Delivery, Trends Biomater. Artif. Organs, Vol 20 (2), pp 101-110, 2007

CHAPTER FOUR

4.0 DISCUSSION OF RESULTS

Prodigiosin eluting stents were submerged in phosphate buffer saline (PBS) solution, at pH 7.4 and 37 ^{0}C in glass test tubes and subjected to manual, periodic shaking. The PBS solutions were replaced with fresh PBS solutions after every three-day and those collected from the test tubes were analyzed for the amount of drug eluted. The amount of drugs released was accounted for using UV-Vis spectrophotometry.

Swelling of the stent coating was observed about 12 hours after the stents were submerged in the PBS solution. The swelling gradually continued for the next 16 hours and was accompanied by an increase in mass. This result indicated water absorption through the micro-porous membrane of the stent.

4.1 PROSCOPE ANALYSIS

Digital USB proscope HR was used to analyze the morphologies of drug coated stents before and during degradation. Proscope micrographs of the surfaces of the prodigiosin coated stents were taken at various intervals upon immersion in the PBS medium. As shown in figure 4.2 (a, b, and c), small discontinuous creases appeared on the surfaces after 12 days of drug elution. Dispersed through the polymer matrix were also clusters of prodigiosin molecules. These creases gradually progressed in size and lead to increasing porous surfaces (Figure 4.2 b). A very similar observation was made by Engineer et al., 2010 when they used scanning electron microscopy (SEM) to analyze the morphological changes of stent coated with drug embedded PLGA (50:50)

[1]. The clusters of drug molecules were as a result of a high concentration of drug on the polymer matrix coupled with incomplete homogenization of drug and polymer.

The proscope micrograph in figure 4.2 (a, b and c) shows the surface morphologies of the coated stents prepared by means of hot-melt process. As discuss in chapter II, polymer degradation refers to the scission of polymer chain in the polymer backbone and hydrolysis is the dominant mode of degradation. Hydrolysis begins with PBS penetrating deeply into the interior areas of the polymer, leading to the cleavage of the covalent bonds holding in place the polymer structure. This alters the polymer's chemical composition and physical parameter such as crystallinity, chain flexibility, cross-linking, chain conformation, molecular weight distribution and branching (Venkatachalam et al., 2012). As clearly seen in figure 4.2 c, water penetration into the polymer has created creases all along its surface thus, allowing greater water penetration and faster polymer degradation. The hydrolysis reaction of PLGA is represented in figure 4.1 on the next page.

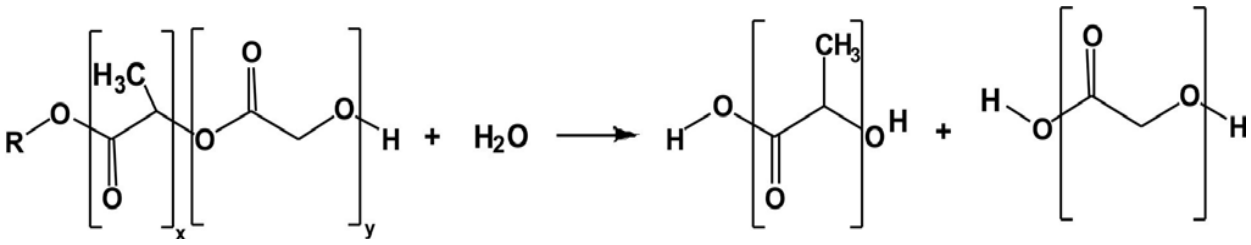
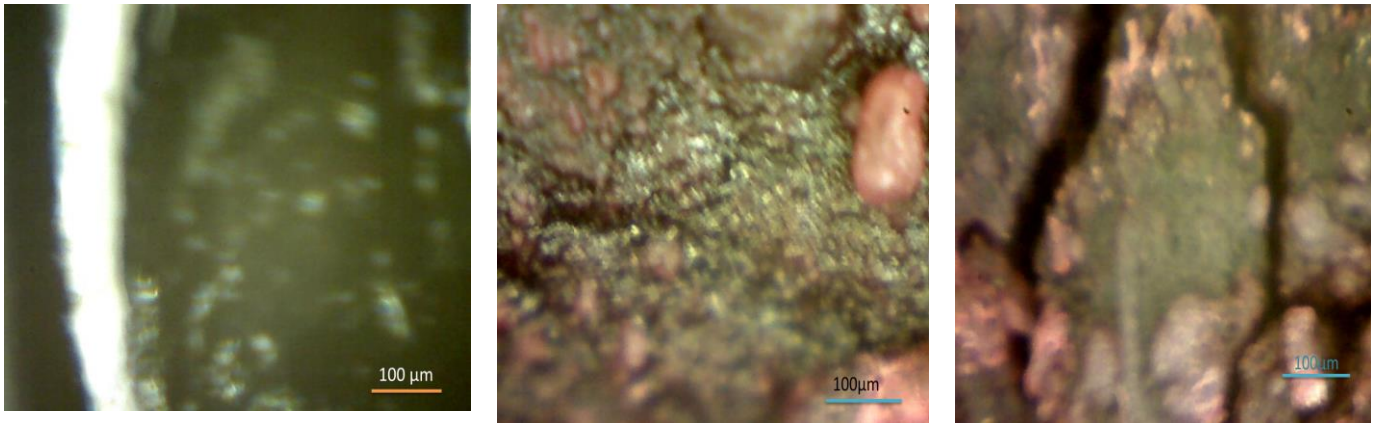


Figure 16 4.1 Hydrolysis of PLGA

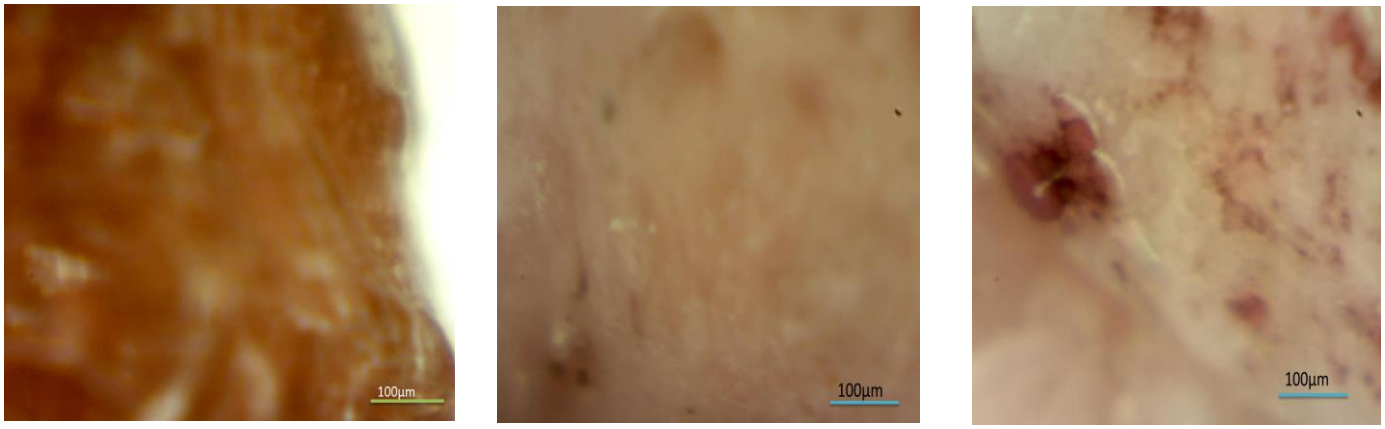
In a related scenario, the micro poles might have erupted from diffusion of drug and the hydrophilic polyvinyl pyrrolidone from the coating and entry of water inward. Vey and Roger have also reported similar degradation behavior of PLGA films [2].



(a) (b) (c)

Figure 17 4.2 Proscope micrograph of stent coating obtained via Hot-Melt process

On the contrary, more uniform drug dispersion is observed in the coating prepared via solvent dissolution method as shown in figure 4.3 below. Also as time progresses, the polymer membrane absorbs water and drug letches out of the polymer as clearly seen in figure 4.3 C. This is a very vital point to consider since the homogeneity of drug in the polymer membrane is a major factor which determines control release. The solvent dissolution method is therefore, a more suitable method for preparing stent coating since it creates the platform for more uniform drug dispersion and hence, uniform drug diffusion.



(a) (b) (c)

Figure 18 4.3 Proscope micrograph of stent coating obtained via solvent dissolution method

4.2 PHASES OF DRUG RELEASE

Also interestingly**,** the release of drug from the biodegradable PLGA was in two folds. A diffusion controlled drug release was observed for the first part of the elution time which was then followed by polymer erosion. Diffusion was said to be the dominant mechanism of drug release for the first 12 days of release of the active agent. A similar result was reported by Engineer at al., 2010 when they studied the release of paclitaxel from 50:50 PLGA coating [1]. During this period, there was absorption of PBS by the polymer membrane and hydrolysis of the functional groups thus, leading to the cleavage of covalent chemical bonds. After 12 days of release, polymer erosion took over as the dominant release mechanism for the rest of the time. Release of degradable products leads to mass loss which is a characteristic for polymer erosion. As opposed to a number of previous Studies which reported that degradation period of PLGA is 4-6 weeks [5,6], degradation of the PLGA matrix began not long after 12 days, for at least one

sample. This was probably due to the presence of the highly hydrophilic PVP cross-linking agent. Furthermore, an initial high release was observed which was then followed by slower release. This initial high drug release rate stage can be attributed to the release of un-dissolved surface connected drug particles [4,6], followed by a gradual release stage attributed to molecular diffusion through the polymer phase [3].

Ideally, a diffusion controlled drug release model of this sort was suggested by (Siepmanna et al. 2001 and Pepass 1985). This model (proposed in chapter II) suggests that solute (in this case, the anti-cancer drug prodigiosin) released from the PLGA membrane can be controlled by diffusion and/or viscoelastic relaxation of swelling. As seen earlier in chapter II, k is a constant relating the structural and geometric characteristics of the controlled release that also incorporates the diffusion coefficient, D; W_t and W_∞ are the weight of the drug released at time t and infinite time respectively, so $\frac{W_t}{W_\infty}$ is the drug release fraction at time t, and n is an exponent that indicates the release mechanism According to Siepmanna and Pepass, the power law relation for this model is given by equation (2.1):

$$\frac{W_t}{W_\infty} = 4\left(\frac{Dt}{\delta^2}\right)^n = kt^n$$

By plotting ln $\left(\frac{W_t}{W_\infty}\right)$ versus ln t, we can obtain the values of n and k.

Also, from equation (2.1), recall that we obtained the diffusion coefficient, D = $\frac{kt\delta^2}{4}$

where δ is the thickness of the PLGA microparticles

According to Wu and Ding, 2004, the rate of polymer erosion can also be given as:

$$\frac{dM}{dt} = -k \qquad (4.1)$$

where M is the polymer mass at any time, t, and k is the kinetic rate constant of the degradation. Rearranging equation (4.1) gives us an equation that relates to the change in mass with as a function of change in time:

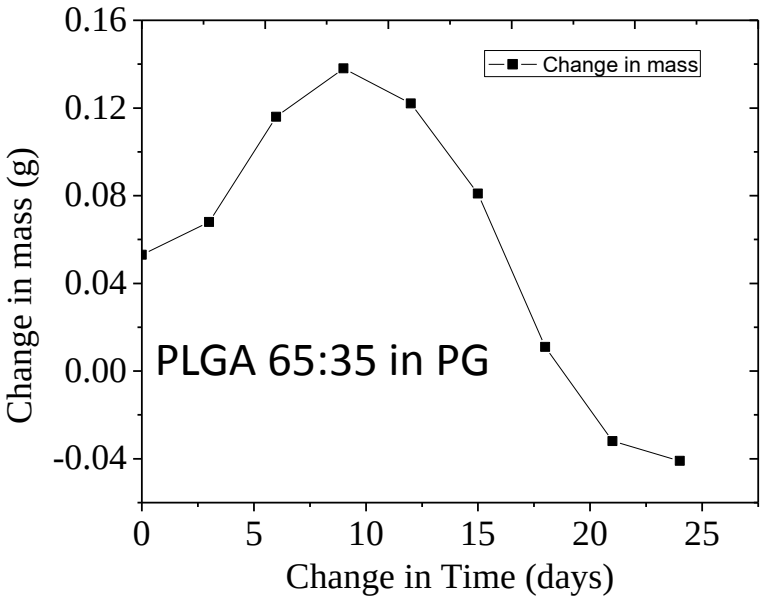
$$\Delta M = -k\Delta t \qquad (4.2)$$

Equation (4.3) is a linear version of equation (4.2), where M_0 is the initial mass of the polymer at time, t.

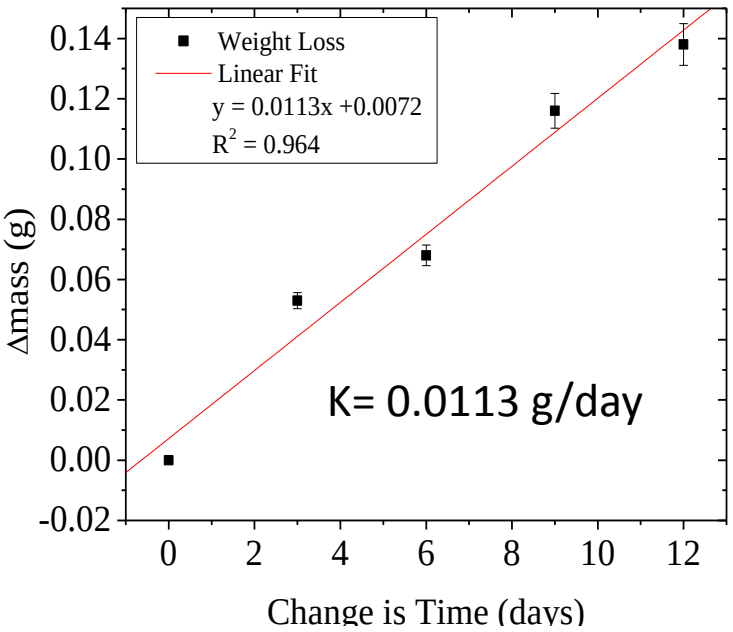
$$\Delta M = M_0 + kt \qquad (4.3)$$

Conversely, equation (4.4) is the exponential version of equation (4.2)

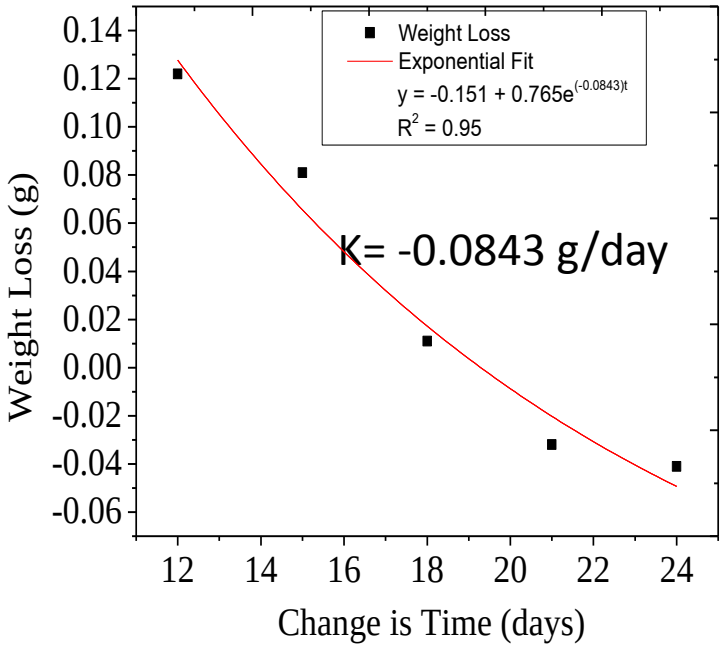
$$\Delta M = M_0 + Ae^{-kt} \qquad (4.4)$$



(a)



(b)



(c)

Figure 19 4.4 Plot of Diffusion and Degradation Controlled Drug Release

Figure 4.4 (a) shows a change in mass of the polymer versus change in time. From the onset, an increase in mass is observed due to water absorption. This increase in mass continued until a saturation point is reached as depicted in figure 4.5 below. The onset of erosion begins at saturation and continues for the rest of the elution time. In the first stage of PLGA degradation marked (a), water penetrates deeply into the interior of the polymer. This is subsequently followed by hydrolysis of the functional group and absorption of water by the PLGA membrane (region marked b). Cleavage of covalent chemical bonds (region c) occurs thereafter. This then leads to breakdown of PLGA into oligomers and monomers (transported from the polymer bulk controlled by diffusion).The release of degradation products either by surface or bulk erosion leads to mass loss which is characteristic of PLGA erosion.

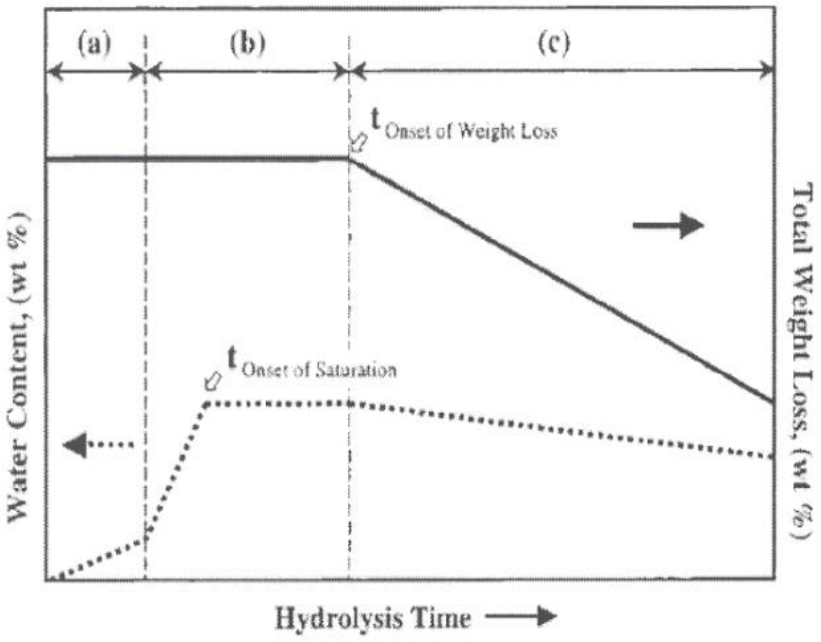


Figure 20 4.5 Schematic of weight gained/loss during PLGA

Figure 4.4 (b) is a linear fit of the portion of figure 4.4 (a) characterized by drug dissolution and diffusion. This linear fit is characterized by equation (4.3) and the kinetic rate constant of degradation was calculated to be 0.0113 g/day. Consequently, figure 4.4 (c) is an exponential fit of the portion of figure (a) characterized by erosion of the PLGA matrix. This curve shows an exponential decay with time as a consequence of mass loss. The kinetic rate constant of degradation, k was likewise calculated as -0.0843 g/day. The negative sign indicates that PLGA erosion is associated with mass loss.

Metters et al., (2000, 2001)[60,61] devised a statistical kinetic model to predict polymer degradation behavior, which says that the probability (P) of any random PLGA unit that has been hydrolyzed is given by:

$$P = 1 - f_{PLGA} = e^{(-k'\Delta m)} \qquad (4.5)$$

where f_{PLGA} is the total fraction of polymer hydrolyzed (equal to the ratios of polymer concentration C/C_o before and after degradation. Using this model, the results of the probability distribution of the change in mass from day 12 to day 27 was validated. According to (Ogunnaike, 2009), $P > 0.05$ is significant at $\alpha = 0.05$ at 95% confidence interval. Since all the probability values were well above 0.05 as required, the results are said to be valid at 95% confidence interval. Table 4.1 shows the change in mass and their corresponding probability values as of day 12. The average of the probability values was calculated as 0.230 as shown in table 4.1 on the next page.

:

Table 4.1

Time /days	Change in Mass (g)	Probability
12	0.138	0.226
15	0.122	0.227
18	0.081	0.230
21	0.011	0.234
24	-0.032	0.237
27	-0.041	0.238
		0.232

Since the amount of drug released at any given time is a function of the drug concentration in the PBS medium, a plot of the cumulative drug concentration should give us an idea of the release profile of the drug. This cumulative release profile shows an initial high drug release followed by a steady release for much of the elution time. This release characteristic was exhibited by prodigiosin for up to 27 days. When compared to paclitaxel, a similar release profile was observed for 15 days even though, the initial concentration of paclitaxel was well above that of prodigiosin. Figure 4.6 shows the cumulative concentration of drug eluted, considering the total drug loading as a function of the immersion time in the release medium.

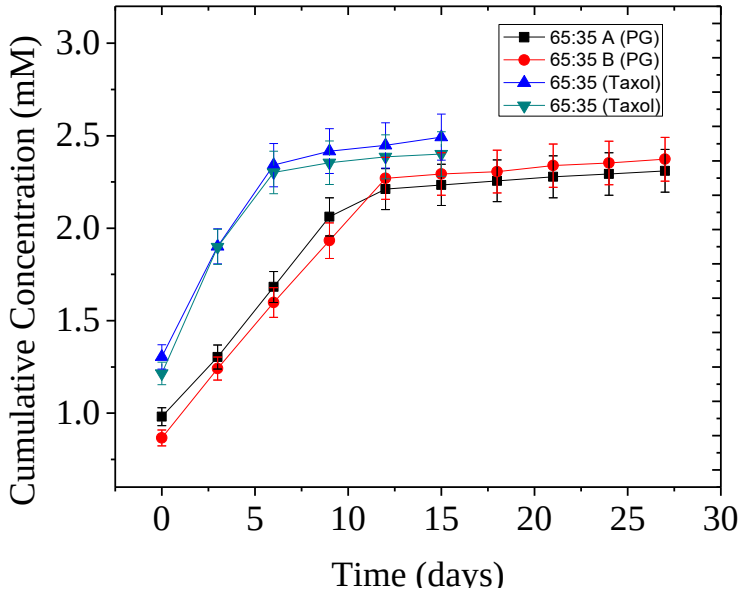


Figure 21 4.6 Plot of Cumulative Concentration versus Time

4.4 Gravimetric Analysis

To determine the percentage of mass lost due to drug eluted, the initial weights (W_0) of coated stents were recorded before placing them in PBS. After 27 days of incubation in PBS and after drying at 37 ^{0}C, stents were again reweighed to gravimetrically ascertain the final weight, W_d. The common factor used to determine the weight loss of the coated stent was the weight of the uncoated stent, Ws. The percentage Mass loss (ML %) was then calculated using equation (4.6) [1].

$$ML\ (\%) = [(W_0\text{-}Ws) - Wd\text{-}Ws)]/(W_0\text{-}Ws) \times 100 \quad (4.6)$$

4.3 UV-VIS SPECTROPHOTOMETRY

For the case of a uniform absorbing medium (solution: solvent and solute molecules that absorb light) the proportion of light radiation passing through it is called the transmittance, T, and the proportion of light absorbed by molecules in the medium is absorbance, A. In current study, the investigation is centered on obtaining the absorbance. As in the case of the samples coated with prodigiosin, all absorbance were taken at a wavelength of 536 nm. The highest absorbance of the prodigiosin was observed at this wavelength. Consequently, all absorbance of the paclitaxel samples were taken at an average wavelength of 205.5 nm. This was the average wavelength value at which the highest paclitaxel absorbance was observed.

Beer-Lambert Law states that Absorbance is proportional to the concentration (**c**) of the absorbing molecules, the length of light-path through the medium (*l*) and the molar extinction coefficient (**ε)**:

$$A = \varepsilon l c \quad (4.7)$$

Below is the standard curve showing a linear fit of drug concentration versus absorbance. The gradient ($1/\varepsilon l$***)*** was calculated as 7.158. From the below plot we can account for the concentration the corresponds to any value of absorbance and vice versa

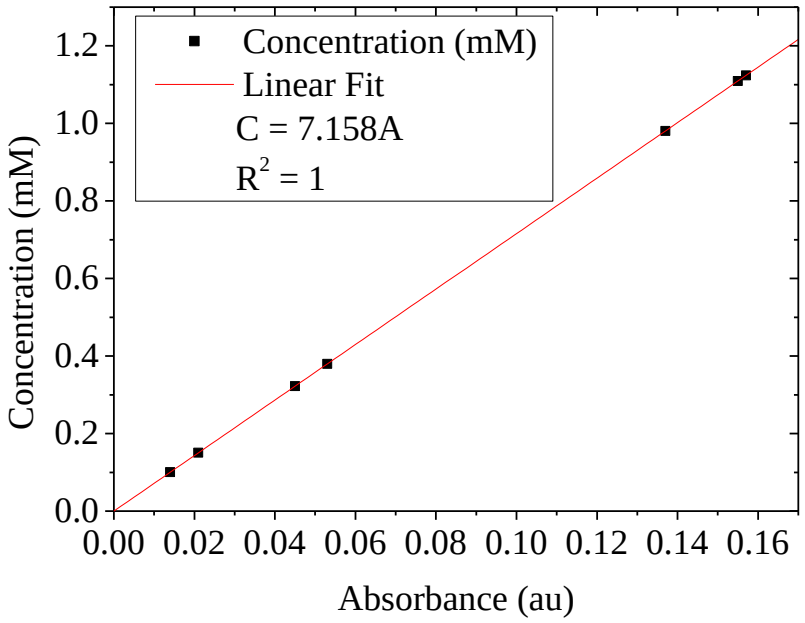


Figure 22 4.7 Linear Fit of Concentration versus Absorbance

References

[1] Engineer C., Parikh J. and Raval A., Hydrolytic Degradation Behavior of 50/50 Poly Lactide-co-Glycolide from Drug Eluting Stents, Trends Biomater. Artif., 2010, 24(3):131-138.

[2] Vey E., Roger C., Meehan L., Booth J., Claybourn M., Miller A.F. and Saiani A., "Degradation mechanism of poly(lactic-co-glycolic) acid block copolymer cast films in phosphate buffer solution", Polym. Degrad. Stab. 93, 1869–1876 (2008)

[3] Hurrell S. and Cameron R.E., "Polyglycolide: degradation and drug release. Part I. Changes in morphology during degradation", J. Mater. Sci. Mater. Med. 12(9), 811–816 (2001).

[4] Raval A., Choubey A., Engineer C., Kotadia H. and Kothwala D., "Novel Biodegradable polymeric matrix coated cardiovascular stent for controlled drug delivery", Trends Biomater. Artif. Organs. 20 (2), 101-110 (2007).

[5] Lewis D.H., In Biodegradable Polymers as Drug Delivery Systems, (Ed.) M. Chasin and R. Langer, Marcel Dekker: New York, 1-43 (1990).

[6] Engineer C., Parikh C. J. and Raval A., "Review on hydrolytic degradation behavior of biodegradable polymers from controlled drug delivery systems", Trends Biomater. Artif. Organs, (2010) (in press).

[7] Giri A.V., Anandkumar N., Muthukumaran G. and Pennathur G. A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil

[9] Kronenthal R.L., In Polymers in Medicine and Surgery, (Ed.) R.L. Kronenthal, Z. Oser and E. Martin, Plentum Press: New York, 119 (1975).

CHAPTER FIVE

5.0 CONCLUSION AND FUTURE WORK

5.1 CONCLUSION

Robust coatings, with uniform drug dispersion were achieved for stents via the solvent dissolution method in comparison to the hot-melt process. The solvent dissolution method which patronized the use of dichloromethane and dimethyl sulfoxide for the dissolution of prodigiosin and paclitaxel respectively proved tougher and more efficaciously than its counterpart formulated by means of the holt-melt process.

For the initial 12 days, drug elution was controlled by dissolution and diffusion phenomena followed by degradation controlled drug release. Diffusion of drug through the micro-porous PLGA membrane was accompanied by the influx of PBS solution into the coating. This lead to enhanced drug dissolution and subsequent release by means of diffusion. The second phase of drug release was governed by polymer erosion which was preceded by hydrolysis and PLGA chain scission.

Higher drug release was observed at the onset, followed by sustained release for much of the elution time. This sustained release at the maximum concentration is anticipated so long that the maximum concentration is within the therapeutic window

Even though, the initial concentration of paclitaxel was well above that of Prodigiosin, the two exhibited similar release trend for the first 15 days.

Gravimetric analysis showed a 26.88% decrease in mass as an evidence of degradation

Molecular weight loss also confirmed drug release by erosion

When compared to paclitaxel, prodigiosin exhibits a lower rate of initial burst.

5.2 FUTURE WORK

Given the clinical success of current generation of DESs, it would be prudent to review the existing technologies being used. This incremental approach rather than a revolution will go a long way in improving the treatment of CVDs while at the same time saving time and resources. A number of options are being considered for this modernized approach. Options include new polymer-coating technology, new stent designs and materials or even completely biodegradable stents. The investigator envisions multi-layer coated stents, in future research work. The various layers would essentially have different characteristic drug release profiles to ensure sustained delivery of active agent over a long period of time. One potential advantage of this scheme lays on the possibility of using different drug concentrations and combinations of different agents. Thus, such a system should regulate the amount of anti-proliferative drug in accordance with the severity of plaque formation as time goes on. Another alternative could incorporate a coating composed of a microporous hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] underlying coating. Hydroxyapatite is a major component of natural bone constituting 70% of its composition. Therefore, Hydroxyapatite is enormously biocompatibility. Encapsulation of the anti-proliferation agent in a biocompatible oil-based formulation can then be loaded into the microporous hydroxyapatite coating. According to In vivo, preclinical data, the expected lifetime of hydroxyapatite coating is between 9 months and 1 year. After that period, it is expected to

completely disappear [1]. The researcher looks forward to the use of modified air suspension coating technique incorporating ultrasonic coating techniques for better coatings and optimum material transfer efficiency. Coatings with uniform thickness are envisaged in future research endeavors. The development of coated surfaces that maintain robust integrity even after crimping and expansion of the stent would also be a step going forward. Future work also seeks to employ higher resolution microscopic techniques and high purity liquid chromatographic (HPLC) techniques to obtain surface morphological composition and surface drug content of the coated stent respectively. This will provide a clearer picture of the accuracy of the technique used to deposit the coatings. Gel permeation Chromatography (GPC) techniques will also be utilized to determine molecular weight and number average molecular weight variations for the polymer. Future generations of commercial DES systems may include stents fabricated completely from bioabsorbable matrix-forming materials. In this light, a major concern in the fabrication of such stent architecture would be the need for the polymer matrix to degrade in a way that maintains its mechanical integrity until the complete endotheliazation of the vessel. Another possibility would be to fabricate bioabsorbable devices whose shapes, when formed into specific shape configurations, can be altered by a dehydration process facilitating delivery of the active agent into the body. The device would then reconfigure to assume its original shape upon rehydration in vivo [1].

5.2.1 NEW STENT COATING TECHNOLOGY

Future drug-delivery coatings for stents will be expected to possess much higher performance efficiency. Any new coating systems will be expected to match the drug-elution profiles for current drugs. If these profiles are matched, it will preserve the genuineness of the outcomes of

clinical trials performed with these drugs and will definitely reduce the burden for approval by the requisite regulatory bodies. New coatings seeking to incorporate new drugs (such as the one being used in this research) for the treatment of CVDs will face with the burden of developing new release profiles, to be proven suitable by clinical trials before being approved by regulatory bodies. A new biodegradable coating should degrade within six to twelve months and the viability of the drug should be unaffected by the degradation process. Moreover, the degradation process must not produce any cytotoxicity or physiologically significant particulate. The adhesion of the coating to the stent strut must be ensured in order to prevent thrombosis. Furthermore, a conceived drug delivery coating must also conform to existing manufacturing requirements including: ability to be applied using current deposition techniques, compatibility with current manufacturing processes, such as stent loading, crimping and sterilization and lastly, compatibility with existing manufacturing work flow [1].

A comprehensive model of the drug release kinetics will certainly be a part of the researcher's future work.

Numerous research endeavors have been director towards attaining a comprehensive understanding of the feasibility of stent implantation in diabetes patients. Patients with diabetes are said to have higher burden of atherosclerosis, smaller coronary arteries, and a higher risk of repeat revascularization after implantation of a bare-metal stent than their counterparts without diabetes [1, 2]. Drug-eluting stents have been widely tested in patients with diabetes and have consistently reduced the rate of restenosis, as compared to bare metal stents (Berry C, 2007).

Current efforts are focused on the selection of the specific type of DESs to be used in patients with diabetes. Future work will also focus on modeling of the drug release rate

References

1 Abizaid A., and Ribamar C. J. Advances in Interventional Cardiology:New Drug-Eluting Stents, An Overview on Biodegradable and Polymer-Free Next-Generation Stent SystemsCirculation: Cardiovascular Interventions.2010; 3: 384-393

2. Berry C. Tardif J.C., Bourassa M.G., Coronary Heart Disease in Patients with Diabetes: part II: recent advances in coronary revascularization. J Am Coll Cardiol 2007; 49:643-56

3. Holmes D.R., Kereiakes, D.J., Garg, S., et al. Stent thrombosis. J Am Coll Cardiol 2010;56:1357-65.