Studies of biodegradable drug-eluting tracheal stents

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STUDIES OF BIODEGRADABLE DRUG-ELUTING TRACHEAL STENTS

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ABSTRACT

A fully biodegradable tracheal stent to prevent occlusion and collapse of the human airway, due to both conditions of tracheal stenosis and tracheomalacia, is an ideal treatment with several advantages. The most important of which are the removal for the need of a second surgical intervention to remove an implanted stent, and the potential for drug-elution from biodegradable stent matrices.

Currently, there is insufficient understanding of the performance of fully biodegradable, drug-eluting tracheal stents, both in-vitro and in-vivo. To achieve better understanding, this project attempted to develop a physical model to study the degradation phenomena of biodegradable polymers under a simulated tracheal environment. In-vitro drug-release studies conducted in a simulated tracheal condition were also performed. Results from these in-vitro studies finally led to the development of prototypical biodegradable tracheal stents that were implanted in rabbits and observed. Correlations between in-vitro and in-vivo performance of such stents were attempted at the end.

It was realised that well-studied bulk-degrading polymers do have the possibility of exhibiting pseudo “surface-eroding” phenomenon when exposed to the unique conditions in the tracheal passage. The addition of certain plasticizers also affects the degradation kinetics of biodegradable polymers in such environments. These observations led to the selection of a candidate material for the fabrication of stents for the animal study.

The drug of interest, mitomycin C (MMC), was studied in relation to its stability in physiological conditions and elution phenomena from a polymer under tracheal

environments. The saturation concentration and diffusion coefficient for MMC in the polymer were elucidated. Attempts to model its release phenomena using currently accepted principles required better understanding of the polymer-drug system and a methodology to correct release results due to MMC instability at longer elution periods.

Lastly, the results from two animal studies suggest that even though the stent material was well-tolerated in the trachea, MMC had to be eluted at efficacious doses for longer time periods. Formulations of MMC both incorporated into the stent body and spray-coated on the outer surface did not inhibit late-stage tracheal restenosis in rabbits. More studies into stabilising MMC and preventing too early drug-depletion was concluded to be the way forward for such a novel fully biodegradable tracheal stent to be in useful service.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

This project was proposed to address the need for a fully biodegradable polymeric tracheal stent to be implanted into the human trachea to address two existing conditions which cause obstruction to the airway and risking respiratory collapse; namely tracheal stenosis and tracheomalacia. Figure 1.1 shows the human respiratory system and all associated organs that permit respiration for normal body functioning.

Figure 1.1. The human respiratory system (courtesy of www.wikipedia.com).

Tracheal stenosis is a fixed airway narrowing which results from prolonged endotracheal intubation, tracheotomy, trauma, infections, tumour or tumour-related treatment and congenital disorders. Surgical intervention may be needed to re-establish a patent airway, with insertion of stents to prevent restenosis. Currently available stent types include silicone and stents which combine a silicone or synthetic outer coating with metal hoops or mesh [1]. Silicone stents are well-tolerated and removable. However, they impair physiologic mucociliary function, trapping airway secretions and mucus plugs, thereby risking life-threatening asphyxia. Silicone stents also have thick walls that narrow the tracheal lumen patency, further limiting their use in younger children with small tracheas.

Tracheomalacia is different from tracheal stenosis in that it is a condition of dynamic airway collapse during expiration, instead of a fixed airway narrowing described above. Briefly, it is caused by structural abnormalities of the tracheal cartilage, a vascular ring applying an undue pressure on the trachea and prolonged endotracheal intubations or infections. Commonly used stents are metallic in nature as they are flexible, have less trapping of secretions and have thinner walls. However, metallic stents are difficult to remove once they are covered by epithelium [2]. They can also fragment, extrude and penetrate into neighbouring structures like the esophagus and large neck vessels.

Tracheal stents made of biodegradable polymers are ideal as they can provide temporary rigidity for a prescribed time and do not need removal in a second surgical procedure. With suitable choice of materials, biodegradable stents can be thin-walled, and allow sustained drug-elution to prevent restenosis [3,4]. Previous in-vivo studies on biodegradable tracheal stents using polydioxanone in rats and poly(L-lactide) and poly(D,L-lactide-co-glycolide) in rabbits have been reported with mixed results [5-8].

Excessive granulation tissue growth, lack of epithelialisation over stent walls, and stent expulsion due to degradation were the main problems. For the in-vitro portion, the stents were merely immersed in buffer solution and their degradation profiles studied. It was found that fragmentation and significant mass loss occurred suddenly, which was undesirable [7,8]. There were interesting attempts by Zilberman et. al, who floated different polymer films on sterile water at 37°C to better simulate the conditions of both water and air interfaces in the trachea. The results showed good mechanical properties and almost linear drug-release profiles over the period of study [9-15]. A mathematical model was also developed and studied to predict the release of dexamethasone from floated polymer films with good correlation [5]. These studies were by far the closest related studies to the current research project described here. However, to achieve accurate in-vitro estimates of the overall bioabsorption period, simulation of the unique tracheal environment (humidified air on the inner surface and an aqueous gel on the outer surface) would be required.

1.2 PROBLEM STATEMENT FOR THE THESIS

Overall, a survey of the literature shows that there are very few studies on fully-degradable tracheal stents, and furthermore, there is no reported work on understanding the details of the degradative behaviour of these stents in a simulated tracheal environment. The main issue that was faced by previous research groups in the area of biodegradable tracheal stenting was the lack of understanding of the degradation process for biodegradable polymers in the tracheal passage. This understanding can be better achieved if a physical model can be elucidated to help predict and control biodegradability on two fronts; namely simulated tracheal tissue (70wt% water) and

flowing humidified air. Once the biodegradability is effectively modelled, other processes such as drug-release from, and epithelialisation on, biodegradable polymers can be easier controlled and understood. The learning from these in vitro studies can be used to optimise the in vivo performance of a fully-degradable stent in the trachea. Thus, the problem statement we propose is:

“There is insufficient understanding of the performance of fully-biodegradable, drug-eluting tracheal stents, both in-vitro and in-vivo.”

1.3 PROJECT OBJECTIVES AND SCOPE FOR THE THESIS

Based on the problem statement, the main objectives for this project were:

- 1) To study and model the degradation phenomena of biodegradable polymers at two different interfaces of 70wt% water (tracheal tissue) and flowing humidified air (inside the tracheal lumen).
- 2) To conduct drug-release studies and model the drug-release profiles corresponding to a simulated tracheal environment.
- 3) To study the deployment and performance of prototypical biodegradable tracheal stents in-vivo and correlate this to in-vitro performance.

To achieve these objectives, the project was divided into two broad sections or study phases (Figure 1.2):

The first section involved all in-vitro work, which was further sub-divided into two areas. The first area of study was to investigate the degradation kinetics of biodegradable

polymers exposed to the two media (gel and air) concurrently. The effects of copolymer and plasticizer types on degradation phenomena / kinetics were elucidated and this enabled a suitable candidate material to be selected to fabricate prototype tracheal stents for the in-vivo study. The second area of study was to investigate in-depth the drug-release phenomena from the selected material, simulating its use in the trachea. The drug of interest, mitomycin C (MMC), was studied in relation to its stability in physiological conditions, release from homogeneously-distributed or spray-coated systems, release into immersed or floated conditions, and loaded at different doses.

The second section involved all in-vivo work and this was also sub-divided into two areas. The selected animal model was New Zealand white rabbits and a disease model employing the use of unipolar diathermy at 35 watts was done to create mucosa injury and induce tracheal stenosis in all rabbits. This was a procedure developed by the NUHS collaborator to give credibility to the performance results of the implanted biodegradable tracheal stents and was proven to be effective in the control group; and almost all other studies in this area did not report on this disease model step. Prototypical tracheal stents fabricated using the selected material from the in-vitro studies were implanted into animal groups consisting of controls, and stents with and without MMC. The first area in this section was a study based on implanted stents made from casting polymer films and rolling these films into tubes. The second area involved improving upon these rolled polymer films by fabricating dip-coated, laser-patterned and spray-coated (with MMC) stents for implantation, and studying the performance of these stents in-vivo.

Figure 1.2. Chart showing the project breakdown and various study sections.

Finally, an attempt was made to correlate the observed in-vitro performance of prototypical biodegradable tracheal stents to their in-vivo performance.

The novelty in this project is that this is the first attempt to use a simulated tracheal environmental model to study the degradation of biodegradable polymers exposed to two different media concurrently, thus enabling a better understanding of the behaviour of biodegradable polymers under tracheal conditions. This is also the first such study to investigate the sustained-release of mitomycin C directly to the trachea to combat tracheal stenosis.

1.4 ORGANISATION OF THE THESIS

Chapter 2 reviews the organ of interest, the human trachea, and the two existing conditions that cause obstruction to the airway, and their current treatments. The

properties and effects of the drug used in this study, mitomycin C, are also presented. On the materials front, this chapter discusses the various accepted degradation and erosion phenomena of biodegradable polymers. Explanations on drug-release mechanisms from biodegradable polymers are also included in this chapter. Finally, more references and corresponding results on research conducted on biodegradable tracheal stenting are presented in greater detail here.

Chapter 3 details the materials, methodologies and equipment used for both the in-vitro and in-vivo parts of this project. All processing conditions, design of the experimental setup and animal study protocols are explained here.

Chapter 4 presents and discusses the results obtained from the study on degradation kinetics and behaviour of various biodegradable polymers and their blends with different plasticizers, when subjected to the experimental setup.

Chapter 5 looks into the drug-release phenomena of MMC either homogeneously distributed or spray-coated onto the selected polymer film or stent. Results from studies into the release of MMC from polymer films immersed or floated on water, and at different doses are discussed here. Stability studies for MMC in physiological conditions are also included.

Chapter 6 discusses the results from all in-vivo experiments conducted in this study. Endoscopic images to determine extent of restenosis in rabbits' tracheas and degradation profiles for explanted stents are presented here. Samplings of histological images are also

included to give some explanation and correlation to results seen from endoscopic images. Lastly, some references to in-vitro results are presented here.

Chapter 7 finally summarises the thesis by stating the main conclusions drawn from this project. Some recommendations and future work are included to give a more holistic picture of this tracheal stent study presently concluded and into the future.

CHAPTER 2

LITERATURE REVIEW

2.1 THE TRACHEA

The air passages of the respiratory system are divided into two functional zones. The respiratory zone is the region where gas exchange occurs, and the conducting zone includes all the anatomical structures through which air passes before reaching the respiratory zone. As presented in Chapter 1 (Figure 1.1), the trachea or more commonly known as the windpipe, is found in the conducting zone [16]. It is located in the neck in front of the esophagus and starts just below the larynx, before ending at the carina where it branches into two bronchi [16,17]. In addition to conducting air into the respiratory zone, the trachea also serves functions like *warming*, *humidification*, *filtration* and *cleaning* of the inspired air (explained in Section 2.1.2) [16]. Figure 2.1 shows an image of the trachea and the relative cross-sections at two different levels, together with the bifurcation angle at the carina.

Figure 2.1. Relative cross-sections of the trachea at different levels and the angle of bifurcation at the carina [18].

The length and diameter of the trachea naturally changes from the individual's infancy to adulthood. Typically, it has an inner diameter of about 20 – 25 mm and a length of about 9 – 15 mm [18]. Figure 2.2 presents the lengths and external diameters of the trachea in a newborn, 10-year old child and an adult.

The trachea is the stiffest airway of both the respiratory and conducting zones, and experience significant deformation at physiological pressures due in part to its radial expansion during inspiration and compression during expiration [19].

Figure 2.2. The lengths and external diameters of the trachea in a newborn, 10-year old child and an adult [18].

2.1.1 Tracheal Rings

The lateral and anterior sides of the human trachea are reinforced by about 15 – 20 incomplete C-shaped cartilaginous rings (sometimes referred to as tracheal rings) which protect and maintain airway patency [18]. These cartilages are connected to each other by ligaments and the Trachealis muscle connects the ends of these incomplete cartilaginous rings. The cartilaginous rings are incomplete to allow the trachea to collapse slightly to allow food to pass down the esophagus, and also to enable the trachea to constrict to increase the air flow rate during coughing. These rings also prevent over-expansion during inspiration and collapse of the airway during expiration, thereby causing the trachea to have extensive dynamic movements throughout a human's lifetime [20]. Tracheal rings measure about 4 mm in depth and 1 mm in thickness [18].

2.1.2 Epithelium Cells

The trachea is lined with pseudostratified ciliated columnar epithelial cells (Figure 2.3), together with other cells known as goblet cells. The term “pseudostratified” is used as all cells come in contact with the base membrane and hence only a single layer of cells exist along the trachea, but the cells’ nuclei are not aligned in the same plane and thus gives a multi-layered appearance of the cells [21]. The goblet cells’ sole function is to secrete mucus (not shown). Figure 2.4 shows a scanning electron microscopy (SEM) image of ciliated cells.

Figure 2.3. Pseudostratified ciliated columnar epithelium (A = line of alignment of columnar cells, B = cilia and C = nuclei) [21].

Figure 2.4. SEM image (X4,400) of a continuous carpet of ciliated cells [11].

Regardless of the temperature and humidity of the ambient air, when inspired air reaches the respiratory zone, it is at a temperature of 37°C (body temperature), and it is saturated with water vapour as it flows over the warm, wet mucous membranes that line the trachea. This ensures that a constant internal body temperature is maintained and that the lung tissue will be protected from desiccation, thereby addressing both *warming* and *humidification* functions of the trachea [16]. Mucus secreted by goblet cells serves to trap

small particles in the inspired air. This mucus is moved along at a rate of 1 – 2 centimetres per minute by cilia projecting from the tops of the epithelium cells (Figure 2.4). There are about 300 cilia per cell that beat in a coordinated fashion to move mucus towards the pharynx, where it can be either swallowed or expectorated, and this addresses the *filtration* and *cleaning* functions of the trachea [16]. It is therefore essential for these linings of cilia to be maintained to serve its protective function even after surgical procedures and / or the introduction of implanted stents. Metallic stents with mesh designs serve this purpose, discussed in detail in Section 2.4.1.

2.2 TRACHEAL STENOSIS

Tracheal stenosis is defined as a fixed airway narrowing which causes airway obstruction in inspiration and expiration [22]. Common symptoms of tracheal stenosis are an inspiratory stridor and / or expiratory wheeze. Almost half of those patients exhibiting such symptoms are misdiagnosed with asthma or chronic bronchitis as they present similar problems such as dyspnea on exertion and wheezing, and most patients do not develop symptoms at rest until their tracheas have been narrowed by about 70%. Mild stenosis is considered less than 70%, moderate at 70 – 90% and severe at greater than 90% [24]. Such an obstruction of the airway, either from a benign or malignant disease, is associated with high morbidity and possible death by asphyxia [24].

Tracheal stenosis is not dependant on gender nor race. There are, however, different leading causal factors according to age. For example, in children, the leading causes are post-intubation stenosis or congenital stenosis. In adults, the leading causes are trauma, systemic inflammatory diseases or tumours [24]. The progression of the disease would

depend on the type of disease. Inflammatory diseases wax and wane, tumours progress with rates dependant on the histopathology and injury stenosis can progress to fixed stenosis in a month (courtesy of Dr. Lynne Lim, NUHS). Figure 2.5 below shows an example of an obstructed airway.

Figure 2.5. Image of tracheal stenosis [25].

2.2.1 Causes of Tracheal Stenosis

The most common cause of tracheal stenosis in adults is internal trauma [24]. This refers to prolonged endotracheal (ET) intubation and tracheostomy (about 30% of all cases) [24,25,26,27], with other causes of tracheal stenosis due to tracheomalacia (described in Section 2.3) and benign or malignant tumours [6,7,17,26]. Stenosis related to endotracheal intubation most commonly occur at the site of the inflatable cuff [26]. For patients who require ET tubes to aid in respiration, when the cuff of the ET tube is blown up such that the pressure in the balloon is greater than 30 mmHg, the capillary perfusion pressure of the trachea is exceeded and causes mucosa ischemia and inflammatory changes. Small ulcerations may eventually cause chondritis and cartilage necrosis. As healing sets in, fibrous and granulation tissue proliferate into the tracheal lumen and leads to stenosis during scarring [12,28]. In contrast to this, tracheostomy-related stenoses are found both at the site of the stoma and at the location of the inflatable cuff [26]. A

tracheostomy is a surgical procedure on the neck to open a direct airway through an incision in the trachea as seen in Figure 2.6.

Figure 2.6. Schematic diagram of a tracheostomy showing; 1 = vocal cords; 2 = thyroid cartilage; 3 = cricoid cartilage; 4 = tracheal ring; and 5 = balloon cuff (courtesy of www.wikipedia.com).

2.2.2 Current Treatments for Tracheal Stenosis

Dilation: This is typically done using serial dilation using various sizes of Jackson bronchoscopes [24]. This is performed as a series of dilations, with increasing balloon diameters until the desired patency is achieved, so as to minimise the risk of rupturing the tracheal wall [7]. However, various studies have been conducted to reveal that mechanical dilation used alone can have a recurrence rate of stenosis, or more commonly termed restenosis, of greater than 90%. Thus, various adjunctive procedures have been developed in order to try to decrease the rate of restenosis [24].

Mitomycin C: This is an example of one of the adjunctive agents used together with mechanical dilation and applied topically. Mitomycin C (MMC) inhibits fibroblast production, thereby reducing the occurrence of tissue granulation and restenosis [24]. More details on MMC are presented in Section 2.5.

Laser: Laser is another adjunctive modality for the treatment of tracheal stenosis and involves the surgical resection of lesions, including radial cuts made with dilation. Some common lasers are the neodymium-aluminium-garnet (Nd-YAG) and CO₂ lasers [17,24]. The granulation tissue or tumour is removed by the process of thermal necrosis or photocoagulation. Thermal necrosis is the process where high energy levels are used to destroy cells. Photocoagulation, on the other hand, uses lower energy levels to heat the tumour and causes it to shrink. This process also diminishes blood flow to the region and allows the tumour to be surgically resected quickly at a controlled bleeding rate [17].

Stenting: Stenting is yet another adjunctive modality for the treatment of tracheal stenosis during endoscopy. Stents for the airway started as early as the 1960s, when Montgomery developed his famous T-tube, a silicone prosthesis in a T-shape that is placed via a tracheostomy to help stent open the airway. Following this in the 1970s, Dumon and Bauer modified Montgomery's T-tube by cutting off the end and making it a long cylinder and placing it in the trachea via an endoscope. In the 1990s, people started taking metallic stents, similar to those used for cardiac stenting, and placing them in the airway [24]. Currently, tracheal stents are implanted into tracheas during chemotherapy / radiation or after laser resection of tracheal granulation tissue and tumours, and act as a structural support while the affected tracheal wall heals and recovers. Both polymeric and metallic stents will be discussed in greater detail in Section 2.4.

Surgery: This is the only definitive management for lesions causing tracheal stenosis if the patient is a surgical candidate. The classic surgical procedure for this is tracheal resection with an end-to-end anastomosis [24,29]. In this procedure, it is important to get a good idea of how long the stricture is and how many areas are involved [24]. Endoscopic treatments as discussed above (dilation, laser and stenting) are preferred for patients with malignant airway stenosis or long-segment benign airway obstructions, rather than resorting to surgical resection [29].

2.2.3 Drawbacks and Complications of Current Treatments for Tracheal Stenosis

Dilation: As mentioned in Section 2.2.2, the rate of restenosis have been shown to be greater than 90% when mechanical dilation is used alone to achieve the desired tracheal patency in a patient with tracheal stenosis [24]. This is because mechanical dilation treats stenosis by forcibly breaking up the fibrotic bands temporarily. As there is tissue damage during this process, there is repeat of the scarring as the tissue damage heals, so restenosis is sometimes worse (courtesy of Dr. Lynne Lim, NUHS). Therefore, dilation is seldom only used alone and other treatment modalities have to be used in conjunction with dilation to achieve reasonable rates of success.

Mitomycin C: MMC, being first used as an ophthalmic treatment for glaucoma, inhibits DNA synthesis and fibroblast proliferation [10]. However, the effect of topically-administered MMC (at short durations of a few minutes) for inhibition of tracheal stenosis is still not completely known. Recent double blind placebo controlled studies that were done on patients actually showed no statistical difference in the rates of recurrence of tracheal stenosis between control and MMC treatment groups [30,31]. Pharmacokinetic

reasons for such observations were not reported, and this was most probably due to the rapid clearance of MMC as it was simply swabbed directly against the tracheal wall.

Laser: This treatment modality has been facing some amount of controversy. Laser has the potential to cause coagulation, necrosis and damage to the normal trachea on either side of the lesion, leading to an extension of the area of stenosis and making further subsequent surgical management more difficult [24].

Stenting: Section 2.4 will discuss in detail the drawbacks and complications of implanting both polymeric and metallic stents after laser resection of tissue / tumours.

Surgery: As explained in Section 2.2.2, before surgery is chosen, the surgeon has to determine how long the stricture is and how many areas are involved. For a surgical technique, a point to note is that the blood supply to the trachea arises laterally. It is important for the surgeon to maintain the dissection of the midline and to try to not disrupt this blood supply. Complications of the surgery include granulation tissue formation at the anastomotic line and this is related to the tension on this line, which the surgeon has to keep in mind to reduce by using absorbable sutures for example. Also, surgery is only possible when the resected segment is less than 6 cm and cannot be performed on patients who have high surgical risk such as severe heart or lung problems or due to old age [17,23,24].

2.3 TRACHEOMALACIA

Tracheomalacia is a process characterised by flaccidity or structural abnormality of the supporting tracheal rings / cartilage, widening of the posterior membranous wall, and reduced anterior-posterior airway calibre. These factors cause tracheal collapse, especially during times of increased airflow such as coughing, crying or feeding [32]. Therefore, tracheomalacia is a condition of dynamic airway collapse during expiration, as compared to fixed airway narrowing in tracheal stenosis as described in Section 2.2 [22]. Symptoms of tracheomalacia include wheeze, cough, stridor, dyspnea, tachypnea, cyanosis, and recurrent respiratory tract infections [26]. All types of tracheomalacia are rare, but infants with abnormal bronchograms who require ventilatory support have been reported to have a high morbidity and mortality, often related to airway obstruction and respiratory failure [22,32].

2.3.1 Causes of Tracheomalacia

Tracheomalacia can be categorised into 3 groups as shown in Table 2.1.

Table 2.1. Types of tracheomalacia based on presentation [32].

Type	Presentation
Type I	Congenital or intrinsic tracheal abnormalities that can be associated with tracheoesophageal fistula
Type II	Extrinsic defects or anomalies, such as vascular ring causing undue pressure on the trachea
Type III	Acquired tracheomalacia that occurs with prolonged intubation or chronic tracheal infections

Broadly speaking, tracheomalacia can be described as *primary*, when cartilaginous rings are congenitally immature / malformed (which is the cause in Type I), and *secondary*, due to degeneration of previously normal / healthy cartilage (which is thought to produce other types) [22,32]. These deficiencies of the supporting cartilage decrease the cartilage-to-muscle ratio. In Type I, tracheomalacia of the airway caused by congenital defects improves by age 6 – 12 months as the structural integrity of the trachea is restored gradually with resolution of the process. In Type II, extrinsic airway compression by a vascular ring or by enlarged great vessels in the presence of a large shunt does not allow normal development in the area of the trachea and can result in permanent airway narrowing in the area of impingement. Interestingly, Type III is the most common etiology of tracheomalacia, where chronic inflammatory processes like prolonged endotracheal intubation or tracheostomy is the cause, similar to tracheal stenosis as described in Section 2.2 [26,32]. Other causes of tracheomalacia include chronic obstructive pulmonary disease, trauma, recurrent infection and polychondritis; and the severity of disease depends on the length, location and degree of narrowing in the affected airway segments [22].

2.3.2 Current Treatments for Tracheomalacia

Non-surgical: Tracheomalacia of the milder primary variety is best treated by non-surgical means, where supportive therapy is provided to most infants. The majority will respond to conservative management, consisting of humidified air, chest physical therapy, slow and careful feedings, and control of infection and secretions with antibiotics. The use of continuous positive airway pressure (CPAP) has been recommended in patients having respiratory distress and is usually successful in patients requiring short-term intervention as the disorder spontaneously resolves. In adults, tracheomalacia may be

caused by chronic obstructive pulmonary disease and CPAP can also be used to keep the airway open and facilitate secretion drainage, while the obstructive disorder is treated first [32].

Surgery: Surgical therapy is only recommended for severe symptoms and failure of conservative therapy or when reflex apnea is present. Surgery includes correction of the underlying cause, tracheostomy, and aortopexy. Tracheal compression caused by tracheoesophageal fistula, mediastinal tumours and vascular rings are some conditions that need to be corrected surgically. In adults, surgical placcation of the posterior wall of the trachea with crystalline polypropylene and high-density polyethylene mesh has been used recently [32].

Tracheostomy: Tracheostomy helps to maintain an airway while the child grows and the trachea regains structural integrity. In adults, it may be effective because the tracheostomy tube might bypass the malacic segment, or the tube itself might splint the airway open. It can be performed as an open procedure or via percutaneous approach. In an open procedure, a transverse incision is made at the third tracheal ring and a lubricated tracheostomy tube is inserted through this opening. The tube is secured to the neck and adjusted so that the distal end is at least 2 cm above the carina. A percutaneous tracheostomy can be performed in the ICU and requires specially designed introducer sets. A 3 cm longitudinal incision is made over the second and third tracheal rings and the introducer set consisting of a dilator, guiding catheter and wire guide are used to position the tracheostomy tube through the larynx. This procedure is inexpensive and easy to learn compared to the usual tracheostomy [32]. Figure 2.7 shows a patient with a tracheostomy tube in place.

Figure 2.7. A patient with a tracheostomy tube in place [21].

Aortopexy: Aortopexy can provide relief of tracheal compression and relieves the external pressure on a flaccid trachea from vascular abnormalities. In this procedure, a single row of monofilament sutures is placed from the arch of the aorta to the undersurface of the sternum and tied down to displace the arch anteriorly. The dissection around the aorta must be avoided to help draw open the lumen of the trachea when aortopexy has been achieved. Therefore, aortopexy attaches the aorta to the sternum, pulling the anterior wall of the trachea forward and preventing its collapse [32].

Stenting: The use of various types of tubes or stents for the management of tracheomalacia is helpful as short-term satisfactory results have been reported with the use of expandable metallic stent placement in patients. Metal stents have been used to manage airway obstruction as they can expand dynamically and preserve mucociliary function. Silicone stents are easy to insert, reposition and remove. Stents have resulted in both subjective and objective improvement, but success is not universal [32]. Both polymeric and metallic stents will be discussed in greater detail in Section 2.4.

2.3.3 Drawbacks and Complications of Current Treatments for Tracheomalacia

Surgery: Similar to surgery conducted for tracheal stenosis, it cannot be performed on patients who have high surgical risk such as severe heart or lung problems or due to old

age. Postoperative care of patients is similar to that of patients undergoing thoracic surgery and patients may need to be monitored closely in an ICU setting because several days may pass before improvement in airway function occurs. Some patients need long-term follow-up care for evaluating the success / failure of the surgical procedure and development of complications [32].

Tracheostomy: Long-standing tracheostomies lead to several complications, which include bilateral vocal cord paralysis; compression and erosion of the innominate artery; formation of granulation tissue which results in protrusion of tracheomalacia; and speech delay in several instances. Another problem with this procedure is that the tracheostomy tube may not support the distal trachea. Complications of percutaneous tracheostomy are bleeding, infection, accidental endotracheal extubation, extratracheal dilator position, esophageal perforation, and mucosal endobronchial flap. Tracheostomy imposes a heavy penalty on the patient and treatment alternatives should be explored [32].

Aortopexy: During aortopexy, as the sutures are placed through the aortic wall, there is an immediate risk of haemorrhage and potential for postoperative aneurysm formation. Deaths have occurred as a result of operative failures, other structural abnormalities, and chronic ventilatory insufficiency. Also, aortopexy treatment failures have been reported for proximal tracheal involvement. This is because the aorta is attached to the sternum in the distal region of the trachea, and proximal tracheomalacia fails to be addressed [32].

Stenting: Section 2.4 will discuss in detail the drawbacks and complications of both polymeric and metallic stents.

2.4 TRACHEAL STENTS

A stent is a medical device designed to serve as a temporary or permanent internal scaffold to maintain or increase the lumen of a body conduit. Both metallic and polymeric stents have been successfully used for the management of tracheal stenosis and tracheomalacia for many years and the results from their use suggest that stenting is a promising method for treating tracheal obstruction [22]. Its increasing popularity is due the simplicity of stent placement and the lack of good alternative approaches to open surgical procedures [28]. It also gives prompt airway relief and is the only immediate treatment for unresectable extraluminal compression as it can provide prompt stabilisation of a threatened airway while the primary tumour is treated with chemotherapy or radiation [33]. A stent is normally inserted whenever the patency of the affected airway remains below 50% of normal after endoscopic therapy with laser and / or dilation [33].

Indications: The potential indications for tracheal stent insertion are [17,33,34,35]:

- Non-resectable tumours
- Complex tracheal stenosis patients who are not suitable surgical candidates due to severe heart or lung problems, or old age
- Congenital defects
- Inflammatory diseases such as tuberculosis or sarcoidosis, while waiting for systemic therapy or pending open surgical resection
- When the patency of the lumen remains low (<50%) even after resection of the tumour or dilation
- Tracheal obstruction due to intrinsic or extrinsic compression in patients who have exhausted curative therapeutic options

- Localised or extensive tracheomalacia regardless of cause
- Anastomotic strictures after lung and heart-lung transplantation
- Airway obstruction due to stenting of the esophagus
- Sealing tracheoesophageal fistulas
- Relief of post-obstructive pneumonia

Classification: Tracheal stents can be broadly classified into three types, namely, polymeric (silicone) tubular stents, metallic wire-mesh stents; and composite stents where the stent is a wire-mesh with a polymer coating [17,33,34,35]. Sections 2.4.1 and 2.4.2 will elaborate on some common commercially available types of stents, advantages and complications in their use.

An ideal tracheal stent should have the following properties: (1) is easy to insert and remove; (2) is made of a biocompatible material so that it does not irritate the airway or precipitate infection; (3) it is designed to prevent tumour in-growth or granulation tissue formation; (4) it is designed to maintain mucociliary clearance or to facilitate mucous clearance; (5) it can be deployed under local anesthesia with conscious sedation; (6) it maintains its placement in the airway without migration during respiration or coughing; (7) it has an expansion force adequate to maintain airway patency against compressive forces without causing pressure to the mucosa that could damage the airway; (8) it is available in different sizes to accommodate various airway sizes and obstructions; and (9) it is economically affordable [28,33,34,35]. So far, such a stent still remains to be developed and each stent has its own advantages and disadvantages, which contribute to its efficacy and complication rate [28,35]. However, stenting still has the outstanding benefit of immediate relief of acute distress from tracheal obstruction within a very short

time and potential developments should include biodegradable stents, and drug-eluting or radioactive-coated stents [4,33].

2.4.1 Metallic Stents

Metallic stents originally designed for use in the vascular system have also been used in tracheal conditions since the mid-1980s [33]. There are generally two types of metallic stents: balloon-expandable and self-expanding stents [28]. The following are some common commercially available metallic stents on the market:

Palmaz Stent (Johnson & Johnson, NJ, USA): This is a stainless steel wire-mesh cylinder where its diameter is determined by the inflation of a balloon after insertion [24,33,34,35]. They are dilated beyond their elastic limits to ensure shape integrity and they do not exert continual expanding pressure on the tracheal walls after deployment [17,33]. The advantages of the Palmaz Stent are its easy placement, precise diameter control and high visibility on X-rays. The disadvantages are its inflexibility, possibility of formation of granulomas in the interstices and the impossibility of its removal upon implantation due to epithelisation within one month [2,24,28,33,34,35]. Other disadvantages reported are that it can only be dilated to diameters of about 11 to 12 mm, thereby restricting its use to pediatric tracheal conditions; inflammatory tissue reactions when the stent was over-dilated; partial collapse of the stents, thereby requiring frequent monitoring and stent adjustment; stent migration; and injury to the tracheal wall during balloon dilation [2,17]. Figure 2.8 shows a picture of the Palmaz Stent.

Figure 2.8. The Palmaz Stent (courtesy of www.cordis.com).

Wallstent (Boston Scientific, MA, USA): Compared to the balloon-expandable Palmaz Stent, the Wallstent is a self-expanding cylindrical mesh made of about 16 to 20 strands of stainless steel wires woven in criss-cross fashion and these wires have to be carefully shaped and bent to maintain a certain mesh pattern. The release of the Wallstent during deployment is carefully controlled using hydraulic pressure within the delivery catheter and foreshortens on radial self-expansion. The advantages of the Wallstent are that it is flexible, exerts only a moderate force on the tracheal wall and can be deployed using a rigid or flexible bronchoscope through the larynx. The major disadvantage of the Wallstent is that the free metallic ends embed in the mucosa for anchorage and stent removal or repositioning becomes very difficult and can be only be performed through laser destruction and subsequent piecemeal wire removal. Other reported disadvantages of the Wallstent include stent migration with models without free metallic ends; poor tissue-stent interaction where granulation tissue is significant; only available in limited lengths and tend to be longer than required; and problem in distal tracheal stenosis as there is a possibility of the stent protruding beyond the carina [2,17,34,35].

The insertion of a permanent metal stent is indicated when the patient is unfit for surgery or when the tracheal strictures are too expensive to resect, or multiple [33]. Table 2.2 summarises the advantages and disadvantages of metallic stents in general [2,17,23,24,25,28,34,35].

Table 2.2. Summary of advantages and disadvantages of metallic stents.

Advantages	Disadvantages
Easily deployed	Poor tissue-stent interaction – tendency to develop tissue granulation
Good flexibility	Difficult to reposition stent
Good expansiveness	Difficult to remove stent due to epithelisation within one month – laser destruction of stent and piecemeal removal of wires needed
Good radial force	Limited sizes available
Less frequent migration than polymeric tubular stents	Dynamic expansion can result in tracheal wall rupture and / or ulceration
Relatively thin walls	Higher cost than polymeric tubular stents
Allows use of both rigid and flexible bronchoscopes	Not capable of preventing tumour or granulation tissue in-growth
Local anaesthetic and outpatient procedure	Care has to be taken when there is active inflammation

2.4.2 Polymeric Stents

The most common polymeric / plastic stents are made of silicone. These stents can only be inserted using a special applicator under general anaesthesia and rigid bronchoscopy. Since Montgomery described the use of a T-tube in 1965, a variety of silicone tracheal stents have been developed and used [33,34,35]. The following describes some of these currently on the market:

Montgomery T-tube (Boston Medical Products, MA, USA): The Montgomery T-tube has a side arm at a right angle near its upper end and serves both as a stent and as a tracheostomy tube in the treatment of subglottic and mid-tracheal stenosis. This was the first effective tube prosthesis and is still in regular use. It is inserted through a tracheostomy stoma / incision to provide support for the upper trachea and larynx, and the removable cap at the external branch allows suction of accumulated mucus and provides ventilation if required. The two main disadvantages of the T-tube are that it requires a tracheostomy incision and there exist a risk of obstruction if the external orifice is uncapped [17,33,34,35].

Dumon Stent (Boston Medical Products, MA, USA): The Dumon Stent, introduced in the late 1980s, has probably been the most commonly used silicone stent to date and because of the vast clinical experience in its use, it is considered the gold standard against which all other tubular stents are measured [17,33]. It is a cylindrical tube made of soft silicone with external studs at regular intervals or a branched shape to prevent its migration in the airway both longitudinally and turning radially, and limit contact with the mucosa. The outer surface of the stent is coated with a non-stick coating and polished to remove all sharp edges, including the ends, to prevent laceration or irritation to the

tracheal wall. The advantages of the Dumon Stent are that it is well tolerated by most patients as majority of them do not complain about any foreign body sensations after the stents have been implanted; and insertion, readjustment and removal of the stents are easily achieved in most cases [17,33]. One major disadvantage of the Dumon Stent is significant stent obstruction with the accumulation of secretions due to impairment of mucociliary clearance and impedance of the cough reflex. Such accumulation of secretions also induces a higher chance of bacterial colonisation [33,34,35]. The thick wall of the Dumon Stent results in a low internal to external diameter ratio such that they cannot be used in small airways. Interestingly, even with the studs and polished surfaces, migration and granulation tissue formation are still frequently encountered which necessitates its removal and replacement and laser resection of the granulation tissue to restore lumen patency [17,33,36]. Other disadvantages of the Dumon Stent include the lack of flexibility in the airway due to its covered design and a silicone wall thickness of 1mm being stiffer (higher modulus) than the trachea, high mucosal pressure and clinical reports of lethal haemorrhage, care needed for tube selection, requirement of a rigid bronchoscope and general anaesthesia [17,24,33]. Figure 2.9 shows a picture of some Dumon Stents.

Figure 2.9. Dumon Stents (courtesy of www.bosmed.com).

Polymeric / silicone tracheal stents are inserted only temporarily due to the many complications in their use and are indicated in the following circumstances [33]:

- When the use of these stents may allow resolution of the stenosis
- When surgery has to be deferred to allow the condition of the patient to improve
- When delay will allow a better assessment of the length and extent of the stricture
- When delay will allow resolution of acute inflammation within the trachea, preparing the airway for permanent stent insertion (i.e. metallic stents)

Table 2.3 summarises the advantages and disadvantages of polymeric / silicone stents in general [17,23,24,25,28,34,35].

Table 2.3. Summary of advantages and disadvantages of polymeric stents.

Advantages	Disadvantages
Inexpensive	Frequent migration
Good tissue tolerance	Granulation tissue formation
Easy to reposition	Thick walls which limit applications
Easy to remove	Less flexible than metallic stents
	Requires rigid bronchoscopy and general anaesthesia
	Cannot be used in short conical stenoses
	Impaired mucociliary clearance which leads to build up of mucous secretions

2.5 MITOMYCIN C

Mitomycin C (MMC) is an anti-tumour antibiotic discovered in the 1950s by Japanese microbiologists in fermentation cultures of the microorganism *Streptomyces caespitosus* [10,37]. It finds its use as a chemotherapeutic, bifunctional alkylating agent that is given as a treatment (usually intravenously) for several types of cancers, including breast, stomach, oesophagus and bladder cancers, due to its ability to inhibit DNA synthesis by crosslinking and fibroblast proliferation [10,11,13,37]. MMC is a purple fluid after being dissolved from odourless blue-violet crystals with a molecular formula of $C_{15}H_{18}N_4O_5$ and a molecular weight of 334.13 g/mol. The systematic name for MMC is 6-amino-1,1a,2,8,8a,8b-hexahydro-8-(hydroxymethyl)-8a-methoxy-5-methyl-azirino[2',3'-3,4]pyrrolo[1,2-a]indole-4,7-dione carbamate (ester) [37,38]. Figure 2.10 shows the molecular structure of MMC.

Figure 2.10. Molecular structure of mitomycin C [10].

2.5.1 Anti-tumour Activity of Mitomycin C

MMC selectively inhibits the synthesis of DNA. MMC crosslinks the complementary strands of the DNA double helix and a single crosslink per genome is enough to cause bacterial cell necrosis, thereby exhibiting its lethality (Figure 2.11). The guanine and cytosine content correlates with the degree of MMC-induced crosslinking [10,37]. Also,

as the activity of MMC is enhanced in hypoxic environments, it has good potential and has been used for localised treatment of solid tumours since a high percentage of viable cancer cells within a solid tumour can be hypoxic [11]. At high concentrations of MMC, cellular RNA and protein synthesis are also suppressed [10].

Figure 2.11. The reductive activation cascade of MMC. The activated MMC attaches DNA twice, resulting in covalent cross-linking of the strands of DNA [10].

2.5.2 Pharmacokinetic Properties of Mitomycin C

Absorption: MMC is rapidly cleared from the serum and is not dose dependent [37]. $C_{\max}$ is 2.4 $\mu\text{g/ml}$ (after 30 mg intravenous (IV) injection), 1.7 $\mu\text{g/ml}$ (after 20 mg IV), and 0.52 $\mu\text{g/ml}$ (after 10 mg IV) [39]. The plasma concentration-time curves show bi-phasic, exponential decline; and absorption after oral administration is very irregular [37].

Metabolism: Metabolism is primarily hepatic, but occurs in other tissues as well. The rate of clearance is inversely proportional to the maximum serum concentration because of saturation of the degradative pathways [39].

Elimination: The major route of elimination is liver metabolism, with approximately 10% of a dose of MMC excreted in the urine [37]. Since metabolic pathways are saturated at relatively low doses, the percent of a dose excreted in urine increases with increasing dose, and this applies similarly to children. The time required to reduce the serum concentration by 50% ($t_{1/2}$) is 17 minutes after a 30 mg bolus injection [39].

2.5.3 Toxicology of Mitomycin C in Animals

MMC has been found to be carcinogenic in rats and mice. At doses about the recommended clinical dose in human, it produces a greater than 100% increase in tumour incidence in male Sprague-Dawley rats, and a greater than 50% increase in female Swiss mice [39].

2.5.4 Toxicology of Mitomycin C in Humans

MMC can reduce the production of white blood cells by the bone marrow (myelosuppression), resulting in a *lowered resistance to infection*. This effect can begin 7 days after treatment has been given and resistance to infection usually reaches its lowest point about 28 days after chemotherapy [11,37].

Bone marrow toxicology is the most common and most serious toxicology, occurring in 605 of 937 patients (64.4%). Thrombocytopenia and / or leukopenia may occur anytime within 8 weeks after onset of MMC therapy with an average time of 4 weeks. Recovery after cessation of therapy was within 10 weeks. About 25% of the thrombocytopenic and leukopenic episodes did not recover [39].

Pulmonary toxicology has occurred infrequently but can be severe and may be life threatening. Dyspnea with non-productive cough and radiographic evidence of pulmonary infiltrates may be indicative of pulmonary toxicology, and MMC therapy should be discontinued. A few cases (4%) of adult respiratory distress syndrome within minutes to hours have been reported in patients receiving MMC in combination with vinca alkaloids [39]. This is because both MMC and vinca alkaloids are anti-cancer agents. If applied systemically, they activate apoptotic signalling pathways even in normal lung tissues and causing hypersensitivity which reduce the gas diffusion between lung tissue and blood capillaries. Hypersensitivity can be even more significant when two chemotherapeutic agents are used concurrently [40].

Other adverse effects of MMC treatment include bleeding due to a reduction of platelets; anaemia due to a low number of red blood cells; loss of appetite; tiredness / fatigue; headache; blurred vision; edema; nausea / vomiting; diarrhoea and pain [37,39]. MMC that leaks into tissue can also damage the tissue in that area, causing a stinging or burning sensation to the patient and ulceration [37].

2.5.5 Stability of Mitomycin C

Unreconstituted MMC stored at room temperature is stable for the lot life indicated on the package, however, exposure to light and excessive heat over 40°C should be avoided [39]. However, MMC is not stable when stored as aqueous solutions. MMC degradation is accelerated by acid, alkali, buffer ions and high temperatures. In acidic conditions, MMC is converted into 1-hydroxy-2,7-diaminomitosenes in an apparent first-order process; and in basic conditions, it is hydrolysed into 7-hydroxymitosane (both reactions shown in Figure 2.12) [37,41].

Figure 2.12. Overall degradation scheme of MMC (acidic and basic conditions) [37].

From Figure 2.12, in the mitosene compounds, it can be seen that the methoxyl group originally present in MMC is extremely labile and easily cleaved under mild acidic (and aqueous) conditions to give one mole of methanol as a by-product [42]. Other important observations from acidic degradation of MMC are that a double bond is introduced between C9 and C9a and the aziridine ring is being opened [37]. It is from these two mechanisms that cause the ultraviolet spectra of the degradation product (mitosenes) to be different from original MMC. It is also noted that only MMC with the aziridine ring intact is biologically active and efficacious, as opposed to biologically inactive mitosenes with open aziridine rings [42]. MMC can be reconstituted with sterile water to a saturation concentration of 0.5 mg/ml and stable for more than 18 days at room temperature; and stability is drug-concentration dependent, with greater stability at higher concentrations [41]. At 0.5 mg/ml, MMC loses 10% potency in water at room temperature after 40 days but loses close to 86% potency just after 1 month at 40°C, clearly showing accelerated degradation at higher temperatures [43]. A combination of MMC (5 mg to 15 mg) and heparin (1000 units to 10000 units) in 30 ml of 0.9% sodium chloride injection is stable for 48 hours at room temperature, with stability inferred if the percent potency reduction

is less than 10% [39,41]. Commercially available MMC is supplied in a lyophilised form containing mannitol or sodium chloride as excipients [37].

MMC activity and degradation are also known to be very **pH-dependent**. Low pH will induce MMC decomposition drastically due to acid catalysis [37,41]. The decrease in MMC detection in culture media as a function time is pH-dependent, with about 24% of MMC degrading after 2 hour exposure at pH 5; compared to less than 2% degradation occurring at pH 6 and 7.4 [44]. It is thus reported and concluded that MMC rapidly degrades in solutions with $\text{pH} < 5$ and acidic MMC dosing solutions for intravesical therapy have diminished value [41,44].

2.5.6 Use of Mitomycin C for the Treatment of Tracheal Stenosis

In tracheal stenosis, MMC is currently administered topically onto the mucosa of the tracheal wall, after surgical resection of granulation tissue or tumours, to decrease the incidence of restenosis by suppressing the production of fibroblasts and scar tissue. However, the effect of MMC for inhibition of tracheal stenosis reported by various groups is still not completely clear and remains controversial. Rahbar et al. reported that topical MMC effectively prevented scar formation in the aerodigestive tract [14]. On the other hand, Smith et al. reported that restenosis and delayed symptom recurrence for the treatment of tracheal stenosis were similar after endoscopic dilation with or without MMC [15]. Also, Shvidler et al. and Hartnick et al. did not observe significant differences in the airways between control and MMC treatment groups [30,31]; and Roh et al. concluded that topical MMC has no benefit when used to prevent tracheal stenosis [45]. It should be noted that these studies were conducted with MMC being only topically applied directly to the trachea for a few minutes, and without the application of stents. In all

previous studies, MMC could only be applied topically for 1 to 5 minutes to avoid blocking the airway during applications [46]. Though most topical applications held 0.1 to 1 mg/ml of MMC, the actual amount (or mass of MMC) delivered this way is unknown.

2.6 DEGRADATION AND EROSION OF BIODEGRADABLE POLYMERS

Polymeric biomaterials can be divided into two main classes according to desired lifetimes: namely biostable and biodegradable. Biostable materials have been used for a long time for both permanent and time-limited applications. However, it was noted that biodegradable systems could be of great use for temporary therapeutic applications in surgery, pharmacology and tissue engineering. Since the first biodegradable sutures made of polyglycolic acid (PGA) were made available in the 1960s, other synthetic polymers have been investigated and are currently referred to as biodegradable, bioabsorbable, bioresorbable and bioerodible [47].

2.6.1 Definitions

Polymer degradation is defined as a deleterious change in the properties of a polymer due to a change in the chemical structure [47]. The process of '*degradation*' describes the chain scission process during which polymer chains are cleaved to form oligomers and finally to form monomers [48]. A **biodegradable polymer** is a polymer in which the degradation is mediated at least partially by a biological system. A **bioabsorbable polymer** is a polymer that can be assimilated by a bio-system. A **bioresorbable polymer** is used to qualify polymers for which elimination of degradation by-products through

natural pathways (metabolisation or kidney filtration) has been proven. Bioresorption implies total elimination of the initial foreign material with no residual side effects. Lastly, a **bioerodible polymer** is defined as a polymer which exhibits a physical loss from the polymer matrix brought about by a variety of physical and chemical processes which are not always specified [47]. More accurately, the process of '*erosion*' designates the loss of material owing to monomers and oligomers dissolving or wearing away of a polymer from the surface [47,48]. The term **biodegradable polymer** is used to denote all film and stent samples used in this study.

2.6.2 Polymer Degradation and Erosion via a Bulk-degrading Process

Polymer *degradation* is the key process of *erosion*. There are two principal ways by which polymer bonds can be cleaved: passively by hydrolysis or actively by enzymatic reaction. For most biodegradable materials, especially synthetic polymers, passive hydrolysis of the hydrolytically unstable backbone is the prevailing and most important mechanism for polymer degradation [48,49]. Figure 2.13 graphically describes the hydrolysis process of generic biodegradable polyesters to produce monomers with acid and alcohol end-groups.

Figure 2.13. Hydrolytic degradation of a polyester (courtesy of Purac Biochem BV).

Hydrolytic degradation of biodegradable polymers occurs in two phases. In the first phase, water rapidly penetrates into the bulk and begins to attack the chemical bonds (Figure 2.13) in the amorphous phase and converting long polymer chains into shorter water-soluble fragments. Since this occurs in the amorphous phase initially, there is a reduction in molecular weight without a loss in physical properties, as the polymer matrix is still held together by the crystalline regions. The reduction in molecular weight is soon followed by a reduction in physical properties, as water begins to fragment the polymer matrix [49]. It is generally accepted that molecular weight drops according to an exponential decay [51]:

$$M_n = M_{n,0} e^{-kt} \quad (1)$$

where $M_{n,0}$ and M_n are the number average molecular weight at time $(t) = 0$ and t respectively, and k is a first-order degradation rate constant. Further complex computational modelling for the degradation of biodegradable polymers via hydrolysis with respect to polymer crystallinity and type of monomer / chemical bond has been performed for three-dimensional biodegradation [52] and by using Monte Carlo calculations [53,54].

In the second phase, enzymatic attack and metabolisation of the fragments occurs, resulting in a rapid loss of polymer mass. This type of degradation, where the rate at which water penetrates the device exceeds that at which the polymer is converted into water-soluble materials, is called bulk erosion and polymers exhibiting such a phenomenon are referred to as **bulk-degrading** polymers [49]. Such polymers are completely intruded by water prior to the start of erosion [50]. Table 2.4 summarises the characteristics of some typical bulk-degrading biodegradable polymers.

Table 2.4. Characteristics of typical bulk-degrading biodegradable polymers [49].

Polymer	Melting point (°C)	Glass transition temperature (°C)	Modulus (GPa)	Degradation time (months)
PGA	225 – 230	35 – 40	7.0	6 – 12
PLLA	173 – 178	60 – 65	2.7	> 24
PDLLA	Amorphous	55 – 60	1.9	12 – 16
PCL	58 – 63	(-65) – (-60)	0.4	> 24
PDS	N.A.	(-10) – 0	1.5	6 – 12
85/15 PDLGA	Amorphous	50 – 55	2.0	5 – 6
75/25 PDLGA	Amorphous	50 – 55	2.0	4 – 5
50/50 PDLGA	Amorphous	45 – 50	2.0	1 – 2

PGA: poly(glycolic acid); PLLA: poly(L-lactic acid); PDLLA: poly(DL-lactic acid); PCL: poly(ϵ -caprolactone); PDS: polydioxanone; PDLGA: poly(DL-lactic-co-glycolic acid).

There are several factors that influence the velocity or rate of hydrolysis, of which, the type of chemical bond, pH, copolymer composition, water uptake and presence of enzymes are the most important. Chemical and physical changes go along with the degradation of biodegradable polymers, like the crystallisation of oligomers and monomers or pH changes [48,52,53]. Some of these factors have a substantial feedback effect on the degradation rate. The most important parameter for monitoring degradation is molecular weight. Other ways of measuring degradation that have been proposed is the loss of mechanical strength, complete degradation to monomers or monomer release [50,52]. These are related but need not necessarily obey the same kinetics. For example, complete degradation of poly(L-lactic acid) is known to take much more time than the loss of tensile strength. Aqueous solutions of lactic acid form spontaneously poly(lactic

acid) oligomers that might affect molecular weight measurements. Also, monomers from copolymers need not be released with identical kinetics during erosion [48].

Effect of the type of chemical bond: It is mainly the type of chemical bond within the polymer backbone that determines the rate of hydrolysis. Anhydride and ortho-ester bonds are the most reactive ones, followed by esters and amides. Reactivities can change greatly upon catalysis or by altering the chemical neighbourhood of the functional group through steric and electronic effects. The influence of steric effects on degradation can be seen with poly(α -hydroxy esters). The slower degradation of poly(lactic acid) is partially due to the voluminous alkyl groups hindering the attack of water [48].

Effect of pH: The pH affects reaction rates through catalysis. Reactions of esters, for example, may change in orders of magnitude due to shifts in pH and catalysis. Ester hydrolysis can be either acid ($\text{pH} < 4$) or base catalysed. In some biodegradable polymers (e.g. PGA and PLGA), it was found that fastest degradation occurred at low (0.2) or high (11.2) pH and breaking strength was highest at neutral pH [48]. This faster chain scission at low pH explains the heterogeneous erosion of poly(lactic acid) due to autocatalysis. The generated monomers, which are carboxylic acids, accelerate polymer degradation by lowering pH. Studies into the degradation of poly(lactic-co-glycolic acid) microspheres revealed evidence of a high concentration of acidic monomers residing at the core and forming a pH gradient towards the edges as acidic by-products diffuse out of the beads. A minimum pH of as low as 1.5 was recorded and such acidic environments have great implications for protein denaturation and drug degradation when used as release devices [55]. The mode of hydrolysis, whether the polymer undergoes *chain-end* or *random*

cleavage of the ester bonds, is determined by pH. Acid-catalysed hydrolysis follows pseudo-zero order kinetics [56,57]:

$$-d[N_{E0}]/dt = k^0/DP_n^0 \quad (2)$$

where $[N_{E0}]$ is the initial concentration of total ester bonds, k^0 is the pseudo-first order rate constant and DP_n^0 is the initial average degree of polymerisation. This shows that the rate of loss of intact ester bonds is independent from the reducing concentration of these ester bonds over time. Zero order kinetics of such degradation proves that hydrolysis occurs via *chain-end* cleavage. In contrast, results from alkaline media show that the rate of ester bond cleavage follows pseudo-first order kinetics [56]:

$$[N_E] = [N_{E0}]e^{-kt} \quad (3)$$

which gives $-d[N_E]/dt = k[N_E]$ (4)

where $[N_E]$ is the concentration of intact ester bonds at time t and k is the pseudo-first order rate constant. Data for first order kinetics predicts that degradation proceeds via *random cleavage*, where all backbone ester bonds are equally reactive and accessible; and these can be observed in base-catalysed hydrolysis of polymers [57].

Effect of copolymer composition: Many properties of the original polymer like crystallinity or glass transition temperature can be altered by introducing a second monomer into the polymer chain. These factors can have indirect effects on the degradation rates of the copolymer. Such changes have been reported for copolymers of

polyanhydrides, and poly(lactic-co-glycolic acid), where the decrease of molecular weight during degradation was found to be accelerated with increasing glycolic acid content. As mentioned previously, this leads to the conclusion that degradation rates depend on the type of chemical bond [48].

Effect of water uptake: Hydrolysis is a bimolecular reaction in which water and the functional group possessing the labile bond are involved. Thus, the reaction rate is determined by the “concentration” of both reaction partners. Hydrophilic polymers take up large quantities of water and this increases their degradation rates. Hydrophobic polymers, on the other hand, exhibit the reverse and slow degradation rates are encountered. The uptake of water is especially important in the area of drug delivery. Some polymers may experience substantial swelling and this may be the decisive parameter for controlling the release of drugs, and may even be more important than polymer degradation [48]. It should be noted, however, that the **total** water content within a polymer bulk is not a rate-determining parameter for hydrolysis. A detailed study into the role of water molecules during hydrolysis postulates that only reactive or “*bound*” water participates in ester bond cleavage, as opposed to “*free*” water occupying the free volume between the polymer chains [58]. An increase in water molecules with hindered rotational freedom increases hydrolysis rates; therefore, the concentration of water as explained above relates only to bound water in the vicinity of ester bonds.

Effect of additives: In cases where polymers tend to degrade too slowly for a specific application, it might be wise to regulate the rate of chemical degradation such as adding excipients to regulate pH. In drug delivery applications, these can be the drugs themselves that are incorporated into the polymers, such as alkaloids and other bases or acids. It has

also been shown that the introduction of acidic and hydrophilic monomers increases the water uptake and enhances autocatalytic degradation, as in the case of poly(lactic acid) [48]. Other reported ways to control the degradation rates of polymers are through; copolymerisation, including block or graft copolymerisation (e.g. polyethylene glycol, dextran to form hydrogels); increasing dielectric constant of the polymer matrix; hydrophilise polymers by adding functional side groups (e.g. -OH, -COOH, -NH₂); and blending (e.g. PEG) [47].

Effect of enzymes: Once bulk-degradation results in both a reduction of molecular weight and physical properties of an implanted polymer, such polymer particles were found to be within the cytoplasm of macrophages, having been phagocytosed by them. Macrophages secrete lysosomal enzymes including hydrolases and these been known to degrade catgut and other similar materials [59]. Zhu et al. have also reported that the in vivo degradation of poly(1,3-trimethylene carbonate) was much faster than in vitro, and concluded that such a phenomenon was due to the specificity of the active site of the enzyme(s) for ester bonds. Control experiments with steric inhibition of access to the active site of the enzyme completely suppressed enzymatic attack [60]. Shirahama et al. confirmed this site / substrate specificity argument by conducting experiments with 3 different enzymes and monitored their effects against polyesters. It was concluded that polymers with high crystallinities and melting points also resisted enzymatic degradation; and such degradation tended to take place in the amorphous regions and uniformly from the polymer surfaces [61].

2.6.3 Polymer Degradation and Erosion via a Surface-eroding Process

All biodegradable polymers share the property of eroding upon degradation. Degradation and erosion are the decisive performance parameters of a device made of such materials undergoing hydrolytic degradation [48]. Bulk (or *homogeneous*) degrading polymers have been explained in Section 2.6.2. There exists a second type of degradation, known as surface (or *heterogeneous*) erosion, whereby the rate at which water penetrates the polymer is slower than the rate of conversion of the polymer to water-soluble materials. Polymers exhibiting such a phenomenon are referred to as **surface-eroding** polymers and such a polymer used as an implant results in the device thinning over time while maintaining its bulk integrity [49]. They lose material from the surface only and get smaller but keep their original geometric shape. The advantage of surface-eroding polymers is the predictability of the erosion process as they exhibit linear erosion profiles, whereas degradation and erosion in bulk-degrading polymers are not confined to the surface of the device and erosion cannot be accurately predicted. However, the size of bulk-degrading polymers will remain constant for a considerable portion of time during its application which might be preferable for certain applications [48]. Figure 2.14 illustrates these two concepts.

Figure 2.14. Relative rates of water permeation and polymer hydrolysis for bulk-degrading and surface-eroding polymers [62].

Surface-eroding polymers are desirable for use as drug delivery devices as the rate of drug release can be related directly to the rate of polymer erosion [48,50]. Polyanhydrides and polyorthoesters are examples of materials that undergo this type of erosion; when the polymer is hydrophobic but the chemical bonds are highly susceptible to hydrolysis [49]. Surface-erosion and bulk-degradation are ideal cases to which most polymers cannot be assigned with absolute certainty. Burkersroda et al. proposed that any polymer can undergo a surface-eroding profile if its matrix dimensions are larger than a critical value L_{critical} , which is derived from the polymer's Deborah number, ϵ . Typical L_{critical} values of surface-eroding polymers like polyanhydrides and polyorthoesters are 75 μm and 0.6 mm respectively; and those of bulk-degrading polymers like poly(α -hydroxy-esters) and polyamides are 7.4 cm and 13.4 m respectively [50].

Polymer erosion is far more complex than degradation as it depends on many other processes such as degradation, swelling, dissolution and diffusion of oligomers and monomers, and morphological changes. Although degradation is the most important process of erosion, other parameters may also become critical in controlling erosion behaviour. Therefore, the successful application of a biodegradable polymer depends upon the knowledge of the erosion mechanism. In drug delivery, swelling and porosity are critical to the rate of release of drugs. Many different indicators have been proposed for erosion, such as molecular weight loss, sample weight loss and changing geometry [50,52,53]. Both degradation and erosion are individual processes for each polymer [48].

Changes in morphology: The first morphological changes during erosion are confined to the polymer surface. With progressive erosion, polymers change to more porous structures as can be detected by mercury intrusion porosimetry. Surface-eroding polymers

like polyanhydrides and polyorthoesters show well defined erosion zones under light microscope [48].

Changes in pH: As described above, the mode and rate of degradation depends strongly on pH [56,57]. Through chain scission, polymers are transformed to oligomers and monomers, which have different functional groups from the polymer. Anhydrides are cleaved into carboxylic acids and esters; similar to poly(lactic acid) which is degraded into alcohols and carboxylic acids. The formation of such by-products affects the pH of the erosion medium and pH gradients in non-stirred buffered degradation media were detected when approaching the eroding surface [48]. An interesting study was conducted on known bulk-degrading polymers of poly(lactic acid) and poly(lactic-co-glycolic acid) degraded at pH > 13. These polymers began to mimic surface-eroding polymers and exhibited linear erosion profiles, and completely degraded after a couple hours, compared to a matter of days and weeks at pH 7.4 and 2 [50]. This goes to show that changes in pH can alter the degradation mechanism of biodegradable polymers substantially.

2.7 DRUG-RELEASE FROM BIODEGRADABLE POLYMERS

Controlled release drug delivery combines well-characterised, reproducible dosage-form design with clinical pharmacology, in particular, steady-state pharmacology. The steady-state biology defines the required input or the desired drug delivery profile. This design involves the application of physical and polymer chemistry to control drug entry into the body within the specifications of the required drug delivery profile, largely independent on external factors. *Sustained release* drug delivery, on the other hand, indicates merely a dosage form that has its dissolution profile extended in time rather than designed and

characterised to achieve a desired drug delivery profile; and subjected to the external environment into which the agent is released [63,64]. The efficacy of a drug in the body to perform its required function safely is determined by its steady state concentration between two levels of the therapeutic index, defined as the ratio of the maximum *toxic* concentration (C_{tox}) over the minimum *effective* concentration (C_{eff}) of the drug. This steady state concentration is given as:

$$C_{ss} = \frac{R_i}{k_{el} V_d} \quad (5)$$

where C_{ss} is the steady state concentration of the drug at the delivery site, R_i is the rate of drug input, k_{el} is the drug elimination rate constant at the site, and V_d is the volume of distribution for the drug. Assuming V_d to be constant, it can be seen that the drug-release rate (R_i) has a direct impact on the concentration of the drug at the delivery site and determines the fundamental efficacy and safety of the drug-delivery device.

Biodegradable polymers as vehicles for controlled and sustained drug delivery are attractive because surgery to remove the implanted device, after depletion of drug supply, is unnecessary [63]. It is thus also possible to program the release of a drug by dispersing it within such polymers, with erosion of the polymer effecting release of drug [64]. Both biodegradable natural polymers (e.g. polypeptides, polysaccharides) and synthetic polymers (e.g. poly(lactic acid), polyanhydrides) have been used in the pharmaceutical industry for many years. Drugs have been dissolved or dispersed uniformly throughout the matrix or incorporated within an erodible reservoir, and drug release is achieved by the degradation of the polymer [63].

Delivery rates from temporal drug delivery systems may be characterised in terms of their kinetics and physical processes. Zero-order systems, for which the release of drug is constant with time, t , are of particular interest due to the predictability of drug release which allows selection of precise efficacious plasma levels; and secondly, zero-order dosage forms have improved efficiency of drug delivery where distribution of the drug into the receptor is much slower than elimination. Zero-order systems can be achieved by using devices of different geometries, non-uniform drug distribution within the polymer matrix and by using surface-eroding polymers [63,64]. However, the most common drug delivery devices exhibit delivery rates that are proportional to $t^{-1/2}$ and this dependence is due to mass transport by sorption / desorption in monolithic systems [63].

2.7.1 Drug-release Mechanisms

Drug release from biodegradable / bioerodible polymers may occur by any of the three basic mechanisms shown in Figure 2.15.

Figure 2.15. Drug release mechanisms from biodegradable polymers [63].

In mechanism A, the drug is attached to the polymeric backbone by a labile bond A. This bond has a higher reactivity towards hydrolysis than the polymer reactivity to breakdown, and the drug is released when bond A is cleaved. Also, it may be toxic when the drug is released with polymer fragments; therefore, the reactivity of bond A should be much higher than bond B. In mechanism B, the drug is in a core surrounded by a biodegradable rate-controlling membrane. This is a reservoir-type device where drug release is controlled by diffusion of the drug across this membrane. The drug-depleted device then erodes and surgical removal is eliminated. Mechanism C describes a homogeneously dispersed drug in a biodegradable polymer. Drug release can be achieved and controlled by diffusion, a combination of both diffusion and erosion, or in rare cases by pure erosion [63]. This type of formulation, where the drug is dispersed homogeneously in a biodegradable matrix, is the most widely investigated system and will be discussed in more detail here.

2.7.2 Drug-release Determined Predominantly by Diffusion

The most popular drug delivery system has been the matrix (or monolithic) system containing uniformly dissolved or dispersed drug (e.g. tablets and granules) because of its low cost and ease of fabrication via a variety of processes like casting, compaction, extrusion and lamination [63]. These advantages outweigh the less desirable feature of declining release rates with time that is characteristic of such systems. The release profile is determined by the loading of the drug and governed mainly by the **diffusion** process, although other processes such as swelling and erosion may also take place at the same time, depending on the nature of the drug and the matrix material [63,64]. The release of a dissolved or dispersed drug from a matrix device involves the presence of a moving diffusional front separating an undissolved core from a partially extracted region. An

increase in diffusional resistance and a decrease in the effective area at this diffusion front during drug release give an inherently Fickian diffusion first-order release behaviour, proportional to $t^{-1/2}$ [63].

There are two principal categories of monolithic devices. A drug that is dissolved in the polymer medium, usually at a low drug-loading, is termed a *monolithic solution*. The device geometry affects drug-release and expressions for the release kinetics from such systems are derived from Crank's laws for desorption [64]:

$$\frac{M_t}{M_0} = 4 \left(\frac{Dt}{\pi l^2} \right)^{1/2} \quad \text{for } 0 \leq \frac{M_t}{M_0} \leq 0.6 \quad (6)$$

where M_0 and M_t are the total mass of drug sorbed and desorbed at time t respectively, D is the diffusion coefficient of the drug in the polymer, and l is the thickness of the device. For the late time approximation, the following expression holds over the final portion of the desorption curve:

$$\frac{M_t}{M_0} = 1 - \frac{8}{\pi^2} \exp \left(-\frac{\pi^2 Dt}{l^2} \right) \quad \text{for } 0.4 \leq \frac{M_t}{M_0} \leq 1.0 \quad (7)$$

The second type of monolithic device is called a *monolithic dispersion*. This system arises if the drug is at a high loading or has a limited solubility in the polymer medium, with only a portion of the drug dissolved and the remainder dispersed as small particles throughout the polymer. As with monolithic solution systems, drug-release is affected by the geometry of the device; and also varies with drug loading. At low loading levels of the drug (0 – 5 vol %), these devices are called *simple monolithic dispersions*. At higher

loading levels of (5 – 10 vol %) and (> 20 vol %), the devices are called *complex monolithic dispersions* and *monolithic matrix systems* respectively [64].

Only simple monolithic dispersions are mentioned here and described by a simple Higuchi model for a slab geometry; and its validity has been experimentally demonstrated numerous times against predicted release rates calculated from the model, starting with [64]:

$$M_0 = \frac{AC_0l}{2} \quad (8)$$

where M_0 is the total mass of drug initially present, A is the total surface area of a slab (both sides), C_0 is the total concentration of drug (dissolved and dispersed) initially present, and l is the device thickness. Based on a pseudo-steady state approximation:

$$M_t = A(DtC_s[2C_0 - C_s])^{1/2} \quad (9)$$

where D and C_s are the diffusion coefficient and solubility of the drug in the polymer respectively. Due to the pseudo-steady state assumption involved, which assumes a linear drug concentration profile in the matrix, Eq. (9) is accurate only when the drug loading is in great excess of the drug solubility ($C_0 \gg C_s$). This gives rise to a simpler form of Eq. (9) in [63,64]:

$$M_t = A(2DtC_sC_0)^{1/2} \quad \text{for } C_0 \gg C_s \quad (10)$$

Combining Eq. (9) and (10) gives the fractional release:

$$\frac{M_t}{M_0} = \frac{2}{l} \left(\frac{2DC_s t}{C_0} \right)^{1/2} \quad (11)$$

Eq. (11) has almost the same form as Eq. (6) for monolithic solutions, but the $t^{1/2}$ relationship holds over the complete release curve, instead of tailing off at late times as seen from Eq. (7). Higuchi's equations describe the dissolution of the drug initially from the surface of the slab and progressively through a thicker drug-depleted layer; and indicate that the amount of drug released follows first-order kinetics ($t^{1/2}$). However, at the limit of the drug loading approaching the drug solubility in the polymer, which occur quite often in delivery systems involving hydrophilic polymers and drugs of high water solubilities (i.e. low C_0/C_s values), Higuchi's equations gives a result 11.3% smaller than the exact solution [63].

2.7.3 Drug-release Determined Predominantly by Erosion

Many biodegradable systems (e.g. esters, anhydrides) have water-labile bonds incorporated into the polymeric chain. Under physiological conditions, the labile bonds are hydrolysed, and the polymeric chain breaks down to oligomers that are easily removed either by the body or the environment, thereby causing **erosion** to take place. The erosion of a polymer can take place due to a variety of mechanisms, namely; cross-link cleavage; ionisation, protonation and hydrolysis of pendent groups; and backbone cleavage, which will be discussed here [63].

The chemical erosion of polymers describes the degradation of insoluble polymers with labile bonds. Hydrolysis of the labile bonds causes scission of the polymer backbone and thus forming low molecular weight, water-soluble molecules which are eroded away.

Polymers which undergo such an erosion mechanism include poly(lactic acid), poly(glycolic acid) and their copolymers, polyorthoesters and polyanhydrides. When the device degrades heterogeneously, also called surface erosion (see Section 2.6.3), the polymer erodes only at its surface and maintains its physical integrity (e.g. device shape, molecular weight) as it degrades. Drug release is confined to the surface of the device and kinetics are predictable, with zero-order release kinetics obtained by careful control over surface-to-volume ratio and device geometry as the polymer undergoes decreasing hydrolysis rates from the surface of the device inward. This reaction is largely confined to the outer layers of the solid device and such devices, however, are difficult to produce [63,64].

For an ideal surface-eroding system (negligible edge effects), the release kinetics of the drug will equal the degradation rate of the polymer. As the degradation boundary moves into the bulk, the drug is released into the surrounding media. For a simple slab containing a uniform concentration of drug, a mass conservation expression is described as [64]:

$$\frac{dM_t}{dt} = BC_0A \quad (12)$$

and

$$M_t = BC_0At \quad (13)$$

where M_t is the mass of drug released from a slab of area A and any time t , and B is the surface degradation rate. The expression for fractional drug release is:

$$\frac{M_t}{M_\infty} = \frac{t}{t_\infty} \quad (14)$$

where M_∞ is the total drug released and t_∞ is the time for total erosion. This gives a direct relationship between the mass (or concentration) of drug loading to release times.

Since the rate of hydrolysis is much higher than the rate of water permeation (as explained in Section 2.6.3), R_w , into a surface-eroding polymer, hydrolysis can be assumed to only occur at the interface at the limiting rate of R_w . Therefore, another simplified way to express Eq. (12) is [63]:

$$R_r = R_w C_0 \quad (15)$$

where R_r is the rate of drug released and C_0 is the initial concentration of the drug in the polymer.

2.8 RESEARCH CONDUCTED ON BIODEGRADABLE TRACHEAL STENTING

Up till now, there have been a number of research groups from around the world who have proposed and conducted research in the area of biodegradable tracheal stenting. Such interest in this specific field stems from the fact that biodegradable tracheal stents do have the potential to meet most, if not all, of the 9 listed characteristics of an ideal tracheal stent as mentioned in Section 2.4. Perhaps the single, most significant advantage of a biodegradable tracheal stent is that it negates the need for a second surgical

intervention to remove the stent after it has served its function both as a support for the diseased and healed airway, and as a drug-eluting device to deliver therapeutic drugs to the affected site. Surgical avoidance is highly regarded as it allows non-surgical candidates to be treated and prevents life-threatening issues such as surgical complications and trauma to arise. Below are summaries of the various research groups who have conducted research on biodegradable tracheal stenting and their findings and challenges, suggesting that stenting is a promising method to treat tracheal obstruction.

Harald Lochbihler et al. (1997) designed and developed a biodegradable tracheal stent made of Vicryl filaments in a homogeneous *polydioxanone* (PDS) melt to study its biocompatibility and degradation. The stents had a wall thickness of 0.2 mm, outer diameter of 2.8 mm and length of 5 mm and were implanted in 35 male Wistar rats. During the first post-operative weeks, 5 rats had to be sacrificed because of inflammatory obstruction of the stented segment due to polyps of granulation tissue at both ends of the implanted stents, possibly caused by mechanic irritation and not the material. After 2 weeks, mucosal defects appeared in the trachea and distinct polyps of granulation tissue were found which caused moderate stenosis in one rat. At the 4th week, the granulations flattened and the stents showed increasing fragmentation. By the 8th week, no stent residues could be detected. There were neither signs of progressing inflammation or tissue incompatibility, nor indications for aspiration of stent fragments [9].

Antti Korpela et al. (1998,1999) developed a self-reinforced *poly(L-lactide)* (PLLA) tracheal stent in the shape of a helical spiral with a wall thickness of 0.7 mm, outer diameter of 5.5 mm and length of 12 mm. These stents were implanted in 9 rabbits to determine the suitability of such a stent. It was reported that the stent lost some

mechanical strength after 6 months and all stents disappeared after 10 months. Complications included compressed tissue causing ulceration of the epithelium, eosinophilia and inflammation (not reported to be caused by material). Epithelium in areas under the spirals of the stents also disappeared. However, foreign-body reaction was minimal and the stents were very well tolerated [6]. In another report published a year later, this same group further expanded on their first experiment by comparing their biodegradable stent with both metallic and silicone stents in addressing tracheal stenosis. 11 rabbits were followed from 13 to 39 weeks and one rabbit had to be sacrificed due to parts of the stent occluding the trachea causing stridor at the 26th week; and another rabbit may have died at the 31st week due to the disintegration of the stent. Complications relating to the stent on the tracheal wall were identical to those reported in their earlier study. It was reported that the silicone stents performed the worst as all rabbits in that group had to be killed due to respiratory problems, and both metallic and their biodegradable PLLA stents were well tolerated with no significant difference observed between these two groups [65].

Thomas C. Robey et al. (2000) designed and developed a “finger-like” biodegradable tracheal stent made of 80:20 *poly(D,L-lactide-co-glycolide)* (PLGA) with a wall thickness of 0.4 mm, outer diameter of 6 mm and length of 20 mm. These stents were tested in vivo in 12 New Zealand white rabbits and 3 stents were used for an in vitro degradation study by immersion in a buffer solution. In the in vitro study, the stents tended to break after 4 weeks, significant mass loss was observed at 5 weeks and all stents were nearly completely eroded / degraded at 15 weeks. The in vivo degradation of the stents was more complex and a combination of degradation and airway expulsion of stent fragments by the rabbits resulted in the early disappearance of the stents. By 6 weeks, the stents were gone

in 7 rabbits and all stents disappeared by 9 weeks. One of the stented rabbits died from fragment occlusion in the airway. In an earlier study by the same group, two rabbits died in the same way. Majority of the other rabbits were able to expectorate the stent fragments. One drawback was the inconsistent contact of the stents with the tracheal wall, resulting in little mucosalisation of the stents. However, there was no incidence of inflammatory reaction to the PLGA stents [8].

Saito et al. (2002) studied the biocompatibility, suitability and mechanical strength of a biodegradable knitted tracheal stent made of *poly(L-lactide)* (PLLA) with a wall thickness of 0.17 mm, outer diameter of 6 mm and length of 12 mm. These stents were implanted in 15 New Zealand white rabbits. After a period of 40 weeks, all the stents disappeared and no rabbit died of airway complications. Histological examination of the implanted sites showed that the ciliated epithelium was preserved and there were numerous blood vessels in the submucosa at 8 weeks. Also reported was that the mechanical strength of the PLLA stents increased as a function of their diameter [7].

M. Zilberman et al. (2001 onwards) prepared biodegradable *poly(L-lactide)* (PLLA) films loaded with a drug, dexamethasone, to conduct in vitro studies on mechanical properties with respect to morphology and degradation and erosion processes for future use as biodegradable tracheal stents. Drug release from the films was also investigated. Generally, two film structures were obtained: (1) A polymer film with large drug tertiary shapes located on its surface; (2) A polymer film with small drug particles and crystals distributed within the bulk. The films were floated on sterile water at 37°C to simulate the conditions in the trachea for the study of their degradation and drug-release kinetics. The PLLA films showed good initial mechanical properties and linear release profiles (which

is very desirable for many controlled release applications) of the drug were obtained for both film structures, with rate of release dependent on the film structure. The films were also rolled into tubular stents (with holes) with wall thicknesses of 0.12 – 0.15 mm, outer diameter of 6 mm and length of 15 mm, and immersed in sterile water. These stents showed good initial radial strength and drug incorporation had only a minor effect on the initial stents' strength. A 20-week in-vitro study of the PLLA stents showed that they were suitable for tracheal support [3,5,66,67,68,69]. In 2005 and 2008, this same group attempted to mathematically model the release of dexamethasone from both amorphous and semi-crystalline films floated on water. Fick's second law of diffusion was evoked to derive the model with assumptions based on changing concentration and diffusion coefficient of the drug in polymer over time; due to drug release, polymer degradation and changes in crystallinity. Generally good fitting to empirical results were obtained and polymer degradation was concluded to affect drug release more than crystallinity changes [70,71].

To sum up, all literature reviewed regarding biodegradable tracheal stenting failed to establish a physical model to closely simulate the trachea for effective studies. Therefore, the current work described here is novel as biodegradable polymer films were exposed to two different media simultaneously and this simulated tracheal conditions as closely as possible, thus enabling more accurate degradative investigation. The effect of sustained-release of mitomycin C directly to the trachea has also not been performed and studied previously.

CHAPTER 3

MATERIALS AND METHODS

3.1 MATERIALS

Three types of biodegradable random copolymers and two types of plasticizers were mainly used in this study. Poly(L-lactide-co- ϵ -caprolactone) 70/30 denoted as PLC, poly(D,L-lactide-co-glycolide) 75/25 denoted as PLGA 75/25; and poly(D,L-lactide-co-glycolide) 53/47 denoted as PLGA 53/47 (with inherent viscosities of 1.62, 0.93 and 1.05 respectively) were purchased from Purac Biochem BV (Gorinchem, The Netherlands). The numbers following the description of the copolymers represent the respective L-lactide/ ϵ -caprolactone and D,L-lactide/glycolide molar ratios of the monomers. The plasticizers of polyethylene glycol (PEG) of molecular weight 2000g/mol was purchased from Merck-Schuchardt (Hohenbrunn, Germany) and triethyl citrate (TEC) was from Sigma-Aldrich Inc. (MO, USA). HPLC-grade dichloromethane (DCM), tetrahydrofuran (THF) and chloroform were purchased from Tedia Company Inc. (OH, USA). A commercially available edible gel composed mainly of cellulose was procured from Aces Foods & Beverages (Malaysia) to achieve the 70wt% water condition in this study. All materials were used without further treatment.

The drug mitomycin C (MMC) was purchased from both Hande Industry and Trade Holdings Limited (Shenzhen, China) and Kyowa Hakko Kirin (Tokyo, Japan). It appeared as violet-blue crystals with a molecular weight of 334.3g/mol.

3.2 FILM SAMPLE PREPARATION

PLC and PLGA 75/25 were selected to be studied as it was predicted that their timeframes for complete degradation in water could be controlled to within 6 weeks to 3 months with addition of various plasticizers, which were close to the preferred timeframe for a tracheal stent in service. Additionally, both these copolymers are known to not elicit any adverse effects on cells and tissue after implantation, and their inherent flexibility is desirable to reduce tracheal cell trauma when used as stent materials. Polymer solutions of PLC and PLGA 75/25 were first prepared by dissolution in DCM. Plasticizers (PEG and TEC) and drug (MMC) were added to the polymer solutions according to the weight percent (wt%) to be achieved. These solutions were homogenised by using a magnetic stirrer for at least 24 hours, before being solution casted into Petri dishes, left in ambient conditions for 24 hours and finally dried in a 37°C vacuum oven for up to 4 weeks. The weights of the starting polymers to be dissolved were calculated from the expected thickness (250µm) of the films in these Petri dishes after drying. All films were dried to a residual solvent content of less than 1%, verified by thermogravimetric analysis (Hi-Res TGA 2950, TA Instruments, USA), and both plasticizer or drug distributions within the films were assumed to be uniform. All samples were analysed using a Modulated Differential Scanning Calorimeter (MDSCTM; DSC 2920 Modulated DSC, TA Instruments, USA) under nitrogen atmosphere at a heating rate of 10°C/min from -10°C to 150°C and a modulation of 1°C/min, to obtain melting endotherms during the first heating profile for initial crystallinity calculations in triplicate. Table 3.1 lists all the polymer blends (with and without MMC) and their useful properties used to develop the film samples for both in-vitro and in-vivo (when rolled into tubes / stents) experiments.

Table 3.1. List of polymer blends and properties.

Polymer / Blend	Estimated degradation time in water (of copolymer only; months)^a	Intrinsic viscosity (of copolymer only; dl/g)^a	Starting Molar Mass (Da)^b	T_g / T_m^c (°C)	Crystallinity^c (%)
PLC	12 – 24	1.62	1.81 x 10 ⁵	8.64 / 97.0	11.9
PLC + 10wt% TEC	12 – 24	1.62	1.64 x 10 ⁵	9.95 / No T _m	Amorphous
PLC + 10wt% PEG	12 – 24	1.62	1.64 x 10 ⁵	8.16 / 93.6	2.16
PLGA 75/25	2 – 6	1.05	6.20 x 10 ⁴	56.0 / No T _m	Amorphous
PLGA 75/25 + 10wt% PEG	2 – 6	1.05	5.64 x 10 ⁴	37.9 / No T _m	Amorphous
PLC + 0.1mg MMC/film	12 – 24	1.62	1.81 x 10 ⁵	N.D. ^d	N.D. ^d
PLC + 1.0mg MMC/film	12 – 24	1.62	1.81 x 10 ⁵	N.D. ^d	N.D. ^d

^a Supplier data^b Determined by Size Exclusion Chromatography (SEC)^c Determined by Modulated Differential Scanning Calorimetry (MDSCTM)^d Not determined

3.3 IN-VITRO STUDIES

3.3.1 70wt% Water / Humidified Air Degradation Study

The polymer samples used for this experiment were obtained from the solution casting process as described in Section 3.2. Film samples measuring 2cm x 2cm were cut from the cast, with average thickness of 250 μ m.

The experimental setup for this study allowed the two opposing surfaces of the samples to be exposed to two different media concurrently; namely 70 ± 2 wt% water (gel-side) and flowing humidified air (air-side). Figure 3.1 shows a disassembled sample holder and Figure 3.2 shows how the 2cm x 2cm polymer film was mounted on a tubular holder. Each tubular holder had a 1.5cm diameter window / hole within which the polymer could be exposed to 70wt% water on one side and flowing humidified air on the other side.

Figure 3.1. A disassembled sample holder.

Figure 3.2. Tubular holder with polymer film mounted.

Figure 3.3 shows an assembled sample holder filled with gel (70 ± 2 wt% water, verified by TGA). The lumen of the tubular holder allows continuous flow of humidified air,

which was assisted by four fans in a sample chamber (Figure 3.4). The fans' speed was set at 600 revolutions per minute to simulate a normal human breathing rate of 1.3m/s in the trachea. This was verified using a portable air-flow meter at several points inside the sample chamber.

Figure 3.3. An assembled sample holder filled with gel.

Figure 3.4. Sample chamber with four fans.

The sample chamber can accommodate 36 sample holders, arranged in 6 x 6 rows / columns as shown in Figure 3.5. Finally, to achieve our desired conditions of 80% humidity (local weather) and temperature of 37°C (physiological temperature); the whole sample chamber was placed into a humidifier (Al-tech Instrumentation and Engineering, Singapore) with the above settings. This is shown in Figure 3.6.

Figure 3.5. Sample holders arranged in the sample chamber.

Figure 3.6. Sample chamber placed inside the humidifier.

The gel in every sample holder was discarded and replenished weekly to prevent mould formation. The polymer films were also removed weekly, rinsed with deionised water and dried in a vacuum oven at 37°C for a week.

Microtomy: To obtain a specific thickness / depth (50µm) of polymer material from either the gel or air sides for molecular weight analyses, a rotary microtome (Leica RM2265, Nussloch, Germany) was used. After drying, 1cm x 1cm portions of the polymer films were cut and mounted on a wooden block using Historesin (Leica, Nussloch, Germany) to allow longitudinal sectioning. Figure 3.7 shows a schematic diagram of a mounted film for sectioning. All slices were obtained in triplicate.

Figure 3.7. Schematic diagram of a polymer film (white) mounted and oriented longitudinally to the microtome knife.

Size exclusion chromatography (SEC): The 50μm polymer slices from both gel and air sides as obtained from above were each dissolved in 2ml of chloroform for molecular weight analyses. An SEC system consisting of an Agilent 1100 Series solvent delivery, sample injector, refractive index detector, and a 300mm x 7.8mm PLgel Mixed-C, 5μm column fitted with a guard column (Agilent Technologies, CA, USA) were used. Chloroform at a flow rate of 1ml/min was used and 50μl was injected per sample. EasiVial PS-M polystyrene standards (Agilent Technologies, CA, USA) were used for calibration. All samples were in triplicate and significance was established using analysis of variance (F-test) by a value of $p < 0.05$.

Titrametry: Saturation water concentration data for both gel and air sides could not be measured from thin 50μm slices of sectioned polymer due to rapid water evaporation in ambient. To achieve this, 1cm x 1cm full-thickness films were cut and either immersed

into gel or directly exposed to humidified air respectively. At specific time-points, a Karl Fischer Titrator (Mitsubishi Moisture Meter CA-06, Tokyo, Japan) was used to measure water concentration. Similar to SEC analyses, all samples were in triplicate and significance was established using analysis of variance (F-test) by a value of $p < 0.05$.

3.3.2 Drug (MMC) Release Study

The MMC-loaded polymer samples used for this experiment were obtained from the solution casting process as described in Section 3.2. Film samples measuring 1cm x 1cm were cut from the cast, with average thickness of 250 μ m. Stent samples had MMC spray-coated at 0.1mg per stent (described in Section 3.4.1).

MMC release studies from 2 different doses were performed to simulate MMC release from drug-loaded stents in 2 different conditions. Films and stents containing MMC at 0.1mg were immersed in 2ml of distilled water in glass vials; and films containing MMC at 1.0mg were immersed in 5ml of distilled water. The volumes of distilled water used were multiples of the maximum solubility of MMC in water (~ 0.5 mg/ml), to ensure sink conditions throughout the MMC release studies. These were placed in a 37°C incubator, with the medium changed weekly to maintain such sink conditions. Experiments with films floated on water were prepared similarly. Cumulative MMC release data were obtained using reversed-phase high performance liquid chromatography (HPLC). The HPLC system consisted of an Agilent 1100 Series solvent delivery, sample injector, UV detector, and a 250mm x 4.6mm reversed-phase C₁₈ column (Agilent Technologies, CA, USA). A mobile phase consisting of water (750ml), methanol (250ml), acetic acid (5ml) and ammonium acetate (1.54g) was delivered at a flow rate of 1ml/min [7]. Samples at

different time points were injected (50 μ l) and measured at a wavelength of 365nm. After the last time point, extraction of any residual MMC in the films was performed by dissolving all films completely in THF and further analysed by HPLC. Release profiles were normalized based on the total loading determined in this manner. Pure MMC stability studies (dissolved at a concentration of 0.05mg/ml in distilled water) were also conducted to investigate the extent of MMC degradation in water at 37°C as the experiment progressed weekly, with UV absorbance data obtained via HPLC as explained above.

3.4 IN-VIVO STUDIES

3.4.1 Stent Fabrication

Two stent designs were used for the first portion of the animal study; namely helical and tubular designs, with both made from the selected candidate material of PLC (Figure 3.8). From the casted and solvent-dried (< 1% residual solvent content measured by thermogravimetric analysis) PLC according to Section 3.2, the helical-shaped stents (without MMC) were fabricated by cutting strips 110mm long and 2mm wide. These strips were coiled tightly around mandrels and heated, then left to cool to 25°C, inserted into Teflon tubes of internal diameter 6mm and annealed in an oven at 55°C for 24 hours to allow them to fully relax their stresses and assume the required outer diameter. The tubular-shaped stents were fabricated by cutting 12mm by 10mm rectangular-shapes from the PLC cast (with and without MMC). Twelve rectangular holes were hand-cut and distributed throughout each of these films, before being coiled around mandrels of diameter 5.5mm. An additional control group utilising commercially-available silicone

stents were used and these were tubes of 1mm wall thickness, 6mm outer diameter and 10mm in length.

Figure 3.8. A) Helical-shaped stent. B) Tubular-shaped stent. C) Tubular shaped stent with 0.1mg MMC.

The stents used for the second part of the animal study were fabricated differently. Solutions of PLC, added with or without MMC, dissolved in DCM were dip-coated using a dip-coater (DC-mono, Nima Technology, England) to produce either homogeneously-distributed MMC or drug-free tubes (Figure 3.9) respectively. These tubes were then sent for laser-patterning using a femtosecond laser source (SIMTech, Singapore), shown in Figure 3.10. The system used was a Ti:sapphire (Clark-MXR CPA 2001) solid state laser using chirped pulse amplification technique. The laser beam had a wavelength of 750nm, pulse frequency of 1kHz, and pulse width at 150fs. The laser beam was delivered to a galvanometer scanner with a focal length of 100 mm, and the beam was focused to a spot diameter of approximately 50 μ m. Laser power (150mW), scanning speed (5mm/s) and number of passes (35) had to be optimized to achieve consistent patterns. A spray-coater (Sono-Tek Medicoat, NY, USA) was used to coat a solution of PLGA 53/47 and 5wt% of MMC in DCM, onto a drug-free laser-patterned stents for the spray-coated MMC stent group. A linear speed of 0.045cm/s, flow rate of 0.05ml/min, revolutions per minute of 20RPM and 12 cycles were set to achieve an average MMC dosage of 0.1mg per stent. These spray-coated samples were left to dry in ambient for 24 hours, before finally dried

in a 37°C vacuum oven for a week. Similar to both the helical and tubular stents, the residual solvent content was determined to be less than 1% by thermogravimetric analysis. A provisional patent application (US Patent Application No. 61/454,858) has been submitted to cover all drug-loadings and laser-patterned stents used in this study.

As mentioned in Section 2.5.6, most topical applications of MMC are between 0.1 to 1mg/ml, but the actual mass of MMC delivered this way is unknown. The dose for therapeutic efficacy of MMC is also unknown; therefore the MMC loading used for all stents in this study was fixed at 0.1mg/stent as this currently is the lowest (and thus safest) dose used clinically.

Figure 3.9. Dip-coated tubes.

Figure 3.10. Laser-patterned stent.

3.4.2 Animal Study Plan

Animal models used for tracheal research have been extensively studied, with the selection of rabbits as the most common model for biodegradable tracheal stent research [72]. Both IACUC (#070/07(A1)08) and OSHE (OSHE/RA/3/04/FOM-466) approvals were obtained for this study, in collaboration with the National University of Singapore (NUS), Otolaryngology – Head and Neck Surgery. All surgical procedures were

performed by the same surgeon in an aseptic manner. In the first part of the animal study, 5 groups of 5 New Zealand white rabbits (weights: 3.5 – 4.0kg) were used. The groups were 1) Control 1 – without stent; 2) Control 2 – commercially available silicone tubular-shaped stent; 3) Biodegradable helical-shaped stent; 4) Biodegradable tubular-shaped stent; and 5) Biodegradable tubular-shaped stent with 0.1mg MMC. One rabbit per group was scheduled to be sacrificed at 3, 9 and 12 weeks post-implantation; leaving 2 rabbits per group as spares.

The second part of the animal study was performed by Innoheart Pte. Ltd. (Singapore). This was to evaluate all fabricated laser-patterned stents. This time, 3 groups of 6 rabbits (weights: 3.5 – 4.0kg) were used. The groups were 1) Control – bare stent without MMC; 2) Homogeneously-distributed 0.1mg MMC laser-patterned stent; and 3) Spray-coated 0.1mg MMC laser-patterned stent. One rabbit per group was scheduled to be sacrificed at 3, 6, 9, 15, 20 and 24 weeks post-implantation; none allocated for spares.

3.4.3 Surgical Techniques and Endoscopic Follow-up Studies

Both first and second parts of the animal study conducted at NUS and Innoheart Pte. respectively observed the same surgical and follow-up procedures as described here.

Each rabbit received ketamine hydrochloride (7.5mg/kg) and xylazine (10mg/kg) intramuscularly for general anesthesia and were spontaneously breathing during the 10 minute surgery. The trachea was exposed through a midline vertical skin incision in the neck, strap muscles were retracted laterally and the midline anterior tracheal wall exposed. A midline tracheal incision was made onto the anterior trachea wall between the

third and seventh tracheal rings. A disease model employing unipolar diathermy at 35 watts was used to create mucosa injury and induce tracheal stenosis circumferentially between the 4th to 6th rings. The stents to be studied were then implanted between the 4th and 6th rings. 5-0 nylon suture was used to prevent the stents from dislodging by placing 2 sutures from the stent to the anterior trachea wall.

All rabbits were observed daily for respiratory distress and well-being. Rabbits with body weight loss of more than 20%, with respiratory distress or anorexia were euthanised. Their airways were evaluated weekly with rigid 2.9mm diameter 0° endoscopes (Karl Storz Endoscopy, MO, USA). Endoscopic examinations were digitally recorded and video frames were paused at a fixed distance from the stent. From the image on the screen, a measuring tape was used to measure the most stenosed distance from this two-dimensional cross-section; and percentage of tracheal stenosis was calculated by assuming all stenosis being concentric. Two independent reviewers viewed the videos and made measurements to score and grade the degree of stenosis, with similar inter-rater and intra-rater concurrence of the severity of tracheal stenosis. For this study, the results of severity of tracheal stenosis were reported in terms of percentage stenosis of the cross-sectional area of the trachea instead of the Cotton-Myer scale as described by Eliashar [12]. This allowed more differentiation between different study groups, compared to the 4 grades of the Cotton-Myer scale. All percentage stenosis reported were the average values from both reviewers and included all surviving rabbits in each group, at each particular time point.

3.4.4 Histology

All histological preparation for the second part of the animal study was performed by Innoheart Pte. Ltd. (courtesy of Dr. Winston Shim, Chief Scientific Officer), for better understanding of the effects of the stent material, design, and drug-release; and to correlate these to in-vitro observations. Tracheal tissues from rabbits that were scheduled to be sacrificed were collected immediately after euthanasia and fixed in 10% neutral-buffered formalin for a minimum of 48 hours, dehydrated through graded alcohol and embedded in paraffin wax. Tissues were trimmed using a rotary microtome (Leica RM2265, Nussloch, Germany). Ten micron thick sections were cut and stained with standard haematoxylin and eosin (H & E) for morphologic evaluation by light microscopy. Three sections were done per trachea (two at stented area, one at non-stented area).

CHAPTER 4

DEGRADATION PHENOMENA OF BIODEGRADABLE COPOLYMERS IN A SIMULATED TRACHEAL ENVIRONMENT

4.1 CRYSTALLINITY DATA

All copolymers (i.e. PLC and PLGA) with and without plasticizers added were characterised specifically with regards to initial crystallinities of the samples before the start of the experiment, in triplicate. In general, crystallinity is related to such properties as rate of water ingress and drug diffusion. The results for MDSC analyses of PLC are presented in Figure 4.1. PLGA 75/25 is fully amorphous and no melting endotherms (and hence no crystallinity data) for all samples based on this copolymer could be obtained.

Figure 4.1. Plots of MDSC curves showing heats of fusion values and crystallinities.

From the melting endotherms presented in Figure 4.1, it may be inferred that all crystals formed within PLC are composed of only L-lactide chains. This is because ϵ -caprolactone crystals have a melting temperature (T_m) of about 60°C and all melting endotherms for PLC commence only after this point (about 70°C); an earlier melting endotherm seen between 30 – 55°C for the PLC + 10wt% PEG plot corresponds to the melting of PEG and is not significant for this study. Even though pure L-lactide crystals melt at a higher temperature of about 180°C, the lowering of T_m for PLC to between 90 – 100°C is due to the 30% molar ratio of ϵ -caprolactone chains / monomers introduced during the commercial copolymerisation process, which disrupts and lowers the crystal perfection of these crystals. Hence, crystallinity measurements for PLC for this study were calculated based on 70% molar ratio of a hypothetical 100% crystallinity value of L-lactide chains of 142 J/g [73], resulting in a value of 99.4 J/g for the L-lactide component of PLC.

As shown, pure PLC has an initial crystallinity of about $11.9 \pm 0.1\%$ and the addition of 10wt% of PEG reduces this to $2.16 \pm 0.2\%$. Furthermore, the addition of 10wt% of TEC totally removes all crystal formation during processing, rendering this sample totally amorphous. These results are expected as any addition of impurities (e.g. plasticizers, fillers) tends to disrupt and hinder the formation of intact polymer crystals during solvent drying or annealing, resulting in the final polymer sample having reduced or non-existent crystal formation. Triethyl citrate, being a much smaller molecule than polyethylene glycol, could have been distributed uniformly throughout the copolymer and prevented any crystallisation process to occur during solvent drying. These observations and implications will be discussed further in Section 4.4.

4.2 DERIVATION OF DEGRADATION KINETICS DATA FROM SEC

To fully utilise Eq. (3) in Chapter 2:

$$[N_E] = [N_{E0}]e^{-kt} \quad (16)$$

and to obtain degradation kinetics data from the samples exposed to both gel and humidified air conditions, the number of ester bonds (N_E) in any given sample at any given time being analysed has to be known or derived. Following which, the first-order rate constants (k) can then be calculated and rates of hydrolysis for both surfaces exposed to the 2 different conditions can be obtained and compared. This was primarily done through the use of size exclusion chromatography (SEC).

Number-average molar masses (M_n) of the various samples ($n = 3$) were obtained from the SEC system. The average number of intact ester bonds (N_E) in any given polymer sample is related to M_n (g/mol) as follows:

$$N_E = [(M_n/M_m) - 1]N_c \quad (17)$$

where M_m (g/mol) is the molar mass of the monomer (PLC: 93g/mol, PLGA 75/25: 56.5g/mol). N_c is the total number of polymer chains in the polymer sample to be analysed and can be expressed as:

$$N_c = (w/M_n)N_A \quad (18)$$

where w (g) is the mass of the polymer sample and N_A is Avogadro's constant. Eq. (17) and (18) can be combined to give the expression:

$$N_E = [(1/M_m) - (1/M_n)]wN_A \quad (19)$$

For unit mass of polymer, this equation reduces to:

$$N_E = [(1/M_m) - (1/M_n)]N_A \quad (20)$$

Therefore the average number of intact ester bonds at any point in time (t) during hydrolysis can be elucidated from M_n data obtained from SEC experiments. Results from Eq. (20) can be substituted into a logarithmic form of Eq. (16) as:

$$\ln(N_E) = -kt + \ln(N_{E0}) \quad (21)$$

and the gradients of plots of $\ln(N_E)$ against t for all samples will yield the first-order rate constants (k) of the samples analysed, if random cleavage of ester bonds occurs as expected for first-order kinetics. The 50 μ m slices obtained via microtomy of both surfaces from gel and air sides of all samples were analysed this way in triplicate (with significance established as described in Chapter 3, Section 3.3.1). As all linearised plots obtained had regression coefficients of at least 0.95 and above, these indicate that the derivation of k -values via SEC as explained above is applicable. The results are presented and discussed in the following 2 sections.

4.3 EFFECT OF TYPE OF COPOLYMER ON DEGRADATION KINETICS

To compare and contrast the degradation phenomena / kinetics of generally hydrophobic and hydrophilic copolymers, PLC and PLGA 75/25 were selected to be studied respectively. These 2 candidates were also considered as their timeframes for complete degradation and known mechanical properties suit tracheal stent applications as described in Chapter 3, Section 3.2.

Figures 4.2 and 4.3 shows M_n vs t and $\ln(N_E)$ vs t plots for the top 50 μ m slices of pure PLC and PLGA 75/25 exposed simultaneously to both 70wt% water and humidified air during a 6-week degradation study timeframe. Data for gel-side and air-side for PLGA 75/25 ended at 3 and 5 weeks respectively as satisfactory microtome slices were not achieved for SEC analyses due to extensive degradation after these time-points. However, the gradients from these samples were still recorded and analysed.

Figure 4.2. Number-average molar mass profiles (M_n) for PLC and PLGA 75/25 (gel and air sides).

Figure 4.3. $\ln(N_E)$ profiles for PLC and PLGA 75/25 (gel and air sides).

From Figure 4.3, it can be seen that PLGA 75/25 has steeper gradients (larger k) for both gel and air sides compared to PLC. This difference is attributable to the higher intrinsic hydrolysis rate for glycolide bonds as compared to L-lactide or ϵ -caprolactone bonds, and agrees well with studies conducted by Pitt et al. [74] who reported increased reactivity to hydrolysis in copolymers containing glycolate linkages. In terms of polymer morphology, PLC is semi-crystalline (as explained in Section 4.1) and this also retards water ingress when contrasted with the fully amorphous PLGA 75/25. The absence of crystallinity also results in an increased concentration of accessible ester bonds for hydrolysis [75]. As we elaborate below, the water ingress rate is important in determining whether or not a water concentration gradient is set up in the film.

Perhaps more important observations from the plots are that the difference in k -values (linear gradients) between the gel and air sides for PLC is not significant, whereas for PLGA 75/25 the k -values are significantly different ($p < 0.05$). This shows that in a

generally more hydrophobic and semi-crystalline polymer such as PLC, water ingress from both gel and air sides into the polymer bulk takes place at such a low rate that hydrolytic degradation kinetics are not affected. Saturation water concentration data from Karl Fischer titrimetry also supports this hypothesis as a value of 1.53 for the ratio of measured saturation water concentration in the gel-side, $[H_2O]_{\infty,g}$, against the air-side, $[H_2O]_{\infty,a}$, is insignificant using analysis of variance (ANOVA, F-test). In contrast, PLGA 75/25 shows divergent k -values as water ingress from the side in contact with 70wt% water (gel) is at a higher rate due to its hydrophilicity and amorphous morphology, thus promoting a higher rate of hydrolytic degradation compared to the air-side. This also means that a water concentration gradient can be achieved from the gel to the air-side, across the whole 250 μ m thick sample, thereby allowing PLGA 75/25 to mimic a pseudo “surface-eroding” profile compared to a more homogeneous degradation profile across the PLC film as glycolide bonds are more labile than both L-lactide and ϵ -caprolactone bonds. A significant value of 1.85 for $[H_2O]_{\infty,g}/[H_2O]_{\infty,a}$ supports the formation of such a water concentration gradient. In separate studies [74,76], it is reported that the absorption of water does decrease the T_g of PLGA (more than generally hydrophobic polymers) which leads to more flexibility and ease for hydrolysis of ester bonds, and this is also observed here.

4.4 EFFECT OF TYPE OF PLASTICIZER ON DEGRADATION KINETICS

In order to accelerate degradation, as well as to manipulate water ingress rates, two plasticizers (TEC and PEG, which are both benign and have a history of use in humans) were used and blended with the two copolymers as presented in Section 4.3. The effects of both TEC and PEG on degradation kinetics of PLC were studied, whereas only data from PEG with PLGA 75/25 could be obtained, since it was not possible to microtome TEC with PLGA 75/25 due to extensive degradation occurring even at early time-points (3rd week onwards).

4.4.1 PLC Blends

Figure 4.4 shows the average k -values for both gel and air sides for pure PLC and its blends with plasticizers, as a bar chart for ease of comparison during the 6-week degradation study timeframe.

Figure 4.4. Comparison of k -values between gel and air sides, for PLC and its blends (* statistically significant, $p < 0.05$).

As seen from the bar chart, k -values for both gel and air sides for PLC with plasticizers are significantly higher than for the respective sides in pure PLC. This clearly shows that the addition of a plasticizer increases the rate of hydrolysis of PLC, as crystallinity is disrupted, and perhaps allows for faster and higher equilibration levels of water inside PLC. Crystallinity data obtained (Figure 4.1) from calorimetry supports this as heats of fusion (and hence crystallinities) decreases or disappears, when TEC and PEG are added to PLC. Figure 4.4 also shows that the difference in k -values between the gel and air sides is not significant for pure PLC (also explained in Section 4.3) and PLC + 10% TEC, but highly significant for the PLC + 10% PEG blend. Triethyl citrate with a relatively low molar mass of 276.28g/mol increases degradation rates uniformly (about 72% more than pure PLC) at both gel and air sides. Thus it is seen that TEC addition does not induce a water concentration gradient in PLC as this is possibly due to the relatively rapid out-leaching of TEC molecules with consequent pore creation on both sides, allowing for fast equilibration of water inside the bulk. Thus no significant difference in k -values is seen

between the gel and air sides and degradation proceeds homogeneously like for pure PLC. A $[H_2O]_{\infty,g}/[H_2O]_{\infty,a}$ value of 1.40, being not significant, also confirms the non-existence of a defined water concentration gradient.

PEG however, being a much larger molecule (molar mass: 2000g/mol), is expected to preferentially leach out from the gel-side creating higher porosity on that side. When water fills the pores, a water concentration gradient forms, which leads to a significant difference in k -values between the gel and air sides as seen and a heterogeneous “surface-eroding” degradation profile can be achieved for this PLC blend. A much higher $[H_2O]_{\infty,g}/[H_2O]_{\infty,a}$ value of 4.67 is obtained and supports this view. These results also agree with a prior study [58] which suggests that a higher amount of “bound” water in the vicinity of ester bonds increases hydrolysis rates. A higher $[H_2O]_{\infty,g}/[H_2O]_{\infty,a}$ value indicates that as more total water is recorded in the polymer bulk (the gel-side in this case), a corresponding increase in bound water is present for chain scission.

4.4.2 PLGA 75/25 Blends

Figures 4.5 and 4.6 shows M_n vs t and $\ln(N_E)$ vs t plots for the top 50 μ m slices of PLGA 75/25 and PLGA 75/25 with 10wt% PEG exposed simultaneously to both 70wt% water and humidified air. Figure 4.5 displays the average gradients (k -values) for these samples as a bar chart for ease of comparison. Similar to PLGA 75/25 as explained in Section 4.4.1, data for gel-side and air-side for PLGA 75/25 + 10wt% PEG also ended at 3 and 5 weeks respectively.

Figure 4.5. Number-average molar mass profiles (M_n) for PLGA 75/25 and PLGA 75/25 + 10wt% PEG (gel and air sides).

Figure 4.6. $\ln(N_E)$ profiles for PLGA 75/25 and PLGA 75/25 + 10wt% PEG (gel and air sides).

Figure 4.7. Comparison of k -values between gel and air sides, for PLGA 75/25 and with PEG (* statistically significant, $p < 0.05$).

Figures 4.6 and 4.7 show that the addition of PEG to PLGA 75/25 also increases the rates of hydrolysis for both gel and air sides compared to without PEG. In this respect, the effect of PEG is similar to its effect on PLC. It is postulated that the rate of hydrolysis for glycolide bonds is generally high and close to the rate of water uptake into the polymer bulk; therefore, an increase in water absorption due to induced porosity when PEG leaches into the gel will lead to a corresponding increase in k -values. The addition of PEG as a plasticizer also reduces the T_g by almost 20°C at the start of the experiment. This drop in T_g leads to more flexibility and ease with which the transition state for hydrolysis of ester bonds may be achieved, and this observation is supported by studies done by Pitt et al. [74,76].

However, in contrast to the results for PLC + 10% PEG, a blend of 10% PEG with PLGA 75/25 does not greatly enhance the difference in k -values as a uniform increase of about 88% for both sides is seen over that of pure PLGA 75/25. An explanation for this is that

the generally hydrophilic pure PLGA 75/25 already intrinsically forms a water concentration gradient in the film (described in Section 4.3) when it is exposed to the differing sides. Hence, even though addition of a hydrophilic plasticizer (PEG) and any induced porosity formed due to its subsequent leaching out into the gel-side that causes $[H_2O]_{\infty,g}/[H_2O]_{\infty,a}$ to increase to a value of 5.51 (i.e. water concentration gradient becomes steeper), the amount of water absorbed for neat PLGA 75/25 was already high and close to twice that of neat PLC (gel-side, via titrametry); and the increment due to PEG addition does not greatly enhance the difference in hydrolysis rates. This therefore means that this difference in k -values will be maintained (i.e. uniform increase for both sides) for PLGA 75/25 regardless of amount of water absorbed at both sides when subjected to such a set up.

Figure 4.8 puts together and summarises the first-order degradation rate constants (k) for all samples as discussed; and Table 4.1 lists these average k -values, linear regression coefficients (R^2), water concentration and crystallinity data for all samples used and described from Sections 4.1 through 4.4.

Figure 4.8. Comparison of k -values between gel and air sides, for all samples (* statistically significant, $p < 0.05$).

Table 4.1: Degradation kinetics, water concentration and crystallinity data.

Polymer / Blend	Side	Average k (week ⁻¹)	Average R^2 (linear)	$[H_2O]_{\infty,g} / [H_2O]_{\infty,a}$ ^b	Crystallinity (%)
PLC	Gel	1.00×10^{-4}	0.95	1.53 ^c	11.9
	Air	0.883×10^{-4}	0.95		
PLC + 10wt% TEC	Gel	1.72×10^{-4}	0.98	1.40 ^c	Amorphous
	Air	1.51×10^{-4}	0.96		
PLC + 10wt% PEG ^a	Gel	1.94×10^{-4}	0.95	4.67 ^d	2.16
	Air	1.18×10^{-4}	0.96		
PLGA 75/25 ^a	Gel	6.01×10^{-4}	0.97	1.85 ^d	Amorphous
	Air	4.75×10^{-4}	0.97		
PLGA 75/25 + 10wt% PEG ^a	Gel	11.4×10^{-4}	0.99	5.51 ^d	Amorphous
	Air	8.90×10^{-4}	0.98		

^a Statistically significant values of k_{gel} / k_{air} obtained ($p < 0.05$).

^b $[H_2O]_{\infty,g}$ and $[H_2O]_{\infty,a}$ represent saturation concentration of water at gel and air sides respectively, obtained by titrametry.

^c No statistical significant difference between $[H_2O]_{\infty,g}$ and $[H_2O]_{\infty,a}$.

^d Statistically significant difference between $[H_2O]_{\infty,g}$ and $[H_2O]_{\infty,a}$ ($p < 0.05$).

4.5 SELECTION OF CANDIDATE MATERIAL FOR IN-VIVO STUDIES

It has been shown that in the case of generally hydrophobic polymers like PLC, a water concentration gradient is not built up between the gel and air sides; therefore degradation tends to proceed homogeneously throughout the polymer bulk. The opposite is true for generally hydrophilic polymers (e.g. PLGA 75/25). Also, the addition of hydrophilic plasticizers to these polymers has quantitatively different effects. In the case of PLC, the addition of PEG or TEC increases rates of hydrolysis for both surfaces as crystallinities are disrupted and reduced; and porosity is assumed to be induced, thereby aiding in ester bond cleavage. However, PEG enhances the difference between hydrolysis rates of the gel and air sides when blended with PLC, compared to TEC which does not. This shows that the out-leaching of the added plasticizer (especially at the gel side), and the postulated resultant porosity, also determine whether heterogeneous degradation takes place. Adding more plasticizer to a generally hydrophilic polymer maintains this difference, as can be seen in PLGA 75/25 blended with PEG.

These results clearly exhibit the possibility of well-studied bulk-degrading polymers exhibiting pseudo “surface-eroding” profiles when exposed to two different environments, as observed in a tracheal passage. Both PLC and PLC + 10wt% TEC exhibit a more homogeneous, bulk-degrading characteristic compared to all other copolymers / blends studied; this homogeneous degradation profile is preferred in tracheal stenting. Due to the relative insensitivity of kinetics to the gel and air sides, PLC was chosen as the candidate material for stent fabrication and implantation into rabbits (results and discussion in Chapter 6) as it possesses better initial mechanical strength compared to

PLC + 10wt% TEC. The modulus of PLC has an average value of 29.5 ± 4.8 MPa using a standard “dog-bone” specimen on a tensile tester (data not shown), compared to current stents made of silicone with a modulus range of only 1 – 5 MPa. Thus, the fabricated stents with an average wall thickness of 0.25mm was deemed to provide structural rigidity when implanted into the trachea. Also, the maintenance of bulk-degrading characteristics (no extensive erosion of material from one side) is preferable for tracheal stent structural integrity and eventual epithelialisation when in service for the desired timeframe of 3 – 6 months.

CHAPTER 5

DRUG-RELEASE PHENOMENA IN A SIMULATED TRACHEAL ENVIRONMENT

5.1 STABILITY STUDY FOR MITOMYCIN C

From literature reviewed and presented in Chapter 2 (Section 2.5.5), it is noted that the presence of water induces mitomycin C (MMC) degradation at varying degrees, depending on the storage and application conditions of the drug solution over time. The storage temperature of dissolved MMC in water apparently plays a significant part in the cleavage of the methoxyl group to form a slightly different molecular structure with the aziridine ring opened and one mole of methanol as a by-product. Also mentioned previously, MMC becomes biologically inactive when the aziridine ring is opened. As UV absorbance using established HPLC parameters will be reduced as decreasing amounts of intact aziridine rings are present, there is therefore a need to investigate the stability (or remaining potency) of MMC at the physiological temperature of 37°C in water, over the time of drug-release study. Figure 5.1 shows the decreasing potency of 0.05mg/ml of MMC in water over time from such a study conducted.

Figure 5.1. Potency of 0.05mg/ml MMC in water over time at 37°C.

As can be seen from the plot, MMC starts to degrade slowly as early as the first day. MMC potency falls to about 50% of its original value after 4 weeks, and continues to drop till about 16% after 12 weeks. This shows that all release data regarding MMC as a sustained release agent and using UV absorbance (e.g. HPLC) to quantify such release requires a theoretical means to correct ever-decreasing absorbance values over time if release of MMC is to be recorded till completion. Efforts to review and establish a methodology to detect and quantify MMC after aziridine ring opening proved unsuccessful. Therefore, even though all cumulative MMC-release data presented in Sections 5.2 through 5.4 reports “intact” or efficacious MMC via HPLC, the percentage potency data derived from this stability study is used to perform a correction to reflect total MMC (efficacious and degraded) release into the selected media and environments. This assumes any percentage reduction in potency of MMC over time is similar across all concentrations in water at 37°C.

5.2 EFFECT OF IMMERSED AND FLOATED CONDITIONS ON MMC-RELEASE

As mentioned in Chapter 4, Section 4.5, PLC was the selected material to be investigated further as a fully biodegradable prototypical tracheal stent due to its favourable mechanical strength and degradation characteristics. The drug currently used to treat tracheal stenosis, MMC, was selected for study of release from PLC matrices.

An MMC-release study was conducted to simulate the uni-directional delivery of drug from a proposed homogeneously-distributed drug-loaded tracheal stent. It was assumed that no drug-release occurs on one side (the interior surface of the stent, where humidified air flows through the stent lumen). However, even though the experimental setup to study the degradation phenomena of polymers in Chapter 4 using gel and flowing humidified air could have been applied here for this drug-release study, attempts to extract MMC released into the gel-side was unsuccessful as the sheer volume of the gel (and water) needed for the setup caused MMC concentrations in solution to be too low; thereby bringing it below the UV detection limits for HPLC.

Therefore, to mimic as closely as possible to tracheal conditions for drug-release, films of PLC loaded homogeneously with 0.1mg (approximately 0.351wt%) of MMC were both immersed and floated on distilled water as described in Chapter 3 (Section 3.3.2), with a schematic diagram similar to a prior study [71].

5.2.1 Efficacious MMC-release (0.1mg/film)

Figure 5.2 presents the cumulative MMC-release data for 3 months for the 2 conditions.

Figure 5.2. Efficacious MMC-release curves for both immersed and floated conditions (0.1mg/film).

To obtain the relevant kinetic data for drug-release, both plots in Figure 5.2 are obtained by a power law curve-fitting according to Eq. (22) by Korsmeyer, Ritger and Peppas [78,79] and similarly used for MMC-release from various biodegradable polymers:

$$M_t/M_o = kt^n \quad (22)$$

where M_t/M_o is the fraction or percentage of total drug (M_o) released at time t , k is a constant depending on the geometry and conditions of the system, and n is the exponent which describes the diffusional release kinetic mechanism or mode of transport. This

expression can be considered to be a more general version of Eq. (11) also presented previously for **monolithic dispersions**:

$$\frac{M_t}{M_o} = \frac{\sqrt{8DC_s}}{\sqrt{C_o l}} t^{0.5} \quad (23)$$

where

$$k \approx \frac{\sqrt{8DC_s}}{\sqrt{C_o l}}$$

It can be clearly seen from Figure 5.2 that Eq. (22) is applicable to predict and obtain the kinetic data (k and n) for efficacious MMC-release due to regression coefficients (R^2) having values close to 1. Interestingly, the diffusional exponents (n) for both immersed and floated drug-release samples appear to be similar, at a value of around 0.27. This shows that even though the release of MMC is predominantly diffusion-controlled, some deviation from Higuchi's principles and non-Fickian diffusion from a slab geometry (expected $n = 0.5$) results. There is a possibility that the degradation of MMC artificially “depresses” these n -values to be less than 0.5 as expected. This deviation is consistent regardless of whether the diffusing species (MMC) is exposed to water at both sides of the slab or to only one side, which also indicates that there is possibly no change in the diffusion coefficient and degradation kinetics of MMC in PLC for both conditions over time, thereby reinforcing Fick's first law. Figure 5.3 more clearly expresses the plots as linear functions of M_t/M_∞ against $t^{0.265}$.

Figure 5.3. Efficacious MMC-release plotted linearly.

Slightly different values of k are obtained when the curves are linearised to a common time-scale ($t^{0.265}$) and made to pass through the origin. From explanations of homogeneously-distributed monolithic dispersions (Chapter 2, Section 2.7.2) and using Eq. (23), the amount or rate of drug released for the fully immersed samples should be twice that of the floated samples for all time points. This is due to the doubling of the surface area for drug release. The assumptions are that there are no changes in the diffusion coefficient (D) of MMC in PLC, and saturation and initial concentrations of MMC in PLC are the same (C_s and C_o , as the same casted polymer is used for both conditions).

A ratio of both values of k gives rise to only about 1.62 ($18.73 / 11.55$), falling short of a value of 2 which is expected from surface area differences. This discrepancy is perhaps due to an imperfect assumption regarding the amount and rate of drug release for the immersed samples to be double that of the floated samples. There is a possibility that if MMC distribution in the film is not perfectly uniform and drug elution occurs from the

edges of the floated samples, a higher $k_{floated}$ value will be obtained. Another possible cause of this discrepancy is the inaccurate assumption that the system is a monolithic dispersion, instead of a **monolithic solution**. With a generally low MMC-loading of 0.351wt%, there is a possibility that a higher percentage of MMC-loaded is dissolved in PLC rather than dispersed in the bulk. The resulting equation for monolithic solutions presented in Chapter 2, Section 2.7.2 is:

$$\frac{M_t}{M_o} = 4 \left(\frac{Dt}{\pi l^2} \right)^{1/2} \quad \text{for } 0 \leq \frac{M_t}{M_o} \leq 0.6 \quad (24)$$

where

$$k \approx 4 \left(\frac{D}{\pi l^2} \right)^{1/2}$$

Substituting the exponent $\frac{1}{2}$ by 0.265, and doubling the value of l to account for only one surface for MMC diffusion under floated condition, as this is the only dimensional parameter; a theoretical calculated ratio of k ($2^{2 \times 0.265}$) results in a value of 1.44. This figure is thus closer to the empirically determined value of 1.62 (error: 11.1%, compared to 19.0% as above), and indicates that it is more correct to describe these films as monolithic solution systems.

As mentioned by other authors previously, attempts have been made to modify Higuchian kinetics by using diffusion coefficients that depend on concentration, polymer degradation and crystallinity. Mixed results were obtained, with polymer degradation (many samples experimented were faster degrading than PLC) being concluded to affect drug-release profiles more than crystallinity changes [71,76,77]. Such a method may not be applicable here for the following reasons:

- 1) PLC is a generally hydrophobic, slow-degrading copolymer and it is assumed that little polymer degradation occurs within the time of drug-release study to affect the diffusion coefficient of MMC in PLC.
- 2) Almost identical diffusional exponents ($n = 0.265$) are obtained for efficacious MMC-release from the 2 experimental conditions, supporting the assumption of a non-changing diffusion coefficient.

5.2.2 Determination of Diffusion Coefficient and Saturation Concentration of MMC in PLC (0.1mg/film)

From the results obtained in Section 5.2.1, both previously unknown values for diffusion coefficient (D) and saturation concentration (C_s) of MMC in PLC can be elucidated if both equations for monolithic dispersions and solutions are used. As Higuchi's equations assume that any diffusional drug-eluting system is simultaneously exposed to the release media at all surfaces, the values for D and C_s were calculated using k -values for the immersed condition in this study.

Starting with Eq. (24) for monolithic solutions, and assuming a $t^{0.265}$ relationship instead of $t^{1/2}$, a diffusion coefficient value of 4.26×10^{-6} cm²/day or 4.93×10^{-11} cm²/s is calculated. This value is reasonable as it lies between established values of drug diffusion coefficients through extremely permeable and impermeable polymers [64].

To obtain the saturation concentration of MMC in PLC, Eq. (23) for monolithic dispersion needs to be used. As before, by assuming a $t^{0.265}$ relationship instead of $t^{1/2}$ and by substituting $D = 4.93 \times 10^{-11}$ cm²/s into the equation, a saturation concentration value

of $C_s = 0.00255 \text{ g/cm}^3$ is obtained. Since C_o for the 0.1mg/film sample is around 0.004 g/cm^3 , this further supports the earlier assumption made that this system behaves more like a monolithic solution and gives clarity with regards to the ratio of k -values as discussed in Section 5.2.1.

5.2.3 Total MMC-release (0.1mg/film)

Due to the instability of MMC in water at physiological temperature (Figure 5.1), a theoretical correction needs to be done for the curves presented in Figure 5.2 to truly reflect the total amount of MMC (efficacious and degraded) released into the media to obtain complete percentage release vis-à-vis to total of amount of MMC loaded per film. Total release of loaded MMC (i.e. 100%) could be achieved / calculated up to 3 months for the immersed condition, with no measurable MMC-release thereafter, thereby validating this method of correction as described. Figures 5.4 and 5.5 display these corrected plots.

Figure 5.4. Total and efficacious MMC-release curves for immersed condition.

Figure 5.5. Total and efficacious MMC-release curves for floated condition.

Both figures exhibit slightly poorer regression coefficients but the underlying equation still holds, with total release completed in 3 months as no residual MMC in the films were detected during drug extraction for the immersed samples. It appears that when total MMC is assumed to be released without any drug degradation occurring, the diffusional exponents (n) for both immersed and floated samples begin to increase. This shows that when total MMC-release is considered for both conditions, its diffusional characteristics agree better with Higuchi's and Fick's laws, Eq. (24), as a closer relationship of drug-release against $t^{0.5}$ is achieved. This supports the earlier postulation that the degradation of MMC reduces the n -values as reflected in the cumulative release curves in Section 5.2.1. With regards to the changes in k for both conditions, linear plots with a common time-scale cannot be established due to differing n .

Finally, it can be seen that a 3-month time-frame is insufficient to witness the complete release of total MMC into the media for the floated condition, which only released up to

about 68% of total dose. With special emphasis on the floated condition to mimic tracheal stent drug-release, *efficacious* MMC-release reached till only about 38% (immersed: 61%) of initial MMC-loaded; and *total* MMC-release reached about 68% (immersed: 100%). These values indicate that higher amounts of MMC need to be loaded for a tracheal stent to successfully prevent stenosis and their implications will be further discussed with in-vivo results in Chapter 6.

5.3 EFFECT OF DRUG-LOADING ON MMC-RELEASE

To investigate the effects of amount of drug-loading on MMC-release kinetics, a another set of experiments were carried out with films of PLC loaded homogeneously with 1.0mg (approximately 3.51wt%) of MMC. These films with ten times the dosage compared to the films in Section 5.2 were studied in accordance to similar conditions. However, since only “intact” MMC is biologically active and is of interest when its release to tracheal cells is sustained, no theoretical correction via potency percentages to determine total MMC are presented; and only efficacious MMC-release results are compared and discussed here.

5.3.1 Efficacious MMC-release (1.0mg/film)

Figure 5.6 presents the cumulative MMC-release data for the 2 conditions.

Figure 5.6. Efficacious MMC-release curves for both immersed and floated conditions (1.0mg/film).

The MMC-release power law curves fitted in Figure 5.6 appears to have different k and n relationships than samples with 0.1mg/film (Figure 5.2). Even though MMC-release still remains predominantly diffusion-controlled, the different diffusional exponents, n , for both conditions imply that the diffusional release kinetics mechanisms or modes of transport are different or greatly affected by the condition for drug-release. Therefore, a direct relationship by assuming that amount or rate of drug release of the floated sample is half that of an immersed sample cannot be applied here as in the previous case in Section 5.2.1. Both k and n need to be considered to give a specific MMC-release percentage at a given time point and such a deviation from the 0.1mg/film samples is attributed to the possibility of drug segregation and agglomeration due to a high loading amount, and this can be microscopically seen in the quality of the MMC-loaded films after casting and drying (Figure 5.10). However, it is more meaningful in this Section to compare 2 different dosages under the same conditions and Figures 5.7 and 5.8 show these plots.

Figure 5.7. Efficacious MMC-release curves for 0.1 and 1.0mg/film (immersed).

Figure 5.8. Efficacious MMC-release curves for 0.1 and 1.0mg/film (floated).

Starting with Figure 5.7 (immersed condition), if the n -value for both curves is assumed to be the same (i.e. similar diffusional release mechanism for 0.1 and 1.0mg/film), from Eq. (23) for *monolithic dispersions*, the ratio of k for the 0.1mg/film sample over the 1.0mg/film sample should be equivalent to:

$$\frac{k_{0.1}}{k_{1.0}} = \frac{\sqrt{C_{1.0}}}{\sqrt{C_{0.1}}} = \frac{\sqrt{10}\sqrt{C_{0.1}}}{\sqrt{C_{0.1}}} = 3.162$$

The corresponding result for the immersed condition gives:

$$18.12 / 6.252 = 2.898$$

and the result for floated condition (Figure 5.8) gives:

$$11.62 / 2.287 = 5.081$$

The results calculated above show that a better agreement to Higuchi's equation is achieved when dosage is increased for the immersed condition, compared to the floated samples. This is expected as all drug-release principles and equations reviewed and used thus far are for samples with all surfaces fully exposed to the release media. With regards to floated conditions, it is observed that MMC-release from the 1.0mg/film samples seem to diffuse out of PLC at a slower rate than expected from the equation at any given time point, thus resulting in a generally low $k_{1.0}$. This is perhaps due to a reduced diffusion coefficient, D , as the dosage might be too high and especially when release is constraint to only one surface. With visible MMC agglomeration on the surface (as can be seen from Figure 5.10 even at a low magnification of 4X) and possibly within the casted films, the drug particles are only exposed to water ingress from one side and large amounts of MMC might be left remaining and prevented from diffusing out if these particles are present within the polymer film and on the air-side. PLC, being a generally hydrophobic polymer with low water ingress rates also exacerbates this problem and lowers the overall

diffusion coefficient value for the floated samples. Figures 5.9 and 5.10 compares both 0.1mg/film and 1.0mg/film microscopic surface images.

Figure 5.9. Surface morphology of 0.1mg/film sample (4X).

Figure 5.10. Surface morphology of 1.0mg/film sample (4X).

As can be seen from the images, MMC crystals and particles are well distributed in PLC for the 0.1mg/film sample, resulting in a higher percentage being dissolved into the polymer and making it more like a *monolithic solution*. The surface morphology of the 1.0mg/film sample shows numerous MMC crystals agglomerated and not well dispersed throughout the sample due to its much higher loading percentage. As explained above for the **floated** release experiment, this results in a lowered diffusion coefficient of MMC recorded empirically.

On the other hand, owing to the same reasoning for MMC particles to be agglomerated and possibly residing more on the surface of the 1.0mg/film sample after solvent drying, the ratio of k for 0.1mg/film over 1.0mg/film appears lower than the expected value (2.898 against expected value of 3.162) because both surfaces are in contact with water. As these particles are diffused out in larger volumes for the 1.0mg/film samples, this

inevitably causes a slightly higher diffusion coefficient (and hence $k_{l,0}$) to be empirically realised and thus making the ratio lower.

To sum up, as MMC dosage is increased by a factor of 10, it appears that the **immersed** condition continues to follow Higuchi's equations for monolithic dispersions closely. In addition to Higuchi's equations being derived specifically for immersed drug delivery systems as explained above, this is also likely due to the fact that the initial drug concentration (0.04 g/cm^3) is much higher ($>15\times$) than the calculated saturation concentration of MMC in PLC (0.00255 g/cm^3). Therefore, combining the results from Sections 5.2.1 and 5.3.1, it is suggested that both dosages cause the samples to lie between monolithic solution and dispersion systems; and never are truly one system or the other exclusively as equations for both are used to derive MMC characteristics in PLC. Results obtained for floated conditions for both 0.1mg/film and 1.0mg/film appear more complex. Inaccurate measurements of diffusion coefficients due to uni-directional drug-release from only one surface negates the use of Higuchi's principles to predict drug-release accurately, even when a dimensional correction is applied to circumvent this. As with Section 5.2.3, with emphasis on the floated condition to mimic tracheal stent drug-release, efficacious MMC-release reached till only about **9.3%** of initial MMC-loaded for the 1.0mg/film samples; but this was clearly more in absolute terms compared to the 0.1mg/film samples (38%).

CHAPTER 6

IN-VIVO PERFORMANCE OF FULLY BIODEGRADABLE TRACHEAL STENTS

6.1 PHASE ONE OF ANIMAL STUDY USING CASTED POLYMER FILMS

As mentioned previously in Chapter 3 (Sections 3.4.1 and 3.4.2), stents fabricated and implanted for the first phase of the animal study consisted of 5 groups of 5 rabbits each, with all rabbits subjected to a disease model to induce tracheal stenosis via excessive scarring during the tissue healing process. This was carried out to evaluate, as realistic as possible, the performance of fully biodegradable stents (with or without MMC) in tracheas after surgical resection of diseased / tumourous regions. The results of the various groups are first described, and for ease of presentation, a summary of the results with discussion is presented at the end of this section.

6.1.1 Control Group 1 – Without Stent

This group was included to act as a control and basically gave two indications for the study methodology. Firstly, the successful formation of induced stenosis during scarring verified that the disease model utilising diathermy was sound and reproducible. Secondly, the extent of stenosis in unstented tracheas, after tracheal stenosis inducement, was used

to compare objectively the performance of the various stents in the other stented groups. It was found that stable tracheal stenosis narrowing the lumen cross-sectional area by up to 75% could be achieved. Stenosis was significant by 3 weeks and stable by 6 weeks post-diathermy injury (Figure 6.1, A).

6.1.2 Control Group 2 – Commercial Silicone Tubular-shaped Stent

Similar to including a control group as described in Section 6.1.1, commercially available silicone stents were used in this group to enable an objective comparison between a current treatment used for tracheal stenosis after surgery and the performance of the various stents in the other groups. In this group, 1 rabbit developed severe post intubation stenosis at the tube cuff site at 3 weeks and this rabbit was euthanised. The remaining 4 rabbits developed cloudy, thick adherent mucus within their stents which resulted in respiratory distress (Figure 6.1, B).

6.1.3 Group 3 – Biodegradable Helical-shaped Stent

Due to the design of the PLC biodegradable helical-shaped stents, profuse tissue reaction in the trachea developed at the non-stented areas of the trachea between the helices of the stent. Amongst all groups, this had the most severe stenosis and mucus trapping in the tracheal lumen (Figure 6.1, C). Two rabbits had to be euthanised at 4 and 6 weeks, and no rabbit survived beyond 6 weeks. This was in stark contrast to all the other groups, where at least 1 rabbit in each group survived up to 12 weeks, and no rabbit had to be euthanised due to excessive tracheal stenosis.

6.1.4 Group 4 – Biodegradable Tubular-shaped Stent

As described in Chapter 3 (Section 3.4.1) regarding the fabrication of PLC tubular-shaped stents, these stents unwound to fit the diameters of the tracheal lumens after implantation. This group had less mucus trapping and airway narrowing compared to the helical biodegradable stents, and the outcomes were similar to that for the commercial silicone tubular-shaped stent group during the first 6 weeks after stenting (Figure 6.1, D). A rabbit in this group died at 8 weeks due to obstruction of the trachea by degraded stent fragments.

6.1.5 Group 5 – Biodegradable Tubular-shaped Stent with MMC

The stent used for this group was similar to the PLC + 0.1mg/film formulation as described previously in Chapter 5 (Section 5.2) and results from in-vitro studies on this system were meant to correlate to observations seen in this group. Amongst all groups, this group had the least calculated tracheal stenosis (Figure 6.2). Sustained-release of MMC from these stents showed efficacy in inhibiting excessive tissue growth. However, 1 stent degraded into two parts and was coughed out by the rabbit at 11 weeks. After which, this resulted in excessive tissue growth and progressive stenosis with blockage of 80% of the tracheal lumen 1 week later.

Figure 6.1. A) Endoscopic findings in Control Group 1 with diathermy injury to trachea without stenting 6 weeks post-injury (40% stenosis). B) Endoscopic findings in Control Group 2 – Commercial Silicone Tubular-shaped Stent at 4 weeks after stent implantation, show mucus trapping throughout the silicone stent narrowing the tracheal airway (45% stenosis). C) Endoscopic findings in Group 3 – Biodegradable Helical-shaped Stent at 6 weeks after stent implantation. Severe tracheal stenosis (99%) and significant mucus trapping was noted between the helical stent coils (non-stented tracheal areas). D) Endoscopic findings in Group 4 – Biodegradable Tubular-shaped Stent at 6 weeks after stent implantation. Tracheal narrowing was similar to Control Group 2 – Commercial Silicone Tubular-shaped Stent, but less than Group 3 – Biodegradable Helical-shaped Stent.

Figure 6.2. Endoscopic findings in Group 5 – Biodegradable Tubular-shaped Stent with MMC at 6 weeks after stent implantation. There was lesser tracheal stenosis compared all other groups.

6.1.6 Extent of Tracheal Stenosis in All 5 Groups

The extent of tracheal stenosis for the 5 groups over the follow-up duration of 12 weeks were recorded and calculated according to the procedure as described for endoscopic follow-up studies in Chapter 3, Section 3.4.3 (Figure 6.3). These values were the average severity of tracheal stenosis, in percentage terms, observed based on all surviving rabbits within a group at that particular week of endoscopy. Tracheal stenosis was most significant in the biodegradable helical stents, followed by the group without stent (Control 1), the biodegradable tubular stents and finally the silicone stents (Control 2). After 12 weeks, tracheal stenosis for the biodegradable tubular stents with MMC was half that of the silicone stents, averaging 10% and 20% of tracheal stenosis respectively.

Figure 6.3. Extent of tracheal stenosis in all 5 groups. Group 5 – Biodegradable Tubular-shaped Stent with MMC had the least tracheal stenosis. Data points with (*) indicate that only 1 surviving rabbit in that group from that week onwards was used for stenosis grading.

6.1.7 Discussion for Phase One of Animal Study

As this initial study was the start of all later animal studies regarding tracheal stenting, careful selection of the animal model had to be decided. The rabbit animal model was chosen here as its airway diameter is very similar to that for a neonate and young pediatric patient [72]. In this group of patients, the risks of airway obstruction are most significant; making wall thickness, mucociliary clearance and risks of granulations and stenosis in relation to implanting tracheal stents even more important. Furthermore, follow-up endoscopy can be performed in rabbits in a similar manner to that for human patients. Tracheal stenosis was also created by controlled diathermy heat injury to simulate the conditions of the injured / diseased trachea that would benefit from stenting in real life, rather than applying the stents to a normal trachea.

The results of the study are summarised here:

- 1) Currently available commercial silicone stents are covered tubes without cut-out holes in the stent. Therefore, granulations and stenosis occur only at the proximal and distal ends of the stents. The silicone stents resulted in tracheal stenosis ranging between 10% and 45% over the period of study (Figure 6.3, B).
- 2) The biodegradable helical stents had the most severe tracheal stenosis, ranging from 22.5% to 99% stenosis (Figure 6.3, C). The reason for adding this group in the study was to evaluate if the trachea could be sufficiently stented to avoid restenosis yet allow unstented areas between the helices of the stent to retain normal mucociliary function. It appeared that the non-stented areas of injured trachea between the helical turns reacted with profuse granulations and this fixed stenosis / scarring eventually.
- 3) For the biodegradable tubular stents, “cut-out” holes were distributed evenly throughout the stent to allow preservation of mucosa even within the stented area of trachea. This preserves healthy tracheal mucociliary activity, improves mucus clearance and tracheal patency. However, for the group with biodegradable tubular stents without MMC-elution, the cut-out holes do allow some granulations and stenosis to surface within the stented area (in the cut-out holes) of the injured trachea. This was the main reason for this group allowing up to 70% stenosis to occur, compared to the totally covered silicone stent group which showed only a maximum of 45% stenosis (Figure 6.3, D).
- 4) The group with the biodegradable tubular stents with 0.1mg MMC-elution performed the best amongst all groups of stents (Figure 6.2). It had the least tracheal stenosis at 10% and the least mucus trapping within the stents at 12 weeks. This was likely contributed by efficacious MMC-elution inhibiting tracheal granulations and stenosis both within the cut-out areas of the tubular stents, and also at the proximal and distal ends of the stents.

This is the first study that investigates the outcomes of a controlled amount of MMC application over a few weeks via drug-elution from a stent. In all previous studies, MMC could only be applied topically for 1 to 5 minutes to avoid blocking the airway during applications; and although most topical application concentrations ranged from 0.1 to 1mg/ml of MMC, the actual amount delivered this way is unknown [46]. With this in mind, and MMC widely known to be cytotoxic at high concentrations, safety and efficacy of the lowest dosage of 0.1mg/stent was selected to be used throughout this study. Referring back to Chapter 5 (Section 5.2), it can be seen that only about 38% of efficacious MMC is released from the PLC film samples under floated conditions up to 12 weeks, which in this case mimics MMC-release from Group 5 (Biodegradable Tubular-shaped Stent with MMC) closely. Assuming that drug delivery from the stent to the trachea is purely uni-directional and no drug loss occurs from the inner surface of the stent, a total “intact” MMC amount of 0.038mg is sufficient to combat tracheal stenosis (since Group 5 performed better than all other groups), if the stent material is carefully chosen and modified to homogeneously degrade completely in 12 weeks; and taking into account MMC potency / degradation kinetics (Chapter 5, Section 5.1). A tabulation of the results for Phase One is presented in Table 6.1.

The limitations in this preliminary study include the small number of rabbits of 5 per group which make statistical analyses less useful and relatively limited histology studies (data not shown). This first phase did, however, provide sufficient data to proceed towards Phase Two of the study with some modifications to stent design and method for drug-loading.

Table 6.1. Summary for Phase One of animal study.

Group	Average extent of tracheal stenosis at 12 weeks (%)	Remarks
Control Group 1 (Without Stent)	65%	Stenosis stable by 6 weeks
Control Group 2 (Commercial Silicone Tubular-shaped Stent)	20%	Granulations and stenosis occur at proximal and distal ends of the stents
Group 3 (Biodegradable Helical-shaped Stent)	99% (at 6 weeks)	Worst stenosis Profuse granulations and fixed stenosis at non-stented areas
Group 4 (Biodegradable Tubular-shaped Stent)	50%	Some granulations and stenosis within the “cut-out” holes
Group 5 (Biodegradable Tubular-shaped Stent with MMC)	10%	Least stenosis and mucus trapping Possibly due to effects of MMC

6.2 PHASE TWO OF ANIMAL STUDY USING DIP-COATED AND LASER-PATTERNED STENTS

Following up from Phase One of the animal study, the second phase was initiated and conducted to improve upon the stents and results achieved, albeit a smaller number of groups and rabbits were used in total (3 groups of 6 rabbits each). As mentioned previously, the same disease model via diathermy was used on all rabbits to induce tracheal stenosis. However, the fabrication and design of the stents were radically different. A dip-coating technique was used to produce covered tubes with consistent wall thickness (about 250 μ m) and a femtosecond laser setup was used to produce

intricately laser-patterned stents as described and shown in Chapter 3, Section 3.4.1 and Figure 3.10. The resultant stent prototypes were uniformly radially stiffer than the rolled polymer films used for Phase One; and with the numerous stent “holes” larger in area and more distributed (i.e. less PLC stent material), it was postulated that easier and quicker epithelialisation over the stent would have occurred during the natural healing process instead of merely preserving mucociliary activity as in the case of hand-cut holes on the polymer films used previously. After epithelialisation, it would be safe (no stent fragments causing occlusion) and convenient (no explantation needed) for the stent material (PLC) to degrade homogeneously without any further undue effects to the healed tracheal wall. It was also these considerations that prompted the selection of PLC as a suitable bulk-degrading material for tracheal stent fabrication, after in-vitro studies (Chapter 4).

In addition to evaluating the performance of this new stent design, 2 different methods of MMC-loading were proposed. The first was to homogeneously load 0.1mg of MMC within the body of the stent, where drug-release would be conceptually similar to Group 5 (Biodegradable Tubular-shaped Stent with MMC) from Phase One. The second method was to spray-coat 0.1mg of MMC together with a faster degrading copolymer (PLGA 53/47) on the outer surface of the stent to be in contact with tracheal cells as described in Chapter 3, Section 3.4.1. These 2 MMC-loaded stents, together with drug-free stents, formed the 3 different study groups for Phase Two. A summary for all in-vivo and in-vitro results with discussion regarding this phase is presented in Section 6.2.6.

6.2.1 Group I – Laser-patterned Stent without Drug

This group acted as a control and was included to compare objectively the performance and restenosis rates of the other 2 groups of stents with 0.1mg MMC. It was observed that good epithelialisation over the stent occurred as early as 1 week post-implantation and this stabilised thereafter. No stents were present from 20 weeks onwards which suggested that complete degradation of PLC in the trachea occurred around that time. However, there was one stent which broke apart at 8 weeks and caused occlusion of the airway leading to rabbit euthanasia at 10 weeks. Figures 6.4 and 6.5 compare a 90% stenosed trachea (from previous Phase One study, Control Group 1) against a selected patent trachea with good epithelialisation over the stent at 2 weeks from this group.

Figure 6.4. Diathermy injury to trachea without stenting (90% stenosis).

Figure 6.5. Selected endoscopic image for Group I at 2 weeks post-implantation shows consistent epithelialisation and patent trachea.

Histological results obtained from this group revealed that there were no giant cells or multi-nucleated cells which suggest no foreign body reactions to the stent. This was maintained throughout the period of study. Mild fibrotic response was also noted around

stent fragments. Figures 6.6 and 6.7 compare histological images of unstented and stented regions of a trachea from the same rabbit at 6 weeks.

Figure 6.6. Histological image of unstented and patent trachea. Staining: H & E.

Figure 6.7. Histological image showing stent fragments and slight fibrotic hyperplasia present, but otherwise a patent trachea (6 weeks). Staining: H & E.

6.2.2 Group II – Laser-patterned Stent with Homogeneously Distributed MMC

The results for this group were markedly different from Group I, mainly due to the presence of MMC. Where epithelialisation occurred rapidly and consistently over the stent as presented in Section 6.2.1, this response was absent here even though the stent design was the same. An explanation for this was that the effect of MMC-elution suppressed the formation of granulation tissue which precedes natural healing and scarring processes, but due to the cytotoxic nature of MMC, this also prevented or delayed epithelialisation. All rabbits survived till the first 3 time points (3, 6 and 9 weeks), but extensive restenosis occurred rapidly between the spaces of the stents after 9 weeks post-implantation and the remaining 3 rabbits did not survive beyond 10 weeks. This could have been because the daily release-rate of efficacious MMC may have been

below the minimum effective concentration, by 9 weeks, to have any therapeutic effect thereafter (also explained in Section 6.2.5). Figures 6.8 and 6.9 present the endoscopic images of 2 different rabbits' tracheas at late time points to reflect such restenosis, with a broken stent in one of the tracheas.

Figure 6.8. Endoscopic image for Group II at 9 weeks post-implantation, shows extensive restenosis (90% stenosed).

Figure 6.9. Endoscopic image for Group II at 10 weeks post-implantation, shows a broken stent and extensive restenosis (95% stenosed).

Histological results obtained from Group II revealed that heightened fibrotic response was seen to subside towards later time points, but increased rapidly after 9 weeks. Similar to Control Group I, there were no giant cells or multi-nucleated cells which suggest no foreign body reactions to the stent. Figures 6.10 and 6.11 compare histological images of unstented and stented regions of a trachea from the same rabbit at 9 weeks.

Figure 6.10. Histological image of unstented and patent trachea. Staining: H & E.

Figure 6.11. Histological image showing stent fragment and fibrotic hyperplasia which compromised tracheal patency (9 weeks). Staining: H & E.

6.2.3 Group III – Laser-patterned Stent with Spray-coated MMC

Consistent with the tissue response seen for Group II, epithelialisation over the stent did not occur in this group. This again was the effect of MMC-elution to the tracheal wall, which prevented and possibly delayed the onset of natural healing processes to take place. Post-implantation, this group did not exhibit any occlusion of the airway up till 6 weeks and performed similarly to Group II. However, because 0.1mg of MMC (as a fast-degrading layer with PLGA 53/47) was sprayed onto a biodegradable material, some drug partitioning may have occurred between PLC and PLGA 53/47; thereby lowering its elution rate and mass of efficacious MMC released, compared to Group II (described in more detail in Section 6.2.5). Therefore, this overall lower dosage allowed excessive tissue growth to occur to cause severe restenosis at earlier time points. All rabbits survived till only 2 time points (3 and 6 weeks), with the remaining 4 rabbits perishing after 8 weeks. This group fared the worst amongst all groups with extensive restenosis

taking place after just 7 weeks. Figure 6.12 displays a montage of 2 different rabbits' tracheas with severe restenosis at 7 and 8 weeks, and their states just a week before.

Figure 6.12. A) and B): Endoscopic findings in Group III for Rabbits A (6 weeks) and B (7 weeks) after stent implantation respectively, both showing encouraging results with generally patent tracheas with little mucus built-up. C) and D): Endoscopic findings for Rabbits A and B one week later respectively. Excessive scar tissue growth resulting in an onset of severe restenosis was observed at 7 and 8 weeks (80% and 95% stenosis). Tracheal patency was highly compromised for all other rabbits in this group after 8 weeks, with none surviving beyond this time point.

Histological results obtained from this group were similar to Group II, except that the incidence of excessive fibrotic hyperplasia occurred earlier from 7 weeks compared to Group II where it started from 9 weeks. This was likely due to a low MMC-elution rate as

explained above. Inflammation was also present at early time points but these gradually taper off after 8 weeks (data not shown). Consistent with both Groups I and II, there were no giant cells or multi-nucleated cells which suggest no foreign body reactions to the stent. Figures 6.13 and 6.14 compare histological images of unstented and stented regions of a trachea from the same rabbit at 8 weeks.

Figure 6.13. Histological image of unstented and patent trachea. Staining: H & E.

Figure 6.14. Histological image showing stent fragments embedded in extensive fibrotic hyperplasia, resulting in severe restenosis (8 weeks). Staining: H & E.

6.2.4 In-vivo and In-vitro Degradation Profiles for PLC

During this Phase Two animal study, all stents were explanted from sacrificed / euthanized rabbits and evaluated for degradation using SEC. The results obtained from SEC were analysed in a similar manner to Chapter 4, Section 4.2, to derive the various degradation kinetics data for comparison between groups. However, it was not possible to obtain thin surface slices of explanted stents via microtomy; therefore the results presented here are data for whole PLC stents immersed in water and explanted stent

fragments. Figure 6.15 shows the degradation plots for stents explanted from all 3 groups and compared against a separate experiment for in-vitro PLC stent degradation in water.

Figure 6.15. Degradation profiles for in-vitro PLC stents and explanted stents.

From the plots, it can be clearly seen that all explanted stents from Groups I to III degrade at faster rates than that for pure PLC stents immersed in water; with stents from Group I (without MMC) degrading with a profile closest to pure PLC. This shows that the first-order degradation rate constants (k) for in-vivo samples are higher than the value obtained experimentally from in-vitro samples, leading to a possible explanation that continuous mucus flow and the dynamic nature of the trachea promote ester bond cleavage. Also, and more likely, with mucus and tracheal cells in contact with the stent, there is a possibility of enzymatic degradation occurring due to the presence of esterase [16,61] which aids in cleavage of ester bonds, hence increasing hydrolysis rates for PLC.

Group II stents appear to degrade faster than those in Group I due to the presence of MMC loaded within the body of the stent. As MMC is eluted out of the stent body, there

is a high possibility of an increase in porosity and thus surface area exposed to water in the trachea. This will cause hydrolysis rates to increase and reflects in higher k -values.

Lastly, Group III stents are seen to degrade faster than those in Group II (even though both MMC-loading is the same at 0.1mg/stent) due to the presence of PLGA 53/47 as the coating material. As SEC is a liquid chromatographic method to obtain average molecular weight data from samples passing through a size-graded column and obtaining times for elution, lower number-average molecular weights (M_n) will be recorded as PLGA 53/47 has a generally lower M_n when compared to PLC. Analysis of data from Group III stents will reflect a lower average amount of remaining ester bonds at any point in time and therefore show a higher hydrolysis rate, resulting in the highest degradation rate constant (k). All data from this degradation study is presented in Table 6.2.

Table 6.2. Degradation kinetics data for Phase Two of animal study.

Sample / Group	Average k (week ⁻¹)	Average R^2 (linear)
PLC Stents in Water	2.05×10^{-4} ^a	0.92
Group I (Laser-patterned Stent Without Drug)	2.63×10^{-4}	0.98
Group II (Laser-patterned Stent With Homogeneously Distributed MMC)	3.12×10^{-4}	0.95
Group III (Laser-patterned Stent With Spray- coated MMC)	4.81×10^{-4}	0.94

^a k -values for PLC exposed to gel and humidified air are 1.00×10^{-4} week⁻¹ and 0.883×10^{-4} week⁻¹ respectively, for comparison (from Chapter 4).

From the first-order degradation rate constants (k) obtained from the plots, it appears that immersing PLC films in water tends to result in a closer value of k to those seen in samples after in-vivo exposure in the trachea; this is compared to a k -value of about $1.10 \times 10^{-4} \text{ week}^{-1}$ obtained from PLC films exposed to both gel and air sides simultaneously, as described in Chapter 4, and analysed via SEC as a whole without gel / air-side sectioning via microtomy (data not shown). Therefore, full immersion of PLC films in water correlates closest to Group I stents' (without drug) degradation profile compared to the experimental setup used for degradation analyses in Chapter 4, for reasons as explained above regarding tracheal conditions.

6.2.5 Comparison Between In-vivo Results and In-vitro MMC-release

Endoscopic and histological results from both Groups II and III are compared with in-vitro MMC-release data to determine correlations between efficacious MMC-release and its effect on preventing tracheal stenosis. Even though the surface areas for drug-release between the PLC films and laser-patterned stents are different however, it can be seen that the surface area parameter is cancelled out (Chapter 2, Eq. (6), (7) and (11)) for monolithic solutions and dispersions, and leaving only the thickness of the device (l) as a dimensional parameter (Note: This does not imply that surface area is not considered. It is used in the calculation for device *volume*, which in turn affects values for drug concentrations, C_o and C_s ; both values for which are known for all films / stents in this study). Also, since both in-vitro PLC films and stent wall thickness are the same at about $250\mu\text{m}$, MMC-release curves can be compared directly for films under floated conditions and Group II stents (homogeneously distributed MMC). As most rabbits survived till 9 weeks in this group, and from results presented in Chapter 5 (Figure 5.5), efficacious and

total MMC-release of 38% and 68% would have occurred respectively. The percentage of efficacious MMC-release is about similar to that seen for Group 5 (Biodegradable Tubular-shaped Stent with MMC) from Phase One and positive results were seen from this group. As such, it is thus possible that the current laser-patterned stent design allowed an excessive growth of granulation tissue to form scarring and cause severe restenosis. This is also confirmed in the endoscopic images (Figure 6.8 and 6.9), compared to the tracheal lumen from Group 5 (Figure 6.2), where the stent design was more “covered” like a tube. Another important observation from this is that if the assumption for MMC-depletion or total degradation occurs around 9 weeks, thereby allowing an onset of excessive granulation tissue growth, there is a possibility that a high percentage of 32% (100% - 68%) of total MMC-loaded risks being lost; with a likelihood of a high amount being cleared away by the continuous mucus flow in the trachea. This shows that drug wastage of close to a third of the amount loaded in such homogeneously distributed drug stents can occur when implanted in the trachea.

Group III performed the worst amongst all groups as can be seen from the tracheal endoscopic observations (and histological images). In-vitro MMC-release from 0.1mg MMC spray-coated stents immersed in water was conducted to determine MMC delivery via such stents (Note: It was not feasible to conduct a floated release or release-into-gel experiment on these spray-coated stents due to stent design and its tubular geometry). The cumulative graph of in-vitro release from the MMC-loaded coating up to 8 weeks is compared against release from PLC films under floated condition and presented in Figure 6.16.

Figure 6.16. Efficacious MMC-release curves from spray-coated stents and floated condition, both with 0.1mg MMC.

From the graph and results obtained, only about 14% of MMC-loaded into the coating of the stent is released into water up to 8 weeks, the time after which extensive restenosis is seen in the rabbits' tracheas for Group III. This compares to about 38% expected to be released from Group II stents (results from floated experiment). We believe that the explanation for the difference in observed release from floated films and from spray-coated stents is as follows:

Most of the MMC release from the spray-coated stent is via a burst release from the coating (which is quite thin). Subsequent release is affected by the PLGA degradation and its effect on MMC stability. It is possible that the lower localised pH caused by molar chain scission of the PLGA further destabilises MMC, so that the amount of efficacious MMC released continues to be very small. Overall, the amount of efficacious MMC available after the second week (or thereabouts) is too low to have any effect on cell proliferation.

PLGA 53/47 is a generally fast-degrading copolymer. As it begins to degrade at earlier times (compared to PLC), a build up of acidic end-groups occurs and results in lowering of the pH. MMC exhibits accelerated degradation at pH below 5 (Chapter 2, Section 2.5.5) and this can have an effect on MMC aziridine ring opening, leading to a reduction in efficacious MMC available for therapy. From Chapter 2, Section 2.7, using Eq. (5):

$$C_{ss} = \frac{R_i}{k_{el} V_d} \quad (25)$$

It can be seen that the steady state concentration (C_{ss}) for MMC in the tracheal cells will be continually decreasing as the drug input rate (R_i) decreases. From Figure 6.16, as the efficacious MMC-release appears to plateau, there is a possibility that the gradient of the MMC-release curve (R_i) for a spray-coated stent causes C_{ss} to be reduced to values below the threshold efficacious concentration (C_{eff}), which is below the minimum effective drug concentration for MMC to prevent granulation tissue growth and combat stenosis, thus resulting in severe restenosis for Group III at earlier time points (8 weeks) compared to Group II (9 weeks).

6.2.6 Discussion for Phase Two of Animal Study

Phase Two of the animal study was planned and executed on the premise that a fully biodegradable tracheal stent should have generally substantial uncovered stent area and cylindrical in nature (as opposed to rolled films) to achieve 3 broad improvements over the tubular stent with hand-cut holes used in the previous study. As mentioned previously; these were to increase the radial strength of the stent, allow more areas for mucociliary activity for natural tracheal clearance, and for easier and quicker epithelialisation over the

stent struts during the natural healing process after tumour resection. The use of laser-machining technology was therefore employed to achieve consistent quality and reproducibility in stent production.

For MMC-loading, the dosage of 0.1mg MMC/stent was maintained as positive results regarding tracheal patency due to suppressed granulation tissue growth and scarring was achieved in the Phase One study. This dosage was selected after a review of current clinical procedures (explained in Section 6.1.7) and was used for both the homogeneously-distributed and spray-coated versions of stents used here.

The results of the study are summarised here:

1) Results from both endoscopic and histological examinations were encouraging for Group I (Laser-patterned Stent without Drug). The rapid epithelialisation observed as early as first week and stabilisation thereafter, seen in all rabbits, suggested that PLC as a stent material was well-tolerated with no foreign body reactions. This also validated the belief that larger uncovered stent areas with lesser stent material would promote the growth of epithelial tissue over the stent struts during healing. Additionally, the generally patent tracheas observed over the study period also pointed to less mucus built-up; which was most likely due to the extensive preservation of mucociliary tissue to both secrete and clear essential mucus for the maintenance of patent airways. However, it was noted that healthy tracheal cells (areas not subjected to diathermy injury) present against the stent (especially at proximal and distal locations) do not readily proliferate and grow to engulf the stent struts. This caused those unepithelialised portions of the stent to degrade and break-apart freely, characteristic of bulk-degrading polymers. This was evident in the rabbit that had to be sacrificed due to stent fragment migration in the airway which caused

severe occlusion at 8 weeks. Nonetheless, there seems to be potential for such a stent to be used to address tracheomalacia (as described in Chapter 2, Section 2.3), where a flaccid trachea requires the use of a temporary structural support without any drug-elution needed.

2) Group II (Laser-patterned Stent with Homogeneously Distributed MMC) was initially postulated to perform better than Group I as it was an improved version of the Group 5 (Biodegradable Tubular-shaped Stent with MMC) stent from Phase One, with a laser-patterned design. This was not to be the case. Even though a lack of epithelialisation was expected during early times due to the anti-proliferative action of MMC, it was meant to proceed normally during the natural healing process once efficacious MMC was fully eluted to suppress granulation tissue growth, or all remaining MMC degraded to become inefficacious (but non-toxic) products. What was observed was that the maintenance of efficacious MMC-elution was needed to be sustained for a longer time period, without which extensive granulation tissue growth leading to fixed restenosis via scarring would occur. Evidence of this could be seen in the histological examinations showing a gradual lowering of both inflammatory and fibrotic responses immediately post-implantation till 9 weeks (i.e. during MMC-elution), and eventual extensive fibrotic hyperplasia once MMC was depleted or became inefficacious. It remains to be seen the optimum dosage and time period for MMC-elution, taking into account the disease model employed here or the condition of the trachea post-surgical resection of tumours.

3) Group III (Laser-patterned Stent with Spray-coated MMC) was initially predicted to be the best performing group when considering factors such as the current clinical practice for MMC application and stent design. As mentioned previously, solutions of MMC at concentrations of 0.1 to 1.0mg/ml were usually held against the injured tracheal site for 1 to 5 minutes (to avoid blocking the airway) clinically with positive results

reported; it was postulated that the elution of 0.1mg MMC to the trachea in a time-frame of about 1 – 2 weeks would suffice to prevent restenosis via granulation tissue growth, yet allowing epithelialisation to naturally occur and accommodate the stent into the tracheal wall thereafter. It was with this in mind that 0.1mg MMC was spray-coated, together with the faster-degrading PLGA 53/47 as a coating material, only on the outer surface of the stent. This would have allowed a rapid drug-elution rate to achieve the above objective, with PLGA 53/47 known to completely degrade in 2 weeks in-vivo. Similar to Group II, the presence of MMC prevented any initial epithelialisation over the stent struts as all tissue growth was inhibited. Tracheal patency was well maintained post-implantation, even better than for Group I, due to continual MMC-elution (firstly from the PLGA 53/47 layer, followed by the PLC stent itself) which gradually reduced inflammation and fibrosis (also seen in Group II) up to 6 weeks. However, taking into account possible extensive MMC degradation due to lowering of pH, it was eventually realised that the efficacious MMC dosage released to the tracheal cells via the diffusional mechanism was highly compromised (described in more detail in Section 6.2.5). This resulted in extensive fibrotic hyperplasia occurring in the trachea from 7 weeks onwards which unfortunately made this group perform the worst. There is a possibility that such stents spray-coated with MMC might not sufficiently prevent the onset of tracheal restenosis due to a low rate of drug-elution in a sustained manner, if such MMC stability issues are not adequately addressed, causing the steady-state concentration of MMC in tracheal cells to fall below a minimum effective value.

To sum up, whilst it was encouraging to note that both the stent material (PLC) and the drug (MMC) were well-tolerated without eliciting any foreign body reactions for all groups studied, the ability of efficacious MMC to be released in a sustained manner for

longer time periods and larger doses is essential to prevent the onset of late-stage tracheal restenosis. This can be addressed in the dosage of MMC loaded, method of incorporating MMC with the stent and even possibly using a different drug (e.g. dexamethasone) which exhibits better stability when used in-vivo, thereby enhancing drug efficacy for longer durations. However, drug studies aside, the laser-patterned stent without MMC seems to be a suitable candidate to address tracheomalacia if further studies can be carried out to validate its safety and efficacy.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

7.1.1 Degradation Phenomena of Biodegradable Copolymers in a Simulated Tracheal Environment

A physical model has been developed to study the degradation kinetics of biodegradable polymers on two fronts, thereby mimicking the unique conditions of the trachea. The use of microtomy to achieve thin longitudinal sections of these polymers enabled a better understanding of degradation behaviour in heterogeneous environments via SEC.

Generally hydrophobic polymers like PLC have a low enough water ingress rate to prevent a distinct water concentration gradient to be built up over the cross-section of the bulk material / film when subjected to the experimental set up; therefore its bulk-degrading characteristic is retained and specific depth analyses of molecular masses from both gel and air sides do not show a significant difference. The opposite is true for generally hydrophilic polymers like PLGA 75/25 as a high water ingress rate causes the material to degrade heterogeneously (pseudo “surface-eroding”) as a distinct water concentration gradient is formed in the experimental setup mimicking the trachea.

The addition of PEG and TEC to PLC increase rates of hydrolysis for both gel and air sides as crystallinities are disrupted and reduced; and porosity is assumed to be induced, thereby aiding in ester bond cleavage. However, PEG enhances the difference between hydrolysis rates of the gel and air sides but TEC does not. This shows that the out-leaching of PEG (especially at the gel side), and the postulated resultant porosity, causes a distinct water concentration gradient to form and therefore heterogeneous degradation takes place even for PLC. Interestingly, adding PEG to a generally hydrophilic polymer (PLGA 75/25) maintains this difference as the increase in water uptake at the gel-side compared to the air-side do not translate to an equivalent increase in the hydrolysis rates for both sides, when an intrinsic water concentration gradient can be built-up in the case for neat PLGA 75/25.

These results express the possibility of well-studied bulk-degrading polymers exhibiting pseudo “surface-eroding” profiles when exposed to two different environments, as observed in a tracheal passage. The methodology to obtain such results for understanding biodegradation behaviour at specific depths is the first such attempt reported thus far, which leads to PLC being the chosen material for fabrication of prototypical fully biodegradable tracheal stents for animals studies due to its relative insensitivity of kinetics to both gel and air sides. Data from this study has been accepted and published as an original research publication [80].

7.1.2 Drug-release Phenomena in a Simulated Tracheal Environment

A stability study for the drug of interest, mitomycin C (MMC), was conducted in water at 37°C to quantify the degradation of MMC when incorporated into a polymer and exposed

to water, and after release into physiological conditions (e.g. in the tracheal wall). The results show that MMC is generally unstable at 37°C as its potency falls to about 50% and 16% after 4 and 12 weeks respectively. Such potency loss is related to the opening of the aziridine ring in MMC and causes UV absorbance to diminish. These reduced UV absorbance values are directly related to MMC degradation and determines the efficacy of MMC in later in-vivo studies.

Comparisons between MMC-release kinetics from PLC films in immersed and floated conditions were done to mimic drug-release phenomena in the trachea, against common methods of quantifying drug-release using Fick's laws and Higuchi's equations. It appears that the accuracy of slight modifications to Higuchi's equations to take into account uni-directional MMC-release to the medium depends largely on the initial concentration of MMC in PLC. With a drug loading of 0.004 g/cm³ (for 0.1mg MMC films), it is more accurate to treat the system as a monolithic solution with a constant diffusion coefficient; and credence is given to these assumptions as expressions for both monolithic solutions and dispersions are utilised to achieve saturation concentration (C_s) and diffusion coefficient (D) values of **0.00255 g/cm³** and **4.93 x 10⁻¹¹ cm²/s** respectively.

Studies into different dosage on MMC-release kinetics reveal that Higuchi's equations are more relevant and accurate for the immersed condition (which was precisely how these expressions were derived). However, owing to the high dosage form of the system (1.0mg/film), slight errors are encountered when correcting for the floated condition. Predominantly using expressions for monolithic dispersions for 1.0mg/film samples, it is postulated that the diffusion coefficient value of MMC through PLC films under the floated condition is artificially lowered from that derived above. An explanation for this is

that a high amount of MMC is constraint to be diffusing out from the film on only one side, thereby reducing this diffusivity value as a lowered amount of drug is recorded in the medium. Microscopic images of the surface morphologies for both 0.1mg/film and 1.0mg/film samples show evidence of drug distribution causing such errors. It is concluded that both dosages in this study do not fall exclusively as a pure monolithic solution or dispersion. A provisional patent application has been submitted to cover various methods and amounts of MMC-loading into different biodegradable polymers / copolymers for use as tracheal stents (US Patent Application No. 61/454,858).

7.1.3 In-vivo Performance of Fully Biodegradable Tracheal Stents

An analysis of the degradation profiles for in-vitro PLC films in water at 37°C and in-vivo explanted stents from the rabbit study shows that all in-vivo stents tend to degrade at higher rates compared to PLC exposed simultaneously to gel and humidified air or in water. It is thus possible to hypothesize that the presence of mucus (with any associated esterase) and the dynamic mechanical actions in the trachea promote ester bond cleavage, hence increasing hydrolysis rates. It is determined that the closest experiment to mimic PLC (or possibly any biodegradable polymer) degradation in the trachea is to simply immerse all samples into water for molecular mass monitoring, which gives maximum water ingress rates. The presence of a drug in the stent also increases hydrolysis rate as porosity might be induced once the drug leaches out, thereby increasing the amount of water uptake and total surface area exposed for ester bond cleavage.

MMC-release results obtained for spray-coated stents revealed that drug-elution rate and amount were consistently lower than from floated films. An explanation for low MMC-

release rates was that the coating material, PLGA 53/47, degraded relatively quickly and lowered pH of the system. Accelerated MMC degradation occurs at pH below 5 and this contributes to lower amounts / rates of efficacious MMC-release. Together with a high amount of drug loss from homogeneously distributed MMC stents, low rates of MMC-release are correlated to the results obtained from the tracheas in the in-vivo animal study.

Lastly, the results for the animal study using the current stent design and drug-loading methodologies were not optimistic, despite clear evidence that PLC was well-tolerated as a stent material in the trachea. Even though the rabbit group with laser-patterned stent without drug performed better than both MMC-loaded groups at later time points, patency was not better especially at early time-points (i.e. MMC did have a positive effect in the initial stages for Groups II and III). Both Groups II and III faced severe restenosis in the tracheas after 9 and 7 weeks respectively, postulated to be the time points for total MMC depletion / degradation or release rates that were too low. Therefore, there is a need for efficacious MMC to be released in a sustained manner for longer time periods and larger doses to prevent late-stage tracheal restenosis. Precise dosage and methodology for MMC-loading onto the stent is needed, and possibly even exploring a different drug exhibiting better stability and efficacy in physiological conditions and for longer durations. Results from this animal study have been accepted and published [36].

7.2 RECOMMENDATIONS

7.2.1 Degradation Phenomena of Biodegradable Copolymers in a Simulated Tracheal Environment

The main limitation to this study as presented in Chapter 4 was the 6-week time frame. As this was the first attempt to utilise a physical model to mimic the trachea and to obtain thin longitudinal surface sections of polymer films for SEC analyses, it was postulated that a good agreement with Eq. (21) in Chapter 4 (Section 4.2) would mean that the derived first-order degradation constants (k) of the various samples would be maintained at all molecular masses till complete degradation; and it was from these initial results (till 6 weeks) that PLC was eventually chosen as a suitable material for fabrication of all stents used. However, as a fully biodegradable tracheal stent is expected to be in service for about 3 – 6 months, it is recommended that longer term studies to analyse the degradation kinetics of the various samples degraded till lower molecular masses to be conducted. This will possibly reveal if there exist critical molecular masses below which the relationship, as expressed in Eq. (21), does not hold. Therefore, polymers suitable to be chosen as tracheal stent materials should have uniform first-order degradation constants regardless of exposure media (i.e. exhibit homogeneous degradation) and should maintain mechanical integrity up to 6 months.

Another limitation to the above study was that the pH of both the gel in contact and within the polymer film samples could not be determined in the course of this experiment. It is noted that changes in pH do play a part in determining whether hydrolysis is via random scission or chain-end cleavage of ester bonds [55,56]. Therefore, even though a

neutral pH for the gel was determined at the start of this experiment, it is a recommendation to continually monitor the pH of the gel throughout the time frame of the study to elucidate and explain any possible changes in degradation kinetics due to environmental changes, especially at later time points when more acidic functional groups are expected to be present from the hydrolysed film samples.

7.2.2 Drug-release Phenomena in a Simulated Tracheal Environment

The assumption used regarding doubling the thickness value (l) of the floated samples, compared to the immersed ones, in Chapter 5 (Section 5.2.1) to account for surface areas differences for MMC-release kinetics using Higuchi's equations still presents a slight deviation for the 0.1mg/film samples. To more clearly compare and validate this, it is a recommendation to prepare and experiment on samples with physical thickness double (or varying) that of those used for the immersed condition. This will allow a more detailed study of thickness variation and its effect on MMC-release especially in a simulated tracheal environment (i.e. floated condition).

For the MMC-release experiments for 1.0mg/film samples, a variation of different drug dosages (e.g. 0.25mg/film, 0.5mg/film, 0.75mg/film) can be prepared and drug-release studies conducted. This will better aid in understanding the differences in diffusional kinetics due to drug dosage and distribution causing the possible changes in diffusion coefficients proposed, and will ultimately shed more light on the deviations seen from Higuchi's equations for drug-release samples under immersed or floated conditions.

7.2.3 In-vivo Performance of Fully Biodegradable Tracheal Stents

An effort to correlate the hydrolytic degradation of PLC in-vivo to PLC in one of the harshest conditions of full immersion in water still seems to fall short. Future studies to mimic in-vivo biodegradable polymer degradation via in-vitro should introduce the presence of enzymes (e.g. cholesterol esterase, lipase B, *Rhizopus delemar* lipase) [65] into the degradation media. In addition to this, with emphasis to this tracheal study, a dynamic mechanical model simulating tracheal movements can be constructed and set up to provide as realistic as possible conditions for polymer degradation in the trachea. This is with the hope that the first-order degradation constants (k) derived from in-vitro experiments can be more accurately correlated to in-vivo results, thus aiding in better material selection and performance especially at longer time periods.

It is a recommendation for experiments to be designed to allow the partition coefficient of MMC between PLC and PLGA 53/47 (in DCM) to be recorded or calculated. This will assist in the understanding of equilibrium MMC distribution between these 2 copolymers when it is spray-coated onto the stent, and will more clearly explain the low cumulative drug-release obtained from the spray-coated stents in this study resulting in unfavourable in-vivo performance after implantation (Group III). In fact, a partitioning study can be conducted for any drug being diffused into 2 (or more) layers of any polymer coating material and this is of importance when understanding drug-release kinetics from spray-coated systems.

Results obtained from the animal study, especially Phase Two, warrant further experiments and testing to address the under-performance of the MMC-loaded stents. Due

to the unstable nature of MMC at 37°C in water, it is recommended that further studies be carried out to maintain the long-term efficacy of MMC via the addition of well-studied excipients such as povidone and cyclodextrin. If this is deemed unfeasible, another future work to sustain the release of efficacious MMC to the tracheal wall will be to look into areas where higher drug-loadings can be achieved on a reasonably-sized stent; without drug distribution, agglomeration and cytotoxicity affecting too much in the predicted drug-release kinetics and its effects on healthy cells.

The replacement of MMC with dexamethasone (DXM) is also proposed to be studied in future. DXM has been known to inhibit granulation tissue growth and is currently being administered orally, intravenously and topically in humans. In-vitro cell culture (preferably with epithelial cell lines) studies are recommended to be conducted on MMC or DXM loaded stents / films to elucidate cell responses and possible minimum effective concentration of drugs for safety and efficacy, before eventual stent fabrication and implantation for testing. It is a belief that both homogeneously distributed and spray-coated systems have the potential to show improved performance, provided effort is made to design specific experiments to better understand the strengths and drawbacks of each when employed as drug-release systems in an organ as unique as the trachea.

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PATENT

1. Subramanian Venkatraman, Anthony Ng, Boey Yin Chiang Freddy, Lynne Lim. (2011). A Biodegradable Tracheal Stent Composition. *US Provisional Patent #61/454,858*.

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